http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (7): 1250-1256, 2006
ISSN 1028-8880

Effect of Different Levels of Citric Acid on Calcium and Phosphorus Efficiencies in Broiler Chicks

[1]A. Nouri Moghadam, [2]J. Pourreza and [2]A.H. Samie
[1]Former Post Graduate of Azad University, of Khurasgan, Isfahan, Iran
[2]Department of Animal Sciences, Isfahan University of Technology, Isfahan 84156, Iran

Abstract: The present experiment was carried out on 405 broiler chicks (Arian strain) over a period of two weeks (from 8 to 21 days of age) to investigate the effects of different levels of dietary citric acid and phosphorus on calcium and phosphorus requirements in broiler chicks and on their performance. The experiment was a 3×3 factorial one including three levels of zero, 1.5 and 3% citric acid as well as three levels of 0.3, 0.35 and 0.4%, respectively dietary phosphorus in a completely randomized design. Thus, 9 experimental diets were prepared, each of which was randomly administered to three groups of the chicks (three replications for each diet). The effects of different levels of citric acid and phosphorus as well as their interactive effects on body weight, daily feed consumption, feed conversion efficiency, feed/gain ratio, weight percentage of body parts, percentage of bone ash, calcium and phosphorus percentages in bone ash and plasma inorganic phosphorus and calcium were assessed and determined. The results showed that the effects of different levels of citric acid on body weight ($p<0.05$), feed consumption, calcium and phosphorus concentrations in bone ash and plasma ($p<0.01$) were significant but that they had no significant effects on bone ash percentage, feed conversion efficiency and feed/gain ratio. The effects of different levels of phosphorus on body weight, daily feed consumption, bone ash percentage, plasma calcium and phosphorus percentages ($p<0.01$) and bone ash phosphorus percentage ($p<0.05$) were significant while no significant effects were observed in the case of feed conversion efficiency, feed/gain ratio and plasma calcium. The interactive effects of citric acid and phosphorus on 21-day body weight, bone ash percentage ($p<0.05$), feed consumption, bone ash calcium percentage, bone ash phosphorus percentage ($p<0.05$), plasma phosphorus and calcium ($p<0.01$) were significant while they had no significant effects on other parameters measured. From the results obtained, it seems that citric acid plays a role in calcium and phosphorus utilized by poultry and may increase the absorption of these minerals.

Key words: Organic acids, calcium, phosphorus, broilers

INTRODUCTION

An important problem in poultry breeding industry is the metabolism of minerals. The importance of dietary minerals has always been appreciated by man since the early days of taming poultry and their industrial breeding. However, despite this old knowledge, there are still a number of unknown or vaguely known issues in mineral diets, especially for fast-growing animals, that need to be investigated.

Minerals are inorganic materials occurring in nature in the form of salts or mixed with organic compounds. The availability of minerals to animals and their metabolic functions largely depend on the compound in which they are part of. Phosphorus, for instance, when combined with phytic acid is only available to regurgitating animals. Chelating agents are selectively absorbed with different elements. These agents release one mineral in favor of another to which they have a higher tendency. This is the reason why despite the adequacy of certain elements in the diet, deficiency symptoms are at times observed (Leeson and Summers, 2001).

Phosphorus is the second most abundant element in animal body, with 80% of phosphorus found in the bones and teeth and the remainder located in the body fluids and soft tissue. High iron, aluminum and magnesium contents when forming insoluble phosphate complexes can lead to reduced phosphorus absorption (Leeson and Summers, 2001). Phytate also makes phosphorus unavailable reducing its absorption (LeeRussel, 1992).

Corresponding Author: A.H. Samie, Department of Animal Sciences, Isfahan University of Technology, Isfahan 84156, Iran

However, a pH level of around 5 increases phosphorus solubility and thus increases its absorption (Broz *et al*., 1994).

Smith and Kabaija (1985) fed birds with alternatively low calcium and phosphorus diets. It was revealed that when the diet was rich in calcium but deficient in phosphorus, feed consumption reduced. They also investigated the effects of various calcium/phosphorus ratios on phosphorus supplement efficiency to find that increasing calcium/phosphorus ratio from 2:1 to 4:1 reduced feed consumption efficiency and body weight.

Frost and Roland (1991) maintained that reducing dietary phosphorus led to a significant reduction in birds' feed consumption such that the lower the phosphorus content, the less feed is consumed. According to them, as a result of phosphorus deficiency, weigh gain rate and bone ash reduces while feed conversion rate increases.

Calcium absorption mainly takes place in the duodenum and depends on body needs. Most calcium compounds are insoluble. The absorption of calcium in the intestines is facilitated and accelerated by agents that make calcium compounds soluble. The acidic conditions in the intestines are responsible for the solubility of calcium compounds and thus increased absorption of calcium. This is while alkaline conditions cause calcium compounds to sediment and, hence, it's reduced absorption (Leeson and Summers, 2001). Many experiments have shown that organic acids are effective in calcium absorption due to their effect in creating acidic conditions. Certain organic acids like citric acid also serve as a chelating agent for calcium (Boling *et al*., 2000a, b; 2003). Dietitians have quite recently discovered that organic chelating agents may be important factors controlling mineral absorption. Certain organic acids are included among chelates (Leeson and Summers, 2001).

Fumaric acid, propionic acid and citric acid are among acids with known effects on weight gain and food efficiency. Introduction of a small amount of these acids into animal feed lowers the pH level and thus ceases microorganism growths. A number of experiments have been carried out to determine the effect of fumaric acid as growth accelerator. Due to its fruit-like taste, fumaric acid increases feed consumption and thus improves weight gain and feed efficiency (Boling *et al*., 2000a, 2001).

Over 70% of plant phosphorus including that in cereals is found as non-absorbable phytic compounds. Different methods can be used to improve their absorbability. One such method is to use phytase which is limited by certain constraints and is not generally used. However, the numerous studies carried out to establish the use of organic acids to increase availability of phosphorus. According to these studies, creation of an acidic environment and formation of complexes with other elements increase phosphorus absorption (Boling *et al*., 2001). One acid used in this method is citric acid which has been used as a chelating agent in bird feed (Boling *et al*., 2000b, 2001, 2003).

Boling *et al*. (2001) performed experiments with soy feed and corn diets to find positive and significant effects of citric acid (at 4 and 6% levels) on weight gain and bone ash as compared with diets lacking the acid. Regarding the fact that phosphorus absorption depends on the dietary calcium/phosphorus ratio, it is clear that these two elements must be studied together. The objective of the present study is to find a way to create the desirable conditions for calcium and phosphorus absorption through the use of an acid in an attempt to provide an economically balanced and suitable bird diet.

MATERIALS AND METHODS

In this experiment, 405 one-day commercial broiler chicks of Arian strain were used. They were grouped in such a way to obtain almost equal average weights across all groups. The chicks were kept in battery cages where the bed floor was covered with wood chips. In each cage were placed common drinking water cups and feed bins. Water and feed were freely available to chicks without any control throughout the study period.

In a completely randomized and factorial design, each of the 9 experimental diets was randomly fed to three groups of chicks (with three replications for each diet). The experiment was continued from day 7 to 21. The diets included three levels of phosphorus (0.35, 0.40 and 0.45% of available phosphorus) and three levels of citric acid (zero, 1.5 and 3% of the diet). All diets were prepared in such a manner that they contained equal levels of energy and protein. The composition of experimental diets (Table 1). At 21 days of age, one hen and one rooster from each treatment were selected and strangled by breaking their necks after taking blood samples. Blood samples were transferred to lab to determine plasma phosphorus and calcium contents. Calcium and phosphorus were determined using Darman Kave Research Lab and Zistchemi Research standard kits, respectively (Baginski *et al*., 1973; Tietz, 1986).

Body weight and feed consumption rate were measured at 7 and 21 days of age. After slaughter, percentage of body weight and percentage of body components weight were measured and then the tibia and femur bones were removed from the body, packed in

Table 1: Composition of experimental diets (7-21 days) according to kg percentage

Ingredients	1	2	3	4	5	6	7	8	9
Corn	60.45	60.11	59.88	56.88	56.62	56.35	53.25	53.00	52.83
Soybean meal	32.3	32.37	32.41	32.65	32.7	32.75	33.01	33.06	33.09
Oyster shell	2.09	1.91	1.72	2.09	1.91	1.73	2.11	1.92	1.74
Dicalcium Phosphate	0.51	0.81	1.10	0.49	0.78	1.08	0.47	0.76	1.06
Soybean oil	1.21	1.36	1.45	2.70	2.80	2.90	4.22	4.32	4.34
Mineral supplement[1]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Vitamin supplement[2]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Fish meal	2.5	2.5	2.5	2.75	2.75	2.75	3	3	3
Salt	0.2	0.2	.0.2	0.2	0.2	0.2	0.2	0.2	0.2
Methionine	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Citric acid	0	0	0	1.5	1.5	1.5	3	3	3
Total %	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Calculated analysis									
Total dietary phosphorus level (%)	0.3	0.35	0.4	0.3	0.35	0.4	0.3	0.35	0.4
Metabolizable energy (kcal kg^{-1})	2900.06	2900.73	2900.22	2899.57	2898.98	2898.05	2899.39	2899.13	2895.51
Crude protein (%)	20.85	20.85	20.85	20.85	20.85	20.85	20.85	20.85	20.85
Energy/Protein ratio	139.09	139.10	139.09	139.06	139.03	138.99	139.05	139.03	138.87

1. Mineral supplement provided per kilogram of diet: manganese, 80 mg; copper, 10 mg; iodine, 0.8 mg; cobalt, 0.25 mg; selenium, 0.3 mg; zinc, 80 mg; iron, 80 mg. Vitamin supplement provided per kilogram of diet: vitamin A, 10000 IU; vitamin D_3, 2500 IU; vitamin E, 10 IU; vitamin B_1, 2.2 mg; niacin, 30 mg; vitamin B_{12}, 0.015 mg; folic acid, 0.5 mg; biotin. 0.15 mg; cholin chloride, 200 mg

nylon bags, indexed and transferred to cool mortuary (+4°C) for storage. To determine ash calcium and phosphorus contents, the bones were then transferred to lab where they were boiled in water and dried in an oven for 24 h following flesh and cartilage removal. The products were then placed in Soxhlet apparatus for 16 h for fat extraction which were then transferred to dry oven and electric furnace for 8 h for treatment to obtain the ash. The ash was then weighed in order to determine the ash percentage of the bones. It was then used to determine the calcium and phosphorus contents using the standard methods recommended by Association of Official Analytical Chemists (AOAC, 1990).

All facilities needed were provided by the Isfahan University of Technology and the experiment was conducted during summer 2005.

Statistical analyses: Data were analyzed using the General Linear Models (GLM) procedure of Statistical Analyses Systems (SAS, 1999). Differences among treatment means were assessed using the Least Significant Difference test (Carmer and Walker, 1985). Various orthogonal single df comparisons were also made to evaluate the significance of main effects and interactions.

RESULTS AND DISCUSSION

The effect of citric acid: The effect of citric acid on body weight, gizzard weight percentage ($p<0.05$), breast weight percentage, daily feed consumption, ash calcium and phosphorus percentages and plasma calcium and phosphorus ($p<0.01$) were significant. However, citric acid was found to have no significant effect on femur weight percentage, liver weight percentage, feed conversion rate, weight gain/feed ratio and ash percentage (Table 2).

Citric acid plays a bonding role for mineral elements and, thus, contributes to the consumption of minerals. Phosphorus, as a mineral, is then made more available when citric acid is used in the diet (Boling *et al*., 2000b, 2001, 2003). This clearly justifies the significant role of citric acid on feed consumption, which through its positive effect on increased daily feed intake causes increased body weight. Breast weight is, on the one hand, a function of the body weight. It, therefore, follows that the effect of citric acid on feed intake and feed consumption also accounts to a certain extent for the effect of other parameters. Citric acid, on the other hand, is a contributor to taste which increases feed intake. Consequently, two mechanisms may be claimed to exist for the effect of citric acid on feed consumption: 1) contributing to mineral consumption and 2) adding taste to feed.

Boling *et al*. (2001) studied the effect of citric acid on calcium requirement in broiler chicks and concluded that growth response to dietary calcium was not affected by citric acid. They also maintained that introduction of citric acid at 6% level caused a slower weight gain increase and in gain/feed ratio.

The inconsistency between the results obtained in this experiment with the finding of Boling *et al*. (2001) regarding growth rate can be attributed to the level of citric acid used, because higher level of citric acid as used by Boling *et al*. (2001) may affect growth rate adversely. They, further, suggested that a level of 6% for citric acid in diets adequately rich in phosphorus was perhaps too high. Another reason for the discrepancy between the results from Boling *et al.* (2001) and those from the present study was the constant level of dietary calcium used. In this experiment Boling *et al*. (2001) did not claim the reason from their studies to be unknown and declared

Table 2: Comparison of average values of body weight, feed consumption, feed conversion rate, weight gain to feed utilization ratio, weight ratio of body components, bone ash percentage, bone ash calcium and phosphorus percentages and plasma phosphorus for the interaction between dietary phosphorus and citric acid

Major effects	21-day old body weight (g)	Breast weight (%)	Femur weight (%)	Gizzard weight (%)	Liver weight (%)	Feed consumption 8-21 days[1]	Feed conversion rate	Weight gain/feed utilization	Bone ash (%)	Ash calcium (%)	Ash phosphorus (%)	Plasma calcium (%)[2]	Plasma phosphorus (%)[3]
Citric acid													
0	374.7^{b}	14.5^{b}	19.5	2.96^{b}	2.48	50.5^{b}	1.62	0.82	50.9	43.7^{c}	18.3^{b}	9.4^{c}	6.9^{b}
1.5	379.2^{ab}	14.9^{ab}	20.6	2.97^{b}	2.49	51.2^{a}	1.61	0.81	50.7	45.2^{b}	17.9^{b}	9.8^{b}	7.0^{b}
3	384.8^{a}	15.9^{b}	20.0	3.06^{a}	2.50	51.4^{a}	1.60	0.82	50.9	47.8^{a}	19.0^{a}	10.9^{a}	7.8^{a}
Dietary phosphorus													
0.3	372.6^{b}	14.9	20.2	2.98	2.48	50.2^{c}	1.62	0.82	49.5^{b}	43.7^{c}	17.5^{b}	9.9	6.5^{c}
0.35	380.2^{a}	15.1	19.8	3.00	2.50	51.0^{b}	1.60	0.82	51.5^{a}	46.1^{b}	18.8^{a}	10.0	7.1^{b}
0.4	385.6^{a}	14.9	20.1	3.01	2.49	51.9^{a}	1.60	0.82	51.5^{a}	46.9^{a}	18.8^{a}	10.2	8.1^{a}
SE	10.7	0.7	1.5	0.08	0.02	1.0	0.03	0.01	1.3	2.6	1.2	0.8	1.0
Sources of variation													
Citric acid (C)	*	**	NS	NS	*	**	NS	NS	NS	**	**	**	**
Dietary phosphorus (P)	**	NS	NS	NS	NS	**	NS	NS	**	**	*	NS	**
Interaction (P × C)	*	NS	NS	NS	NS	**	NS	NS	*	**	**	**	**

1.Grams/chicken/day, 2. Mg/deciliter, 3.Mg (%), a-c: Average values with different superscripts in each column indicate significant differences among them at 0.05 (%), NS, * and ** indicate no significant and significant at 5 and 1% levels, respectively

that perhaps the results from their study could be attributed to the desirable level of available phosphorus.

Table 2 indicated that increasing citric acid resulted in an increase in phosphorus availability therefore a part of increased growth rate and better feed conversion could be the result of this increased phosphorus availability.

The results obtained are in agreement with the finding of Ahmed *et al.* (1990) who showed that use of citrate increased performance traits, leg bone weight and its ash.

Comparing the means of the measured parameters for different levels of citric acid (Table 2), it is clear that the best responses for body weight, breast weight percentage, gizzard weight percentage and daily feed consumption is observed to a level of 3% citric acid. However, these responses showed no significant differences for breast weight percentage and daily feed consumption from the 1.5% citric acid. This is while the response for gizzard weight percentage was significant only for a 3% citric acid level ($p<0.05$). The reason may be reduced feed concentration and pH level due to utilization of highest citric acid level. This may have effects on increasing muscular movements of the gizzard.

Generally speaking, a 1.5% citric acid level entailed a suitable response in the case of body weight, breast weight percentage and feed consumption. Giesting and Easter (1985) in their study of the effects of organic acid supplements on pig performance showed that growth efficiency ($p<0.05$) was influenced by each (propionic, fumaric and citric) acid used. The results obtained from the present study agreed well with those from both Boling *et al.* (2000a,b) and Giesting and Easter (1985).

From the Table, it is observed that the best responses for ash calcium and phosphorus percentages, as well as for plasma calcium and phosphorus obtained for citric acid at a 3% level. The 1.5% citric acid level also yielded responses for ash phosphorus and plasma phosphorus percentages similar to those for no citric acid diets. The positive effect of citric acid on better utilization of dietary calcium and phosphorus are obvious, by higher bone ash calcium and phosphorus and also higher plasma level of calcium and phosphorus due to added citric acid. These results in not in agreement with the finding of Boling *et al.* (2000a, b) who reported no any improvement in tibia ash due to added citric acid. The inconsistence between the results may be due to the criteria measured. The results suggest that plasma calcium and phosphorus and also bone ash calcium and phosphorus may be better criteria for evaluating the effect of citric acid, on mineral utilization.

The effect of dietary phosphorus: Table 2 shows that the dietary phosphorus on body weight, feed consumption, bone ash percentage, ash calcium percentage, plasma phosphorus ($p<0.01$) and ash phosphorus percentage ($p<0.05$) had significant effects while it had no significant effects on feed conversion rate and plasma calcium content. Neither did the dietary phosphorus have any significant effects on body component weight ratios nor on gain/feed consumption ratio. The significant effect of dietary phosphorus on body weight seems to be more related to increased feed consumption proportionate to higher phosphorus content. Although dietary phosphorus had no significant effect on feed conversion rate, increased phosphorus levels improved the observed feed conversion rate.

In the experiments performed by Perney *et al.* (1993), dietary phosphorus levels had significant effects on feed conversion rate. However, no such results were observed in our study. This may be due to the use of over 50% of inorganic (available) phosphorus in the present study which prevented different levels of phosphorus to show their significant effects.

Table 2, shows the highest and the lowest responses of body weight to dietary phosphorus were observed for dietary phosphorus levels of 0.4 and 0.3%, respectively. The highest response for daily feed consumption belonged to a 0.4% level of dietary phosphorus while the lowest belonged to a 0.3% level. The responses of body weight for both levels of 0.35 and 0.4% dietary phosphorus were the same (380.2 and 385.6 g, respectively). However, a 0.3% total phosphorus level was different from all other levels (372.6 g). This was due to increased feed consumption with increasing dietary phosphorus levels.

In their study of the effects of three dietary calcium/ total phosphorus ratios, Liu *et al.* (1998) reported that reduced calcium/total phosphorus ratio linearly increased the average daily weight gain during the study period ($p<0.03$) and that generally, weight gain/feed consumption ($p<0.001$) and phosphorus absorption increased toward the end of their experiment period. These findings are in good agreement with our findings to the effect that body weight gain and feed consumption were influenced by different levels of dietary phosphorus but the gain/consumption ratio was not significantly affected by dietary phosphorus. The reason for the results are attributed to high dietary calcium concentrations as high concentrations reduce phytic phosphorus consumption in animal diets due to the formation of insoluble calcium-phytate.

The best responses for bone ash percentage and bone ash phosphorus percentage were obtained for a phosphorus level of 0.4% which had no significant differences with the 0.35% level. The best responses to the dietary phosphorus levels for ash calcium and plasma phosphors percentages belonged to the 0.4% level of total dietary phosphorus.

The results from Liu *et al.* (1998) showed that low calcium/phosphorus ratios linearly increased bone strength and bone ash weight. They found that diets with level calcium concentrations had a negative effect on phosphorus utilization which was due to the formation of the calcium-phytate complex. Generally, it seems that the 0.4% level of dietary phosphorus provide the best responses in the measured parameters.

Interactions: The interaction of dietary phosphorus and citric acid on body weight, bone ash [percentage ($p<0.05$), daily feed consumption, ash calcium and phosphorus percentages, plasma calcium and phosphorus ($p<0.01$) were significant but not in the case of other parameters.

Table 3 presents the comparison of average values for the interactions of dietary phosphorus levels with citric acid levels on the parameters measured. Overall, the lowest responses during the experimental period belonged to the 0.3% level of phosphorus in the diet without citric acid. The highest response for the interaction on body weight belonged to the 0.4% level of the diet without citric acid and the 0.3% phosphorus in the diet with 3% citric acid. In the light of these findings, citric acid seems to be effective in the utilization of dietary (phytic) phosphorus. The best response in the case of daily feed consumption belonged to the 0.4% level of phosphorus and the 1.5% level of citric acid, which definitely showed no significant difference with the 0.4% phosphorus in the diet without citric acid and the 0.4% phosphorus in the diet with 3% citric acid. In this case, the effect of dietary phosphorus was more conspicuous. The lowest response for daily feed consumption belonged to the no citric acid level and the 0.3% dietary phosphorus. These results agreed well with those from other studies (Boling *et al.*, 2000b; 2001, 2003).

Boling *et al.* (2000b) showed the positive and significant interactive effect of citrate with Ca/available P ratio on weight gain. It was in this relation that the strong bonding role of mineral elements was recognized.

According to Table 3, the highest response for the interactive effect on bone ash percentage, ash calcium percentage and on plasma calcium belonged to the 3% citric acid and 0.35% dietary phosphorus levels. For the plasma phosphorus, the highest response belonged to the 3% citric acid and 0.4% phosphorus levels while for the ash phosphorus percentage, the highest response belonged to 1.5% citric acid and 0.4% dietary phosphorus levels.

Using two levels of dietary phosphorus (0.2 and 0.45%) and 4 levels of citric acid (0, 2, 4 and 6%), Boling *et al.* (2001) observed that increasing the dietary phosphorus level resulted in a corresponding increase in growth and bone ash. Increases in weigh gain and in leg ash was observed as a result of increased citric acid, which agreed well with the results from the present study.

In another study, Boling *et al.* (2001) used six levels of phosphorus (0.2, 0.25, 0.3, … and 0.45) with two levels of citric acid (0 and 6%), Adding citric acid to low phosphorus diets (0.2 to 0.3%) increased the weight gain and leg bone ash while the effect of citric acid at high available phosphorus was not significant.

The results of the requirement study is not in agreement with the above findings, combination of 3% citric acid and 0.4% available phosphorus resulted higher bone ash calcium and phosphorus and plasma calcium and phosphorus (Table 3). The difference between results

Table 3: Comparison of average values of body weight, feed consumption, feed conversion rate, weight gain to feed utilization ratio, weight ratio of body components, bone ash percentage, bone ash calcium and phosphorus percentages and plasma phosphorus for the interaction between dietary phosphorus and citric acid

P×CA interaction	21-Day old body weight (g)	Breast weight (%)	Femur weight (%)	Gizzard weight (%)	Liver weight (%)	Feed consumption 8-21 days[1]	Feed conversion rate	Weight gain/feed utilization	Bone ash (%)	Ash calcium (%)	Ash phosphorus (%)	Plasma calcium (%)[2]	Plasma phosphorus (%)[3]
$C_1 \times P_1$	361.0^{c}	14.3	20.0	2.9	2.47	49.2^{e}	1.65	0.81	50.2^{c-e}	40.7^{d}	17.7^{c}	9.4^{b}	6.3^{e}
$C_1 \times P_2$	375.7^{ab}	14.8	18.5	3.0	2.50	50.5^{cd}	1.6	0.82	50.9^{b-d}	43.9^{c}	17.3^{cd}	9.5^{b}	6.9^{de}
$C_1 \times P_3$	387.3^{a}	14.3	20.0	3.0	2.49	51.8^{ab}	1.59	0.82	51.6^{bc}	46.4^{b}	19.9^{a}	9.7^{b}	7.4^{cd}
$C_2 \times P_1$	369.3^{bc}	14.5	20.0	2.7	2.49	50.0^{d}	1.63	0.81	48.8^{e}	43.9^{c}	16.6^{d}	9.3^{b}	6.9^{de}
$C_2 \times P_2$	382.7^{ab}	15.3	20.9	3.0	2.50	51.4^{bc}	1.60	0.82	51.1^{bc}	44.4^{c}	17.5^{cd}	9.4^{b}	6.3^{e}
$C_2 \times P_3$	385.7^{a}	15.0	20.8	2.9	2.49	52.2^{a}	1.61	0.81	52.2^{b}	47.2^{b}	19.7^{a}	10.7^{a}	7.9^{bc}
$C_3 \times P_1$	387.3^{a}	15.8	20.5	3.1	2.49	51.3^{bc}	1.57	0.83	49.4^{de}	46.3^{b}	18.3^{bc}	11.0^{a}	6.3^{e}
$C_3 \times P_2$	382.3^{ab}	15.0	20.0	3.0	2.50	51.1^{bc}	1.60	0.82	54.4^{a}	49.9^{a}	19.4^{ab}	11.2^{a}	8.2^{ab}
$C_3 \times P_3$	384.7^{a}	15.5	19.5	3.1	2.50	51.7^{ab}	1.60	0.82	50.8^{b-d}	47.3^{b}	19.2^{ab}	10.6^{a}	8.9^{a}
SE	10.7	0.7	1.5	0.08	0.02	1.0	0.03	0.01	1.3	2.6	1.2	0.8	1.0
Sources of variation													
Citric (C)	*	**	NS	NS	*	**	NS	NS	NS	**	**	**	**
Dietary phosphorus (P)	**	NS	NS	NS	NS	**	NS	NS	**	**	*	NS	**
Interaction (C × P)	*	NS	NS	NS	NS	**	NS	NS	*	**	**	**	**

1. Grams/chicken/day, 2. Mg/deciliter, 3. Mg (%) a-e: Average values with different superscripts in each column indicate significant differences among them at 0.05 percent. NS, * and ** indicate no significant and significant at 5 and 1% levels, respectively. C_1, C_2 and C_3: Zero, 1.5 and 3% levels of citric acid, respectively. P_1, P_2 and P_3: 0.3, 0.35 and 0.4% levels of dietary phosphorus

is relatively related to the conditions and quantity of citric acid and phosphorus, gut microflora, dietary composition, duration of experiment, chick strain and finding criteria used for evaluation.

For better evaluation of the effect of citric acid on mineral utilization it is suggested that other criteria such as plasma parameter can leads to precise results.

Considering the results from the present study and the interactions between dietary phosphorus levels and different levels of citric acid, it may be claimed that citric acid influenced the improvement of dietary phosphorus utilization and suitable responses were observed at low dietary phosphorus levels in the presence of citric acid (interactive effect).

It seems that in addition to its calcium bonding role and preventive role in the formation of calcium phosphate and calcium phytate complex, citric acid has other roles. Regarding the fact that pH level in the digestive system can help the hydrolysis of phytic acid rings and can activate internal phytase, the findings from the present study indicate that citric acid in diets with phosphorus contents lower than levels recommended by NRC played a more conspicuous and decisive role. Given the environmentally destructive effects of waste matter with phosphorus compound residues and the limitations in using phytase, the use of citric acid is highly recommended in order to reduce dietary phosphorus levels. Additionally, the use of citric acid has a positive added effect on animal performance which awaits further research. From the results obtained from this study, however, a 3% citric acid level is recommended.

REFERENCES

Ahmed, S.H., A.T. Cantor, H. Johnson and A.Y. Robert, 1990. Relationship of dietary aluminum, phosphorus and calcium to phosphorus and calcium metasbolism and growth performance of broiler chicks. Poult. Sci., 64: 966-971.

AOAC., 1990. Official Methods of Analysis. 1st Edn., Association of Official Analytical Chemists, Washington, DC., pp: 88.

Baginski, E.S., S.S. Marie, W.L. Clark and B. Zak. 1973. Direct microdetermination of serum calcium. Clin. Chim. Acta, 46: 49-54.

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

Boling, S.D., M.W. Douglas, J.L. Snow,C.M. Parsons and D.H. Baker, 2000b. Citric acid does not improve phoephorus utilization in laying hens fed a corn-soybean meal-based diet. Poult. Sci., 79: 1335-1337.

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

Boling, S.D., M.W. Douglas, R.B. Shirley, J.L. Snow, A.B. Coreless and C.M. Parsons. 2003. The effect of various dietary levels of citrate in phosphorus-deficient diets fed to laying hens. Poult. Sci., 80: 1-75.

Broz, J., P. Oldale and A.P. Voltz, 1994. Effect of supplemental phytase on performance an phosphorus utilization in broiler chikens fed a low phospohorus diet without addition of inorganic phosphates. Brit. Poult. Sci., 35: 273-280.

Carmer, S.G. and W.M. Walker, 1985. Pairwise multiple compariSons of treatment means in agronomic research. J. Agron. Educ., 14: 19-26.

Frost, T.J. and D.A. Roland, 1991.The influence of various calcium and phosphorus levels on tibia strenth and egg shell quality of pullets during peak production. Poult. Sci., 70: 963-969.

Giesting, D.W. and R.A. Easter. 1985. Response of starter pigs to supplementation of corn-soybean meal diets with organic acids. J. Anim. Sci., 60: 1288-1294.

LeeRussel, M.C., 1992. Minerals in Animal and Human Nutrition. Academic Press. INC. London.

Leeson, S. and J.D. Summers, 2001. Scott`s Nutrition of the Chicken. 4th Edn. Guelph, Ontario, Canada.

Liu, J., D.W. Bollinger and D.R. Ledoux. and T.L. Veum, 1998. Lowering the dietary calcium to total phosphorus ratio icreases phosphorus utilization in low-phosphorus corn-soybean meal diet supplemented with microbial phytase for growing-finishing pigs. J. Anim. Sci., 76: 808-813.

Perney, K.M., A.H. Cantor, M.L. Straw and K.L. Herkelman, 1993. The effect of dietary phytase on growth performance and phosphorus utilization of broiler chicks. Poult. Sci., 72: 2706-2714.

SAS Institute, 1999. SAS User's Guide. Release 8.2 Edn. SAS Institute Inc. Cary. NC.

Smith, O.B. and E. Kabaija, 1985. Effect of high dietary Ca and wide calcium phosphorus ration in broiler diets. Poult. Sci., 64: 1713-1720.

Tietz, N.W. (ED.), 1986. Textbook of Clinical Chemistry, W.B. Saunders Co., Philadelphia, pp: 1351.