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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

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ABSTRACT

An experiment was conducted to study the effect of phytase and citric acid on the growth performance, phosphorus, calcium and nitrogen retention with sixty 1 day old mixed sex broiler chicks for 42 days. The experiment consists of four treatments with three replicates and five chicks in each replicate. The treatments were: T1-control diet (0.3% available phosphorus), T2-control diet with 2% citric acid, T3-control diet with 800 U phytase enzyme, T4-control diet with 800 U phytase plus 2% citric acid. The results revealed that the weight gain of the chicks was significantly higher ($p<0.05$) for groups that received phytase and phytase plus citric acid supplementation. Feed intake and feed conversion ratio were significantly higher for group that received both phytase and citric acid. Phytase and citric acid supplementation individually and in combination significantly improved phosphorus retention. Calcium and nitrogen retention were significantly higher for groups that received supplementation of phytase and phytase plus citric acid. No significant difference in phosphorus and calcium content of toe samples were observed between broilers of different diets.

Key words: Enterobacteriaceae phytase, citric acid, broiler, mineral retention, growth performance, available phosphorus

INTRODUCTION

Poultry rations are based largely on cereal grains and oilseeds meals. Unfortunately, around two thirds of the phosphorus in cereal grains and oilseed meals are present in the form of phytic acid and some other minerals such as Zn, Cu, Co, Fe and Ca to form phytate phosphorous, which are not available for poultry and most of them are excreted in the litter (Cosgrove, 1966). Phytic acid is considered as an anti nutritional factor as they reduce the availability of cations (Sohail and Roland, 1999), carbohydrates, aminoacids (Ravindran *et al*., 1999), enzymes (Sebastian *et al*., 1998) and also lowers fat digestibility (Ravindran and Bryden, 1999).

Phytase, (myo-inositol hexaphosphate phosphohydrolases) are enzymes that can hydrolyze phytic acid. Initially, poultry diets were supplemented with exogenous phytase to reduce the inclusion of inorganic P and allow better utilization of phytate-P (Nelson *et al*., 1971). However, later studies demonstrated that the benefits of using dietary phytases are not restricted to improving mineral retention, but also may improve performance, energy and amino acid availability (Cowieson *et al*., 2006).

Organic acids have been evaluated numerous times for their efficacy in improving growth performance and P utilization in pigs and chickens (Jongbloed *et al*., 2000). Pileggi *et al*. (1956) confirmed the earlier studies of Shohl (1937) that addition of a citric acid/sodium citrate mixture (1:1) to rachitogenic rat diets prevented rickets in rats and further demonstrated that the beneficial effects of citrate were only observed in rats fed phytate containing diets. Boling *et al*. (2000) showed that citric acid is efficacious in improving phytate-P utilization in chicks fed corn-soybean meal (SBM) diets containing no supplemental phosphorus.

This study was undertaken to investigate the influence of phytase and citric acid both independently and simultaneously on growth performance and mineral utilization in broiler chicks.

MATERIALS AND METHODS

Four corn soya based diets were prepared and were supplemented with commercial citric acid and Enterobacteriaceae derived phytase. Diets were formulated to contain the same nutrient density except for phytase and citric acid (Table 1). Four experimental diets were as follows: T1-control diet (0.3% available phosphorus), T2-control diet with 2% Citric Acid (CA), T3-control diet with 800 U phytase enzyme, T4-control diet with 800U phytase plus 2% CA.

A total of 60 day old straight run broiler chicks were used for 42 days during October-December, 2009. Chicks were housed in deep litter pens maintained on a 23 h constant light schedule. They

Table 1: Ingredient and chemical composition (g kg^{-1}) of diet as fed to the broilers

Items	Starter diet	Finisher diet
Ingredients		
Maize	585.000	640.000
Soyabean meal	346.000	281.000
Calcite/LSP	17.000	15.000
Dicalcium phosphate	10.000	10.000
Rice bran oil	34.000	46.000
Threonine	0.180	0.260
Methonine	2.630	2.310
Lysine	1.740	1.850
Sodium bicarbonate	0.530	0.650
Salt	3.600	3.280
Vitamin premix	0.001	0.001
Calculated composition (%)		
ME Mcal kg^{-1}	3.070	3.200
C. Protein	21.500	19.000
C. Fibre	3.240	2.960
Calcium	0.950	0.890
T. Phosphorus	0.540	0.530
A. Phosphorus	0.300	0.300
Analyzed composition (%)		
C. Protein	22.000	21.500
Calcium	1.000	1.010
A. Phosphorus	0.330	0.350

Mineral mixture added at the level kg^{-1} feed supplied manganese: 81 mg, Zinc: 78 mg, Iron: 30 mg, Iodine: 3 mg, Copper: 3 mg and Cobalt: 1.5 mg. Vitamin AB2D3K added at the level kg^{-1} feed supplied vitamin A: 16500 IU, B2: 10 mg, D3: 3200 IU and vitamin K: 2 mg. Vitamin B complex added at the level kg^{-1} feed supplied, thiamine 2.8 mg, pyridoxine 5.6 mg, Niacin 42 mg, cyanocobalamine 28 mcg, vitamin E 28 mg, calcium D pantothenate 28 mg and folic acid 2.8 mg, calcium 30.1 mg

were individually weighed and randomly allocated to different diets, thus each of dietary treatments had three replications in which 5 birds were assigned. The chicks were fed with weighed quantity of feed *ad libitum* and had free access to water. Feed intake was measured weekly while mortality was recorded daily.

A metabolic trial was conducted for a period of three days (days 33, 34 and 35) to check for phosphorus (P), calcium (Ca) and nitrogen (N) retention. Excreta from each cage was collected daily for 3 days and stored in plastic bags at -20°C. The N content of the excreta was determined in fresh material. Excreta samples were dried at 100°C and grounded to pass a 1 mm sieve. Feeds and excreta were analyzed for proximate components, P, Ca and N as per the procedure described by AOAC (1990).

The birds were chosen randomly from each treatment and killed by cervical dislocation on day 42. Toe samples were obtained as per the procedure described by Yi *et al.* (1996). They were used to determine the amount of ash, P and Ca (AOAC, 1990).

The collected datas were subjected to analysis of variance (one way ANOVA) at 5% significance level.

RESULTS

No health problems were associated with the use of the enzyme throughout the experiment. Mortality during the trial was within acceptable levels and was not related to dietary treatments.

Effects of phytase and CA supplementation individually and simultaneously on performance of broiler chicks are shown in Table 2. The body weight gain of broilers was significantly ($p<0.05$) higher in T3 and T4 throughout the study period as compared to T1 and T2. Among the treatments, the group T4 that received both phytase and CA had significantly higher feed intake during 1-21 days. Significant difference was observed in feed conversion ratio between T1 and T3, T4 during 21-42 days. Significant difference in weight gain and feed intake were observed between T1 and T2 throughout the study period.

The effect of enzyme and CA supplementation on dry matter, crude ash, Ca, P and N retention were shown in Table 3. The percentage of P and Ca retention were significantly higher ($p<0.05$) for T4 compared to other groups. N retention was significantly higher for T3 and T4 groups. There is no significant difference in Ca and N retention between T1 and T2.

The effect of phytase and CA supplementation on the toe ash, Ca and P contents of 42 days old broilers were shown in Table 4. In the present study, the P and Ca deposition in the toe sample were not significantly affected by phytase or CA supplementation.

Table 2: Effect of phytase and citric acid supplementation on Mean (±SE) weight gain, feed intake and feed conversion ratio of broilers fed corn soybean meal diet for 42 days

Treatment groups	Weight gain (g)		Feed intake (g bird^{-1})		Feed conversion ratio	
	1-21 days	21-42 days	1-21 days	21-42 days	1-21 days	21-42 days
T1	465.9±21.15^{a}	1014±65.35^{a}	727±14.15^{a}	1585±38.97^{a}	1.51±0.01^{c}	1.56±0.04^{b}
T2	561.7±20.35^{b}	1184±41.61^{b}	840±12.17^{b}	1826±12.86^{b}	1.49±0.01^{bc}	1.62±0.03^{c}
T3	701.6±5.72^{c}	1767±94.23^{c}	993±17.68^{c}	2533±53.73^{c}	1.42±0.03^{a}	1.48±0.03^{a}
T4	717.6±9.67^{c}	1838±35.33^{c}	1038±10.21^{d}	2711±50.85^{d}	1.45±0.03^{ab}	1.47±0.03^{a}

Values given in each cell is the mean of ten observations. Mean within a column with no common superscript differ significantly ($p<0.05$)

Table 3: Effect of phytase and citric acid supplementation on drymatter, total ash, total phosphorus, calcium and nitrogen retention in broilers fed corn soybean meal diet

			Mineral retention (%)		
Treatment groups	Dry matter (%)	Total ash (%)	Total phosphorus	Calcium	Nitrogen
T1	63.36±1.94	27.46±0.05	39.01±0.55^{a}	35.80±0.13^{a}	10.48±0.04^{a}
T2	64.28±1.36	28.04±0.01	40.05±0.16^{b}	36.12±0.23^{a}	10.75±0.58^{a}
T3	64.63±1.39	29.51±0.01	41.65±0.12^{c}	40.09±0.30^{b}	13.02±0.64^{b}
T4	64.71±5.41	30.24±0.01	42.86±0.28^{d}	44.50±0.32^{c}	13.18±1.07^{b}

Values given in each cell is the Mean (±SE) of five observations. Mean within a coulumn with no common superscript differ significantly ($p<0.05$)

Table 4: Effect of phytase and citric acid supplementation on toe ash, phosphorus and calcium content of toe samples of broilers fed corn soybean meal diet

Treatment groups	Toe ash (%)	Phosphorus (%)	Calcium (%)
T1	14.98±0.40	8.57±0.42^{a}	24.32±0.24^{a}
T2	18.78±0.42	10.72±0.66^{ab}	25.12±1.55^{ab}
T3	23.80±0.36	11.32±1.26^{ab}	28.36±2.63^{ab}
T4	24.11±0.70	12.50±1.04^{b}	30.26±1.52^{b}

Values given in each cell is the Mean (±SE) of five observations. Mean within a coulumn with no common superscript differ significantly ($p<0.05$)

DISCUSSION

Supplementation of phytase and CA independently and simultaneously in the basal diet improved the body weight gain of broiler chicks from 1-21 days of age. However, higher body weight gain was recorded in T4. The present findings are in agreement with Boling *et al.* (2000) who also observed a significant increase in weight gain in chicks when phytase plus CA was added to the diet. It was also reported that phytase supplementation of 2500 FTU in chicks increased body weight gain by 10.2% (Pirgozliev *et al.*, 2007). Significant increase in body weight gain of chicks was observed when CA was supplemented in diets containing 0.2% available P (Boling-Frankenbach *et al.*, 2001). Atapattu and Nelligaswatta (2005) as well reported that CA could have a positive effect on body weight gain when diets are low in available phosphorus.

In the present study, supplementation of diets with phytase and CA clearly increased the feed intake and show lower feed conversion ratio in the broilers. Pirgozliev *et al.* (2007) has reported that phytase supplementation in the diet caused 11.2% increase in feed intake by the chicks. Many researchers have observed an improvement in feed intake due to phytase supplementation (Sebastian *et al.*, 1996; Cabahug *et al.*, 1999; Bingol *et al.*, 2009). Rafacz-Livingston *et al.* (2005) has explained that the feed intake was increased by CA in NHC and commercial boiler chicks. Cowieson *et al.* (2006) observed a better feed conversion ratio with phytase supplementation in corn-soybean-based diets. Increase in feed intake and body weight gain of phytase and CA supplemented groups may due to the fact that phytase may have increased the availability of nutrients (Dilger *et al.*, 2004) decreased passage rate of feed stuffs in digestive tract and degraded the cellwall of ingredients (Viveros *et al.*, 2000). Inclusion of CA reduced the pH levels of the feeds and digesta of the crop and gizzard. Therefore, it can be assumed that CA itself and/or low pH environment created by it stimulate the feed intake (Atapattu and Nelligaswatta, 2005) and therefore resulted in body weight gain.

The effect of phytase plus CA diet on P and Ca retention was significantly higher ($p<0.05$) compared to other groups. Brenes *et al.* (2003) has reported an increase in P and Ca retention by 600 U phytase plus 2% CA. Al-Sharafat *et al.* (2009) has observed that adding microbial phytase in combination with CA decreased the P disappearance in the crop content of laying hens. The mechanism by which phytase and CA increase P and Ca retention is that CA combines with dietary Ca and reduces the formation of highly indigestible Ca phytate complexes. Maenz (2001) suggested that one or more weak phosphate groups of the phytic acid have a higher affinity for protons than Ca and Mg. Inclusion of CA thus make phytic acid partially protonized and prevents the formation of insoluble Ca phytate complexes. Phytase which is active at low pH hydrolyze phosphate groups bound to phytate molecules. Furthermore, low intestinal pH (created by CA) increases the solubility and absorption of P and Ca in the small intestine (Overland *et al.*, 2002) thereby the amount of retained P and Ca was increased. When, the responses to single addition of these two compounds in the present study are compared with previous research, the data herein are in agreement with previous results for CA (Boling *et al.*, 2000; Boling-Frankenbach *et al.*, 2001) and phytase (Santos *et al.*, 2008; Dilger *et al.*, 2004).

The effect of phytase and CA individually and in combination on N retention were investigated. Significantly higher N retention was observed in boilers fed phytase alone and phytase plus CA. Dilger *et al.* (2004) have also observed a significant difference in N retention in boilers when phytase was added to the diet. Sands *et al.* (2001) has also observed that microbial phytase supplementation improved N retention in pigs.

Although, supplemental phytase individually and in combination increased the percentage of P and Ca retention, no significant effect on percentage of toe P and Ca was observed. Similar results were also obtained by Ravindran *et al.* (2001), Akyurek *et al.* (2005) and Musavi *et al.* (2009). Ahmad *et al.* (2000) has also observed that P and Ca concentration of the bone was not affected by phytase supplementation.

In conclusion, these results indicated that the addition of phytase improved the growth performance and increased Ca, P and N retention in chicks. Inclusion of CA along with phytase caused an increase in mineral utilization. Proper combination of CA and phytase may represent a practical solution to improve growth performance, phytate P and other mineral utilization.

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