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Effects of Citric Acid and Microbial Phytase Supplementation on Performance and Phytate Phosphorus Utilization in Broiler Chicks

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An experiment was conducted to investigate the effects of supplementation of broilers diet with both citric acid and microbial phytase on performance criteria and utilization of phytate phosphorus (P) in broiler chicks from day old to 49 d of age. The experiment was carried out using a completely randomized design with factorial arrangement of 3×2 (0, 2.5 and 5 percentage of citric acid and 0 and 500 IU of phytase enzyme per kg). Experimental diets were formulated so that had 0.2% lower available P than positive control diet. Four replicates of 15 chicks per each were fed experimental (6 diet) and positive control diet. Weight gain (WG), feed consumption (FC), alkaline phosphatase activity, plasma Ca and P concentration, tibia ash and liver, spleen and abdominal fat weight and also mortality were measured. Supplementation of low P diet with citric acid significantly improved WG and feed: gain ($P<0.05$), decreased activity of alkaline phosphatase ($P<0.01$) and increased plasma P concentration ($P<0.01$), but had no significant effect on feed intake, tibia ash and plasma Ca concentration. Microbial phytase significantly ($P<0.05$) improved weight gain and feed: gain in low P diet and also a significant ($P<0.01$) interaction between citric acid and phytase was observed throughout the experimental period.

Key words: alkaline phosphatase, broiler, citric acid, phytase

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Introduction

The major ingredients used in poultry feeds are of plant origin. About two third of the phosphorous (P) in these feedstuff is present as phytate P, which is poorly utilized by poultry. The inability of poultry to utilize phytate P causes both economic and environmental problems. Physical methods such as soaking, drying, germination (Jongbloed *et al.,* 1991), supplementation of diets with exogenous microbial phytase (Kornegey, 2001) and vitamin D (Mitchel and Edwards, 1996) have found to be effective in increasing phytate hydrolysis. Several authors (Boling *et al.,* 1998, 2000, 2001; Brenes *et al.,* 2003) have found that citric acid alone or in combination with phytase increased the phytate hydrolysis in chicken. It is hypothesized that citric acid complex with Ca and reduces the formation of more stable Ca-phytate complexes. Alternatively, citric acid may change the intestinal pH for better phytase activity. Theoretically these supplements could have synergistic or additive effect. Interaction between microbial phytase and vitamin D analogs (Biehle *et al.,* 1995; Mitchel and Edwards, 1996) and phytase and citric acid (Boling *et al.,* 2000) have been assessed and the results showed that there may be a synergistic effect. The objective of this study was to investigate the effects of supplementing diet with both phytase and citric acid on the performance of broiler chicks, activity of alkaline phosphatase (ALP) and utilization of phytate P in corn soy meal based diet.

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Materials and Methods

A total of 420 feather sexed (male: female, 50:50) Ross 308 day old broiler chicks randomly assigned to 28 groups of 15 chicks per each, so that the initial weight and weight distribution were similar among the groups. Each floor pen contained one bell-shaped waterer, one hand-filled hanging feeder and a brooding light. The temperature was maintained at 32±1℃ in the first week and reduced by 3℃ per week to 21℃. Feed and water were provided ad libitum and a continuous lighting schedule were used through the experiment. The experiment was carried out using a completely randomized design with factorial arrangement of 3×2 (0, 2.5 and 5 percentage of citric acid and 0 and 500 IU of phytase enzyme per kg). A positive control diet was also used according to National Research Council (NRC) (1994) recommendations. A basal diet (negative control) was formulated to had 0.2% lower available P (aP) than positive control diet. Broiler chicks were fed the following diets throughout the experiment:

1) positive control (PC) with NRC (1994) recommended aP level, 2) negative control (NC) with 0.2% lower aP than PC diet and without addition of phytase and citric acid, 3) NC+500 unit microbial phytase per kg of diet, 4) NC+2.5% citric acid, 5) NC+2.5% citric acid+500 unit microbial phytase per kg of diet, 6) NC+5% citric acid, 7) NC+5% citric acid+500 unit microbial phytase per kg of diet. All the diets were kept isocaloric and isonitrogenous, and formulated to meet or exceed the NRC (1994) recommendations. Diets were provided in the mash form. Citric acid was supplied as monohydrate citric acid with 92% purity, and phytase (Natuphos® 500, BASF Corp., Mt. Live, Nj) source also had 10,000 unit active phytase per gram. Ingredient composition and the calculated nutrient composition are given in the Table 1. Body weight and feed consumption were measured for each pen at 21, 42 and 49 d of age. At the end of experimental period, 2 birds per pen were selected randomly and blood samples were taken from their wing vein to measure serum ALP, Ca and P using an automated chemistry analyzer of Zest Shimi Kit (Ziest Chem., Diagnostica, Cat No.10–508, 5256). Relative liver and spleen weight were also measured in 2 birds per pen and tibia bones of these birds were thawed to remove any trace of flesh, solvent extracted to remove fat and then shed in a muffle furnace at 550℃ for 8 hours. Daily mortality rate also were measured. Data were analyzed using general linear model procedure of SAS® (SAS Institute, 1990) software and significant differences among treatments were separated using Duncan's multiple range test.

Results and Discussion

Weight gain (WG), feed consumption (FC) and feed: gain of broiler chick fed different levels of citric acid, with or without phytase supplementation, are presented in Table 2. Results indicated that dietary supplementation with citric acid, significantly improved WG at 21 ($P<0.0003$), 42 ($P<0.0001$) and 49 ($P<0.0002$) d of age. However, addition of 5% citric acid compared to 2.5% citric acid had no further benefits. Faster growth was associated with more efficient utilization of feed in citric acid fed groups. Our findings support the thesis given by Boling *et al.* (2000, 2001) that citric acid could have a positive effects on growth performance when diets are low in aP and high Ca: aP ratio. When Ca: aP ratio is low, the release of additional P causes Ca deficiency by creating unfavorable Ca: aP ratio. The aP level used in this experiment was 0.2% and the Ca: aP ratio was 4.5 during the first 21 days. The mechanisms by which citric acid increases phytate hydrolysis is/are not clear. One possible mechanism is that it combines with dietary Ca and thus reduces the formation of highly indigestible Ca phytate complexes. Maenz (2000) suggested that one or more weak phosphate groups of the phytic acid have a higher affinity for protons than Ca and Mg. At acidic condition citric acid may serve as a proton donor and thus make phytic acid partially protonized and prevents the formation of insoluble Ca phytate complexes. Second possible mechanism could be that citric may alter the pH profile of the gastrointestinal tract making a more favorable envi-

Table 1. Ingredients and nutrient composition (g/kg) of experimental diets in starter period

Ingredients	Treatment						
	1	2	3	4	5	6	7
Corn	616.5	623.2	622.2	583.7	582.6	544.1	543.1
Soybean meal (44%)	338.5	337.2	337.4	344.8	345.1	352.3	352.5
Soybean oil	4.9	2.9	3.2	7.5	7.8	12.1	12.4
Oyster shell	15.2	22.6	22.6	22.5	22.5	22.4	22.4
Dicalcium phosphate	13.8	3	3	3.1	3.1	3.2	3.2
Salt	4.1	4.1	4.1	4.1	4.1	4.1	4.1
Premix[1]	5	5	5	5	5	5	5
DL-Methionine	2	2	2	2	2	2.1	2.1
Citric acid (92%)	—	—	—	27.3	27.3	54.7	54.7
Phytase[2]	—	—	0.5	—	0.5	—	0.5
Calculated Analysis (data on dry matter)							
ME (kcal/kg)	2950	2950	2950	2950	2950	2950	2950
Crude protein	202.5	202.5	202.5	202.5	202.5	202.5	202.5
Available P	4	2	2	2	2	2	2
Total P	6.5	4.5	4.5	4.5	4.5	4.5	4.5
Calcium	9	9	9	9	9	9	9
Methionine+cystine	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Lysine	10.7	10.7	10.7	10.7	10.7	10.7	10.7

[1] Vitamin and mineral mix supplied/kg diet: vitamin A, 11000 IU; vitamin D_3, 1800 IU; vitamin E, 11 mg; vitamin K_3, 2 mg; Vitamin B_2, 5.7 mg; Vitamin B_6, 2 mg; vitamin B_{12}, 0.024 mg; Nicotinic acid, 28 mg; folic acid, 0.5 mg; pantothenic acid, 12 mg; choline chloride, 250 mg; Mn, 100 mg; Zn, 65 mg; cu, 5 mg; Se, 0.22 mg; I, 0.5 mg; Co, 0.5 mg.
[2] Natuphos® (BASF Crop., Mt. Olive, NJ) was used to supply 500 U microbial phytase per kilogram of diet.

Table 2. Effects of Dietary levels of citric acid, microbial phytase, on weight gain (WG), feed consumption (FC), and feed conversion ratio (FCR) in broiler chicks from 0 to 7 weeks of age

Treatment		Weight gain (g)				Feed consumption (g)				Feed conversion ratio (FCR)			
Citric acid (%)	Phytase (U/kg)[1]	1–21	21–42	42–49	1–49	1–21	21–42	42–49	1–49	1–21	21–42	42–49	1–49
0	(0) PC[2]	464[a]	1114[a]	408[ab]	1987[a]	836[a]	2776	1025	4637[a]	1.80[b]	2776	2.51[b]	2.33[b]
0	(0) NC[3]	300[d]	718[d]	293[c]	1311[d]	793[ab]	2433	1062	4289[ab]	2.66[a]	2433	3.65[a]	3.25[a]
0	500	368[c]	852[c]	382[b]	1604[c]	786[ab]	2363	945	4095[b]	2.12[b]	2363	2.47[b]	2.55[b]
2.5	0	369[c]	863[c]	368[b]	1602[c]	760[b]	2420	949	4129[b]	2.05[b]	2420	2.56[b]	2.57[b]
2.5	500	432[ab]	998[ab]	436[a]	1867[ab]	805[ab]	2524	1090	4420[ab]	1.85[b]	2524	2.49[b]	2.36[b]
5	0	393[bc]	963[b]	420[ab]	1777[b]	772[b]	2573	1040	4386[ab]	1.97[b]	2573	2.47[b]	2.47[b]
5	500	408[bc]	968[b]	401[ab]	1777[b]	805[ab]	2605	1066	4478[ab]	1.97[b]	2605	2.66[b]	2.52[b]
Pooled SEM		15.5	30.1	15.4	44.1	17.9	139.9	43.5	148.3	0.09	139.9	0.08	0.08
Main effects													
Citric acid	0	334[b]	785[b]	337[b]	1458[b]	790	2398	1004	4192	2.39[a]	2398	3.06[a]	2.90[a]
Phytase	2.5	400[a]	931[a]	402[a]	1734[a]	782	2472	1019	4274	1.95[b]	2472	2.56[b]	2.47[b]
	5	400[a]	966[a]	410[a]	1777[a]	789	2589	1053	4432	1.97[b]	2589	2.53[b]	2.49[b]
	0	354[b]	848[b]	360[b]	1563[b]	775	2475	1017	4268	2.23[a]	2475	2.89[a]	2.76[a]
	500	403[a]	940[a]	406[a]	1749[a]	799	2497	1034	4331	1.98[b]	2497	2.54[b]	2.48[b]
		probabilities											
Citric acid		0.0003	0.0001	0.0002	0.0001	0.9113	0.3984	0.4781	0.2688	0.0007	0.0012	0.0001	0.0006
Phytase		0.0007	0.0019	0.0009	0.0002	0.1546	0.8471	0.6195	0.6002	0.0072	0.0131	0.0002	0.0020
Phytase× Citric acid		0.1192	0.0620	0.0028	0.0091	0.3880	0.8177	0.0240	0.2685	0.0442	0.0016	0.0001	0.0044

[a–c] Means in columns with no common superscript differ significantly ($P<0.05$).
[1] Natuphos® (BASF Crop, Mt. Olive, NJ) was used to supply 500 U microbial phytase per kilogram of diet.
[2] PC = Positive control group.
[3] NC = Negative control group.

ronment for phytase from intestinal, plant or microbial origins.

As expected, phytase supplementation also resulted in significantly ($P<0.01$) better WG and feed: gain, but neither citric acid nor phytase had any significant effect on FC (Table 2). The following mechanism are thought to be involved in the improvement of performance by phytase: 1) liberation of P from phytate salt (Qian *et al.,* 1996; Sebastian *et al.,* 1996), 2) enhance digestibility of starch (Knuckles and Betschart, 1987) or availability of protein and amino acids (Selle *et al.*, 2000) and 3) increase efficiency of utilization of myo-inositol (final product of phytate dephosphorylation) and other material which liberated from phytate complex (Simons *et al.,* 1990).

Dietary supplementation of citric acid ($P<0.0093$) and phytase ($P<0.0587$) significantly reduced ALP activity (Table 3). Although 2.5% citric acid did not lead to any change in plasma P concentration, as did phytase ($P<0.0033$), 5% citric acid significantly ($P<0.0095$) increased plasma P concentration (Table 3). ALP is Zn^{++} containing metaloenzyme that has a key roll in bone mineralization. Decreased blood aP level by any reason, will increase ALP activity. Viveros *et al.* (2002) and Brenes *et al.* (2003) reported that decreasing aP level of diet increased ALP activity. Phytase and citric acid through the mechanism mentioned above facilitate liberation of phytate P and so increase plasma P concentration (as observed in our study) and resulted in decreased ALP activity. As Boling *et al.* (2001) showed that citric acid did not improve Ca availability; plasma Ca concentration was not also affected by either phytase or citric acid in our study (Table 3). Phytase significantly increased tibia ash, but citric acid increased it only numerically (Table 3). Since low level of aP will decreased tibia ash (Lesson *et al.,* 2000; Viveros *et al.,* 2002; Bernes *et al.,* 2003), it seems phytase and citric acid exert their effects on tibia ash by increasing availability of phytate P. Several studies have previously shown higher tibia ash in phytase and citric acid supplemented low P diets (Ahmad *et al.,* 2000; Lesson *et al.,* 2000; Snow *et al.,* 2004).

As reported by Simons *et al.* (1990), supplementation of diet with both citric acid ($P<0.0018$) and phytase ($P<0.0087$) significantly decreased mortality rate in our study, but had no effect on liver, spleen and abdominal fat relative weight (Table 3).

Significant interaction was observed between citric acid and phytase so that a combination of citric acid and phytase resulted in better WG ($P<0.009$), improved feed: gain ($P<0.004$), decreased ALP activity ($P<0.02$) and increased plasma P concentration ($P<0.06$) than solely

Table 3. Effects of Dietary levels of citric acid, microbial phytase, on alkaline phosphates (ALP), calcium (Ca), phosphorus (P), tibia ash, carcass yield, liver percentage, spleen percentage, abdominal fat percentage, mortality percentage in broiler chicks from 0 to 7 weeks of age

Treatment		ALP	Ca	P	Tibia ash	Carcass	Liver	Spleen	Abdominal	Mortality
Citric acid (%)	Phytase (U/kg)[1]	(U/L)	(mg/dl)	(mg/dl)	(mg/g)	yield (%)	(%)	(%)	fat (%)	(%)
0	(0) PC[2]	956[a]	9.24	6.17[a]	41.93[a]	71.43	2.11	0.163[a]	2.60	9.09[b]
0	(0)NC[3]	933[ab]	9.78	3.93[bc]	29.14[d]	70.76	2.13	0.110[b]	2.02	51.51[a]
0	500	939[ab]	10.45	3.93[bc]	39.44[ad]	69.08	2.28	0.130[b]	2.15	12.12[b]
2.5	0	926[abc]	10.33	3.12[c]	35.08[bc]	69.74	2.02	0.123[b]	2.14	15.15[b]
2.5	500	861[d]	9.74	5.44[ad]	38.64[abc]	70.82	2.18	0.100[b]	2.19	3.03[b]
5	0	886[cd]	10.37	4.65[bc]	32.86[cd]	70.32	2.18	0.117[b]	2.25	3.03[b]
5	500	900[bcd]	10.13	6.57[a]	37.38[abc]	71.39	1.94	0.114[b]	2.88	9.09[b]
Pooled SEM		12.5	0.6	0.4	1.8	0.9	0.1	0.009	0.3	5.8
Main effects										
Citric acid	0	936[a]	10.11	3.93[b]	34.29	69.92	2.20	0.012	2.08	31.81[a]
	2.5	893[b]	10.03	4.28[b]	36.86	70.28	2.10	0.112	2.17	9.09[b]
	5	893[b]	10.25	5.61[a]	35.12	70.86	2.06	0.116	2.56	6.06[b]
Phytase	0	915	10.16	3.90[b]	32.36[b]	70.27	2.11	0.116	2.14	23.23[a]
	500	900	10.11	5.31[a]	38.48[a]	70.43	2.13	0.115	2.41	8.08[b]
					probabilities					
Citric acid		0.0093	0.9329	0.0095	0.3708	0.0018	0.4613	0.6978	0.3321	0.0018
Phytase		0.1887	0.9113	0.0033	0.0012	0.0087	0.7767	0.8186	0.3383	0.0087
Phytase× Citric acid		0.0213	0.5605	0.0671	0.1677	0.5679	0.1909	0.1121	0.6441	0.5679

[a–c] Means in columns with no common superscript differ significantly ($P < 0.05$).
[1] Natuphos® (BASF Crop, Mt. Olive, NJ) was used to supply 500 U microbial phytase per kilogram of diet.
[2] PC = Positive control group.
[3] NC = Negative control group.

addition of them. The results obtained here suggest that phytase and citric acid may have some additive or synergistic effects in poultry, but further research is needed to define the optimal levels of the two compounds when fed together. A proper combination of citric acid and phytase may represent a practical solution to improving phytate-P utilization and decreasing P levels in poultry excreta.

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