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Effects of Dietary Available Phosphorus and Microbial Phytase on Growth Performance, Carcass Traits, Serum Minerals and Toe Ash Content in Broiler Chicks

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ABSTRACT

Effect of dietary phosphorus and microbial phytase was investigated on the growth performance, carcass, toe ash and serum Ca and P parameters using 160 Ross-308 broiler chicks. For this purpose, phosphorus low (0.329, 0.265 & 0.219%) and very low (0.251, 0.200, 0.145%) levels of available phosphorus (AP) were used for starter, grower and finisher periods, respectively. two Microbial phytase (MP) was used at 400 and 800 FTU/kg levels in each period. Experiments were laid in 2x2 factorial arrangements with 4 replications and 10 birds per each replicate. The interactive effect of AP×MP for growth performance, serum Ca and P and toe ash content was not significant ($p>0.05$); whereas it was significant ($p<0.01$) for carcass and liver percent. Data of main effects indicated that lowering AP levels caused a decrease ($p<0.01$) in weight gain and worse FCR ($p<0.01$) at the starter, grower and overall of experiment period (0-49 d), but 800 FTU/kg of MP improved ($p<0.01$) weight gain and FCR at said periods. Carcass, breast, thighs and toe ash percent was affected ($p<0.01$) by very low AP and decreased. MP @ 800 FTU/kg increased ($p<0.05$) toe ash percent. An increase in Ca and decrease in P levels of blood serum was observed ($p<0.01$) with very low AP. Results indicated that interaction effects of AP×MP could not affect the growth performance, toe ash, serum minerals and carcass traits except carcass percent and make it better in birds fed low AP diets.

Key Words: Phosphorus; Microbial phytase; Carcass; Toe ash; Serum; Broiler

INTRODUCTION

The phytic acid content in plant-based feed ingredients is of great importance as this acid has a high phosphorus (P) content (28.2%), but poultry have a poor ability to utilize phytate P (NRC, 1994; Ravindran *et al.*, 2006), because they do not produce significant amounts of intrinsic phytase enzyme to hydrolyze phytate molecule (Smith *et al*., 2004; Chivandi *et al.*, 2010). The availability of P from cereal grains is largely determined by percentage of P in grain bound as phytate, which can vary between 50 and 58% of total P (Leytem *et al.*, 2008). Phytic acid, myo-inositol phospated on all of its hydroxyl groups can bind minerals and proteins ionically in aqueous medium (Zaghari, 2009). Therefore, poultry diets must supplement with inorganic P sources to meet the nutritional requirements of birds, which leads to increased poultry production cost (Leytem *et al.*, 2008; Abo-Omar & Sabha, 2009; Han *et al.*, 2009). Moreover, high levels of phosphorus are excreted in animal feces and this is hazardous for environment especially in areas of intensive livestock operations (Abo-Omar & Sabha, 2009; Ebrahim-Nezhad *et al.*, 2010) resulting in eutrophication that negatively affects aquatic ecosystems (Chivandi *et al.*, 2010).

Phytase enzymes are used to solve phytic acid or phytate problems. Phytase can help to improve the availability of bound phosphorus in phytate and reducing P levels in excreta by approximately one-third without depressing performance (Nelson, 1967). Phytase is a phosphatase that is a capable of catalyzing the release of phosphorus from phytate (Musapuor *et al.*, 2006). Many factors influence the response to an enzyme additive like enzyme specificity, concentration of the substrate, dosage of the enzyme, type of ingredient and it's quality, level of nutrients in diet and age of animal (Cowieson *et al.*, 2006; Francesch & Geraert, 2009). It has been demonstrated that microbial phytase supplementations enhance the phytic acid hydrolysis and increase the release of mineral and proteins, which are bound to phytic acid (Zaghari, 2009), significantly improve phytate P utilization in poultry diets

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(Selle & Ravindran, 2007; Hughes *et al.*, 2009), increase P retention and tibia ash, has positive effects on weight gain, feed intake, feed conversion rate and reduce endogenous loss (Selle & Ravindran, 2007; Francesch & Geraert, 2009). The objective of this study was to determine the effect of microbial phytase on the growth and carcass performance, toe ash content and serum Ca and P levels of broiler chicks.

MATERIALS AND METHODS

Birds and diets: Total of 160 days-old broiler chicks from Ross 308 strain were individually weighed and randomly allocated to the four treatment groups with four replications of 10 birds per each. The experiment was assigned to a 2×2 factorial arrangement with two available phosphorus (AP) levels and two levels of added microbial phytase (MP). Experimental diets were formulated according to NRC (1994) recommendations but the nutrient concentrations in diets were modified on the basis of metabolizable energy content. Birds were fed experimental diets for starter (0-21 d), grower (22-42 d) and finisher (43-49 d) periods. Treatments 1 and 2 with low AP (LAP) had a 20% deficient in AP in starter and grower, and 25% in finisher period in contrast with NRC recommendations; also treatments 3 and 4 with very low AP (VLAP) had a 40% deficient in AP in starter and grower, and 50% in finisher period in contrast with NRC recommendations. Low (0.329, 0.265 & 0.219%) and very low (0.251, 0.200, 0.145%) levels of available phosphorus (AP) were used for starter, grower and finisher periods, respectively.

Natuphos® 1000 FTU, derived from *Aspergillus Niger* was used to supply 400 and 800 FTU microbial phytase (MP) per kilogram of diet, therefore the MP was added on top to experimental diets. In treatments 1 and 3 added 400 FTU/kg also for treatments 2 and 4, 800 FTU/kg of MP. Chicks were under uniform management conditions. They had free access to feed and water and light provided according 23 h light and 1 h dark throughout the experimental period.

Growth performance: Averages of feed intake (AFI) and body weight gain (AWG) were measured weekly and feed conversion rate (FCR) were used to determine the growth performance at the end of starter, grower, finisher and overall of experimental period (0-49 d).

Carcass traits: At the end of experiment, final body weight was taken, then two birds from each pen (each bird as a replicate), were randomly selected and tagged. Birds were fasted (water *ad libitum*) for 8 h on day 49 then birds were weighed and slaughtered by serving both of the right and left carotid artery and jugular vein in a single cut and bled for 180s. After slaughter, carcass weight measured on the chilled carcass after removal of feather, head, lungs, GIT, liver, kidney, abdominal fat, dissected and collected. Carcass (CAP), breast meat (BMP), thigh (TP), intestine (IP), liver (LP) and abdominal fat (AFP) were calculated as the percentage of fasted live body weight.

Toe ash: For determination of toe ash content, the toe samples were excised from left foot of individual bird. Talons and soft tissues were removed then samples were dried at 100°C for 24 h. Dried samples were passed through a flame heating process (coaling), weighed and ashed in a muffle furnace at 550°C for 8 h. Toe ash was expressed as a percentage of dry weight.

Blood samples procedures: At day 49, 2 mL blood samples were collected from brachial vein of chicks selected to carcass traits measurements, for determining serum minerals (Ca & P) content. Collected blood samples were allowed to clot and then centrifuged at 3000×g (Beckman Avanti J.) for 10 min. Serum Ca and P parameters were measured on auto-analyzer (ALCYON 300-Abbott, USA) using commercially available kits.

Statistical analysis: Data were subjected to analysis of variance using the general linear models procedures of SAS software (SAS Institute, 2003). Significant differences among treatment means were determined at $P < 0.05$ by Duncan's multiple-range test.

RESULTS AND DISCUSSION

Growth performance: The effects of AP levels and MP supplementation on growth performance are summarized in Table II. Data of main effects showed that very low available phosphorus (VLAP) decreased AFI ($p<0.01$) at the end of grower (42 d) and overall of experimental period (0-49 d). Birds fed VLAP diets had a lower ($p<0.05$) AWG at the end of starter (21 d), also AWG decrease ($p<0.01$) observed at the end of grower, finisher (49 d) and overall of experimental period. FCR increased ($p<0.01$) in all the periods in birds fed VLAP diets. The inclusion of 800 FTU/kg diet improved ($p<0.01$) AWG and FCR parameters in starter, grower and overall of experiment period. The interactive effect of AP×MP for growth performance was not significant ($p>0.05$) at any stage.

Boling *et al.* (2000) reported that deficiencies in AP cause decrease in BW, AFI and increase in FCR. Adding phytase to LAP corn soybean meal diets significantly improve the weight gain and FCR of broilers (Xing-Dong *et al.*, 2002; Dilger *et al.*, 2004). These results are in agreement with the present findings. An increase in AWG, AFI, FCR and retention of P has also been reported earlier (Ghasemi *et al.*, 2006; Han *et al.*, 2009) with phytase supplementation in poultry. The increase in BW is a result of increase in FI that improve by phytase addition. Zong-fu *et al.* (2007) indicated that supplemental phytase reduce AFI and FCR in starter, FCR in grower and AFI and FCR at the overall of period but there was no significant effect of phytase and AP on growth performance.

Carcass traits: The effect of AP on carcass parameters showed that VLAP resulted in an increase ($p<0.01$) in low value parts of carcass (FP, HP & LP) with no effects on GP and AFP, but it caused a decrease ($p<0.01$) in CAP, BMP and TP. Levels of MP had not significant effect on carcass

Table I: Ingredients and composition of experimental diets

Ingredient and composition	Starter (0-21 d)				Grower (22-42 d)				Finisher (43-49 d)			
	T_1[1]	T_2	T_3	T_4	T_1	T_2	T_3	T_4	T_1	T_2	T_3	T_4
	(%)											
Ground yellow corn	64.66	64.66	64.77	64.77	70.99	70.99	71.08	71.08	71.62	71.62	71.72	71.72
Soybean meal (48% CP)	31.76	31.76	31.83	31.83	25.83	25.83	25.89	25.89	23.48	23.48	23.51	23.51
Sunflower oil	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.08	2.08	2.08	2.08
DCP	1.00	1.00	0.58	0.58	0.70	0.70	0.35	0.35	0.48	0.48	0.08	0.08
Oyster shell	1.58	1.58	1.82	1.82	1.61	1.61	1.81	1.81	1.57	1.57	1.84	1.84
Sodium chloride	0.35	0.35	0.35	0.35	0.30	0.30	0.30	0.30	0.24	0.24	0.24	0.24
Vitamin mix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Trace mineral mix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.15	0.15	0.15	0.15	0.07	0.07	0.07	0.07	0.03	0.03	0.03	0.03
L-Lysine HCL	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Microbial phytase[4]	0.04	0.08	0.04	0.08	0.04	0.08	0.04	0.08	0.04	0.08	0.04	0.08
Calculated analysis												
ME, kcal/kg	2946	2946	2952	2952	3011	3011	3016	3016	3156	3156	3160	3160
CP (N × 6.25)	21.18	21.18	21.22	21.22	18.81	18.81	18.85	18.85	17.71	17.71	17.73	17.73
ME/CP	139.12	139.12	139.08	139.08	160.03	160.03	159.94	159.94	178.25	178.25	178.24	178.24
Calcium	0.913	0.913	0.915	0.915	0.846	0.846	0.847	0.847	0.777	0.777	0.795	0.795
Available phosphorus	0.329	0.329	0.251	0.251	0.265	0.265	0.200	0.200	0.219	0.219	0.145	0.145
Ca/AP	2.77	2.77	3.64	3.64	3.19	3.19	4.23	4.23	3.51	3.51	5.47	5.47
Methionine	0.48	0.48	0.48	0.48	0.37	0.37	0.37	0.37	0.32	0.32	0.32	0.32
Lysine	1.108	1.108	1.111	1.111	0.949	0.949	0.951	0.951	0.881	0.881	0.882	0.882
TSAA	0.82	0.82	0.82	0.82	0.68	0.68	0.68	0.68	0.61	0.61	0.61	0.61

1) Birds in each period fed T_1 and T_2 diets were containing low available phosphorus (LAP) and T_3 and T_4 were containing very low available phosphorus (VLAP)

2) The vitamin premix supplied the following per kilogram of complete feed: vitamin A, 4,500 IU (retinyl acetate); cholecalciferol, 1,000 IU; vitamin E, 25 IU (dl-a-tocopheryl acetate); vitamin B12, 0.02 mg; menadione, 1.5 mg; riboflavin, 3 mg; thiamine, 1.5 mg; pantothenic acid, 5 mg; niacin, 20 mg; choline, 150 mg; folic acid, 0.5 mg; biotin, 0.5 mg; pyridoxine, 2.5 mg

3) The mineral premix supplied the following per kilogram of complete feed: manganese (MnSO4\H2O), 60 g; zinc (ZnO), 40 mg; iron (FeSO4\7H2O), 80 mg; copper (CuSO4\5H2O), 8 mg; selenium (Na2SeO3), 0.2 mg; iodine (Iodized NaCl), 0.8 mg; cobalt (CoCl2), 0.4 mg

4) Natuphos® 1000 FTU/g (BASF Crop., Mt. Olive, NJ) derived from *Aspergillus niger* was used to supply 400 and 800 FTU microbial phytase per kilogram of diet; therefore the basal diet was prepared and phytase was added on top to experimental diets containing

Table II: Effects of dietary available phosphorus and microbial phytase levels on growth performance from 0-49 d of age

					Growth Performance											
	AP (%)			MP (FTU/kg)	AFI				AWG				FCR			
Treatment	0-21	22-42	43-49		21 d	42 d	49 d	0-49 d	21 d	42 d	49 d	0-49 d	21 d	42 d	49 d	0-49 d
					(g/b)								NU			
1	0.329	0.265	0.219	400	764.7	2816.5	1342.5	4923.7	511.9	1554.8	668.1	2734.8	1.49	1.81	2.01	1.80
2	0.329	0.265	0.219	800	799.5	3037.9	1313.8	5151.1	551.0	1719.4	658.1	2928.5	1.45	1.77	2.00	1.76
3	0.251	0.200	0.145	400	767.4	2379.3	1269.8	4416.4	480.3	1241.1	496.3	2217.7	1.60	1.92	2.61	1.99
4	0.251	0.200	0.145	800	795.62	2511.0	1238.8	4545.4	519.9	1351.8	552.8	2424.4	1.53	1.86	2.29	1.88
SEM					12.24	58.05	23.07	74.44	9.31	39.98	21.56	61.97	0.01	0.01	0.08	0.02
Main effects																
AP																
0.329-0.265-0.219[1]					782.1	2927.2^a	1328.1	5037.4^a	531.5^a	1637.1^a	663.1^a	2831.7^a	1.47^b	1.79^b	2.01^b	1.78^b
0.251-0.200-0.145[2]					781.5	2445.2^b	1254.3	4480.9^b	500.1^b	1296.4^b	524.5^b	2321.0^b	1.56^a	1.89^a	2.45^a	1.93^a
MP																
400					766.0	2597.9^b	1306.1	4670.1	496.1^b	1397.9^b	582.2	2476.2^b	1.55^a	1.86^a	2.31	1.90^a
800					797.6	2774.4^a	1276.3	4848.3	535.4^a	1535.6^a	605.4	2676.4^a	1.49^b	1.81^b	2.15	1.82^b
Source of variance					*P-value*											
AP effect					NS	<0.001	NS	<0.001	0.025	<0.001	0.003	<0.001	<0.001	<0.001	0.014	<0.001
MP effect					NS	0.011	NS	NS	0.007	0.001	NS	0.003	<0.001	<0.001	NS	0.013
AP × MP					NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

AFI: Average feed intake, AWG: Average weight gain, FCR: Feed conversion rate

AP: Available phosphorus, MP: Microbial phytase, g/b: grams per bird, NU: no unit

1) LAP: Low available phosphorus

2) VLAP: Very low available phosphorus; amounts are AP% respectively for starter, grower and finisher periods

SEM: Standard error of the mean, based on pooled estimate of variance, *P-value*: Probability value

a,b: Means followed by different letters within columns are different by Duncan's multiple range test in 0.05 significance level

Table III: Effects of dietary available phosphorus and microbial phytase levels on carcass traits and toe ash content

	AP (%)			MP (FTU/kg)	CAP	BMP	TP	FP	HP	LP	GP	AFP	Toe Ash
Treatment	**0-21**	**22-42**	**43-49**						**(%)**				
1	0.329	0.265	0.219	400	67.46[a]	30.47	26.15	4.54	0.57	2.04[b]	1.57	2.04	15.36
2	0.329	0.265	0.219	800	66.47[ab]	29.62	25.83	4.49	0.58	2.22[b]	1.73	1.94	15.95
3	0.251	0.200	0.145	400	63.65[c]	27.41	24.94	5.11	0.69	2.72[a]	1.67	2.06	13.62
4	0.251	0.200	0.145	800	65.33[b]	28.86	25.16	4.81	0.63	2.54[a]	1.51	1.81	14.35
SEM					0.30	0.30	0.15	0.06	0.01	0.05	0.04	0.06	1.25
Main effects													
AP													
0.329-0.265-0.219[1]					66.97[a]	30.04[a]	25.99[a]	4.51[b]	0.57[b]	2.13[b]	1.65	1.99	15.65[a]
0.251-0.200-0.145[2]					64.49[b]	28.13[b]	25.05[b]	4.96[a]	0.66[a]	2.63[a]	1.59	1.94	13.98[b]
MP													
400					65.56	28.94	25.54	4.82	0.63	2.38	1.62	2.05	14.49[b]
800					65.90	29.24	25.50	4.65	0.61	2.38	1.62	1.88	15.15[a]
Source of variance									*P-value*				
AP effect					<0.001	0.005	0.006	<0.001	0.001	<0.001	NS	NS	<0.001
MP effect					NS	NS	NS	NS	NS	NS	NS	NS	0.046
AP × MP					0.018	NS	NS	NS	NS	0.010	NS	NS	NS

CAP: Carcass percent, BMP: Breast meat percent, TP: Thighs percent, FP: Feet percent, HP: Heart Percent, LP: liver percent, GP: Gizzard percent, AFP: Abdominal fat percent; Carcass traits are as a percentage of fasted body live weight before slaughter

AP: Available phosphorus, MP: Microbial phytase

1) LAP: Low available phosphorus and 2) VLAP: Very low available phosphorus; amounts are AP% respectively for starter, grower and finisher periods

SEM: Standard error of the mean, based on pooled estimate of variance, *P-value*: Probability value

a,b: Means followed by different letters within columns are different by Duncan's multiple range test in 0.05 significance level

traits ($p>0.05$). Interactive effect of AP×MP was significant ($p<0.01$) only for CAP and LP that is resultant of AP effect. T1 had a higher and T3 had a lower percent of carcass. T3 and T4 had higher amounts of LP.

Scheideler (2000) reported that overall carcass yields were not affected by AP level, but leg quarter weights were significantly higher in broilers fed LAP plus phytase in diets. Preston *et al.* (2000) and Pillai *et al.* (2006) showed that phytase addition can significantly increase percentage of the important parts of carcass compared to P-deficient diets; however, this is not confirmed by Angel *et al.* (2007) and our results. In contrast, Abo-Omar and Sabha (2009) indicated that phytase enzyme supplementation had no effect on thighs, legs, wings and breast. It is important to maintain adequate P during the entire growth period of the bird, as a continuous sub-marginal level of P from the onset of feeding inhibits growth and results in carcass defects (Yan *et al.*, 2004).

Toe ash: The main effect data indicated that decrease in dietary AP content caused a significant ($p<0.01$) reduction in toe ash content. MP @ 800 FTU/kg resulted in an increase ($p<0.05$) in the toe ash content. The interaction between AP and MP levels was not significant ($p>0.05$) for toe ash content (Table III).

Toe ash has been used as a measure of P status (Broz *et al.*, 1994). Ravindran *et al.* (1995) reported that body weight gain and toe ash are more sensitive than tibia ash as response criteria for P bioavailability assay in 21 days-old broilers. An increase in the toe/tibia/bone ash content by the supplementation of phytase has also been reported earlier (Silversides *et al.*, 2004; Rezaei *et al.*, 2007; Han *et al.*, 2009; Zaghari, 2009). Our findings endorse the previous findings for poultry and for other species like pekin ducks (Orban *et al.*, 1999) and turkeys (Atia *et al.*, 2000).

Table IV: Effects of dietary available phosphorus and microbial phytase levels on serum minerals

	AP (%)			Phytase (FTU/kg)	Ca	P
Treatment	**0-21**	**22-42**	**43-49**		**(mg/dl)**	
1	0.329	0.265	0.219	400	10.30	4.31
2	0.329	0.265	0.219	800	9.74	4.89
3	0.251	0.200	0.145	400	10.81	3.74
4	0.251	0.200	0.145	800	11.16	3.43
SEM					0.13	0.15
Main effects						
AP						
0.329-0.265-0.219[1]					10.02[b]	4.60[a]
0.251-0.200-0.145[2]					10.99[a]	3.58[b]
MP						
400					10.56	4.03
800					10.45	4.16
Source of variance					*P-value*	
AP effect					<0.001	0.001
MP effect					NS	NS
AP × MP					NS	NS

AP: Available phosphorus, MP: Microbial phytase, Ca: Calcium, P: Phosphorus

1) LAP: Low available phosphorus and 2) VLAP: Very low available phosphorus; amounts are AP% respectively for starter, grower and finisher periods

SEM: Standard error of the mean, based on pooled estimate of variance, *P-value*: Probability value

a,b: Means followed by different letters within columns are different by

Blood Factors: The main effect data of AP indicated that Ca and P in serum had a significant difference ($p<0.01$) as the LAP diets had a lower Ca and higher P amounts in blood serum than VLAP. There were however, no significant differences ($p>0.05$) observed at MP and AP×MP effect for serum Ca and P (Table IV).

Some studies indicated that phytase supplementation can increase significantly serum P but with no differences in

serum Ca concentration (Scheideler, 2000; Ghasemi *et al.*, 2006). Some reports are available on increase in serum P and decrease in Ca by adding phytase in the poultry diets (Viveros *et al.*, 2002; Shirley & Edwards, 2003; Onyango *et al.*, 2004; Han *et al.*, 2009). Serum level of P is a result of the homeostatic regulation of P, significant lowering of P level may be indicative of low body P reserves as was, perhaps, the case in the VLAP and LAP diets (Onyango *et al.*, 2004).

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