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Effect of Phytase on the Growth Performance, Calcium and Phosphorus Availability of Broilers

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Abstract: This study was carried out to determine the effects of phytase on the growth performance, bone mineralization, digestion and metabolization of nutrientsubstance in Avian broilers. Each of 400, 1 day old male Avian broilers and 400, 1 day old female Avian broilers was randomly divided into 5 groups (Group I, available phosphorus-adequate group without phytase; Group II, available phosphorus-deficient group without phytase; Group III, available phosphorus-deficient group with 250 U kg^{-1} phytase; Group IV, available phosphorus-deficient group with 500 U kg^{-1} phytase; Group V, available phosphorus-deficient group with 750 U kg^{-1} phytase). Each group included 4 replicates, each with 20 broilers. The results showed that the effect of phytase on the growth permance in male broilers was more obvious than that of female. Dry tibia weights of male and female broilers fed with Group II and III diets were decreased compared with Group I; Group IV and V were increased compared with Group I. Tibia phosphorus contents of male and female broilers fed with Group II-V were decreased compared with Group I. Tibia calcium contents of male broilers fed with Group II and V diets were increased by compared with Group I; Group III and IV were decreased compared with Group I. Tibia calcium contents of female broilers fed with Group II, IV and V diets were increased compared with Group I; Group III was decreased compared with Group I. Apparent digestibility coefficients of total phosphorus of Group II-V in male and female broilers were increased compared with Group I. Phosphorus concentrations of faeces of Group II-V in male and female broilers were decreased compared with Group I. Apparent digestibility coefficients of calcium Group II-V in male and female broilers were increased compared with Group I. The result showed that phytase added to available phosphorus-deficient broiler diet could improve the growth performance, bone mineralization as well as calcium and phosphorus availability.

Key words: Phytase, growth performance, calcium availability, phosphorus availability, broilers

INTRODUCTION

Phosphorus is an important element in the feeding of pigs and poultry. Phytic acid is the main storage form of phosphorus in many plant-derived feeds such as corn, cereals and soybean and monogastric animals have insufficient endogenous mucosal phytase to effectively digest dietary phytate (Nelson, 1967). Consequently, most of the phytate-bound phosphorus in the diet can not be utilized by monogastric animals. Phytate can also bind other nutrients and digestive enzymes in the gut thereby reducing nutrient digestibility and availability to the body (Lenis and Jongbloed, 1999). Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate.

Addition of microbial phytase to monogastric diets reduces the amount of P getting into the environment by improving its utilization (Waldroup, 1999). Phytase has been shown to improve utilization of other minerals such as Fe, Mg and Zn in pigs (Adeola, 1995; Adeola *et al*., 1995) and chickens (Biehl *et al*., 1995; Sebastian *et al*., 1996).

Microbial phytase in diets has also been reported to improve protein and amino acid digestibilities in the chicken (Biehl and Baker, 1997; Namkung and Leeson, 1999; Shaw *et al*., 2010, 2011). The main objective of the current trial was to evaluate the effects of a microbial phytase feed additive on broiler growth performance, phosphorus utilization and calcium utilization.

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MATERIALS AND METHODS

Phytase: The experimental microbial phytase was derived from *Aspergillus ficuum* and had an analyzed enzyme activity of 2500 phytase units/g. One phytase unit (FTU) is defined as the quantity of enzyme required to liberate 1 μmol of inorganic P/min at pH 5.5 from an excess of 7.5 μM sodium phytate at 37°C.

Experimental design and diets: Each of 400, 1 day old male Avian broilers and 400, 1 day old female Avian broilers was randomly divided into 5 groups, respectively. Each group included 4 replicates, each with 20 broilers. Group I was available phosphorus-adequate group without phytase (1-21 days, 0.42% available phosphorus; 22-42 days, 0.36% available phosphorus). Group II was available phosphorus-deficient group without phytase (1-21 days, 0.33% available phosphorus; 22-42 days, 0.28% available phosphorus). Group III was available phosphorus-deficient group with 250 U kg^{-1} phytase (1-21 days, 0.33% available phosphorus; 22-42 days, 0.28% available phosphorus). Group IV was available phosphorus-deficient group with 500 U kg^{-1} phytase (1-21 days, 0.33% available phosphorus; 22-42 days, 0.28% available phosphorus). Group V was available phosphorus-deficient group with 750 U kg^{-1} phytase (1-21 days, 0.33% available phosphorus; 22-42 days, 0.28% available phosphorus). All diets were corn-soybean meal based and formulated to meet or exceed NRC recommendations for the 0-21 days old broiler (NRC, 1994) with the exception of dietary P (Table 1).

Housing and management: Broilers were provided *ad libitum* access to water and dietary treatments from days 1-42 and battery temperatures were maintained at 32 and 27°C from days 8-14 and 15-21, respectively. Individual body weight of broilers and feed consumption per cage were recorded, mortality was monitored on a daily basis.

Digestion experiment and excreta collection: Chromic oxide (0.5 g kg^{-1} DM) was added to the diets as an exogenous outside indicator and the diets containing chromic oxide were given to broilers during a 4th day adaptation period (days 36-39). In the following 3 days (days 40-42), excreta were collected twice daily from all chickens in 4 pens per treatment. Excreta collected per pen during the 3 days were pooled and represented 1 replicate. Contaminants such as feathers and scales were removed carefully before excreta were stored in closed containers at -20°C immediately after collection to prevent microbial fermentation. On day 43, 4 chickens, respectively from each replicate were killed by a lethal dose of sodium pentobarbital injected intraperitoneally for slaughter experiment.

Growth performance: At 42 days of age, chicks weight and feed intake were recorded by cage. Feed to gain ratio was then calculated from the ratio of feed intake to body weight gain (per cage) for the 42 days of experimental treatment. Mortality was checked twice daily and weight of dead chickens was used to correct feed consumption and feed to gain ratio (Table 2).

Bone mineralization: Ca and P concentrations were measured in the left tibia. The tibia were cleaned from adherent tissue at sampling and pooled by cage (4 replicates/diet), defatted (24 h in ether), dried (105°C) for 12 h and weighed. Bones were ashed (550°C for 14 h) and ash weight was calculated relative to tibial dry weight.

Table 1: Diet composition and nutrition level

	1-21 days					22-42 days				
Ingredient (%)	I	II	III	IV	V	I	II	III	IV	V
Corn	51.45	51.45	51.45	51.45	51.45	53.45	53.45	53.45	53.45	53.45
Soybean meal	32.50	32.50	32.50	32.50	32.50	30.00	30.00	30.00	30.00	30.00
Bran	8.00	8.00	8.00	8.00	8.00	10.00	10.00	10.00	10.00	10.00
Fish meal	2.00	2.00	2.00	2.00	2.00	0.00	0.00	0.00	0.00	0.00
Bean oil	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Stone powder	0.70	1.10	1.10	1.10	1.10	1.15	1.50	1.50	1.50	1.50
Calcium phosphate	1.30	0.75	0.75	0.75	0.75	1.30	0.75	0.75	0.75	0.75
Premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Zeolite powder	0.00	0.20	0.20	0.10	0.00	0.00	0.20	0.20	0.10	0.00
Phytase	0.00	0.00	0.10	0.20	0.30	0.00	0.00	0.10	0.20	0.30
Nutrition level										
ME (MJ kg^{-1})	12.00	11.99	11.99	11.99	11.99	11.94	11.93	11.93	11.93	11.93
Crud protein (%)	20.60	20.60	20.60	20.60	20.60	18.78	18.78	18.78	18.78	18.78
Calcium (%)	0.89	0.89	0.89	0.89	0.89	0.87	0.86	0.86	0.86	0.86
Available phosphate (%)	0.42	0.33	0.33	0.33	0.33	0.36	0.28	0.28	0.28	0.28
Lysine (%)	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09	1.09
Methionine (%)	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45

Table 2: Effect of phytase on gain and the ratio of feed to gain in broilers

Gender	Ingredient	I (0 U kg^{-1})	II (0 U kg^{-1})	III (250 U kg^{-1})	IV (500 U kg^{-1})	V (750 U kg^{-1})
Male	Average gain (kg)	1.96±0.012^{bc}	1.88±0.072^{bc}	2.03±0.052^{b}	2.12±0.047^{a}	2.16±0.056^{a}
	Feed/gain	1.77±0.100^{a}	1.85±0.070^{a}	1.68±0.030^{b}	1.62±0.080^{bc}	1.63±0.070^{bc}
Female	Average gain (kg)	1.85±0.019^{b}	1.72±0.071^{bc}	1.84±0.038^{bc}	1.88±0.020^{b}	1.95±0.033^{a}
	Feed/gain	1.87±0.048^{b}	1.95±0.039^{a}	1.81±0.042^{bc}	1.80±0.030^{cd}	1.73±0.032^{e}

Values within rows with different superscript letters are significantly different ($p<0.05$)

The resultant ash was solubilized on a sand heater (300°C 15 min) in 10 mL 6 N HCl and 30 mL demineralized water. The solution was transferred after filtration (ashless filters) into a 100 mL volumetric flask. About 10 mL of a lanthanum chloride solution were added and volumetric flask was completed to 100 mL with demineralized water.

Calculations: Apparent nutrient retention and digestibility values were calculated by using the index method with the equation:

$$CADx = 100\% (C_1/C_2) \times (P_2/P_1) \times 100\%$$

CADx = The apparent nutrient digestibility coefficient
C_1 = The concentration of chromic oxide present in the diet
C_2 = The concentration of chromic oxide present in excreta
P_1 = The nutrient concentration present in the diet
P_2 = The nutrient concentration present in excreta

All values for C_1, C_2, P_1 and P_2 are expressed as percentage of dry matter.

Statistical analysis: The datas were subjected to statistical analysis for interpretation of results by using analysis of variance technique with completely randomized design. Treatment means were compared by using the Duncan multiple range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Growth performance: Average gain of male broilers fed with Group II diet was improved by 4.08% ($p>0.05$) compared with Group I; the ratio of feed to gain of Group II was decreased by 4.52% ($p>0.05$) compared with Group I; average gain of Group III-V were increased by 3.57 ($p>0.05$), 8.16 ($p<0.05$) and 9.89% ($p<0.05$) compared with Group I, respectively the ratio of feed to gain of Group III-V were decreased by 5.08 ($p>0.05$), 8.47 ($p<0.05$) and 9.89% ($p<0.05$) compared with Group I, respectively. Average gain of male broilers fed with Group II diet was improved by 4.08% ($p>0.05$) compared with Group I; the ratio of feed to gain of Group II was decreased by 4.28% ($p>0.05$) compared with Group I; average gain of Group III-V were increased by -0.54 ($p>0.05$), 0.62 ($p>0.05$) and 5.50% ($p>0.05$) compared with Group I, respectively the ratio of feed to gain of Group III-V were decreased by 3.21 ($p>0.05$), 3.74 ($p<0.05$) and 7.53% ($p<0.05$) compared with Group I, respectively. The result showed that the effect of phytase on the growth performance in male broilers was more obvious than that of female.

Bone mineralization: Dry tibia weight of male broilers fed with Group II diet was decreased by 13.53% ($p<0.05$) compared with Group I; dry tibia weights of Group III-V were increased by 12.86 ($p<0.05$), 24.08 ($p<0.05$) and 26.26% ($p<0.05$) compared with Group II, respectively; dry tibia weights of Group III-V were increased by -2.41 ($p>0.05$), 7.29 ($p>0.05$) and 9.18% ($p<0.05$) compared with Group I, respectively. Dry tibia weight of female broilers fed with Group II diet was decreased by 10.92% ($p<0.05$) compared with Group I; dry tibia weights of Group III-V were increased by 7.70 ($p<0.05$), 14.05 ($p<0.05$) and 22.57% ($p<0.05$) compared with Group II, respectively; dry tibia weights of Group III-V were increased by -4.06 ($p>0.05$), 1.60 ($p>0.05$) and 9.19% ($p<0.05$) compared with Group I, respectively.

Tibia phosphorus content of male broilers fed with Group II diet was decreased by 9.25% ($p<0.05$) compared with Group I; tibia phosphorus content of Group III-V were increased by 0.77 ($p>0.05$), 9.23 ($p<0.05$) and 8.27% ($p<0.05$) compared with Group II, respectively; tibia phosphorus content of Group IV and V had no significant differences to that of Group I ($p>0.05$). Tibia phosphorus content of female broilers fed with Group II diet was decreased by 6.15% ($p<0.05$) compared with Group I; tibia phosphorus content of Group III-V were increased by 0.30 ($p>0.05$), 0.91 ($p>0.05$) and 4.42% ($p<0.05$) compared with Group II, respectively; tibia phosphorus content of Group V had no significant differences to that of Group I ($p>0.05$).

Tibia calcium content of male broilers fed with Group II diet was increased by 7.19% ($p>0.05$) compared with Group I; tibia calcium content of Group III-V were decreased by 7.63 ($p>0.05$), 8.47 ($p>0.05$) and 10.90% ($p<0.05$) compared with Group II, respectively; tibia calcium content of Group III-V were decreased by 0.99, 2.07 and 4.49% ($p>0.05$) compared with Group I, respectively. Tibia calcium content of female broilers fed with Group II diet was increased by 6.08% ($p<0.05$)

compared with Group I; tibia calcium content of Group III-V were increased by -4.35, 0.23 and 1.91% ($p>0.05$) compared with Group II, respectively; tibia calcium content of Group III-V were increased by 1.46 ($p>0.05$), 6.32 ($p>0.05$) and 8.10% ($p<0.05$) compared with Group I, respectively (Table 3).

Apparent digestibility of calcium and phosphorus: Apparent digestibility coefficient of total phosphorus of Group II in male broilers was increased by 28.03% compared with Group I ($p<0.05$); apparent digestibility coefficients of total phosphorus of Group III-V were increased by 6.91, 14.87 and 17.00% ($p<0.05$) compared with Group II, respectively; apparent digestibility coefficients of total phosphorus of Group III-V were increased by 36.88, 47.07 and 49.80% ($p<0.05$) compared with Group I, respectively. Apparent digestibility coefficient of total phosphorus of Group II in female broilers was increased by 25.12% compared with Group I ($p<0.05$); apparent digestibility coefficients of total phosphorus of Group III-V were increased by 7.59, 15.14 and 19.57% ($p<0.05$) compared with Group II, respectively; apparent digestibility coefficients of total phosphorus of Group III-V were increased by 34.62, 44.06 and 49.61% ($p<0.05$) compared with Group I, respectively.

Phosphorus concentration of faeces of Group II in male broilers was decreased by 31.40% compared with Group I ($p<0.05$); phosphorus concentration of faeces of Group III-V were decreased by 8.37, 18.03 and 20.61% ($p<0.05$) compared with Group II, respectively; phosphorus concentration of faeces of Group III-V were decreased by 37.13, 44.00 and 45.58% ($p<0.05$) compared with Group I, respectively. Phosphorus concentration of faeces of Group II in female broilers was decreased by 30.13% compared with Group I ($p<0.05$); phosphorus concentration of faeces of Group III-V were decreased by 8.49, 16.87 and 23.88% ($p<0.05$) compared with Group II, respectively; phosphorus concentration of faeces of Group III-V were decreased by 36.07, 41.79 and 46.76% ($p<0.05$) compared with Group I, respectively (Table 4).

Phytase added to available phosphorus-deficient diet could increase apparent digestibility of calcium. Apparent digestibility coefficient of calcium Group II in male broilers had no significant difference to Group I ($p>0.05$); apparent digestibility coefficients of calcium of Group III-V were increased by 14.57, 19.27 and 13.74% ($p<0.05$) compared with Group II, respectively; apparent digestibility coefficients of calcium of Group III-V were increased by 16.79, 21.58 and 15.95% ($p<0.05$) compared with Group I, respectively; the differences among Group III-V were not significantly ($p>0.05$). Apparent digestibility coefficient of calcium Group II in female broilers had no significant difference to Group I ($p>0.05$); apparent digestibility coefficients of calcium of Group III-V broilers were increased by 4.09 ($p>0.05$), 10.54 ($p<0.05$) and 1.88% ($p<0.05$) compared with Group II, respectively; apparent digestibility coefficients of calcium of Group III-V were increased by 9.03 ($p<0.05$), 15.76 ($p<0.05$) and 2.77% ($p>0.05$) compared with Group I, respectively; the differences among Group III-V were not significantly ($p>0.05$).

Growth performance: Phytase added to available phosphorus-deficient diet could increase average gain and decrease the ratio of feed to gain in broilers. The improvement in growth performance of broilers fed on phytase can be attributed to the release of mineral from the phytate mineral complex and the utilization of inositol by the bird (Simons *et al*., 1990) or increased starch digestibility (Knuckles and Betschart, 1987). The same can also be due to the increased availability of proteins because phytate also complexes with proteins making them less available. Phytate protein complexes are less subject to proteolytic digestion than the same protein

Table 3: Effect of phytase on tibia development in broilers

Gender	Ingredient	I (0 U kg^{-1})	II (0 U kg^{-1})	III (250 U kg^{-1})	IV (500 U kg^{-1})	V (750 U kg^{-1})
Male	Tibia weight (g)	17.00+1.81^{b}	14.70+0.92^{c}	16.59+1.02^{b}	18.24+2.06^{ab}	18.56+1.50^{a}
	Tibia Ca (%)	11.13+0.38^{ab}	11.93+1.24^{a}	11.02+0.21^{ab}	10.90+1.05^{ab}	10.63+0.33^{b}
	Tibia P (%)	5.73+0.15^{a}	5.20+0.46^{b}	5.24+0.28^{b}	5.68+0.08^{a}	5.63+0.07^{a}
Female	Tibia weight (g)	15.02+1.38^{b}	13.38+0.74^{c}	14.41+1.01^{b}	15.26+1.05^{ab}	16.40+0.25^{a}
	Tibia Ca (%)	12.34+1.00^{c}	13.09+0.42^{a}	12.52+0.39^{ac}	13.12+0.26^{abc}	13.34+0.38^{ab}
	Tibia P (%)	6.99+0.12^{a}	6.56+0.07^{b}	6.58+0.15^{b}	6.62+0.05^{b}	6.85+0.06^{a}

Table 4: Effect of phytase on apparent digestibility of calcium, phosphorus and phosphorus concentration of faeces in broilers

Gender	Ingredient (%)	I (0 U kg^{-1})	II (0 U kg^{-1})	III (250 U kg^{-1})	IV (500 U kg^{-1})	V (750 U kg^{-1})
Male	CAD Ca	49.47+1.070^{b}	50.43+0.960^{b}	57.78±2.130^{a}	60.15±2.050^{a}	57.36±1.780^{a}
	CAD TP	42.81+1.860^{d}	54.81+1.260^{c}	58.60±0.720^{b}	62.96±0.770^{a}	64.13±0.540^{a}
	Faeces P	0.51+0.017^{a}	0.35+0.010^{b}	0.32±0.006^{c}	0.29±0.006^{d}	0.28±0.004^{d}
Female	CAD Ca	50.84±1.840^{c}	53.25±1.120^{bc}	55.43±0.520^{b}	58.86±2.310^{a}	52.25±0.720^{c}
	CAD TP	42.03±1.460^{d}	52.59±1.050^{c}	56.58±0.880^{b}	60.55±1.840^{a}	62.88±1.970^{a}
	Faeces P	0.52±0.013^{a}	0.37±0.008^{b}	0.34±0.006^{c}	0.30±0.014^{d}	0.28±0.015^{e}

Values within rows with different superscript letters are significantly different ($p<0.05$)

alone. So, it can be postulated that phytase-liberated proteins from the complex making them more available to the birds.

Average weight gain of male broilers fed with Group II diet was decreased by 4.08% ($p>0.05$) compared with Group I; Group III-V were increased by 3.57 ($p>0.05$), 8.16 ($p<0.05$) and 9.89% ($p<0.05$) compared with Group I, respectively. The result showed that in the available phosphorus condition of this trial, 250 U kg^{-1} phytase was not enough for male broilers, 500 U kg^{-1} phytase was sufficient and the effect of the larger dose of phytase was not remarkable. Average weight gain of female broilers fed with Group II and III diets were decreased by 7.03 ($p>0.05$) and 0.54% ($p>0.05$) compared with Group I, respectively; Group IV and V were increased by 0.62 ($p>0.05$) and 5.50% ($p<0.05$) compared with Group I, respectively. The result showed: in the available phosphorus condition of this trial, 250 and 500 U kg^{-1} phytase was not enough for female broilers and the effect of 750 U kg^{-1} phytase was obvious.

Similarly, phytase could improve the utility condition of feed. The ratio of feed to weight gain of male broilers fed with Group II diet was increased by 4.52% ($p>0.05$) compared with Group I; Group III-V were decreased by 5.08 ($p>0.05$), 8.47 ($p<0.05$) and 7.68% ($p<0.05$) compared with Group I, respectively. The ratio of feed to weight gain of female broilers fed with Group II diet was increased by 4.28 ($p<0.05$) compared with Group I; Group III-V were decreased by 3.21 ($p>0.05$), 3.74 ($p<0.05$) and 7.53% ($p<0.05$) compared with Group I, respectively.

Improvement in weight gain and the ratio of feed to gain when supplemental phytase is added to broiler diets has been shown in a number of studies. Denbow *et al.* (1995) reported increased gain and feed intake but not feed eficiency when phytase was supplemented to corn-soybean meal-based diets fed to 1 day old chicks for 21 days. Selle *et al.* (2003) reported that in days 1-14, phytase could increase weight gain, feed intake and feed utility ratio. In the study of Simons *et al.* (1990), phytase addition (1500 FTU kg^{-1}) to diets containing 4.5 g kg^{-1} total P increased weight gain (733 g versus 338 g) and feed efficiency (1.50 versus 1.85) of broilers from 0-24 days of age. Subsequently, Cabahug *et al.* (1999) reported that phytase addition (400 and 800 FTU kg^{-1}) to 2.3 g kg^{-1} non-phytate-P diets increased weight gain (18.8), feed intake (9.0%) and feed efficiency (7.9%) of broilers from 7-25 days of age.

The effect of phytase on growth performance was influenced by gender. Sebastian *et al.* (1996) reported that the effect of phytase on the growth performance in male broilers was more obvious than that of female. In this experiment 500 U kg^{-1} phytase increased weight gain of male broiler 8.21 and only 2.12% in male broilers; 750 U kg^{-1} phytase increased weight gain of male broiler 9.89 and only 4.88% in male broilers. The 500 U kg^{-1} phytase decreased the ratio of feed to gain of male broiler 8.47 and only 3.78% in male broilers.

Bone mineralization: Bone is the mainly storage organ of phosphorus. If the phosphors contained in diet can not meet the requirement of the animal, it will be released from bone to satisfy with body. It would effect the quality of bone and furthermore the growth performance of animals would decreased. Denbow *et al.* (1995) reported improved tibia ash and shear force and stress when 1 day old broilers were fed a soybean meal-based semi purified diet supplemented with phytase for 21 days. The result of this trial also showed that phytase could improve bone mineralization. Dry tibia weights of male broilers fed with Group II-V diet were increased by -13.53 ($p<0.05$), -2.41 ($p>0.05$), 7.29 ($p>0.05$) and 9.18% ($p<0.05$) compared with Group I, respectively; dry tibia weights of female broilers fed with Group II-V diet were increased by -10.92% ($p<0.05$), -4.06% ($p>0.05$), 1.60% ($p>0.05$) and 9.19% ($p<0.05$) compared with Group I, respectively.

Phyatse could increase tibia phosphors and calcium contents showed bone mineralization improvement. Ravindran *et al.* (1995) showed that bone mineralization criteria are more sensitive indicators of P status in the bird than are growth criteria. Hence, as P is a major component of the skeletal system, this observation reiterates that bone mineralization requirements are met before growth when P nutrition is the focus. The present results showed that the effect of phytase on bone mineralization was also influenced by gender. Sebastian *et al.* (1996) reported that phytase increased female broilers tibia ash content 42.25% and male only 21.18%. The conclusion of Sebastian *et al.* (1996) showed that the effect of phytase on the bone mineralization in female broilers was more obvious than male. But the present results were reverse to the obove conclusion. In the present study, dry tibia weights of male broilers fed with Group IV and V diet were increased by 7.29 ($p>0.05$) and 9.18% ($p<0.05$) compared with Group I, respectively; dry tibia weights of female broilers fed with Group IV and V diet were increased by 1.60 ($p>0.05$) and 9.19% ($p<0.05$) compared with Group I, respectively. The phenomena might be effected by the ratio of calcium and phosphors which required for further research.

Apparent digestibility of calcium and phosphorus: In many cereals, seeds and legumes phosphorus is mainly stored as phytic acid, a form that is not assimilated by animals. The increasingly demanding on controlling phosphorus pollution have intensified the phytase

research. Nelson *et al.* (1971) indicated that from 50-100% of the phytate-bound P in corn-soybean meal diets could be released by phytase supplementation, depending upon the level of phytase used. Denbow *et al.* (1995) reported that P released by phytase ranged from 31-58% for 250-1,000 units of phytase kg^{-1} of feed. Simons *et al.* (1990) indicated that >60% of the P was released by addition of phytase. Yi *et al.* (1996) estimated that >37% of the phytate P in soybean meal was released by addition of 1,000 units of phytase/kg of diet. Waldroup (1999) calculated that approximately 50% of the phytate-bound P in a corn-soybean meal diet was released by phytase. Thus, phytase could increase the utilized of phosphorus. Simons *et al.* (1990) indicated that addition of phytase to low-P diets increases the availability of P to over 60 g/100 g and the amount of P in the droppings decreased by 50 g/100 g when compared to a low-P diet without enzyme supplementation. In this study, apparent digestibility coefficients of total phosphorus of Group II-V in male broilers were increased by 28.03, 36.88, 47.07 and 49.80% ($p<0.05$) compared with Group I, respectively; Group II-V in female broilers were increased by 25.12, 34.62, 44.06 and 49.61% ($p<0.05$) compared with Group I, respectively. These findings are similar to those of many other researchers (Simons *et al.*, 1990; Broz *et al.*, 1994; Schoner *et al.*, 1993; Yi *et al.*, 1996) who gave P deficient maize and soyabean meal diets to chickens.

Phytase liberates calcium from the Ca-phytate complex and as the availability of P increases, the availability of calcium also increases because both are part of the same complex. Schoner *et al.* (1993) reported utilization of calcium could be improved by the addition of phytase to broiler diet and Schoner also indicated 500 U kg^{-1} phytase could replace 0.56 g calcium. Sebastian *et al.* (1996) showed that calcium utilization was increased by 19.3% by 600 U kg^{-1} phytase. In the present study, apparent digestibility coefficients of calcium Group II-V in male broilers were increased by 1.94 ($p>0.05$), 16.79 ($p<0.05$), 21.58 ($p<0.05$) and 15.95% ($p<0.05$) compared with Group I, respectively; Group II-V in female broilers were increased by 4.74 ($p>0.05$), 9.03 ($p<0.05$), 15.76 ($p<0.05$) and 2.77% ($p>0.05$) compared with Group I, respectively. Improvements in the utilization of Ca by supplemental phytase have been reported by Mitchell and Edwards (1996) and Zyla *et al.* (1996).

CONCLUSION

The result showed that phytase added to available phosphorus-deficient broiler diet could improve the growth performance, bone mineralization, digestion and metabolization of calcium and phosphorus.

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