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Effects of Microbial Phytase on Performance and Bone Strenght in Broiler Chickens

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Abstract: An experiment was conducted to study the effects of microbial phytase, metabolizable energy and available phosphorus on performance and carcass characteristics and mineral content of tibia bone in broiler chickens. Five hundred and forty days old ross male broiler chicks allocated to 9 treatments in a 2×2×2 factorial arrangement with two levels of phytase enzyme (0 and 500 FTU), two levels of metabolizable energy (recommended and 106 kcal kg^{-1} less than recommended) and available phosphorus (50 and 75% of recommended level) and a control group (with recommended phosphorus and no enzyme supplementation), with 4 replicates of 15 chicks in a completely randomized experimental design. The use of phytase enzyme had significant effect on feed intake, body weight gain (whole period), breast and thigh percentage of body weight ($p<0.05$). There were significant differences among energy effects on feed intake, body weight gain and FCR ($p<0.05$). The effect of diet containing 75 and 50% of recommended available phosphorus on feed intake, body weight gain, FCR, breast and thigh percentage and Ca and P content of tibia bone were significantly different ($p<0.05$). The Interaction of energy and phosphorus effect on feed intake, body weight gain, FCR and P content of tibia bone were significant ($p<0.05$). Results of this experiment revealed that the use of phytase enzyme in diets containing 75% of recommended available phosphorus had the best performance without considering energy.

Key words: Phytase, phosphorus, energy, growth performance, tibia bone

INTRODUCTION

Phosphorus is an essential element involved in energy metabolism of poultry and necessary for normal appetite, feed intake, FCR, bone development and general health. Approximately, 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 phosphorus, which are not available for poultry and are excreted in the litter. Phytate in its native state, constitutes complexes with various cations, protein, lipids and starch (Cosgrove, 1966). These complexes may decrease the activity of digestive enzymes with a subsequent decrease in the digestibility of dietary phosphorus and energy and protein. Phytase, myo-inositol hexaphosphate phosphohydrolase is the enzyme that release P from phytate (Gibson and Ullah, 1990). Supplemental microbial phytase has been reported to improve dietary phytate P availability (Biehl *et al*., 1995; Denbow *et al*., 1995; Mitchell and Edwards, 1996). Several recent studies from Australia have evaluated the influence of phytase on energy utilization. Of sorghum-soybean meal based diets (2810 vs 2870 kcal kg^{-1}) when phytase was added to broiler diets. Phytate also forms low-soluble complexes with bi-and trivalent minerals calcium, magnesium, iron and zinc, lowering their bioavailability and influencing the bone formation process. Numerous investigations with broilers fed maize-soybean meal based diets have shown that supplemental phytase has a beneficial influence on the bone mineralization process in general (Qian *et al*., 1996; Denbow *et al*., 1998; Sohail and Roland, 1999). Tibia ash and mineral content, density, length and strength increased in broilers fed diets with supplemental phytase. It was reported that mineral-phytate complexes might prevent lipid utilization and by preventing of formation of mineral-phytate complexes, phytase might reduce the degree of soap formation in the gut and enhance the utilization of energy derived from lipids (Ravindran *et al*., 2001). This experiment was conducted to study the effect of microbial phytase, dietary energy and phosphorus levels on performance and bone strength of broiler chickens.

MATERIALS AND METHODS

A total of 540 days old Ross 308 male broiler chicks were used to investigate the effects of phytase and dietary energy on performance, Ca and P content of tibia

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Table 1: Composition (%) of experimental diets (0-42 days)

	Starter					Grower					Finisher				
Ingredients (%)	C[2]	1	2	3	4	C[2]	1	2	3	4	C[2]	1	2	3	4
Corn grain	51.3	52.4	51.9	54.7	54.2	60.2	61.1	60.7	63.5	62.9	61.2	62.4	61.9	64.7	64.2
Soybean meal	39.9	39.6	39.7	39.2	39.3	31.51	43.3	4.31	31.0	31.0	29.3	29.1	29.0	28.6	28.7
Poultry BP	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Soybean oil	2.31	1.89	2.1	0.08	0.28	2.2	1.8	1.4	1.8	1.4	3.8	3.4	3.5	1.5	1.7
Dical. Phos.	1.93	0.47	1.19	0.47	1.18	1.7	0.4	1.06	0.38	1.05	1.7	0.38	1	0.38	0.99
Oyster shells	1.04	2.01	1.53	2.01	1.54	0.9	1.8	1.4	1.8	1.4	0.94	1.7	1.3	1.7	1.3
Common salt	0.3	0.3	0.3	0.3	0.3	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
Vit + Min[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Dl-methionine	0.33	0.33	0.33	0.29	0.29	0.25	0.25	0.25	0.22	0.22	0.15	0.15	0.15	0.15	0.15
L-lysine	0.28	0.28	0.28	0.28	0.28	0.24	0.2	0.25	0.18	0.22	-	-	-	-	-
Sodium Bica.	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Zeolite	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Coccidiosetate	-	-	-	-	-	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Calculated nutrient content															
ME (kcal g^{-1})	2.85	2.85	2.85	2.75	2.75	2.95	2.95	2.95	2.87	2.87	3.05	3.05	3.05	2.97	2.97
CP (%)	22.5	22.5	22.5	22.5	22.5	19.5	19.5	19.5	19.5	19.5	18.5	18.5	18.5	18.5	18.5
Calcium (%)	0.94	0.94	0.94	0.94	0.94	0.84	0.84	0.84	0.84	0.84	0.8	0.8	0.8	0.8	0.8
Avail. Phos. (%)	0.47	0.23	0.35	0.23	0.35	0.42	0.21	0.31	0.21	0.31	0.4	0.2	0.3	0.2	0.3
Sodium (%)	0.16	0.16	0.16	0.16	0.16	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Potassium (%)	0.95	0.95	0.95	0.95	0.95	0.81	0.81	0.81	0.81	0.81	0.77	0.77	0.77	0.77	0.77
Chlorine (%)	0.27	0.27	0.27	0.27	0.27	0.24	0.24	0.24	0.24	0.24	0.2	0.2	0.2	0.2	0.2
Arginine (%)	1.43	1.43	1.43	1.43	1.43	1.2	1.2	1.2	1.2	1.2	1.14	1.14	1.14	1.14	1.14
Lysine (%)	1.39	1.39	1.39	1.39	1.39	1.17	1.17	1.17	1.17	1.17	0.93	0.93	0.93	0.93	0.93
Methionine (%)	0.67	0.67	0.67	0.67	0.67	0.55	0.55	0.55	0.55	0.55	0.45	0.45	0.45	0.45	0.43
Met + Cys (%)	03.1	03.1	03.1	1	1	88.0	88.0	88.0	86.0	86.0	76.0	76.0	76.0	74.0	74.0
Tryptophn (%)	0.26	0.26	0.26	0.26	0.26	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21	0.21
Threonine (%)	0.82	0.82	0.82	0.82	0.82	0.71	0.71	0.71	0.71	0.71	0.68	0.68	0.68	0.68	0.68

[1]Supplied per kilogram of feed: 7, 500 IU of vitamin A, 2, 000 IU of vitamin D3, 30 mg of vitamin E (all-rac- α-tocopheryl acetate), 15 μg of vitamin B12, 2 mg of vitamin B6, 5 mg of vitamin K, 5 mg of vitamin B2, 1 mg of vitamin B1, 40 mg nicotinic acid, 160 μg of biotin, 12 mg of calcium pantothenate, 1 mg of folic acid, 20 mg of Fe (ferrous sulfate), 71 mg of Mn (manganese oxide), 100 μg of Se (sodium selenite), 37 mg of Zn (zinc oxide), 6 mg of Cu (copper sulfate), 1.14 mg of I (potassium iodide), 400 μg of Co (cobalt sulfate) and 4 mg of butylated hydroxytoluene. [2] Control group, with recommended energy and phosporus level and not supplemented with phytase

bone. Dietary treatments (Table 1) including the control group according to the Ross broiler requirement manual and others was conducted as a 2×2×2 factorial arrangement of treatments with 2 levels of Phytase (0 and 500 FTU kg^{-1}), ME (control and 106 kcal kg^{-1} less than control) and available phosphorus (50 and 75% of requirement) with 4 relicates of 15 birds each. The birds were exposed to a continuous lighting of 24 h of photoperiod. Initial room temperature was 32°C and was then gradually decreased according to usual brooding practices. Waterers were fixed in pens such a way that the birds were able to eat and drink conveniently. All diets were corn-soybean based. Feed and water were given *ad libitum* and were supplied to the experimental birds. Production performance such as body weight gain and feed intake were measured weekly while, mortality was recorded daily. Feed conversion ratio was corrected for mortality by including the weight gain of the dead birds in the body weight gain. Two birds per pen, with an average live body weight of each replicate group were selected at 42 days fasted, weighed and killed in slaughterhouse to determine carcass traits such as eviscerated carcass, abdominal fat, liver, breast, thigh weights and then left tibias were removed, tibias were dried to constant weight at 105°C, defatted and then ashed in a furnace at 550°C. Ash was dissolved in concentrated HCl for mineral determination. Phosphorus content of tibias measured with Spectrophotometer system and calcium content with Atomic Absorption Spectrophotometer system.

Statistical analysis: Main effects (Phytase, Energy and Phosphorus) levels and their interactions were analyzed by variance analysis using the procedure described by the SAS Institute (1999). Duncan (1955) multiple range test was used to separate the significant differences between treatment mean values ($p<0.05$). The Duncan test was used to compare of treatments with control birds.

RESULTS AND DISCUSSION

Composition (%) of experimental diets from 0-42 days are shown in Table 1 and effects of phytase supplementation on performance of broiler chicks are shown in Table 2. Adding phytase to diet had significant effect on feed intake and body weight gain measured in

Table 2: Effects of phytase, phosphorus and energy levels and interaction between them on the performance of chickens

Main and interaction effects	FI (g/bird/day)	BWG (g/bird/day)	FCR (g:g)
Phytase			
0 FTU	82.40^{b}	40.61^{b}	2.02
500 FTU	84.6^{a}	42.19^{a}	2.02
Energy			
Recommended	85.71^{a}	43.59^{a}	1.97^{b}
3.5%<recomm.	81.09^{b}	40.59^{b}	2.07^{a}
AV. phosphorus			
50% of recomm.	87.55^{b}	36.45^{b}	2.17^{b}
75% of recomm.	88.43^{a}	46.35	1.91^{a}
Phytase x Energy			
0 FTU x recomm. energy	84.47	42.14	2.01
500 FTU x recomm. energy	86.92	45.04	1.92
0 FTU ×3.5%<recomm.	80.92	39.83	2.08
500 FTU ×3.5%<recomm.	81.28	40.14	2.05
Phytase x Phos.			
0 FTU ×50% of recomm.	76.97	35.04	2.20
500 FTU ×75% of recomm.	80.14	37.85	2.10
0 FTU ×50% of recomm.	87.83	46.21	1.90
500FTU ×75% of recomm.	86.19	46.50	1.91
Phos. x Energy			
Recommended ×50% of recomm.	83.19^{c}	41.45^{b}	2.01^{b}
Recommended ×75% of recomm.	88.21^{a}	45.70^{a}	1.92^{bc}
3.5%<recomm. ×50% of recomm.	75.8^{d}	33.00^{c}	
3.5%<recomm. ×75% of recomm.	86.38^{ab}	46.19^{a}	1.84^{c}
Phos. x Energy x Phytase			
0 FTU ×recomm. Energy ×50% of recomm.	80.52^{c}	38.3^{c}	2.09^{c}
500 FTU ×recomm. Energy ×50% of recomm.	85.85^{ab}	44.5^{ab}	1.92^{d}
0 FTU ×recomm. Energy ×75% of recomm.	88.45^{a}	45.9^{a}	1.92^{d}
500 FTU × recomm. Energy ×75% of recomm.	88.09	45.47^{a}	1.93^{d}
0 FTU ×3.5%<recomm. ×50% of recomm.	75.60^{cd}	32.11^{dc}	2.36^{a}
500 FTU ×3.5%<recomm. ×75% of recomm.	76.02^{cd}	33.88^{a}	2.24^{ab}
0 FTU ×3.5%<recomm. ×75% of recomm.	86.23^{ab}	47.58^{a}	1.81^{d}
500 FTU ×3.5%<recomm. ×75% of recomm.	86.52^{ab}	46.26^{a}	1.87^{d}
SEM	1.074	1.003	0.030

^{abc}Values in column with no common superscripts differs significantly ($p<0.05$)

whole period (0-42 days) of the experiment ($p<0.05$). These results are in agreement with the findings of previous studies (Dilger *et al*., 2004; Onyango *et al*., 2004). The phytase may have increased the availability of nutrients (Dilger *et al*., 2004) decreased passage rate of feed stuffs in digestive tract and degraded the cell wall of ingredients and thus, improve of feed intake and weight gain (Viverous *et al*., 2000). Aksakal and and Bilal (2002) reported that add of phytase to broiler chicken diet increased feed intake but there was no significant effect on FCR. Viverous *et al*. (2000) similarly reported an increase in feed intake simultaneously body weight, effect of phytase supplementation on FCR of broiler chicks was not significant. Ravindran *et al*. (2001) reported that the addition of phytase to corn-soybean meal diet released more phytate due to the fact that corn-soybean diet has a high concentration of phytate. The results revealed that there were significant differences on feed intake, body weight gain and feed conversion ratio ($p<0.05$) of birds fed diet with different level of energy (Table 2). The birds fed diet contained 75% available phosphorus had improved better than birds fed 50% available phosphorus diet on feed intake, body weight gain, feed conversion ratio, thigh and breast percent ($p<0.05$). In other study, the 50% available phosphorus diet fed to broiler chickens caused to decrease the production performance (Viverous *et al*., 2000). Phosphorus has important role in bone, blood, metabolism of energy, carbohydrates, protein, fatty acid transportation, membrane phospholipids and enzymatic processes, therefore, the higher level of phosphorus could improve performance rather than lower level. Negative effects of increasing in Ca/P proportion from 1.2 : 1-2:1 on body weight gain, phosphorus retention and tibia phosphorus have been shown by Qian *et al*. (1997).

Table 3 showed the effects of phytase, phosphorus and energy levels on mineral released from tibia bone in broiler chickens. Effects of phytase levels on the ash, Ca and P percent of tibia bone was not significant ($p>0.05$), but the effect of phosphorus levels was significant ($p<0.05$) as shown in Table 3. The interaction effects of energy and phosphorus on P content of tibia bone was significant (p>0.05). The non significant effect of phytase on tibia bone minerals may be because of insufficient amount of phytase (500 FTU) or young chicks used in this experiment as stated before (Scheideler and Ferket, 2000). In this experiment, the 75% phosphorus could mineralized the bone and tibia bone during the period. Results of

Table 3: Effects of phytase, phosphorus and energy levels on mineral released from tibia bone in broiler chickens

Main and interaction effects	Tibia ash (%)	Calcium in tibia bone (%)	Phosphorus in tibia bone (%)
Phytase			
0 FTU	45.64	15.26	8.07
500 FTU	42.71	15.07	8.24
Energy			
Recommended	43.23	14.38	8.35
3.5%<recomm.	42.02	15.03	8.10
AV. phosphorus			
50% of recomm.	41.69^{b}	14.65a	7.96^{b}
75% of recomm.	43.66^{a}	15.81a	8.35^{a}
Phytase x Energy			
0 FTU x recomm. energy	43.55	15.56	8.08
500 FTU x recomm. energy	44.70	15.20^{a}	8.62
0 FTU ×3.5%<recomm.	43.40	14.98	8.02
500FTU ×3.5%<recomm.	42.64	15.07	8.17
Phytase x Phos.			
0 FTU ×50% of recomm.	41.74^{b}	14.78^{ab}	7.81
500 FTU ×75% of recomm.	41.65^{b}	14.52^{b}	8.12
0 FTU ×50% of recomm.	44.54^{a}	15.74^{a}	8.34
500 FTU ×75% of recomm.	43.78^{a}	15.87^{a}	8.36
Phos. x Energy			
Recommended ×50% of recomm.	43.00^{a}	14.99^{ab}	8.21^{abc}
Recommended ×75% of recomm.	43.46^{a}	15.77^{a}	8.50^{ab}
3.5%<recomm. ×50% of recomm.	39.53^{b}	14.00^{b}	7.52^{c}
3.5%<recomm. ×75% of recomm.	43.48^{a}	16.06^{a}	8.68^{a}
Phos. x Energy x Phytase			
0 FTU x recomm. Energy ×50% of recomm.	43.88^{a}	14.91	7.99^{abc}
500 FTU x recomm. Energy ×50% of recomm.	42.11^{a}	15.08	8.42^{ab}
0 FTU x recomm. Energy ×75% of recomm.	43.62^{a}	16.22	8.17^{abc}
500 FTU x recomm. Energy ×75% of recomm.	43.29^{a}	15.32	8.82^{a}
0 FTU ×3.5%<recomm. ×50% of recomm.	39.05^{b}	14.04	7.24^{c}
500 FTU ×3.5%<recomm. ×75% of recomm.	41.34^{ab}	13.96	7.79^{abc}
0 FTU ×3.5%<recomm. ×75% of recomm.	43.76^{a}	15.93	8.81^{a}
500 FTU ×3.5%<recomm. ×75% of recomm.	43.93^{a}	16.19	8.55^{ab}
SEM	0.330	0.213	0.105

[abc]Values in column with no common superscripts differs significantly (p<0.05)

Table 4: Effects of phytase, phosphorus and energy levels and interaction between them on the carcass traits in chicks

Main and interaction effects	Carcass[1] yield (%)	Thigh[2] (%)	Breast[2] (%)	Abdominal fat (%)	Liver (%)
Phytase					
0 FTU	68.97	35.7^{b}	32.3^{b}	1.86	3.41
500 FTU	69.31	36.1^{a}	34.3^{a}	1.83	3.19
Energy					
Recommended	69.33	35.95	33.5	1.93	3.53
3.5%<recomm.	68.43	35.93	33.4	1.85	3.25
AV. phosphorus					
50% of recomm.	68.86	35.4	33.02^{b}	1.79	3.33
75% of recomm.	67.42	36.3^{a}	33.69^{a}	1.90	3.10
Phytase × Energy					
0 FTU × recomm. energy	68.03	35.73	32.53^{b}	1.97	3.29
500 FTU ×recomm. energy	67.64	36.16	34.47^{a}	1.89	3.21
0 FTU ×3.5%<recomm.	69.03	35.72	32.44^{b}	1.79	3.34
500 FTU ×3.5%<recomm.	68.50	36.14	34.37^{a}	1.92	3.16
Phytase x Phos.					
0 FTU ×50% of recomm.	68.74	35.21^{d}	31.88^{c}	1.79	3.24^{ab}
500 FTU ×75% of recomm.	68.98	35.71^{c}	34.16^{a}	1.78	3.42^{a}
0 FTU ×50% of recomm.	69.20	36.21^{b}	32.82^{b}	1.94	3.23^{ab}
500 FTU ×75% of recomm.	67.84	36.53^{a}	34.55^{a}	1.87	2.97^{b}
Phos. x Energy					
Recommended ×50% of recomm.	67.91	35.48^{b}	33.15	1.76	3.40
Recommended ×75% of recomm.	68.75	36.42^{a}	33.85	2.10	3.10
3.5%<recomm. ×50% of recomm.	69.03	35.41^{b}	33.06	1.92	3.40
3.5%<recomm. ×75% of recomm.	67.83	36.32^{a}	33.74	1.78	3.11

Table 4: Continue

Main and interaction effects	Carcass[1] yield (%)	Thigh[2] (%)	Breast[2] (%)	Abdominal fat (%)	Liver (%)
Phos. x Energy x Phytase					
0 FTU × recomm. Energy ×50% of recomm.	68.340	35.210[d]	32.050[dc]	1.850	3.350
500 FTU × recomm. Energy ×50% of recomm.	68.480	35.740[bcd]	34.250[ab]	1.660	3.450
0 FTU × recomm. Energy ×75% of recomm.	67.810	36.240[ab]	33.010[bc]	2.090	3.240
500 FTU × recomm. Energy ×75% of recomm.	67.800	36.590[a]	34.690[a]	2.120	2.970
0 FTU ×3.5%<recomm. ×50% of recomm.	69.010	35.230[d]	31.960[d]	1.740	3.410
500 FTU ×3.5%<recomm. ×75% of recomm.	68.050	35.750[dc]	34.160[ab]	2.100	3.390
0 FTU ×3.5%<recomm. ×75% of recomm.	68.710	36.210[abc]	32.910[dc]	1.830	3.270
500 FTU ×3.5%<recomm. ×75% of recomm.	69.060	36.530[a]	34.580[a]	1.740	2.940
SEM	0.123	0.095	0.207	0.072	0.059

[abc]Values in column with no common superscripts differs significantly (p<0.05), [1]: As a percent of live weight, [2]: As a percent of carcass weight

Table 5: Effects of treatments on performance criteria, Ca and P content of tibia bone in broiler chicks

Treatments	FI (g/b/w)	BWG (g/b/w)	FCR (g:g)	Tibia Ca (%)	Tibia P (%)
Control	3896.4	2135.8	1.82	16.25	8.75
0 FTU x recomm. Energy ×50% of recomm.	3382.2*	1610.6*	2.09*	14.91	7.99
500 FTU×recomm. Energy ×50% of recomm.	3606.7*	1871.6*	1.92	15.08	8.42
0 FTU × recomm. Energy ×75% of recomm.	3715.0*	1930.9*	1.92	16.22	8.17
500 FTU × recomm. Energy ×75% of recomm.	3696.0*	1914.2*	1.93	15.32	8.82
0 FTU ×3.5%<recomm. ×50% of recomm.	3175.8*	1349.2*	2.36*	14.04	7.24
500 FTU ×3.5%<recomm.× 75% of recomm.	3193.9*	1423.8*	2.24*	13.96	7.79
0 FTU ×3.5%<recomm.× 75% of recomm.	3622.9*	1998.3*	1.81	15.93	8.18
500 FTU ×3.5%<recomm.× 75% of recomm.	3634.3*	1943.0*	1.87	16.19	8.55
P-value	p<0.05	p<0.05	p<0.05	ns	ns

*Values in column differs with control group significantly (p<0.05)

Table 2 showed that interaction effects of energy and phosphorus on FI, BWG and FCR was significant (p<0.05) and in 2 levels of energy, the birds fed 75% available phosphorus, had better growth performance than other groups probably it was because the role of phosphorus in structure of ATP, ADP and cheratin phosphate. Table 4 showed the effects of phytase, phosphorus and energy levels and interaction between them on the carcass traits of chicks.

Results showed that phytase could increase the breast and thigh percent significantly (p<0.05) and this results previously reported by many of researches. The improvement in breast and thigh yield is because of increase the releasing the amino acids from phytate complex.

Ruthrerford *et al.* (2004) reported that with supplementation of feed with 500 FTU phytase, apparent digestibility of amino acids increased from 81-89%. In Table 5, all treatments compared with control group and significant differences in parameters between them with control group are marked.

CONCLUSION

In this experiment, hydrolysis of phytate phosphorus with adding microbial phytase caused to improve in growth performance of broiler chickens and use of phytase enzyme in diets containing 75% available phosphorus showed best performance without considering energy and differences between main and interaction effects of phytase, phosphorus and energy levels was not significant (p>0.05).

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