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EFFICACY OF MICROBIAL PHYTASE AT DIFFERENT LEVELS ON GROWTH PERFORMANCE AND MINERAL AVAILABILITY IN BROILER CHICKEN

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ABSTRACT

A 4-week feeding trial with 120 day-old broiler (Hubbard) chicks was conducted to determine the efficacy of microbial phytase (Natuphos®) at different levels on growth performance and mineral availability in broiler chicks fed a low (0.35%) Phosphorus (P) diet. Four levels of microbial phytase (0, 250, 500 and 750 U / kg diet) were added to diets containing M.E. 3000 Kcal/kg and CP 21 %. Phytase supplementation increased ($P<0.05$) body weight, feed intake, FCR and dressing percentage at 28 day. Supplementation of phytase @ 500 U/kg diet to the low phasphorous diet significantly ($P<0.05$) improved toe ash percentage. Microbial phytase improved ($P<0.05$) apparent availability of P, Ca, and Mg. Supplementation of phytase @ 250 U/kg diet was the most economical as cost/kg live weight was lesser than 500 and 750 phytase U/kg diet supplementation. The results showed that microbial phytase supplementation to a low-P diet improved growth performance and apparent mineral availability in broiler chickens.

Key words: phytase, broiler, phosphorus, calcium, magnesium

INTRODUCTION

Phosphorus (P) is the second most abundant mineral in the animal body, approximately 80 percent of which is found in the bones and teeth (NRC, 1994). The most important quantitative function of P along with calcium is the formation and maintenance of bones. The remaining 20 percent of P is widely distributed in the fluids and soft tissues of the body, where it serves a range of essential functions. Phosphorus is very important for health, growth, production and reproduction. Its role in energy metabolism, synthesis of sugars and maintenance of acid-base balance in the body fluids is of paramount importance. Both the quantity and availability of dietary P is of critical importance for growing birds.

Plant materials are the major constituents of poultry diets. About two-third of the P of plant origin is present as phytic acid in the form of myo-inositol hexakisdihydrogen phosphates (NRC, 1994). The ability of chicken to utilize phytate P is generally considered poor due to the lack of endogenous phytase enzyme. Abnormal bone development is one of the most obvious signs of a P deficiency. P deprivation leads to rachitic bones, osteoporosis, osteomalacia and osteodystrophy, which result from the abnormal growth and arrangement of cartilaginous and bone cells that are due to incomplete or irregular ossification or demineralization. At the same time, P deprived bones are shorter and have reduced mass and mineral contents (Edwards *et al.* 1983). Phytic acid has strong chelating potential and forms a wide variety of insoluble salts with di and trivalent cations (Ca, P, Mg, Zn, Cu, Co, Mn and Fe) at neutral pH (Reddy *et al.*, 1982). Phytate complexes with metals and precipitates at the pH of intestine and potentially render the mineral unavailable.

The phytate P content may, on average, vary between 56-68% of the total P content in the commonly used cereals (corn, wheat, sorghum, barley and oats), which are the major components of the poultry feed, and from 61-70% of the total P in soybean meal and cotton seed meal. However, the variation in the phytate P contents is higher in protein rich feedstuffs like soybean and rapeseed meal. Poultry birds are capable of digesting and utilizing phytate P from zero to over 50% (Ravindran *et al.*, 1999). This low biological availability of phytate P has boosted the demand for mineral P supplementation.

The addition of microbial phytase has provided another practical way of releasing the phytate bound P for utilization and reducing P excretion. Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate. Microbial phytase improves dietary phytate P utilization and may release all phytate bound minerals.

Supplementation of diets with microbial phytase derived from *Aspergillus niger* increases availability and retention of phytate P and Zn in chicks as measured by ash content of bone (Sebastian *et al.*, 1996a and Qian *et al.*, 1997, Ahmad *et al*., 2000). Phytase supplementation to low-P diets improves performance; and mineral (P, Ca, Mg and Zn) utilization in broiler chicken (Viveros *et al.*, 2002). Current evidences suggest that added phytase may also have positive effect on protein digestion and utilization (Ravindran *et al.*, 1999) and also increases the apparent metabolizable energy in broilers (Ravindran *et al.*, 2001). Phytase could partially hydrolyze viscous carbohydrates such as glucans and pentosans in certain cereal grains, increases the bioavailability of starch (polysaccharides) and proteins (Ravindran *et al.*, 1995). More exclusively hydrolyzes fibrous material, usually not degraded by natural enzymes in animals thus releasing more nutrients. One of the greatest contributions of phytase supplementation is to reduce mortality at the lower levels of nonphytate P (Waldroup *et al.*, 2000).

Therefore, this study was planned with the objective to investigate the effects of phytase supplementation on the performance of broiler chicks consuming low available phosphorus diets and to determine the availability of minerals (P, Ca and Mg).

MATERIALS AND METHODS

In this trial one hundred and twenty day-old broiler (Hubbard) chicks were used to investigate the effects of phytase supplementation on performance of broilers and minerals availability. The chicks were wing-banded for identification and randomly divided into 4 experimental group having 3 replicates comprising 10 chicks each. Each replicate of the chicks was placed in a separate pen.

Four broiler rations (A, B, C and D) were formulated using computer software UFFDA (Pesti *et al.*, 1992). Experimental birds were divided in following four dietary treatments (Table 1): 1) Diet A + O U of phytase/kg of diet, 2) Diet B+250 U of phytase/kg of diet, 3) Diet C + 500 U of phytase/kg of diet, 4) Diet C + 750 U of phytase/kg of diet. All the diets were iso caloric (ME 3000 kcal) and isonitrogenous (21% CP) but contained low levels of phosphorus (Table 1). A phytase unit (U) is defined as the quantity of enzyme that liberates 1 µmol of inorganic P/min form 1.5 mM of sodium phytate at pH 5.5 and 37^{o}C. All dietary P levels were formulated below the current NRC (1994) recommendations of 0.45% non phytate phosphorus (nP) to provide an opportunity for a measurable response to phytase additions.

The chicks were housed and reared under standard managemental conditions. Temperature was maintained at 35^{o}C in the brooding house during first week of the trial and lowered by 3°C each week till 29°C, which was maintained during remaining period. Fresh water was provided to the chicks round the clock. Light was provided 24 hours during experimental period. Broiler rations were fed *ad libitum* to the chicks from 1-28 days of age.

Body weight and feed intake were recorded per replicate at weekly intervals. The data recorded for body weight gain and feed intake were used to calculate feed conversion ratio of each experimental unit on weekly basis. Economics of each dietary treatment was also calculated.

Excreta of the chicks from each experimental unit, were collected weekly using polyethylene sheets to analyze the apparent mineral availability.

At the end of the experiment, two birds from each replicate unit were picked up randomly and slaughtered to calculate dressing and toe ash percentage. Toe ash percentage was also used as response criteria for P retention. Toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones. The left and right middle toes of one bird within a pen were obtained, yielding two samples of toes per pen. The clipped toes were cleaned of any waste material, but were left intact otherwise. No flesh, skin, or nail was removed.

Table 1. Ingredient Composition of Experimental Diets

Ingredient (%)	A	B	C	D
Maize	46.14	46.14	46.14	46.14
Rice polishing	9.00	9.00	9.00	9.00
Soybean meal	24.27	24.27	24.27	24.27
Canola meal	7.00	7.00	7.00	7.00
Corn gluten 60%	5.00	5.00	5.00	5.00
Vegetable oil	3.16	3.16	3.16	3.16
Molasses	2.00	2.00	2.00	2.00
Limestone	1.58	1.58	1.58	1.58
Di-calcium phosphate	1.10	1.10	1.10	1.10
Premix*	0.50	0.50	0.50	0.50
DL-Methionine	0.12	0.12	0.12	0.12
Natuphos (U/ kg)	0	250	500	750
Nutrient Composition (calculated)				
Metabolizable energy (kcal/kg)	3020	3043	3000	3000
Crude protein (%)	20.9	21.35	21.00	21.20
Calcium (%)	0.99	0.99	1.00	1.00
Av. phosphorus (%)	0.35	0.35	0.35	0.35
Total phosphorus (%)	0.55	0.55	0.55	0.55
Phytate phosphorus (%)	0.20	0.20	0.20	0.20
Crude fiber	3.90	3.90	3.90	3.90
Linoleic acid	3.02	3.02	3.02	3.02
Methionine	0.50	0.50	0.50	0.50
Lysine	1.08	1.10	1.10	1.10
Threonine	0.78	0.79	0.80	0.81

*Supplied per kilogram of diet: retinyl acetate, 1,816µg; cholecalciferol, 132µg;dl-α-tocopheryl acetate, 53 mg; menadione sodium bisulfate complex, 1.5mgg; riboflavin, 15mg; dl-calcium pantothenate, 19.4; niacin, 52.8; cyanocobalamin, 22 µg; choline chloride, 2025mg; biotin, 0.62mg; folic acid, 6.2 mg; thiamin HCl, 16 mg; pyridoxine HCl, 6.2 mg; ethoxyquin, 100mg; virginiamycin, 5.8mg. Levels of trace minerals per kilogram of diet: manganese, 88mg; zinc, 95mg; iron, 100mg; copper, 12.5mg; iodine, 4mg; and selenium, 0.6mg.

Analytical Methods: Proximate analysis of feed and feces per replicate unit was made by standard procedure (A.O.A.C., 1990). The toe samples were dried to a constant weight at 105°C and then ashed in a muffle furnace at 600°C for four hours. Toe ash was expressed as a percentage of dry weight. Afterwards, the ash was wet-digested using a nitric acid: perchloric acid mixture (5:3 vol/vol). The Ca, P and Mg contents of toe ash were determined by Atomic Absorption Spectrophotometer.

Statistical Analysis: The experiment was laid down under Completely Randomized Design.

The technique data thus collected were subjected to analysis of variance (ANOVA) and the differences among the means were tested by applying Duncan Multiple Range Test (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Weight gain: The average weight gain of chicks fed on different rations is mentioned in Table 2. The highest weight gain (1014.03 grams) was observed in chicks fed on ration C containing phytase @ 500 U/kg while lowest weight gain (847.46 grams) was recorded in chicks fed on ration A containing no phytase. Analysis of variance showed significant (P<0.05) difference between weight gain of chicks fed on different rations. Ration A as compared to those fed on rations B, C, and D. However, non-significant difference was observed between the weight gains of chicks fed on rations B, C, and D. Phytase supplementation increased the body weight gain of chicks as the phytase level in diets increased from lower to higher level at low available P (0.35%).

Similar observations were recorded by Sebastian *et al.* (1996b), Mitchell and Edwards (1996), Qian *et al.* (1997), Cabahug *et al.* (1999) and Shirley and Edwards (2003). They reported that the growth rate on low P diets containing microbial phytase was improved. The improvement in the growth performance observed in chicks fed phytase supplemented rations may be due to;) the release of minerals from phytate mineral complex;) the utilization of inositol by birds;) phytase enzyme improved the digestibility of dry matter and CP in feed, as suggested by Ravindran *et al.* (2000, 2001).

The results indicated that a decrease to 0.1 % available P than recommended level (NRC, 1994) in broiler ration with addition of phytase is acceptable. The same observation were reported by Sohail and Roland (1999) and Yan *et al.* (2001) who also reported that P level in broiler ration could be reduced by 0.1 % by the supplementation of phytase.

Feed Consumption: The results of feed consumption of chicks fed on different rations are presented in Table 2. The highest feed consumption (1726.77 grams) was observed in chicks fed on ration C containing 500U/kg phytase while lowest feed consumption (1561.99 grams) was observed in chicks fed on ration A containing no phytase unit. Analysis of variance showed significant (P<0.05) difference between feed consumption of chicks fed on different rations. The results indicated that phytase improves the feed consumption.. The response to phytase addition at low P level in this study agree with the findings of Ravindran *et al.* (1995), Sebastian *et al.* (1996b), Mitchell and Edwards (1996), Qian *et al.* (1997), Yan *et al.* (2001), Viveros *et al.* (2002) and Shirley and Edwards (2003) who reported that feed intake and growth rate increased with the supplementation of phytase enzyme at low available P in broiler diets.

Feed Conversion Ratio: The best FCR 1.70 was observed in chicks fed on ration C containing 500 U/kg phytase while the poorest FCR 1.84 was recorded in chicks fed on ration A containing no phytase (Table 2). The results of present experiment revealed non-significant difference in FCR of chicks fed on rations B, C, and D. However, the FCR of chicks fed on phytase supplemented rations B, C, and D was significantly improved as compared to ration A. The results of this study are in line of finding of Ravindran *et al.* (1995), Sebastian *et al.* (1996b), Mitchell and Edwards (1996), Qian *et al.* (1997), Cabahug *et al.* (1999), Van *et al.* (2000), Viveros *et al.* (2002) and Shirley and Edwards (2003) who reported that phytase supplementation improve the feed conversion ratio.

Dressing percentage and Toe ash percentage: The maximum dressing percentage (60.77%) was observed in chicks fed on ration C containing 500U/kg phytase while minimum dressing percentage (58.68%) was recorded in chicks fed on ration A containing no phytase (Table-2). Analysis of variance revealed a significant (P<0.05) difference between dressing percentage of chicks fed on ration A as compared to those fed on rations B, C, and D. Whereas there was a non-significant difference between the dressing percentage of chicks fed on rations B, C, and D. The results of dressing percentage of the present study showed improvement with supplementation of phytase enzyme but the differences were nonsignificant between birds fed on rations B, C, and D. Similar observation was recorded by Viveros *et al.* (2002) who concluded that the use of phytase enzyme improved dressing% of broiler chicks.

Toe Ash Percentage: The maximum toe ash percentage (11.99%) was observed in chicks fed on ration C containing 500U/kg phytase while minimum toe ash percentage (10.89%) was recorded in chicks fed on ration A containing no phytase. There was a significant (P<0.05) difference between toe ash percentage of chicks fed on ration C as compared to those fed on rations A, B, and D. However, non-significant difference was observed between the toe ash percentage of chicks fed on rations A, B, and D. Similar observations were recorded by Roberson and Edwards (1994), Ravindran *et al.* (1995), Sebastian *et al.* (1996b), Mitchell and Edwards (1996), Qian *et al.* (1997), Cabahug *et al.* (1999), Van *et al.* and (2000).

Apparent availability of Minerals: The effect of phytase supplementation on P availability in broiler chicks fed on different rations are summarized in Table 3. The results of present study indicated that phytase supplementation significantly ($P<0.05$) improved the availability of P Ca and Mg in the chicks fed on rations B, C and D as compared to those fed on ration A which agree with the results of previous studies of Ravindran *et al.* (1995), Sebastian *et al.* (1996b), Mitchell and Edwards (1996), Qian *et al.* (1997), Cabahug *et al.* (1999), Yan *et al.* (2000), Viveros *et al.* (2002) and Shirley and Edwards (2003).

Table 2. The effect of phytase supplementation on weight gain, feed consumption, FCR, dressing percentage, and toe ash percentage of broilers (0-4 wk)

Diet	Wt. Gain (g)	Feed consumed (g)	FCR	Dressing %	Toe ash %
A	847.46	1561.99	1.84	58.68	10.89
B	995.26	1705.12	1.71	60.60	11.18
C	1014.03	1726.77	1.70	60.77	11.99
D	991.23	1710.83	1.73	60.65	11.56

Table 3. The effect of phytase supplementation on apparent availability of minerals in broilers (0-4 wk)

Diet	Phosphorus %	Calcium %	Magnesium %
A	43.81	38.14	21.80
B	61.60	42.26	27.92
C	63.15	44.35	28.58
D	63.68	43.93	28.34

Economics of experimental diets: The cost of one kg of live weight (excluding the cost of day-old chick) was Rs. 22.50, 21.07, 21.14, and 21.60 in the chicks fed on ration A, B, C and D, respectively. It has been observed that as the level of phytase enzyme increases from lower to higher, the cost per kg of the experimental ration increases.

Table 4. Economics of experimental diets.

	A	B	C	D
Cost/ kg diet (Rs.)	12.21	12.30	12.41	12.51
Cost/ kg live wt.	22.50	21.07	21.14	21.60

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