Effects of Phytase Supplementation on the Performance of Broiler Chickens Fed Maize and Wheat Based Diets with Different Levels of Non-phytate Phosphorus

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ABSTRACT : An experiment was conducted to evaluate the effects of phytase supplementation on the growth performance, nutrients utilization and bone mineralization in broiler chickens. Day-old broiler chicks (n=480) were equally devided into eight treatment groups and fed maize or wheat based isocaloric, isonitrogenous and isocalcium diets having two non phytate phosphorus (NPP) concentrations (0.50% and 0.30%) and two phytase levels (0 and 500 phytase units/kg diet) in a 42 days growth trial. Maize based dietary treatments were MC (NPP 0.50%, MN (NPP 0.30%), MNP (MN+500 units of phytase) and MCP (MC+500 units of phytase), whereas wheat based experimental diets were WC (NPP 0.50%), WN (NPP 0.30%), WNP (WN+500 units of phytase) and WCP (WC+500 units of phytase). The NPP levels were maintained by dicalcium phosphate. Reduction in dietary NPP depressed live weight gain and feed intake and increased feed conversion ratio (FCR). Phytase supplementation to low NPP (0.30%) diets significantly ($p<0.05$) improved the growth performances of broilers. The supplementation to low NPP diets allowed complete, safe and economic replacement of dietary inorganic P (dicalcium phosphate) to reduce feed cost per kg live weight gain of broilers. Reduction in dietary NPP did not affect retention of nutrients except phosphorus (P) but had a significant ($p<0.05$) depression in tibia ash and minerals (Ca, P) concentration in serum and tibia ash. Phytase supplementation at low NPP level was effective ($p<0.05$) in improving the retention of dry matter, Ca and P and Ca and P concentration in serum and tibia ash. However, the supplementation was not effective at high level of NPP (0.50%). There were no significant ($p>0.05$) differences in carcass quality among dietary treatments. The response of phytase was greater in low NPP and maize based diets as compared with high NPP and wheat based diets, respectively. The results show that phytase supplementation to low NPP (0.30%) diets improved the growth performance, relative retention of nutrients (N, Ca and P) and minerals (Ca, P) status of blood and bone in broiler chickens, with a better efficacy in maize based diets. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1642-1649)***

Key Words : Broiler, Phytase, Maize, Wheat, Non- Phytate Phosphorus (NPP), Nitrogen, Calcium, Growth Performance

INTRODUCTION

Phytic acid (*myo*-inositol 1, 2, 3, 4, 5, 6-hexikis dihydrogen phosphate) is a potent anti-nutritional factor present in plant-derived feeds, which binds 60 to 80% of the total phosphorus (P). Phytic acid not only forms an insoluble complexes with various di-valent and tri-valent cations (Reddy et al., 1982; Oberleas and Harland, 1996) but also has the ability to form complex with protein (O'Dell and Boland, 1976), proteolytic enzymes (Singh and Krikorian, 1982; Caldwell, 1992), starch and (α-salivary amylase (Thompson and Yoon, 1984) and fatty acid in the gastrointestinal tract (Lesson, 1993), thereby, lowering their utilization in poultry (Ravindran et al., 1995). A major portion of poultry diets consists of plant-derived feed ingredients, therefore, phytic acid assumes a considerable significance in poultry nutrition. Phytase (EC 3.1.3.8, *myo*-inositol hexaphosphate phosphohydrolase) is an enzyme which catalyses the stepwise removal of inorganic orthophosphate from the phytic acid (Nayni and Markakis, 1986). Concentration of the enzyme in the small intestine of the chicken is too low to degrade phytate, effectively (Maenz et al., 1997). In recent years, the availability of microbial phytase has provided a practical way of releasing the phytate bound P and alleviating other anti-nutritional activity of phytic acid.

Maize is generally used as a source of energy in poultry ration. A comparable nutrients composition of wheat and its surplus production in India, has provided it as an alternative of maize. In addition, wheat is rich in vegetal phytase (1,193 phytase units/kg) (Eeckhout and dePaepe, 1994), which may have an added advantage as it can act upon phytate of other ingredients as well (Scheurmann et al., 1988). Maize and wheat contains 0.24% and 0.27% phytate P, respectively (Ravindran et al., 1995). Most of the studies regarding the effect of phytase supplementation in poultry feeding have been conducted using maize based ration. Correspondingly, studies utilizing wheat based diet is limited. The present experiment was conducted to evaluate the comparative performance of broilers fed phytase supplemented maize or wheat based rations.

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Table 1. Percent proportions of feed ingredients or supplements in the starter mashes (0-4 weeks)

Ingredients/supplements (%)	Treatments							
	MC	MN	MNP	MCP	WC	WN	WNP	WCP
Maize	40.00	40.00	40.00	40.00	-	-	-	-
Wheat	-	-	-	-	45.00	45.00	45.00	45.00
Soybean meal	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Groundnut cake	11.00	11.00	11.00	11.00	10.00	10.00	10.00	10.00
Rice polish	12.00	12.00	12.00	12.00	12.00	12.00	12.00	12.00
Deoiled rice polish	8.70	9.20	9.20	8.70	7.70	8.20	8.20	7.70
Fish meal	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Dicalcium phosphate	1.10	-	-	1.10	1.10	-	-	1.10
Calcium carbonate	0.90	1.50	1.50	0.90	0.90	1.50	1.50	0.90
Lysine	0.05	0.05	0.05	0.05	-	-	-	-
DL-methionine	0.07	0.07	0.07	0.07	0.04	0.04	0.04	0.04
Others[1]	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Phytase[2]	-	-	0.020	0.020	-	-	0.020	0.020
Chemical composition (on dry matter basis)								
Metabolisable energy (kcal/kg)*	2,800	2,812	2,812	2,800	2,803	2,816	2,816	2,803
Crude protein (%)**	22.91	22.99	22.99	22.91	22.96	23.04	23.04	22.96
Lysine (%)*	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Methionine (%)*	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Calcium (%)**	1.21	1.20	1.20	1.21	1.21	1.20	1.20	1.21
Total phosphorus (%)**	1.00	0.82	0.82	1.00	0.99	0.79	0.79	0.99
Non-phytate phosphorus (%)**	0.50	0.31	0.31	0.50	0.50	0.30	0.30	0.50
Feed cost (Rs./100 kg)*	689	671	682	700	679	661	672	690

* Calculated values, ** Analyzed values.

[1] Others include common salt (300 g), Avet TM (Each 100 g contained Mn 10.8 g, Zn 10.4 g, Fe 4.0 g, Cu 400 mg and Co 200 mg)-50 g, Indomix BE (Each gram contained vit. B_1 4 mg, vit. B_6 8 mg, vit. B_{12} 40 mg, niacin 60 mg vit. E 40 mg and calcium pentothenate 40 mg)-10 g, Indomix A+B_2+D_3+K (Each gram contained vit. A. 82,500 IU, vit. D_3 12,000 IU, vit. B_2 50 mg and vit. K 10 mg-25 g, Cygro (1% maduramycin ammonia)-50 g and lincomix (4.4% lincomycin)-10 g.

[2] Each gram phytase (Bio- Feed Phytase® CT, NOVO Nordisk, A/S Denmark) contained activity of 2,500 FYT. One FYT is defined as the amount of enzyme that releases one µmole inorganic phosphate per minute from 5.0 mM sodium phytate at pH 5.5 and 37°C.

MATERIALS AND METHODS

Animal and diet

Four hundred and eighty, day-old Hubbard broiler chicks were purchased from a commercial hatchery, wing banded and weighed individually. The birds were distributed equally into 24 floor pens (20 birds per pen) following completely randomized design. Three pens were assigned to each treatment and raised upto six week of age under standard and similar managemental conditions. Feed ingredients used in the formulation of experimental diets were obtained in bulk and were analyzed for the proximate constituents (AOAC, 1995), phytic acid and phytate P (Haugh and Lantzsch, 1983). The non-phytate phosphorus (NPP) was calculated by subtracting the phytate-P from the total P. Eight isocaloric, isonitrogenous and isocalcium experimental diets were formulated to meet BIS (1992) requirement except NPP and phytase enzyme. (Table 1 and 2). There were two NPP levels tested i.e 0.50% and 0.30%. The NPP source used was dicalcium phosphate (DCP) containing 22.01% Ca and 17.21% P. Phytase enzyme (Bio-Feed Phytase® CT, NOVO NORDISK, Denmark) was added in the diets at the rate of 20 g/100 kg diets to provide 500 phytase units (FYT) per kg of diet. One FYT is defined as the quantity of enzyme which releases one µmole of inorganic phosphate per minute from 5.0 mM sodium phytate at pH 5.5 and 37°C. Maize based dietary treatments were MC (NPP 0.50%, MN (NPP 0.30%), MNP (MN+500 FYT phytase) and MCP (MC+500 FYT phytase), whereas wheat based experimental diets were WC (NPP 0.50%), WN (NPP 0.30%), WNP (WN+500 FYT phytase) and WCP (WC+500 FYT phytase). Birds were given a starter diet (Table 1) from 0 to 4 weeks of age and a finisher diet (Table 2) from 5 to 6 weeks of age. Diets and water were fed *ad. libitum*. Individual live weight gain (LWG) and feed intake on pen basis were recorded at fortnightly interval and feed conversion ratio (FCR) were calculated for the same periods. Mortality and clinical health status of the birds were monitored daily.

Economics of phytase supplementation

Relative economics was calculated at the end of sixth week. The information about the prices of major feed ingredients used in the rations was obtained from the Department of Animal Feed Technology, CCS Haryana Agricultural University, Hisar, India and prices of feed supplements were considered as per the rate list of respective manufacturer. Total feed intake, cost of feed

Table 2. Percent proportions of feed ingredients or supplements in the finisher mashes (5-6 weeks)

Ingredients/supplements (%)	Treatments							
	MC	MN	MNP	MCP	WC	WN	WNP	WCP
Maize	52.00	52.00	52.00	52.00	-	-	-	-
Wheat	-	-	-		55.00	55.00	55.00	55.00
Soyabean meal	14.00	14.00	14.00	14.00	12.00	12.00	12.00	12.00
Groundnut cake	10.00	10.00	10.00	10.00	11.00	11.00	11.00	11.00
Rice polish	10.00	10.00	10.00	10.00	10.60	10.60	10.60	10.60
Deoiled rice polish	5.60	6.10	6.10	5.60	3.00	3.50	3.50	3.00
Fish meal	6.00	6.00	6.00	6.00	6.00	6.00	6.00	6.00
Dicalcium phosphate	1.20	-	-	1.20	1.20	-	-	1.20
Calcium carbonate	0.90	1.60	1.60	0.90	0.90	1.60	1.60	0.90
Others[1]	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47
Phytase[2]	-	-	0.020	0.020	-	-	0.020	0.020
Chemical composition (on dry matter basis)								
Metabolisable energy (kcal/ kg)*	2,900	2,911	2,911	2,900	2,904	2,905	2,905	2,904
Crude protein (%)**	20.10	20.17	20.17	20.10	20.09	20.21	20.21	20.09
Lysine (%)*	1.00	1.01	1.01	1.00	1.10	1.07	1.07	1.10
Methionine (%)*	0.38	0.38	0.38	0.38	0.40	0.40	0.40	0.40
Calcium (%)**	1.20	1.21	1.21	1.20	1.22	1.20	1.20	1.22
Total phosphorus (%)**	0.95	0.76	0.76	0.95	0.93	0.74	0.74	0.93
Non-phytate phosphorus (%)**	0.50	0.30	0.30	0.50	0.50	0.30	0.30	0.50
Feed cost (Rs./100 kg)*	676	657	668	687	673	654	665	683

* Calculated values, ** Analyzed values.

[1] Others include common salt (300 g), avet TM (each 100 g contained Mn 10.8 g, Zn 10.4 g, Fe 4.0 g, Cu 400 mg and Co 200 mg)-50 g, indomix BE (Each gram contained vit. B_1 4 mg, vit B_6 8 mg, vit. B_{12} 40 mg, niacin 60 mg vit. E 40 mg and calcium pentothenate 40 mg)-10 g, indomix A+B_2+D_3+K (Each gram contained vit. A. 82,500 IU, vit. D_3 2,000 IU, vit. B_2 50 mg and vit. K 10 mg-25 g, Cygro (1% maduramycin ammonia)-50 g and lincomix (4.4% lincomycin)-10 g.

[2] Each gram phytase (bio-feed phytase® CT, NOVO Nordisk, A/S Denmark) contained activity of 2,500 FYT. One FYT is defined as the amount of enzyme that releases one μmole inorganic phosphate per minute from 5.0 mM sodium phytate at pH 5.5 and 37°C.

consumed and the average LWG of a broiler per treatment during the research trial was recorded and economics in terms of feed cost (Rs./kg LWG) were calculated and compared among treatments to know the benefit of phytase supplementation. The cost of starting (Table 1) and finishing mashes (Table 2) were calculated separately.

Metabolic trial

A metabolic trial was conducted during 6th week of the experiment (40-42 days). Four birds per replicate were selected at random and were housed in 24 different metabolic cages. After four days of an adjustment period, a measured amount of feed was offered and daily feed consumption by the birds was recorded on cage basis. Excreta voided were collected in every 24 h from each pen in a separate wide mouth plastic bottle. At the end of collection period, excreta of each pen were thoroughly mixed and weighed. Representative samples of the excreta were dried in an oven at 60°C and ground to pass through 1 mm sieve. These ground excreta samples were analysed for total nitrogen (N), Ca and P contents (AOAC, 1995). All calculations were expressed on dry matter (DM) basis. The difference in the nutrient content of the feed consumed and of the faeces voided was used to calculate the retention of nutrients (N, Ca and P) on percentage DM basis.

Serum analysis

After the metabolic trial (42th day), nine birds (3 birds per replicate) were randomly selected and sacrificed by cervical dislocation. Blood samples were collected in clean, dry and non-heparinized test tubes. Blood samples were allowed to clot at room temperature and sera were obtained. Sera collected from the samples were analyzed for the Ca (Gitelman, 1967) and inorganic P content (Fiske and Subba Raw, 1925).

Bone analysis

Left tibia of the slaughtered birds used for blood sampling was removed. After removing any adhering soft tissue, tibiae were defattened by soaking in the petroleum ether for 48 h. The samples were dried to a constant weight at 100°C and then ashed at 600°C for 12 h in a muffle furnace for the determination of bone ash, which was expressed on dry weight of the tibia. Bone ash and its Ca and P were analyzed (AOAC, 1995) and expressed as a percentage of dry and fat free tibia ash.

Carcass quality

At the end of the experiment, nine birds per dietary treatment (three from each replicate) were randomly selected. The birds were kept off feed for 12 h prior to their sacrifice, but the drinking water was provided. Immediately

Table 3. Effect of phytase supplementation on the growth performance and comparative economics of broilers

Parameters	Treatments							
	MC	MN	MNP	MCP	WC	WN	WNP	WCP
Live weight gain (g)								
(0-4 wks.)	983.23^{f}±6.66	817.99^{b}±11.36	933.95^{d}±12.40	990.06^{f}±13.89	954.00^{e}±19.86	775.46^{a}±13.42	886.92^{c}±22.37	952.99^{e}±14.38
(0-6 wks.)	1,764.18^{e}±13.74	1,426.58^{a}±14.43	1,680.00^{d}±23.58	1,769.92^{e}±26.77	1,612.66^{c}±12.44	1,435.50^{a}±33.64	1,587.20^{b}±22.41	1,633.73^{c}±17.89
Feed intake (g)								
(0-4 wks.)	1,877.96^{de}±18.77	1,644.16^{b}±17.86	1,709.12^{c}±14.71	1,900.92^{e}±24.94	1,869.84^{d}±14.92	1,550.92^{a}±16.08	1,694.20^{c}±20.59	1,877.20^{de}±11.08
(0-6 wks.)	4,110.60^{a}±26.37	3,380.99^{b}±28.87	3561.60^{c}±39.57	4,141.60^{d}±16.05	3,822.00^{e}±40.66	3,444.00^{f}±38.71	3,602.49^{g}±44.34	3,855.00^{h}±30.03
Feed conversion ratio (FCR)								
(0-4 wks.)	1.91^{b}±0.00	2.1^{e}±0.01	1.83^{a}±0.01	1.92^{b}±0.01	1.96^{c}±0.00	2.00^{de}±0.01	1.91^{b}±0.01	1.97^{cd}±0.02
(0-6 wks.)	2.33^{c}±0.02	2.37^{cd}±0.01	2.2^{a} ±0.01	2.34^{c}±0.01	2.37^{cd}±0.01	2.40^{d}±0.01	2.27^{b}±0.01	2.36^{cd}±0.02
Feed cost/kg wt. gain (Rs.)	15.89	16.01	14.30	16.22	16.02	16.11	15.13	17.42

$^{a-h}$ Values in a row bearing different superscripts differ significantly ($p<0.05$).

Table 4. Effect of phytase supplementation on nutrients utilization by broilers

Parameters	Treatments							
	MC	MN	MNP	MCP	WC	WN	WNP	WCP
Retention (%)								
Dry matter	66.50^{c}±0.33	70.08^{d}±0.21	71.65^{e}±0.83	71.80^{e}±0.62	62.21^{a}±0.23	64.32^{b}±0.72	66.18^{c}±0.16	68.88^{d}±0.30
Nitrogen	53.54^{ab}±0.78	54.12^{bc}±0.16	58.26^{ef}±0.74	59.18^{f}±0.72	51.78^{a} ±0.36	52.40^{ab}±1.10	55.85^{cd}±0.85	56.37^{de}±0.47
Calcium	42.81^{b}±0.73	40.75^{b}±0.10	44.78^{c}±1.12	42.70^{b}±0.44	40.95^{b}±0.47	38.02^{a}±0.65	45.43^{c}±0.43	41.60^{b}±0.58
Phosphorus	50.40^{a}±0.77	56.67^{c}±0.58	65.80^{f}±0.47	61.45^{d}±0.52	51.72^{a}±0.39	54.31^{b}±0.18	64.21^{e}±0.26	60.84^{d}±0.38
Serum concentration (mg/dl)								
Calcium	11.20^{b}±0.44	10.31^{a}±0.36	11.46^{b}±0.26	10.80^{ab}±0.42	11.31^{ab}±0.27	10.35^{a}±0.28	11.09^{ab}±0.11	11.02^{ab}±0.09
Phosphorus	5.86^{bcd}±0.13	4.26^{a}±0.26	5.14^{b}±0.07	5.69^{bc}±0.50	6.64^{de}±0.33	5.97^{bcd}±0.23	6.33^{cde}±0.09	6.93^{e}±0.14

$^{a-f}$ Values in a row bearing different superscripts differ significantly ($p<0.05$).

after recording their live weights, the birds were slaughtered by severing the jugular vein and allowed to bleed completely following 'halal' method. The birds were then subjected to scalding at about 130°F for 30 seconds. The feathers were removed completely leaving the skin intact. Later their heads were separated at atlanto-occipital joint and shanks were cut out at hock joints and dressed weight recorded. Eviscerations were done by removing crop, gullet, trachea and preen glands. A horizontal cut was made at the rear of the keel bone. Thereby, the breast was a little upturned and pushed forward, exposing the viscera along with the visceral organs, which were then removed completely by pulling. The lungs were scrapped off and the heart, liver and gizzard constituting giblets removed carefully from the viscera. The gall bladder was recovered carefully from the liver with least damage. The gizzard was then opened, the contents washed out and inner epithelial lining discarded. The heart was made free from blood and adhering vessel. The eviscerated weight was recorded as the weight of carcass together with giblets. The weight of giblets and their constituting organs were also taken separately. All the weights related to carcass traits were expressed as the percentage of live weight.

Statistical analysis

The experimental results were subjected to an analysis of variance on the basis of pen means. The significance of differences between the treatment means was determined using Duncan's multiple range test (Kramer, 1956).

RESULTS

Growth performance and relative economics

The effects of different dietary treatment on growth performance of broiler are summarized in Table 3. Live weight gain, feed intake and feed efficiency depressed significantly ($p<0.05$) when dietary NPP were decreased from 0.50% to 0.30% in both maize and wheat based diets. However, at the same level of NPP (0.30% or 0.50%), the performance of broilers fed maize based ration was significantly superior than those on wheat based rations. Feed intake of different dietary treatments did not show any regular pattern. FCR was also significantly ($p<0.05$) affected by dietary NPP level. It was poorest (2.40 kg feed/kg LWG) in wheat based low NPP diet. In general, lowering dietary NPP increased FCR, indicating poor efficiency of feed utilization. Phytase supplementation to low dietary NPP significantly ($p<0.05$) improved the performance of broilers, however, it was not comparable to that of control treatments containing adequate dietary NPP (0.50%). The supplementation to adequate NPP did not show any improvement over the respective control groups, suggesting no significant effect of phytase supplementation at higher NPP level.

It was observed that the complete replacement of DCP by phytase reduced the cost of starter (Table 1) and finisher mashes (Table 2) by Rs. 7.00 and Rs. 8.00, respectively. Relative economics (feed cost/kg weight gain) followed the similar trend of weight gain. Maize based control diet was

Table 5. Effect of phytase supplementation on bone mineralisation and carcass quality of broilers

Parameters	Treatments							
	MC	MN	MNP	MCP	WC	WN	WNP	WCP
Tibia Characteristics								
Dry weight (% of live wt.)	$7.96^{a}\pm0.18$	$7.38^{a}\pm0.21$	$7.96^{a}\pm0.18$	$8.38^{a}\pm0.32$	$8.11^{a}\pm0.05$	$7.17^{a}\pm0.33$	$7.82^{a}\pm0.36$	$8.37^{a}\pm0.04$
Ash (% of DM)	$47.57^{c}\pm0.21$	$43.17^{a}\pm0.68$	$47.57^{c}\pm0.21$	$51.5^{de}\pm0.72$	$52.19^{e}\pm0.75$	$45.86^{b}\pm0.34$	$48.74^{c}\pm0.26$	$52.22^{e}\pm0.40$
Calcium (% of ash)	$35.35^{a}\pm0.20$	$35.32^{a}\pm0.23$	$36.78^{bc}\pm0.39$	$37.08^{c}\pm0.61$	$36.02^{ab}\pm0.10$	$35.86^{ab}\pm0.16$	$37.05^{c}\pm0.27$	$37.50^{c}\pm0.16$
Phosphorus (% of ash)	$16.89^{b}\pm0.57$	$15.50^{a}\pm0.25$	$17.07^{bc}\pm0.19$	$17.75^{bcd}\pm0.24$	$17.50^{bcd}\pm0..25$	$15.95^{a}\pm0.20$	$17.89^{cd}\pm0.13$	$18.16^{d}\pm0.29$
Carcass traits (% of live weight)								
Dressed weight	$80.72^{a}\pm0.11$	$79.13^{a}\pm0.44$	$78.62^{a}\pm0.58$	$80.53^{a}\pm0.22$	$78.99^{a}\pm0.63$	$79.49^{a}\pm1.01$	$78.89^{a}\pm1.16$	$78.83^{a}\pm0.63$
Eviscerated weight	$73.02^{b}\pm0.97$	$70.70^{a}\pm0.51$	$72.57^{ab}\pm0.18$	$71.74^{ab}\pm0.71$	$70.92^{ab}\pm0.87$	$71.21^{ab}\pm0.73$	$70.44^{a}\pm0.56$	$70.55^{a}\pm0.50$
Giblets weight	$4.09^{a}\pm0.07$	$4.22^{a}\pm0.11$	$4.11^{a}\pm0.22$	$4.06^{a}\pm0.10$	$3.89^{a}\pm0.19$	$4.32^{a}\pm0.24$	$4.15^{a}\pm0.19$	$3.93^{a}\pm0.13$

$^{a-f}$ Values in a row bearing different superscripts differ significantly ($p<0.05$).

more economical than wheat based diets. Broiler production was most economical due to phytase supplementation in low NPP maize based diet. However, the supplementation in high NPP diets was a costlier affair without any beneficial effect. Total mortality during the trial was considered acceptable (3.43%) and the deaths were not related to dietary treatments.

Retention and serum concentration of nutrients

The effects of phytase supplementation on the serum concentration and retention of as a percentage of unit intake is summarized in Table 4. Dietary NPP had no influence on N retention but supplemental phytase significantly ($p<0.05$) increased average nitrogen retention, with no further improvement due to supplementation in high NPP diets. Nitrogen retention was greater in maize based rations than those based on wheat. Calcium retention at high NPP level was numerically, but not significantly ($p>0.05$) higher than that of the low NPP treatment. Reduction of dietary NPP from 0.50% to 0.30% improved the retention of P by birds in both maize and wheat based treatments. Phytase supplementation improved ($p<0.05$) dry matter, Ca and P retention, with greater effects in low NPP and maize based diets. In general, there was no improvement in retentions due to the supplementation in high NPP diets (0.50%). Reducing dietary NPP depressed minerals (Ca and P) concentration in broilers. Serum analyses (Table 4) showed a significant increase in Ca and inorganic P content as a result of phytase supplementation. An increased level of serum P was obtained at 0.30% NPP level with phytase, however, this value did not significantly differ from the value obtained at 0.50% NPP without phytase. The effect of supplementation was more pronounced in low NPP maize diet as compared to high NPP and wheat based diets. The supplementation at higher NPP did not produce any significant effect on the mineral status of the birds.

Bone mineralizations and carcass quality

The effects of phytase supplementation at different dietary NPP levels on minerals content of tibia bone and carcass quality of birds are summarized in Table 5. Dry weight of tibia (% of live weight) did not show any significant difference ($p>0.05$) among treatments, but numerically, phytase supplementation improved the weight of tibia. It was maximum (8.38%) in phytase supplemented diets having high NPP. Ash content and Ca and P content of tibia ash significantly ($p<0.05$) depressed due to reduction of NPP from 0.50 to 0.30%.

Phytase supplementation increased total ash content of tibia and its minerals (Ca and P) percentage. The tibial ash concentrations of the birds fed on control and low NPP plus phytase diets were not significantly ($p>0.05$) different. The highest tibia ash were recorded in birds fed on the normal NPP plus phytase diet. Carcass traits (dressed, eviscerated and giblet weight) as a percentage of live weight were not influenced by dietary NPP and/or phytase supplementation.

DISCUSSION

The study presented herein shows the effects of microbial phytase supplementation (500 units/kg diet) on growth performance, retention of nutrients and mineral utilization in broilers. Lowering NPP levels in broiler diets decreased feed intake and weight gain and increased FCR. Diets deficient in NPP are associated with decreased gain and feed intake (Denbow et al., 1995). It is evident that maintaining optimum Ca: NPP ratio resulted in better performance of broilers. Supplemental phytase to low NPP diets improved those parameters. Similar observations have been reported by Simons et al., 1990, Cabahug et al., 1999, Rama Rao et al., 1999, Lim et al., 2001; Zyla et al., 2001. An improved growth performance and complete replacement of DCP by phytase at low NPP reduced feed cost per kg LWG. However, the supplementation at higher dietary NPP was unable to reduce the broiler production due to higher cost of ration.

The improvement in growth performance observed in the chickens fed phytase were associated with increased feed intake and feed efficiency, which might be due to the release and utilization of P from the phytate- mineral complex (Qian et al., 1996; Sebastain et al., 1996) or utilization of inositol (Simons et al., 1990) or increase starch digestibility (Knuckles and Betschart, 1987) or increased utilization of protein and amino acids (Ravindran et al., 2000) or overall utilization of nutrients (Miles and Nelson, 1974).

Phytate-Ca forms insoluble complex with starch and fatty acids in the gastrointestinal tract of broilers, which depress the digestibility of carbohydrate and lipid (Rama Rao and Reddy, 2001). Therefore, supplemental phytase had positive effects on dry matter digestibility by releasing bound organic nutrients (Ravindran et al., 2000). Improved nitrogen digestibility due to added phytase has been reported in several studies (Kornegay, 1996; Sebastian et al., 1997; Ravindran et al., 2000). The interaction of phytic acid and protein forms phytate-protein complex, which are insoluble and less subject to attack by proteolytic enzymes than the uncomplexed protein (Cheryan, 1980). The phytate also binds with the major proteolytic enzyme, trypsin (Caldwell, 1992) eventually leading to lowered digestibilties of nitrogen and amino acids. It is therefore, likely that when phytase hydrolyses the ester bonds to release P from the phytic acid molecule, it will also release the phytate bound protein and removes the negative effects of phytic acid on proteolytic enzymes, thus, increasing the digestion and absorption of protein and amino acids (Ravindran et al., 2000).

Phosphorus retention improved as dietary NPP level reduced. An increase in P retention at low dietary NPP may be explained by the homeostasis mechanism of body that increases retention and absorption of P at low dietary intake as compared to normal dietary P (Allen and Wood, 1994).

Phytase treatments of diet has been reported to increase minerals (Ca, P) retention and its concentration in serum and tibia ash of broilers by various workers by various workers (Sebastian et al., 1996; Ahmad et al., 2000; Ravindran et al., 2000; Lim et al., 2001).

Phytate being a strong acid, can form various salts with essential minerals, thus, reducing their solubilities and ultimately their absorption (Sandberg and Svanberg, 1991). When phytate is hydrolyzed by microbial phytase, it may release all constituents minerals, *myo*-inositol and inorganic phosphate (Wodzinski and Ullah, 1996). Increase in P retention and its concentration in serum and bone gives evidence of phytate P utilization by microbial phytase, as Mitchell and Edward (1996) suggested that bone ash and serum P are the most sensitive and reliable methods for determining the animal's P need. Improvement in Ca retention and Ca content in tibia ash was expected because phytase liberates Ca from the Ca-phytate complex and as the availability of P increased, the availability of Ca also increased and both were deposited in the bones (Simon et al., 1990). It is known that P and Ca together accounts for more than 50% of the bone ash content (Qian et al., 1996).

Phytase treatment of diets has been reported to increase the tibia ash content by many workers (Nelson et al., 1971; Sebastian et al., 1996; Lim et al., 2001). It is considered to be a good indication of increased bone mineralization associated with phytase supplementation and consequently increased P and Ca availability.

A better performance of broilers fed maize based diets than those of wheat based diets was reported by Peng et al. (2003). It has been established that wheat contains vegetal phytase (1,193 units/kg) (Eckhout and dePaepe, 1994) but the better performance of broilers on maize based rations in comparison to wheat containing rations could be explained by the fact that in addition to phytate wheat contains another antinutritional factor, arabinoxylans (AX), a non-starch polysaccharides at levels of 5.27 to 8.18% of dry matter depending on cereal variety and growing conditions (Annison, 1990). The digestibility of AX varies from 4 to 19%, depending on age of broilers (Bolton, 1955). The soluble fractions of AX were shown to increase digesta viscosity and decrease apparent metabolisable energy (AME), ileal digestibility of starch, protein and lipid resulting in poor performance of birds (Annison, 1993; Maisonnier et al., 2001). Van der Klis (1993) observed a reduced mineral absorption at higher intestinal viscocities due to water soluble AX of wheat. It is known that the availability of plant P depends on the feed stuffs (Cromwell, 1980) and the phytase activity appears to depend upon the cultivar and/or drying and storage condition of cereal seeds. Genetic characteristic could also play a role in the determination of the phytase activity in wheat (Barrier-Guillot et al., 1996). Phytase in dry seeds is inactive but during germination, phytase in seeds is activated and hydrolyses phytate to release P to meet the requirement of growing plants. Furthermore, lots of new phytase are biosynthesized during germination, so, the activity of phytase increases in the course of germination (Ma and Shan, 2002), but not in dormant stage of seeds. Plant phytase (EC 3.1.3.26) has optimum pH of about 5.0, therefore, it fails to have the activity at low pH while microbial phytase (EC 3.1.3.8) is active over a wide pH range (Wodrinski and Ullah, 1996).

Control diets containing normal levels of P satisfy the birds requirements whereas phytase treatment of this diet increased both P and Ca contents which were not fully retained by the birds and led to excessive amounts being excreted. No significant improvement due to phytase supplementation at high concentration of inorganic P suggested that high NPP might have inhibit phytase activity because of a negative feed back mechanism (Irving and Cosgrove, 1974; Wise, 1983). Zhang et al. (2000) reported that the bio-availability of phytase P was linearly improved with dietary available P reduction.

It is concluded that microbial phytase supplementation (500 units/kg diet) to low non-phytate P (0.30%) maize or wheat based diets improved the growth performance, retention of nutrients (N, Ca and P) and minerals (Ca and P) content of serum and bone ash. The supplementation to practical broiler diets allowed the complete and safe replacement of usual dietary addition of inorganic phosphate to economize broiler production. Type of diets and dietary P had a significant effect on the response to supplemental phytase. The optimum performance were achieved at the 0.30% non-phytate P in maize based diets supplemented with microbial phytase in comparison to wheat based diets.

REFERENCES

AOAC. 1995. Official Methods of Analysis, 16th edn. Association of Official Analytical Chemists Washington, DC.

Ahmad, T., S. Rassol, M. Sarwar, A. Haq and Z. Hasan. 2000. Effect of microbial phytase produced from a fungus Aspergillus niger on bioavailibility of phosphorus and calcium in broiler chicken. Anim. Feed Sci. Techol. 83:103-114.

Allen, L. H. and R. J. Wood. 1994. Calcium and Phosphorus. In: Modern Nutrition in Health and Disease. (Ed. R. S. Goodhardt and M. E. Shils), Lea & Febiger, Philadelphia, PA. pp. 144-163.

Annison, G. 1990. Polysaccharide composition of Australian wheats and the digestibilities of their starches in broiler chicken diets. Aust. J. Exp. Agric. 83:183-186.

Annison, G. 1993. The role of wheat non-starch polysaccharides in broiler nutrition. Aust. J. Agric. Res. 44:405-422.

BIS. 1992. Poultry Feeds Specification, 4th Review. Bureau of Indian Standards. Manak Bhavan, 9, Bahadur Shah Zafar marg, New Delhi.

Barrier-Guillot, B., P. Casado, P. Maupetitt, C. Jondeville, F. Gatel and M. Latbier. 1996. Wheat phosphorus availability. I-*In vitro* study: Factors affecting endogenous phytasic activity and phytic phosphorus content. J. Sci. Food Agric. 70:62-68.

Bolton, W. 1955. The digestibility of carbohydrate complex by birds of different ages, J. Agric. Sci. 46:420.

Cabahug, S., V. Ravindran, W. L. Bryden and P. H. Selle. 1999. Response of broilers to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus levels. I. Effects on broiler performance and toe ash content. Br. Poult. Sci. 40:660-666.

Caldwell, R. A. 1992. Effect of calcium and phytic acid on the activation of trypsinogen and the stability of trypsin. J. Sci. Food Agric. 40:43-46.

Cheryan, M. 1980. Phytic acid interactions in food systems. CRC Critical Reviews in Food Science and Nutrition. 13:297-302.

Cromwell, G. L. 1980. Biological availability of phosphorus for pigs. Feedstuffs. March (3):38-42.

Denbow, D. M., V. Ravindran, E. T. Kornegay, Z. Yi and R. M. Hulet. 1995. Improving phosphorus availability in soybean meal for broilers by supplemental phytase. Poult. Sci. 74: 1831-42.

Eeckhout, W. and M. de Paepe. 1994. Total phosphorus phytate-phosphorus and phytase activity in plant feedstuffs. Anim. Feed Sci. Techol. 47:19-29.

Fiske, C. H., Y. Subba Row. 1925. The colorimetric determination of phosphorus. J. Biol. Chem. 66:375-400.

Gitelman, H. J. 1967. An improved automated procedure for the determination of calcium in biological specimens. Anal. Biochem. 18:521-531.

Haugh, W. and H. J. Lantzsch. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. J. Sci. Food Agric. 34:1423-26.

Irving, G. C. J. and D. J. Cosgrove. 1971. Inositol phosphate of microbial origin. Some properties of the partially purified phytase of *Aspergillu ficcum* NRRL 3135. Aust. J. Biol. Sci. 27:365-368.

Knuckles, B. E. and A. A. Betschart. 1987. Effect of phytate and other myo-inositol phosphate esters on alpha-amylase digestion of starch. J. Food Sci. 52:719-721.

Kornegay, E. T. 1996. Effectiveness of Natuphos phytase on protein and amino digestibility and nitrogen retention of poultry. In: Phytase in Animal Nutrition and Waste Management (Ed. M. B. Coelho and E. T. Kornegay). BASF Corporation, Mount Olive, NJ. pp. 493-514.

Kramer, C. Y. 1956. Extension of multiple range test to group means with unequal number of replicates. Biometrics. 12:307-310.

Lesson, S. 1993. Recent advances in fat utilization by poultry. In: Recent Advances in Animal Nutrition in Australia. (Ed. D. J. Farrell). University of New England, Armidale, Australia. pp. 170-181.

Lim, H. S., H. Namkung, J. S. Um, K. R. Kang, B. S. Kim and I. K. Paik. 2001. The effect of phytase supplementation on the performance of broiler chickens fed diets with different levels of non-phytate phosphorus. Asian-Aust. J. Anim. Sci. 14(2):250-257.

Ma, Xi and A. Shan. 2002. Effect of germination and heating on phytase activity in cereal seeds. Asian-Aust. J. Anim. Sci. 15 (5):1036-1039.

Maenz, D. D., C. M. Engele-Schaan, H. L. Classen. 1997. Endogenous phytase activity in the small intestinal brush border membrane of broiler chicks and laying hens. Poult. Sci. 76(suppl.):71.

Maisonnier, S., J. Gomer and B. Carre. 2001. Nutrient digestibility and intestinal viscocities in broiler chickens fed on wheat diets, as compared to maize diets with added guar gum. Br. Poult. Sci. 42:102-110.

Miles, R. D. and T. S. Nelson. 1974. The effect of enzymatic hydrolysis of phytate on the available energy content of feed ingredients for chicken and rats. Poult. Sci. 53:1714-1717.

Mitchell, R. D. and H. M. Edwards. 1996. Effects of phytase and 1, 25-dihydroxycholecalciferol on phytase utilization and the quantitative requirement for calcium and phosphorus in young broiler chickens. Poult. Sci. 75:95-110.

Nayni, N. R. and P. Markakis. 1986. Phytases. In: Phytic acid: Chemistry and Applications (Ed. E. Graf), Pitlatus Press, Minneapolis. pp. 101-110.

Nelson, T. S., T. R. Shieh, R. J. Wodzinski, J. H. Ware. 1971. Effect of supplemental phytase on the utilization of phytate phosphorus by chicks. J. Nutr. 101:1289-1294.

O'Dell, B. L. and A. D. Boland. 1976. Complexation of phytate with protein and cations in corn germ and oil seed meals. J. Agri. Food Chem. 24(4):804-808.

Oberleas, D. and B. F. Harland. 1996. Impact of phytate on nutrients availability. In: Phytase in Animal Nutrition and

Waste Management (Ed. M. B. Coehlo and E. T. Kornegay). BASF Corporation, Mount Olive, NJ. pp. 10-34.

Peng, Y. L., Y. M. Guo and J. M. Yuan. 2003. Effect of microbial phytase replacing partial inorganic phosphorus suplementation and xylanase on the growth performance and nutrient digestibility in broiler fed wheat based diets. Asian-Aust. J. Anim. Sci. 16(2):239-247.

Qian, H., E. T. Kornegay and D. M. Denbow. 1996. Phosphorus equivalence of microbial phytase in turkey diets as influenced by calcium to phosphorus levels. Poullt. Sci. 75:69-81.

Rama Rao, S. V., V. R. Reddy and R. S. Reddy. 1999. Enhancement of phytate phosphorus availability in the diets of commercial broilers and layers. Anim. Feed Sci. Technol. 79:211-222.

Rama Rao, S. V. and V. Ramasubba Reddy. 2001. Phytic phosphorus for eco-friendly products. Poult. International 40: 46-52.

Ravindran, V., W. L. Bryden and E. T. Kornegay. 1995. Phytates: Occurrence, bioavailability and implications in poultry nutrition. Poult. Avian Bio. Rev. 6(2):125-143.

Ravindran, V., S. Cabahug, G. Ravindran, P. H. Selle and W. L. Bryden. 2000. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorous levels. II. Effects on apparent metabolisable energy, nutrient digestibility and nutrient retention. Br. Poult. Sci. 41:193-200.

Reddy, N. R., S. Sathe and D. K. Salunke. 1982. Phytates in legumes and cereals. Adv. Food Res. 28:1-92.

Sandberg, A. S. and H. M. Svanberg. 1991. Phytate hydrolysis by phytase in cereals. Effects of *in vitro* estimation of ron availability. J. Food Sci. 56:559- 566.

Scheurmann Von, S. E., H. L. Lantzsch and K. H. Menke. 1988. *In vitro* and *in vivo* experiments on the hydrolysis of phytate. 2. Activity of plant phytase. J. Anim. Physiol. Anim. Nutr. 60:64.

Sebastian, S., S. P. Touchburn, E. R. Chavez and P. C. Lague. 1996. Efficacy of supplemental microbial phtase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. Poult. Sci. 76:1760-1769.

Sebastian, S., S. P. Touchburn, E. R. Chavez and P. C. Lague. 1997. Apparent digestibility of protein and amino acids in broiler chickens fed a corn-soybean diet supplemented with microbial phytase. Poult. Sci. 76:160-1769.

Simons, P. C. M., H. A. J. Versteegh, A. W. Jongbloed, P. A. Kemme, P. Slump, K. D. Bos, M. G. E. Wolters, R. F. Beudeker and G. J. Verschooor. 1990. Improvement of phosphorus availability by microbial phytase in broilers and pigs. Br. J. Nutr. 64:525-540.

Singh, M. and A. D. Krikorian. 1982. Inhibition of trypsin activity *in vitro* by phytate. J. Agri. Food Chem. 30:799-800.

Thomson, L. U. and J. H. Yoon. 1984. Starch digestibility as affected by poly phenols and phytic acid. J. Food Sci. 49:1228-1229.

Van der Klis, J. D., M. W. A. Versteegh and A. Van Voorst. 1993. Effect of soluble polysaccharides (carboxy methyl cellulose) on the the absorption of the minerals from the gastrointestinal tract of broilers. Br. Poult. Sci. 34:985-997.

Wise, A. 1983. Dietary factors determining the biological activity of phytase. Nutr. Abstr. Rev. 53:791-806.

Wodzeniski, R. J. and A. H. J. Ullah. 1996. Phytase. Adv. Appl. Microbiol. 42:263-303.

Zhang, Z. B., E. T. Kornegay, J. S. Radcliffe, D. M. Denbow, H. P. Veit and C. T. Larson. 2000. Comparison of genetically engineered microbial and plant phytase for young broilers. Poult. Sci. 79:709-717.

Zyla, K., J. Korleski, S. Swiatkiewcz, D. R. Ledoux and J. Piironen. 2001. Influence of supplemental enzyme on the performance and phosphorus excretion of broliers fed wheat based diets to 6 weeks of age. Anim. Feed Sci. Technol. 89:113-118.