ISSN 1682-8356
ansinet.org/ijps

INTERNATIONAL JOURNAL OF

POULTRY SCIENCE

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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

International Journal of Poultry Science 6 (3): 201-206, 2007
ISSN 1682-8356

Effect of Microbial Phytase in Soybean Meal Based Broiler Diets Containing Low Phosphorous

M.K. Mondal, S. Panda and P. Biswas
Department of Animal Nutrition, West Bengal University of Animal and Fishery Sciences,
37, Kshudiram Bose Sarani, Kolkata-700 037, India

Abstract: A six wk feeding experiment was conducted with d-old four hundred broiler chicks (Ven Cobb) to determine the effects of microbial phytase (Allzyme) supplementation in soybean meal based broiler diet containing low phosphorous. These birds were randomly divided into four dietary treatment groups of 100 broilers each. Each treatment group was further sub-divided into five replicates of 25 broilers per replicate. The treatments groups were control; low phosphorus; low phosphorus plus 250 PU phytase/kg diet; low phosphorus plus 500 PU phytase/kg diet. There were significant effects of dietary treatments on body weight, body weight gain, feed intake and feed conversion ratio at 0 to 42 days. The body weight and the body weight gain of the broilers fed the control and low phosphorus plus phytase diet were heavier ($p<0.05$) than other treatment. Feed conversion ratio of broiler fed on low phosphorus plus phytase 500 PU/kg was significantly better ($p<0.05$) than that of broilers fed on low phosphorus phytase supplementation had no effect on broiler mortality. Concentration of blood metabolites were unaffected ($p>0.05$) by the dietary treatments. Supplemental phytase (500 PU/kg) maintained the same plasma concentration of Ca and P as found in control whereas the concentration of Fe. Mn and Zn was unaffected by the dietary treatments. The percentage of tibia ash, Ca and phosphorus and retention of Ca and P was significantly increased by the addition of microbial phytase to low phosphorus diet. This study demonstrates that microbial phytase can compensate the untoward effect of low phosphorus levels in the soybean meal based broiler diet.

Key words: Phytase, performance, phosphorous, broiler chicken

Introduction

Protein part of a poultry ration is met up mainly with vegetable proteins plus little amount of animal protein (Alam *et al*., 2003). In poultry ration, protein costs involve about 45 per cent of the total feed cost (Lester, 1989). Among vegetables protein sources, soybean meal is comparatively cheaper and available in India throughout the year. Its nutritive value is quite fine when compared with other plant protein sources. But this mostly available and cheaper soybean meal is not suitable for using in higher amounts in poultry diet, because of its some antinutritional factors like phytate phosphorus, trypsin inhibitors, non-starch polysaccharides, oligosaccharides and lectins (Acamovic, 2001; NRC, 1994) which decrease feed consumption, growth rate and feed utilization. The major portion of phosphorus in soybean is in the form of phytate, which is largely unavailable in monogastric animals like poultry. These adverse effects of soybean meal could possibly be overcome by dietary supplementation of exogenous phytase (Bozkurt *et al*., 2006) which improve the availability of phytate-bound phosphorus and to reduce the phosphorus levels in effluent from intensive livestock operations. In addition to reducing phosphorus availability, phytates can also chelate divalent cations such as Ca, Mg, Fe, Zn, Cu, Mn and also can reduce protein availability (Ravindran *et al*., 2001; Bedford and Schulze, 1998). Diets deficient in P depressed growth rate and feed efficiency (Fernandes *et al*., 1999; Li *et al*., 2000), but microbial phytase supplementation has been shown to relieve the detrimental effects. Studies have shown that exogenous dietary phytase improves phytate phosphorus utilization and enhanced over all performance in broilers (Atia *et al*., 2000; Waldroup *et al*., 2000; Bozkurt *et al*., 2006). So there is a chance to study the effect of microbial phytase in soybean meal based broiler diets containing low phosphorous.

Materials and Methods

Birds and experimental design: Four hundred d-old commercial broilers (Ven Cobb) were randomly divided into four dietary treatment groups of 100 broilers each. Each treatment group was further subdivided into four replicates of 25 birds. All broilers were fed a typical commercial broiler starter diet for the first 3 wks of the experiment followed by finisher diet up to 6 wks. The treatment groups were control diet; a low phosphorous diet; low phosphorous plus 250 PU phytase (Allzyme)/kg diet and a low phosphorous plus 500 PU phytase/kg diet.

Housing and management: Chicks were housed in a battery type California cages which were cleaned thoroughly with formaldehyde and potassium

Table 1: Ingredients and chemical composition of starter and finisher diet

Attributes	Starter		Finisher	
	Basal	Low P	Basal	Low P
Ingredients, % air dry basis				
Maize	55.1	55.1	65.0	65.0
Soybean Meal	39.0	39.0	31.0	31.0
Sesame Cake	3.0	3.0	-	-
Soybean Oil	-	-	1.0	1.0
Di-Calcium Phosphate	1.5	0.75	1.5	0.75
Lime stone powder	0.20	0.20	0.20	0.20
Deoiled rice bran	0.64	1.39	0.74	1.49
Iodized Salt	0.10	0.10	0.10	0.10
Vitamin premix	0.02	0.02	0.02	0.02
Trace mineral mixture	0.10	0.10	0.10	0.10
Choline Chloride (60%)	0.05	0.05	0.05	0.05
Lincomycine (1%)	0.03	0.03	0.03	0.03
DL-Methionine	0.15	0.15	0.15	0.15
L-Lysine	0.05	0.05	0.05	0.05
Maduramycin	0.05	0.05	0.05	0.05
Mould inhibitor	0.01	0.01	0.01	0.01
Chemical composition[a]				
Crude protein, %	20.05	20.14	18.35	18.43
Ether extract, %	3.68	3.77	5.02	5.11
ME, Kcal/kg[b]	2830	2835	2910	2915
Calcium, %	1.22	1.05	1.10	0.94
Total P, %	0.65	0.52	0.63	0.50
Available P, %[b]	0.46	0.31	0.45	0.30

† Composition of each Kg trace mineral mixture : Cu, 15g.; Co, 02g.; Fe, 60g.; Zn, 80g.; Mn, 80g.; I, 02g.; Se, 0.3g.; Mo, 0.1g.
‡ Each Kg contains : Vitamin A- 80 MIU; Vitamin D_3- 12 MIU; Vitamin E- 70g; Vitamin K_3 -8g; Vitamin B_1- 6.4g; Vitamin B_2- 40g; Vitamin B_6-12.8g; Vitamin B_{12}-160mg; Nicotinic acid- 80g; Vitamin B_5- 115g; Folic acid-4g; Biotin-24mg. [a]Assayed value.
[b]Calculated on the basis of standard values applicable under Indian condition (Singh and Panda, 1996).

permanganate solution three days prior to arrival of birds. The d-old chicks were offered electrolyte solution upon arrival. Birds were maintained on a 24 hours constant light schedule. The brooding temperature was maintained close to their requirement, first by heating device for 3 days following arrival of chicks. Then no additional heating was required as the summer room temperature was found appropriate up to 3 weeks and finally by turning cooler fan during day time for the last 3 weeks of rearing period. The birds were vaccinated against Ranikhet disease and Infectious Bursal Disease on d 7, 14 and 21 and provided antibiotic for the first 5 days as per recommendation.

Details of the feeding regimens: The chicks were offered maize soybean meal based diet (broiler starter and broiler finisher in mash form). These diets were formulated to meet or exceed the BIS (1992) nutritional requirement of broiler chicken. The diets were fortified with mineral and vitamin premix as per the standard stipulated by the Bureau of Indian Standard for broiler chickens (1992). Ingredients and chemical composition of basal diet were presented in Table 1. Total amount of feed offered during 24 hours to a replicate under a specific treatment groups was divided into 3 equal proportions. The amount and timing of feed was adjusted in such a way that the birds consume the whole of the diet offered at any one time. As a result hardly any residue can be obtained from the replicate after a days feeding. The standard techniques of the proximate analysis were used to determine nutrient content of experimental diets (AOAC, 1995).

Record keeping: Individual body weight and feed consumption of broilers from all pens were measured at the 0, 21 and 42 day of age. Mortality of each pen was recorded on a daily basis. Feed conversion ratio was adjusted according to the feed consumption of the dead broilers. Body weight was recorded before offering feed. Body weight gain was obtained by calculation.

Metabolism trial: A metabolic trial of 3 days duration was conducted during the last three days of 6 week of feeding trial. During the metabolic trial total amount of feed consumed and total amount of excreta voided from each replicate of the individual experimental group was quantified. The excreta from each replicate was collected in a previous weighed clean and dry petridish and was oven dried at 100 ± 2^{0}C for subsequent estimation of Ca and P.

Chemical analysis of feed and faeces samples: The dietary ingredients were dried at 70^{0}C for 12 hrs in a hot air oven and ground to pass through a 1 mm sieve, were analyzed for DM, Crude protein and ether extract (AOAC, 1995). Calcium concentration of feed and faeces samples was determined by Flame Atomic Absorption Spectrophotometer (A Analyst 100, Perkin- Elmer Inc., USA). Phosphorous in the feeds and faeces were determined colorimetrically (AOAC, 1995).

Collection, processing and analysis of blood samples: Two birds from each replicate were slaughtered on d 43 by severing the carotid artery and jugular vein and blood samples were collected for analyses of plasma glucose, proteins (total proteins, albumin), cholesterol, enzymes {aspertate aminotransferase (AST) and alanine aminotransferase (ALT)} and minerals (Ca, P, Fe, Mn and Zn). Blood samples (approx. 10 ml) collected in heparinized vacuitainer tube (Becon Dickinson India Pvt. Ltd., New Delhi, India) for biochemical study. Immediately after collection, tubes were placed in an ice bath and transported to the laboratory. Plasma was harvested subsequently by centrifuging the whole blood samples at 3000 rpm for 15 min in centrifuge machine. The heparinized plasma samples were stored at -20^{0}C in Eppendorf tubes and analyzed subsequently. Plasma glucose, total protein, albumin, cholesterol, ALT and AST were analyzed in the Automatic Blood Analyzer (Microlab

Table 2: Effect of microbial phytase supplementation on the performance of broiler

Attributes	Age, day	Control	Low P Phytase/kg	250 PU Phytase/kg	500 PU	SEM	P
Body weight, g	0	39.18	39.22	39.45	38.75	0.52	0.125
	21	660.52^{a}	601.32^{c}	621.37^{b}	643.84^{a}	17.21	0.034
	42	2186.3^{a}	2112.6^{c}	2141.5^{b}	2168.2^{a}	19.24	0.026
Body weight gain, g	0-21	621.34^{a}	562.1^{c}	581.92^{b}	604.73^{a}	18.54	0.039
	0-42	2147.1^{a}	2073.4^{c}	2102.1^{b}	2129.5^{a}	19.37	0.015
Feed intake, g	0-21	950.3^{a}	925.6^{b}	925.4^{b}	935.7^{a}	23.25	0.021
	0-42	4012.2^{a}	4065.4^{b}	4051.2^{b}	4002.2^{a}	32.34	0.036
FCR, g/g	0-21	1.52^{a}	1.64^{c}	1.59^{b}	1.54^{a}	0.02	0.015
	0-42	1.86^{a}	1.95^{c}	1.92^{b}	1.87^{a}	0.02	0.027
Survivability, %	0-42	99.21	99.31	99.24	99.34	0.24	0.138^{a-c}

Means within row with no common superscripts differ significantly (P<0.05).

200, E-Merck India Ltd., Mumbai, India) using commercial kit (Transasia Bio-Medical Ltd., Ringanwada, Daman, India). Plasma minerals except P were determined as per the method described by Sandel (1950) and modified by Arenza *et al*. (1977) utilizing atomic absorption spectrophotometer (Perkin Elmer A Analyst 100). Plasma P was estimated by the method described by Fiske and Subbarow (1925).

Collection, processing and analysis of bone samples: At 43 days of age, two broilers per replicate, representative of the mean body weight, were killed by cervical dislocation; the right tibia of each broiler was removed and stored in a freezer at -18^{0}C for studying bone length and breadth, total ash and mineral (Ca, P and Zn) content. Tibias were cleaned by removing adhering tissue, then dried at 105^{0}C for 24 h and extracted with ether, dried again and reweighed. The length and breadth of dry fat-free bones were calculated by using slide calipers. Bones were burned in a muffle furnace at 600^{0}C for 6 h. Bone mineral concentration was determine by following the same procedure of minerals estimation of feeds and faeces.

Statistical analysis: The data were analyzed using the General Linear Models procedure of SPSS (1997). Significant differences between treatment means were separated using the Duncan's multiple range test.

Results and Discussion

Performance: The effects of microbial phytase supplementation on the performance of broiler are shown in Table 2. Reducing total phosphorus level from 0.65% to 0.52% during starter and 0.63% to 0.50% during finisher significantly depressed body weight at d 21 and 42, and body weight gain at d 0 to 21 and 0 to 42 compared with control diet. This lower body weight was due to deficiency of phosphorus in broilers fed 0.52% and 0.50% phosphorus level, which was slightly below the recommended level for broilers during starter and finisher period (NRC, 1994). This effect of phosphorus deficiency was also reported in broilers (Sohail and Roland, 1999; Fernandes *et al*. 1999; Bozkurt *et al*., 2006) and ducks (Orban *et al*., 1999). However, phytase supplementation at the level of 500 PU/kg diet to the starter and finisher diet ameliorated this negative effect but the level of 250 PU/kg diet failed to achieve the body weight of control. Broilers fed 0.50% phosphorus weighted 2112.6 g as compared with 2168.2 g for broilers receiving 0.50% phosphorus plus phytase at d 42. Phytase supplementation to low phosphorus diet also improved the body weight gain of broilers either the at 0 to 21 or 0 to 42 day intervals. These results were in agreement with those of Qian *et al*. (1997), Huff *et al*. (1998), Namkung and Leeson (1999), Zyla *et al*. (2000) and Bozkurt *et al*. (2006) which reported that the growth rate and feed conversion ratio of broilers fed the low phosphorus diets containing microbial phytase are comparable with or even better than those obtained for broilers fed the standard phosphorus diets. These results supported the concept that phytase was improving phosphorus availability and phosphorus level can be lowered in soybean based broiler starter and finisher diets added phytase.

Phytase supplementation to the low phosphorus diet at 500 PU/kg improved (P<0.05) feed conversion ratio of broilers at both d 21 and 42 compared with low phosphorus diet without phytase and low phosphorous plus 250 PU/kg. The current study supports the observations of Huff *et al*. (1998), Sohail and Roland (1999), Ravindran *et al*. (2001) and Bozkurt *et al*. (2006) who reported that phytase supplementation to broiler grower diets caused numerical improvements in feed efficiency of broilers fed a phosphorus deficient diet compared to phosphorus adequate diet fed without phytase. Feed intake and feed efficiency of broilers fed diet containing phytase (500 PU/kg) were also similar to those broilers fed control diet containing dicalcium phosphate. The results indicate that phytase at 500 PU/kg released phytate phosphorus that was adequately utilized for growth in a similar manner as would phosphorus supplied by dicalcium phosphate. But this effect of phytase supplementation was not encountered at the level of 250 PU phytase/kg diet. Similar results were observed for the broiler, duck and turkey diets respectively (Huff *et al*., 1998; Orban *et al*., 1999; Atia *et*

Table 3: Effect of microbial phytase supplementation on blood metabolites of broiler

Attributes	Control	Low P	250 PU Phytase/kg	500 PU Phytase/kg	SEM
Glucose, mg/dl	174.67	175.33	176.02	175.06	5.78
Total protein, mg/dl	7.07	6.69	6.75	6.85	1.32
Albumin, mg/dl	2.47	2.53	2.47	2.47	0.86
Cholesterol, mg/dl	93.11	93.42	93.67	93.52	3.42
Aspertate aminotransferase, U/l	86.33	87.33	85.00	84.33	4.41
Alanine aminotransferase, U/l	6.12	8.21	6.67	7.33	2.92

al., 2000). This result suggests that phytate phosphorus released by phytase was sufficient to meet starter and finisher broiler's growth requirements. The survivability of birds during the experimental period did not differ significantly. The survivability of all dietary treatment groups was nearer to 100%. It indicates that dietary supplementation had no detrimental effect on survivability. Survivability results of birds during entire experimental period indicated that enzyme supplementation had no effect on mortality. This result coincides with the finding of Alam *et al*. (2003); Lan *et al*. (2002). They also reported no effect of phytase supplementation on survivability results.

Blood metabolites: Concentration of plasma glucose, proteins, cholesterol and enzymes of broiler chicken supplemented with microbial phytase presented in Table 3. Effect of phytase supplementation to a low P diet has no effect on plasma glucose, proteins and cholesterol. No significant effects of treatments were found on plasma alanine aminotransferase (ALT) and aspertate aminotransferase (AST). Concentration of these plasma enzymes signified that the birds were apparently healthy throughout the experimental period which can also be correlated with the findings of survivability.

Plasma minerals: Reducing total P level in the diet significantly decreased plasma Ca and P concentration but this reduction amended when phytase was added in the diet at the level of 500 PU/kg (Table 4). Supplemental phytase at the level of 250 PU/kg failed to achieve the level of plasma Ca and P as found in control. Concentration of plasma Fe, Mn and Zn were unaffected by the dietary treatment. Similar findings were also reported in broiler breeder (Bhanja *et al*., 2005). In another study Sebastian *et al*. (1996) reported microbial phytase increased the plasma P by 15.7% and reduced ($p <= 0.05$) the Ca concentration by 34.1%, but had no effect on plasma concentrations of Cu or Zn in broilers where the findings regarding the Ca concentration contradict with the present findings.

Length, breadth and mineral content of tibia: The effects of microbial phytase supplementation to low phosphorus diet on length, breadth and mineral content of tibia are presented in Table 5. Reduction of P level in the diet reduced ($p<0.05$) length and breadth of tibia compared to control but this effect amend when phytase was added in the diet at the 500 PU/kg. The improvement of length and breadth of tibia by supplementing phytase is indicative of deposition of mineral in tibia. The percentage of tibia crude ash was significantly increased by the addition of dietary phytase, an observation that agrees with the previous studies dealing with broilers (Sabestian *et al*., 1996; Zyla *et al*., 2000), Pekin ducks (Orban *et al*., 1999) and turkeys (Atia *et al*., 2000). However, as it was reported in some experiments (Fernandes *et al*., 1999; Harter-Dennis and Sterling, 1999; Bozkurt *et al*., 2006), dropping dietary phosphorus level decreased tibia ash, also in the current study. Phytase supplementation to diets increased the content of Ca and P in the tibia compared with diet containing low phosphorus. Such an improvement in ash and phosphorus percentage in tibia was described by Sabestian *et al*. (1996) as a good indication of increased availability of phosphorus from phytase-mineral complex by the action of phytase. The response of tibia characteristics such as tibia ash and phosphorus content to dietary levels of microbial phytase in the present study was similar to previous observations reported in broilers and ducks, in which dietary phytase phosphorus was found to increase tibia ash and phosphorus percentage (Orban *et al*., 1999; Sohail and Roland, 1999). Bone Zn level was increased ($p<0.05$) when phytase was added in the diet. This finding corroborates with the findings of Zanini and Sazzad (1999) where they reported increased the concentration of Ca and Zn in the tibiae by phytase supplementation. Phytate can reduce Zn availability by chelateing divalent Zn and phytase supplementation can release this Zn which increases its concentration in bone.

Calcium and phosphorous retention: The improvement in Ca and P retention was found when phytase was added in low phosphorus diet indicate phytase reduced the amount of inorganic phosphorus in starter and finisher diet of broiler (Table 5). The report of Simons *et al*. (1990) showed that the availability of phosphorus increased to over 60% and the amount of phosphorus in manure decreased by 50% when microbial phytase was added to low phosphorus diets. The phytase supplementation improved P availability resulting in low

Table 4: Effect of microbial phytase supplementation on plasma minerals of broiler

Attributes	Control	Low P	250 PU Phytase/kg	500 PU Phytase/kg	SEM	P
Calcium, mg/dl	10.82[a]	9.59[c]	9.99[b]	10.52[a]	0.28	0.045
Phosphorous, mg/dl	7.17[a]	6.36[c]	6.63[b]	6.97[a]	0.12	0.036
Iron, ìg/ml	4.68	4.25	4.70	4.88	0.14	0.025
Manganese, ìg/ml	0.62	0.60	0.64	0.69	0.02	0.034
Zinc, ìg/ml	3.84	3.78	3.77	3.87	0.15	0.038

[a-c]Means within row with no common superscripts differ significantly (P<0.05).

Table 5: Effect of microbial phytase supplementation on physical characteristics of bone, bone minerals and mineral retention of broiler

Attributes	Control	Low P	250 PU Phytase/kg	500 PU Phytase/kg	SEM	P
Physical characteristics						
Length, cm	12.27[a]	12.05[a]	12.05[a]	12.33[a]	0.18	0.032
Breadth, mm	17.11[a]	16.31[b]	16.96[a]	17.46[a]	0.66	0.003
Bone minerals						
Total ash, %	31.04[ab]	29.21[c]	30.54[b]	33.56[a]	1.11	0.002
Calcium, %	26.15[b]	25.76[c]	26.03[b]	26.78[a]	0.24	0.024
Phosphorous, %	19.51[a]	18.50[c]	19.03[b]	19.86[a]	0.39	0.036
Zinc, mg/kg	134.35[a]	130.47[c]	132.40[b]	134.75[a]	0.21	0.028
Mineral retention						
Calcium, %	67.2[a]	58.7[c]	66.6[b]	67.7[a]	0.46	0.042
Phosphorus, %	55.7[a]	52.7[c]	54.6[b]	55.8[a]	0.33	0.035

[a-c]Means within row with no common superscripts differ significantly (P<0.05).

P excretion (Um *et al*., 2000) and increased retention of Ca, P (Lim *et al*., 2001). These findings affirm the present findings.

The results of this study suggest that microbial phytase in broiler starter and finisher diet enhanced the availability of phosphorus that supported the growth performance, increased tibia ash and phosphorus content, retention of Ca and P. The results of this study showed that the incorporation of phytase in the soybean based broiler starter and finisher diet improved Ca and phosphorus availability. The increasing of phosphorus availability may reduce the amount of phosphorus that would be excreted in the manure, thus reducing the environmental pollution potential.

It can be concluded that with 500 PU of microbial phytase/kg, dietary phosphorus can be reduced to 0.52 and 0.50 in starter and finisher diet of broiler without affecting performance and overcame the depression of growth rate observed on the low phosphorus diet.

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