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Effect of Locally Produced Phytase on Growth of Layer Chicks

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Abstract.- The most active and thermo-stable fungus *Aspergillus niger* was isolated and selected as fermenting agent to produce economical microbial phytase on defatted rice polishings through solid-state fermentation (SSF). Maximum enzyme activity was obtained at 100 µg /ml after 96 hours of incubation, 40% moisture level and 37°C. This enzyme phytase thus produced was biologically evaluated on 180 growing layers of 9 weeks of age. Group-A (control group) was without phytase supplementation, however, supplemented with 0.4% non-phytate phosphorus provided by di-calcium phosphate (DCP, 1.10%). In groups B, C and D, the phytase levels were kept at 1000, 1500 and 2000 µg/kg, respectively. The DCP was added 50% less in all groups as compared to control group. Total feed consumption, weight gain, feed conversion ratio of 18 weeks aged pullets in groups A, B, C and D were 2920, 2862, 2914 and 2931g; 543.15, 518.37, 549.70 and 568.00g and 5.38, 5.52, 5.30 and 5.16, respectively. The toe ash percentage was 11.9, 11.2, 12.1, and 12.7%, respectively and there was significant difference ($P<0.05$) among different experimental pullet groups. The growth performance was non-significant ($P>0.05$) among all groups although there was significant availability of phosphorus 42.67, 50.00, 54.68 and 60.67%, respectively in groups A, B, C and D. There was linear increase ($P<0.05$) in percentage of phosphorus availability with increase in level of phytase.

Key Words: Solid- state fermentation, phytase, *Aspergillus niger*, broiler, growth performance, phosphorus availability

INTRODUCTION

Phosphorus (P) is an essential element for the growth and survival of poultry. Cereal and cereal byproducts contain P which is only partially available because most of the P is present in phytate form. Phytic acid [myo-inositol (1, 2, 3, 4, 5 6) hexakis phosphate] is the major storage form of P (approximately two-thirds of the P) in cereals (Erdman and Poneros-Schneier, 1989). It has strong chelating properties and forms insoluble complexes with essential mineral elements such as calcium, zinc, magnesium and iron thus decreasing there bioavailability (Fox and Tao, 1989). Presence of phytic acid also has negative influence on the solubility of proteins and the function of pepsins can be expected because of the ionic binding between basic phosphate groups of phytic acid and protonized amino acids, such as lysyl, histidyl, and arginyl residues (Singh and Krikorian, 1982). Phytate P is not absorbed in the digestive system of birds due to the absence of the phytase in their intestines (Swick and Ivey, 1992). Inorganic or nonphytate P is therefore added in the feed to meet the bird dietary requirement. Phytase (myo-inositol hexaphosphate hydrolase), an enzyme of microbial origin, can increase the availability of phytate P. Phytase activity has been found most frequently in fungi (Zyla, 1992) and *Aspergillus* in particular (Hirabayashi *et al.*, 1998; Howson and Davis, 1983). It hydrolyses phytic acid to myo-inositol and phosphoric acid (Wodzinski and Ullah, 1996). Supplementation of animal feedstuff with phytase will increase the bioavailability of phosphate, decreasing P pollution in area of intensive animal production. Phytase also improves nitrogen absorption in laying hens (Van der Klis and Versteegh, 1996) and improves nitrogen and amino acid digestibilities in broilers (Ravindran *et al.*, 1995, 2001). Rice polishings is an excellent feed ingredient for poultry. It is a byproduct of rice milling, have high amount of total P (1.3%), out of which its availability to poultry is only 0.14% because most of the P present in rice polishings is in phytate form (Hubbell, 1990). It contains about 12% protein, 14% ether extract, 7.6% crude fiber and vitamin B complex (Rao and Reddy, 1986). Very few experiments have been conducted to study the non-phytate phosphorus (NPP) requirements of laying chicks during their growth stage (Punna and

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Roland, 2000). The ability of poultry to utilize the phosphorus bound in the phytic acid is generally assumed to be poor (Punna and Roland, 2000). The availability of microbial phytase has not only provided a practical way of releasing the phytate bound P for avian utilization but also have positive effect on protein digestion and utilization (Cabahug *et al.*, 1999). This study was conducted with the objective to produce phytase enzyme by fermentation of utilizing rice polishings with *Aspergillus niger* under optimal conditions and to test its potential to provide phosphorus by conducting a feeding trial on growing layers.

MATERIALS AND METHODS

Bio-production of phytase

The most active fungus *Aspergillus niger* (Lie and Stahl, 2000) was isolated and selected as fermenting agent to produce microbial phytase on rice polishing solid-state fermentation (SSF) technique as it is more economical for commercial enzyme production (Krishna and Nockes, 2001). The culture of *Aspergillus niger* was maintained on Potato Dextrose Agar (PDA) slants by periodic transfer and stored at 42°C as described by Singh *et al.* (1992). Ten milliliters of Sporulation medium (agar 2g, dextrose 2g, and potato infusion 20ml/100 ml of water) was placed in each autoclaved test tube aseptically. These tubes were then plugged with cotton and were placed in slanting position for 24 h. The tubes without contamination were inoculated with spore suspension and incubated at 30˚C until a uniform growth of organism took place. Later, these tubes were placed at 10˚C in a refrigerator. Spores from old cultured slants were wetted with 5 ml of sterile Manoxal Di-octylester (MOT) solution. Supernatant containing spore's suspension was decanted off aseptically. The agar surface was washed twice with sterile MOT solution. The combined washing was made to 50 ml with distilled water and shaken with glass beads to break the clumps of spores. Two hundred gram of defatted rice polishings was poured in two liter capacity conical flask. Distilled water was added to attain 40% moisture in the medium. The flask was plugged with cotton, autoclaved for 30 min at room temperature and inoculated at 37ºC with spore suspension. The enzyme activity was determined at 48, 72, 96 and 120 hours of incubation using Dvorakova (1998) technique (Table I). The flasks after 96 h of incubation were selected on the basis of enzyme activity, removed from incubation chamber and phytase enzyme was extracted with 2% $CaCl_2$ solution. The culture filtrate was passed through a four fold cotton cloth and crude filtrate was stored in a refrigerator.

Table I.- Phytase activity at various stages of incubation.

Incubation duration (hours)	Enzyme activity (µg)
48	78
72	87
96	100
12	98

Biological trial

Four iso-caloric and iso-nitrogenous layer diets *i.e.* A, B, C and D were formulated and machine mixed according to NRC, 1994 containing four levels of phytase 0, 1000µg, 1500µg, and 2000µg phytase units per kilogram feed, respectively (Table II). The diets were biologically tested on 180 growing layers of 9 weeks of age till 18 weeks of age. These birds were randomly divided into 20 experimental units, containing nine birds each. All experimental diets were randomly assigned to the birds and there were three replicates on each diet. Group-A (control group) contained commercially recommended level of non-phytate phosphorus (NPP-0.4%) provided by di-calcium phosphate (DCP, 1.10%) and without phytase supplementation. In groups B, C and D, the phytase levels were kept at 1000, 1500 and 2000 µg/kg, respectively. Whereas, DCP was added 50% less in groups B, C and D as compared to control group (A).

The feed consumption, weight gain and feed conversation ratio of birds from each experimental group were measured at the end of each weak during nine weeks of experimental period. The excreta voided from each experimental group was separately collected during the last (18^{th}) week of experiment. The droppings, which were retained on the wire screen floor of the cage, were also collected. Efforts were made to remove every bit of

feathers from the droppings to avoid contamination. The excreta from various groups were weighed, oven dried at 80°C at beginning and then gradually decreased to 60°C till complete drying. The dried excreta were ground to 60-mesh size, stored. Finely ground samples (0.5 g) from each test material were used for analyzing total P according to AOAC (1990).

Table II.- Composition of experimental layer grower ration.

Feed ingredients (%)	Groups (Phytase µg/kg of diet)			
	A (0)	B (1000)	C (1500)	D (2000)
Corn	25.00	25.00	25.00	25.00
Rice polishing	30.00	30.00	30.00	30.00
Rice tips	18.24	18.64	18.64	18.64
Soybean meal	10.74	10.66	10.66	10.66
Guar meal	4.00	4.00	4.00	4.00
Fish meal	5.00	5.00	5.00	5.00
Oil	0.38	0.24	0.24	0.24
Molasses	3.00	3.00	3.00	3.00
Di-calcium phosphate	1.10	0.55	0.55	0.55
Calcium Carbonate	1.54	1.91	1.91	1.91
Vit. & mineral supplement	1.00	1.00	1.00	1.00
Phytase	0	1000µg	1500µg	2000µg
Chemical composition				
Metabolizable Energy (kcal/kg)	2850	2850	2850	2850
Crude Protein %	16.5	16.5	16.5	16.5
Ether Extract %	5.57	5.43	5.43	5.43
Crude Fiber %	3.25	3.25	3.25	3.25
Calcium %	0.90	0.90	0.90	0.90
Phosphorous %	0.40	0.31	0.31	0.31
Lysine	0.80	0.80	0.80	0.80
Methionine	0.30	0.30	0.30	0.30

At the end of experiment toes of three birds, one from each replicate, of each experimental unit were obtained by severing the right thumb toe through the joint between the second and third tarsal bone from the distal end. The composite samples of the toes were dried to a constant weight at 100˚C and then put in a muffle furnace at 600˚C for 6 has to get ash percentage (Potter, 1988).

Statistical analysis

The data collected of research *parameters* were subjected to statistical analysis using completely randomized design (CRD) and their comparisons of means were studied by least significant difference (LSD) technique (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

The results on the various parameters recorded in this study have been discussed as under.

Growth parameters

The growth parameters like feed consumption, weight gain and feed conversion ration were studied during growing phase (9-18 weeks) of experimental layers kept in metallic cages with control feeding (Table III). The overall feed consumption (46g/pullet/day) in all groups were less than normal values because of summer stress. The present study revealed that feed consumption, weight gain and feed conversion ratio of growing pullets of different groups (A, B, C and D) fed on iso-caloric and iso-nitrogenous experimental diets did not differ significantly (P>0.05) among groups.

Table III.- Effect of phytase supplementation on pullets (9-18 weeks) growth performance

Diets	Feed consumption (g/pullet)	Weight gain (g/pullet)	Feed conversion ratio
A (Control)	2920 [a]	543.15 [a]	5.38[a]
B (1000µg phytase/kg of feed)	2862 [a]	518.37 [a]	5.52 [a]
C (1500µg phytase/kg of feed)	2914 [a]	549.70 [a]	5.30 [a]
D (2000µg phytase/kg of feed)	2931 [a]	568.00 [a]	5.16[a]

Same superscripts show non-significant difference (P > 0.05) with in column

The growth performance of layer pullets fed diets containing phytase was statistically similar to those fed diets containing DCP (NPP-0.4%). The results suggest that phytate P released by phytase was sufficient to meet the layer pullets growth

requirement similar to P supplied as inorganic source. It also indicates that diet of group B with 50% DCP supplemented with lowest level of phytase (1000 µg/kg of diet) was sufficient for optimum growth performance of layer pullets. This level of DCP and phytase provided the P level required to pullets; therefore, higher levels of phytase addition in diets did not perform any significant improvement in their feed consumption and growth rate. Scheideler *et al.* (2001) reported that low non-phytate phosphorus (NPP) had a negative effect on feed intake which was reversed by the addition of phytase. Similarly, Viveros *et al.* (2002) reported that performance of chicks fed supplemental phytase with 0.35% and 0.27% NPP were comparable with those of chicks fed the control diet that contained normal levels of NPP (0.45 and 0.37%, respectively). The authors further reported that the growth promoting effect of P caused by phytase can be partially attributed to the increased concentration of myo-inositol, the final product of phytate dephosphorylation, and to the release minerals from complexes with phytic acid. It may partially or completely replace inorganic phosphorus with phytase in the diet and reduces P excretion in excreta up to 50% and enhances bioavailability of Ca, Zn and Fe (Lie and Stahl, 2000). Similarly, it could also be due to possible increase of starch digestibility (Knuckles and Betschart, 1987) or an increased availability of protein (Selle *et al.,* 2000). This increased digestibility may be explained by the fact that phytate-protein complex were cleaved by phytase (Nair *et al.*, 1991). The growth performance was not affected (P>0.05) in chicks fed diets with phytase relative to those fed the control diet in chicks.

Dilger *et al.* (2004) fed 500, 750, or 1000 microbial phytase units/kg diet to chicks and found that weight gains of chicks were similar between phosphorus-adequate and phytase-supplemented diets. Ceylan *et al.* (2003) fed different levels of phytase to layers and reported that phytase supplementation reversed the adverse effects of P deficiency and further improved the feed conversion ratio. Miles *et al.* (2001) also found non-significant difference in feed intake among different levels of phytase (300, 450, 600 FYT/Kg of diet) (One FYT- the amount of enzyme that liberates 1 micro mole inorganic ortho-phosphate per min. under the following conditions: pH 5.5; temperature 37°C; substrate: sodium phytate in a concentration of 0.0050 mole/l.). Rice polishings was used as a major ingredient as it is one of the high phytate containing ingredients of poultry diet. Non-significant difference (P>0.05) from control group indicates that phytase successfully librated the P from phytate present in rice polishings. These results are also in line with the findings of Keshavarz (2003) who fed three levels of low NPP to growing pullets and reported that the least NPP was sufficient to support the body weight gain for the entire growing period in the presence of phytase. Um and Paik (1999) found that retention of fiber, fat, and minerals increased after phytase supplementation to a diet containing low levels of NPP. The results are similar to Nair *et al.* (1991) who found that increased P retention by phytase resulted in a reduction of P excretion by about 13%. Yan *et al.* (2003) reported that addition of phytase and reduction in dietary phosphorus content should aid in reducing phosphorus excretion without impairing performance.

Availability of phosphorus

The addition of phytase significantly (P<0.05) increased the hydrolysis of phytate P, thereby its excretion decreased markedly. There was a linear increase (P < 0.05) in the availability of P in birds fed diets A to D containing gradient increase in phytase (Table-IV). This resulted in increase in the availability of P from 8 to 18%. These results are in line with the findings of Keshavarz (2000) who observed that phytase had significant effect on P retention and increased phytate P retention by 15%. Boiling *et al.* (2000) found approximately 50% decrease in excreta P in laying birds fed 0.1% AP + 300 U/kg phytase compared with those fed 0.45% available phosphorus.

Similarly, Pallauf and Rimbach (1997) reported that an average of 2/3 of phosphorus in cereals, legume seeds and oil seeds is bound as phytate which is least available to poultry. However, with the aid of microbial phytase, significant improvement in P utilization can be achieved in poultry while sparing mineral P supplements and decreasing P excretion without adverse effect on performance.

Table IV.- Effect of phytase supplementation on feed P availability (%)) during 9-18 weeks

Diets	Feed Intake g/pullet/day	Phosphorus Intake g/pullet/day	Phosphorus Excreted g/pullet/day	Phosphorus availability (%)	Toe Ash[1] (%)
A (Control)	46.00[a]	0.1558	0.0893	42.67[a]	11.9[c]
B (1000 µg phytase/kg diet)	45.00[a]	0.1191	0.0596	50.00[b]	11.2[d]
C (1500 µg phytase/kg diet)	46.00[a]	0.1260	0.0571	54.68[c]	12.1[bc]
D (2000 µg phytase/kg diet)	46.00[a]	0.1284	0.0505	60.67[d]	12.7[ab]

Different superscripts show significant difference ($P < 0.05$) with in the column
[1]At the end of experiment toe of one bird from each replicate was used to determine ash percentage

Toe ash

Toe ash has been shown to a good indicator of phosphorus status and is accurate in determining P availability of diets for poultry. The toe ash percentage was significantly higher ($P < 0.05$) in birds of group D (phytase level 1500 µg/kg feed) as compared to birds fed in group B but was comparable ($P > 0.05$) to groups A & D (Table IV). These results are in agreement with the findings of Perney *et al.* (1993) and Dilger *et al.* (2004); they found linear increase in toe ash with supplementation of phytase and reported that phytase improved bone mineralization in chicks. In this study, the addition of phytase linearly increased the percentage of ash. The magnitude of increment was greatest for the first addition of P or phytase and then tended to plateau with further additions. Ravindran *et al.* (2001) also found that toe ash contents ($P < 0.001$) were increased as the P or phytase was added to the low-P diet.

Livability

The treatments did not affect the livability of pullets. Only two birds died during the experiment probably due to environmental factors. The results of present study are in agreement with the findings of Yan *et al.* (2003) who observed that the lowest level of NPP with or without phytase supplementation was sufficient for livability.

CONCLUSIONS

The present study concluded that phytase could be used to replace minerals sources of P; however, further investigations are required to decrease the DCP level more to 50% so that phytase can show its full potential.

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