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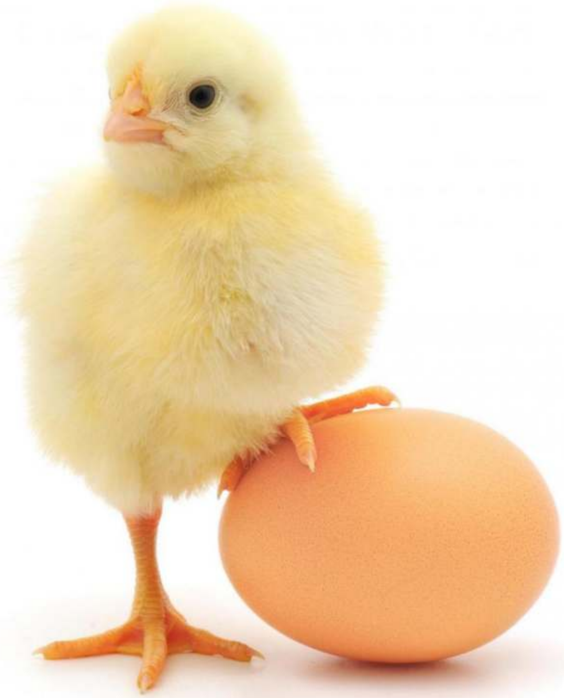

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Phytase with Synergistic Herbs: An Option to Reduce Environmental Pollution by Partial Replacement of Inorganic Phosphorus in Broiler Ration

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Abstract: Supplementation of phytase alongwith certain synergistic herbs (Ayufytase) was assessed on growth performance, phosphorus excretion and bone mineralization in 6 weeks trial using 90 day old Vencob broiler chicks fed corn-soyabean based diet. Chicks were randomly divided into three equal groups (n = 30), each having three replicates. Composition of experimental diets for all three groups were designed in a way that group T_0 was offered standard basal diet formulated as per NRC (1994) requirements containing Dicalcium Phosphate (DCP) as the source of inorganic phosphorus and without any phytase supplementation, T_1 was offered basal diet with supplemental Ayufytase@100gm/tonne of feed replacing 50% of DCP, T_2 was offered basal diet with Ayufytase@100gm/tonne of feed replacing 65% of DCP. Feed intake and body weight gain was recorded on weekly basis. A metabolic trial of three days was conducted at the end of the trial to estimate percent (%) Phosphorus (P) excreted in faeces. Among bone mineralization parameters; tibia ash, toe ash, phosphorus %, tibiotarsal and robusticity index were determined at 6^{th} week. The results of treatment group (T_1 and T_2) reveals significant ($p \leq 0.05$) improvement in feed intake, mean weekly body weight gain, phosphorus retention and bone mineralization parameters than control group. It can be concluded that Ayufytase is helpful to reduce environmental pollution caused by excretory Phosphorus by birds most efficiently if replaced with inorganic sources of phosphorus in basal ration at 50% level.

Key words: Broiler, phosphorus, phytase, tibia ash, mineralization, growth performance

INTRODUCTION

Dietary requirements for Phosphorus (P) and its availability in feedstuffs of plant origin are key issues in poultry nutrition. Phosphorus from plant sources is only 30 to 40% available (Perney *et al.*, 1993), because much of the P is in the form of phytate (*myo*-inositol hexaphosphate) and is poorly used by poultry. Vegetable feeds contain significant amount of this mineral, however, 50-80% of phosphorous is bound in phytates that cannot be broken down by endogenous enzymes in poultry (Anon, 1997). In order to become available to broiler chicks, phosphorous from vegetable sources must be hydrolyzed, with phytase as a catalyst, to inositols and inorganic phosphates which are readily absorbed in digestive tract (Annison and Choct, 1993). Non-ruminant animals, such as pig and poultry, lack significant amounts of endogenous phytase in gastrointestinal tract to hydrolyze phytic acid and release bound phosphorus (Cooper and Gowing, 1983; Maenz and Classen, 1998) which is a cause of environmental pollution, Diets are usually supplemented with an inorganic source of P (Sebastian *et al.*, 1998) to meet dietary requirements of phosphorus. This supplementation is not only expensive but also fails to address the problem of over-supplementation, leading to potential environmental P pollution (Ravindran *et al.*, 1998). Various management and nutritional approaches are being followed in an effort to reduce the P content in the manure of high producing livestock and poultry to a minimum. As a result of economic and environmental concerns, there is renewed interest in using phytase to reduce the need for inorganic P supplements and to improve utilization of P present in feedstuffs. Phytase is myoinositol hexaphosphate hydrolase and it belongs to phosphate mono-ester group. There are three major sources of phytase; plant, animal and microbial origin. Phytate P from plant sources can efficiently be made available to simple-stomach animals by using microbial phytase (Nelson *et al.*, 1996). The enzyme phytase (*myo*-inositol hexakisphosphate-3 and 6-phosphohydrolase) from yeasts, fungi and bacteria hydrolyzes phytate P into inorganic P and inositol phosphate. Supplementation of animal feeds with microbial phytase increases P availability. This result has been observed in broilers (Kiiskenen *et al.*, 1994; Mitchell and Edwards, 1996; Sohail and Roland, 1999), swine (Young *et al.*, 1993) and turkeys (Ledoux *et al.*, 1995; Qian *et al.*, 1996). Many herbs are known to positively influence the absorption and assimilation of minerals (Calcium and Phosphorus) such as *Cissus quadrangularis, Zingiber officinale, Lepidium sativum, Terminalia arjuna, Cestrum diurnum and Uraria picta*

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Table 1: Experimental broiler diets in control (T_0) and treatment groups (T_1 and T_2)

	Control (T_0)		Treatment (T_1)		Treatment (T_2)	
Ingredients (g/kg)	Starter	Grower and Finisher	Starter	Grower and Finisher	Starter	Grower and Finisher
Maize	515	600	515	600	537	600
Soya	310	260	307	264.90	307	270
SFM	120	60	127.5	70	125	69.4
Oil	20	45	15	45	15	45
DCP	30	30	15	15	10.5	10.5
test product (Ayufytase)	--	--	0.1	0.1	0.1	0.1
salt	5	5	5	5	5	5
Crude protein (CP%)	22.62		22.89	19.75	22.83	19.97
Crude fibre (CF%)	5.05		5.207	4.04	5.166	4.06
Total calcium (Ca)	1.3455	0.78	0.83705	0.88	0.8479	0.77
Total phosphorus (P)	0.92	0.48	0.60751	0.62	0.6139	0.56

Trace minerals were added @ 100 gm/100kg of feed. Vitamin premix (Vit A, D3,E, K and vit B complex) were added @ 50gm/100 kg of feed

(Sankaran *et al.*, 1964). These herbs act in the intestinal tract and help in better absorption and utilization of plant phosphorus. Recently a new formulation (Ayufytase) has been originated, which is a combination of Phytase and synergistic herbs. The Phytase in the Ayufytase is produced by filamentous fungi *Trichoderma reecei* which is genetically engineered by the inclusion of the gene of *Pichia pastoris* and Glycosylation process. An experiment was conducted to assess growth performance, phosphorus absorption and bone mineralization efficacy of Ayufytase by partially replacing inorganic phosphorus in broiler ration.

MATERIALS AND METHODS

To study the effect of phytase supplementation in broiler chicken, a feeding trial was carried out in the College of Veterinary and Animal Sciences, Udgir, Dist. Latur, Maharashtra, India. The trial involved 90 day old Vencob broiler chicks, which were randomly divided into three equal groups and housed in deep litter system under controlled ambient temperature. Each group had three replicates of 10 chicks each. The trial lasted for 42 days. Birds had *ad libitum* access to feed and water. Composition of trial diets for all the groups were designed in a way that group T_0 was offered standard basal diet formulated as per NRC (1994) requirements having 100% Dicalcium Phosphate (DCP) as the source of inorganic phosphorus and without any source of phytase, T_1 was offered basal diet with supplemental Ayufytase@100gm/tonne of feed replacing 50% of DCP, T_2 was offered basal diet alongwith Ayufytase @100gm/tonne of feed replacing 65% of Dicalcium Phosphorus (DCP). Detailed composition of three different experimental feeds is given in Table 1. The test product Ayufytase is the combination of fungal phytase alongwith synergistic herbs (*Cissus quadrangularis, Zingiber officinale, Lepidium sativum, Terminalia arjuna, Cestrum diurnum and Uraria picta)* that helps in better absorption and utilization of plant phosphorus.

Growth and performance: Initial body weight was registered at day one of experiment and later the chicken were weighed at weekly intervals till 42nd day of experiment, at the same hour and before offering daily ration. The chicks were weighed by each replicate of all the treatments. Feed intake of each individual group was recorded on weekly basis. Feed efficiency was calculated on the basis of body weight gain and total feed intake. During the trial, feed intake and excreta output was measured for three consecutive days at the end of experiment to estimate phosphorus retention. The excreta for phosphorus estimation was collected at 9:00 h. everyday (day 39th, 40th and 41st) in plastic bags and stored at -20°C for analysis (Yi *et al.*, 1996).

Bone mineralization studies: At the termination of the study representative birds from each group were sacrificed and left tibias of five birds per replicate were removed. Tibias were dried to constant weight at 105°C for 24 h, defatted and then ashed in a muffle furnace at 550°C for 6 h in porcelain crucible and the percentage of tibia ash was determined relative to the dry weight of the tibia. Toe samples were obtained by severing the middle toe through the joint between the second and third tarsal bones from the distal end. The left and right middle toes of chicks were pooled. The toe samples were dried at 100°C and then ashed in a muffle furnace at 600°C for 4 h to determine amount of ash (Yi *et al.*, 1996). In addition to it, Calcium was determined by a titrimetric method (ISO 6490-1, 1985), phosphorus by a spectrometric method (ISO 6491, 1998). Tibio-Tarsal Index and Robusticity Index was calculated as per formulas:

Tibiotarsal Index = Diaphysis diameter - medullary canal diameter/diaphysis diameter X 100

Robusticity Index = Bone length/cube root of bone weight.

Statistical analysis was done by ANOVA as per the method given by Snedecor and Cochran (1994), using complete randomized block design. The level of significance was set at ($p \leq 0.05$).

Table 2: Effect of dietary Ayufytase supplementation on Average weekly feed consumption (gm) in different groups

Age in weeks	Groups Control (T_0)	Treatment (T_1)	Treatment (T_2)
0	-	-	-
1	125.67	126.18	130.22
2	312.85	328.59	315.98
3	546.60	559.33	556.77
4	877.25	907.51	840.41
5	1011.75	1045.92	999.25
6	1025.49	1021.55	1019.49
Mean±SE	649.94±155.22^a	664.85±157.66^b	643.69±151.10^a

Means bearing different superscripts differ significantly at ($p \leq 0.05$)

RESULTS AND DISCUSSION

Growth and performance: The effect of phytase supplementation on body weight gain, feed efficiency and feed intake are presented in (Table 2 and 3). Mean weekly feed consumption in treatment group (T_1) was found to be 664.94 g which is highest amongst the three groups under study (Table 2). The addition of fungal phytase alongwith synergistic herbs has been observed to increase the feed intake indicative of potential of test product in enhancing availability of nutrients. In agreement with these result, it was reported that the addition phytase in poultry diets increased feed intake (Jalal and Scheideler, 2001; Punna and Roland, 1999). The mean weekly body weight gain (Fig. 1) and final body weight was significantly ($p \leq 0.05$) higher in group T_1 followed by T_2 when compared with control fed basal diet without any phytase. Mean body weight at the end of 6th week was found to be significantly ($p \leq 0.05$) higher in the T_1 (2407.70±80.22 g) than T_2 (2314.50±65.08 g) and control (2100.05±95.80) group (Table 3). Mean FCR in both the treatment groups were almost similar (1.61 and 1.60) and significantly ($p \leq 0.05$) low as compared to the control birds (1.77). The results of growth and performance parameters were observed to be better in T_1 group supplemented with Ayufytase replacing 50% DCP in feed than T_2 group supplemented with Ayufytase replacing 65% f DCP in feed. The results obtained in T_0 are in accordance to those reported by (Viveros *et al*., 2002; Yan *et al*., 2001; Shirley and Edwards, 2003) that broilers fed Non Phytate Phosphorus diet typically result in reduced feed intake and perhaps a mildly adverse effect on body weight gain and FCR and the performance was restored upon addition of phytase. Junepere *et al*. (2004) also reported that addition of exogenous phytase to phosphorus deficient diets improved animal performance to levels comparable to chicks fed positive control diet. The improvement in body weight and performance in broilers by the addition of Ayufytase in basal ration might be due to greater availability of P, especially *myo*-inositol, released by phytate hydrolysis or dephosphorylation and release of trace elements or minerals from complexes with phytate.

Phosphorus retention: The effect of Ayufytase supplementation on phosphorus retention was significant. The amount of retained phosphorus was increased while that of excreted phosphorus was decreased in treatment groups and the apparent availability of phosphorus was significantly ($p \leq 0.05$) higher in group T_1 followed by T_2 (Table 4) indicating the efficacy of product in mineral retention. The findings are consistent with some other studies (Yi *et al*., 1996; Van dr Klis and Versteegh, 1996). In addition, phytase supplementation to poultry feed has been shown to increase P availability from 35-60% and reduced excretion by 42% (Simons *et al*., 1990). In present study, it was evident that replacement of 50% of DCP with Ayufytase has been proven more efficacious in phosphorus retention.

Bone mineralization:

Phosphorus	18.87±0.09^a	20.20±0.23^b	19.43±0.23^a
Ash %	31.19±0.50^a	34.44±0.54^b	32.71±0.41^b

Tibia Ash and phosphorus %: Tibia phosphorus % was significantly ($p \leq 0.05$) higher in T_1 (20.20±0.23) followed by T_2 (19.43±0.23) compared to control (19.43±0.23) which signifies role of Ayufytase in promoting bone mineralization (Table 5). The Tibia ash % (one of the important parameters to assess mineral retention) in control group was 31.19±0.50 which increased ($p \leq 0.05$) significantly to 34.44±0.54 in group T_1 followed by 32.71±0.41 in group T_2. The results are suggestive of the efficacy of Ayufytase in significantly ($p \leq 0.05$) better phosphorus retention in bones when it is replaces DCP@ 50% in basal ration. These findings are in accordance with certain other scientific findings suggestive of increase in the mineral content of tibia due to presence of microbial phytase in basal diet (Sohail and Roland, 1999). Pertaining to the role of herbs in Ayufytase formulation and its supplementation to T_1 and T_2 groups, results in the present study are well corroborated to the findings of Sankaran *et al*. (1964)

Table 3: Effect of dietary Ayufytase supplementation on overall growth and performance of broiler chicken during 6 weeks experimental period

Parameters	Control (T_0)	Treatment (T_1)	Treatment (T_2)
Mean Weekly body weight gain (gm)	343.30±64.98^a	394.57±84.49^b	379.04±75.65^b
Final body weight at 6th week (gm)	2100.05±95.80^a	2407.70±80.22^b	2314.50±65.08^b
Total feed intake (gm)	3890±22.87^a	3990±47.86^b	3860±71.39^a
Feed efficiency (FCR)	1.768±0.17^a	1.605±0.12^b	1.600±0.12^b

Means bearing different superscripts differ significantly at ($p \leq 0.05$)

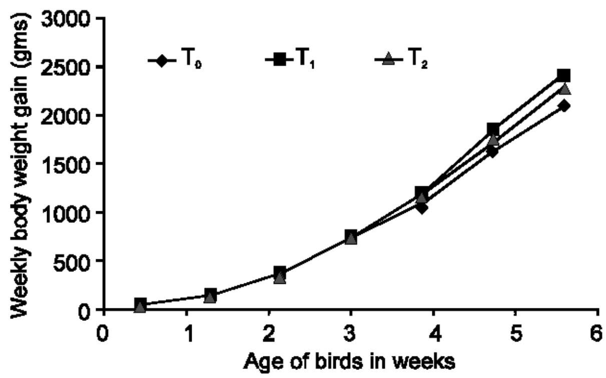

Fig. 1: Effect of dietary Ayufytase supplementation on Average weekly live weight (gm) of birds in different groups

Table 4: Phosphorus retention % in control (T_0) and treatment groups (T_1 and T_2)

	P retention after 6th Wk		
Groups	P in feed	P excreted	Retention (%)
Control (T_0)	0.95	0.60	36.84
T_1	0.65	0.30	53
T_2	0.50	0.25	50

Udupa and Prasad (1964) and Singh and Udupa (1962) who reported that certain herbs namely *Cissus quadrangularis, Lepidium sativum, Uraria picta* etc. increase uptake as well as utilization of dietary minerals (Ca and P) which helps in collagen formation and bone mineralization. The bone ash content have been used as the indices of bone strength because bone mineralization provides compressional strength to bones (Nelson *et al.*, 1971). Increased bone ash suggests an improvement in bone mineralization due to increased P and Ca utilization, which was caused by the liberation of inorganic P and Ca from the phytate molecule by the phytase enzyme in the gastrointestinal tract (Perney *et al.*, 1993; Qian *et al.*, 1996). In addition to above results, No significant difference in the values of toe ash and phosphorus % was recorded among treatment and control groups.

Tibiotarsal and robusticity index: Values in the treated groups were significantly ($p \leq 0.05$) lower in treatments than control (Table 6). Lower is the Tibiotarsal and Robusticity index, it indicates better retention of minerals and higher bone mineralization. According to Reisenfeld, (1972) and Seedor *et al.* (1991), higher is the bone weight/bone length and density of bone, lower is the tibiotarsal and robusticity index. These results of tibiotarsal index and Robusticity index suggest that supplemental phytase increases the availability of P and Ca and promotes the growth and development of the bone.

Table 5: Effect of dietary Ayufytase supplementation on bone mineralization parameters (Average Tibia and Toe Phosphorus and Ash %) in different groups

		Groups		
Parameters		Control (T_0)	Treatment (T_1)	Treatment (T_2)
Tibia	Phosphorus	18.87±0.09^a	20.20±0.23^b	19.43±0.23^a
	Ash %	31.19±0.50^a	34.44±0.54^b	32.71±0.41^b
Toe	Phosphorus	0.72±0.02^a	0.79±0.01^a	0.73±.01^a
	Ash %	12.45±0.25^a	12.64±0.04^a	11.49±0.15^a

Means bearing different superscripts differ significantly at ($p \leq 0.05$)

Table 6: Effect of dietary Ayufytase supplementation on bone strength indices (tibiotarsal and robusticity index) in different groups

	Groups		
Parameters	Control (T_0)	Treatment (T_1)	Treatment (T_2)
Tibiotarsal index	34.12±2.32^a	30.96±2.86^b	31.04±2.42^b
Robusticity Index	4.27±0.06^a	3.91±0.09^b	4.19±0.06^b

Means bearing different superscripts differ significantly at ($p \leq 0.05$)

Conclusion: Present study provided the evidence that the fungal phytase alongwith synergistic herbs could improve broiler growth, performance, bone strength by promoting mineralization in addition to improving utilization of phytate phosphorus and phosphorus retention. It can be concluded that Ayufytase is helpful to reduce environmental pollution caused by excretory Phosphorus in non-ruminant animals most efficiently if replaced with inorganic sources of phosphorus in basal ration at 50% level. The findings were also supported with the results of morphometric indices (tibiotarsal and robusticity index).

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