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Research Article
Effects of Phytase Super Dosing on Performance, Plasma Mineral Contents and Bone Mineralization in Broiler Chicken

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Abstract

Objectives: This experiment evaluated the effect of phytase super dosing on performance, tibia bone quality and serum biochemistry of broiler chicken. **Materials and Methods:** A total of 96 day-old chicks were distributed randomly into four treatment groups: D0, D1, D2, D3 with four replicates per treatment (6 chicks per replicate). The treatments were control diet (D0), control diet +500 FTU phytase kg^{-1} (D1), control diet +1500 FTU phytase kg^{-1} (D2) and control diet +2500 FTU phytase kg^{-1} (D3), that were fed to the birds from day 13-28. Birds were offered a commercial starter diet from day 0-12. **Results:** The different levels of phytase had no significant effect on body weight gain (BWG) and feed intake (FI). Supplementation of 1500 FTU phytase kg^{-1} of diet showed better ($p<0.05$) FCR than those received 2500 FTU phytase kg^{-1} of diet. Diet with 1500 FTU phytase kg^{-1} increased ($p<0.05$) the serum concentration of phosphorus (P) and total protein (TP). Inclusion of 1500 FTU phytase kg^{-1} of diet increased ($p<0.05$) the calcium (Ca) content of tibia. Diets supplemented with 500 and 1500 FTU phytase kg^{-1} reduced ($p<0.05$) the heart weight but increased ($p<0.05$) the drumstick weight of birds. Supplementation of 500 and 1500 FTU phytase kg^{-1} diet significantly reduced the total feed cost, production cost and increased the total profit kg^{-1} live bird. **Conclusion:** Supplementing diets with 500 and 1500 FTU phytase kg^{-1} improved the overall production performance of broiler chickens and consequently enhanced economic profitability.

Key words: Phytate, phytase, bone quality, cost-benefit, carcass, serum

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The plant-based poultry diet contains an important antinutrient factor called phytate (IP6) which is the major storage site of phosphorus (P). Phytate not only limits the availability of phosphorus (P) but also other minerals and nutrients like protein, carbohydrate, etc[1]. Poultry is unable to hydrolyze phytate due to a lack of effective endogenous phytase activity[2]. Moreover, the endogenous intestinal phytase poorly hydrolyzes the phytate due to the different pH levels and cation concentrations of the gastrointestinal tract (GIT) of poultry[3]. Therefore, exogenous phytase is routinely added to the poultry diet to improve the availability of phytate-bound minerals and nutrients. A significant amount of literature has already reported the beneficial effect of conventional dose of phytase (500 FTU kg^{-1}) on growth performance, nutrient utilization and bone quality of broiler chickens[1,4-7].

The corn-soybean-based poultry diet contains around 28% phytate which stores 60-80% of the total P[8]. It has been reported that 500 FTU kg^{-1} of phytase could only hydrolyze 62% of the total phytate and released only 0.15% phytate-P[9]. Due to several extrinsic and intrinsic factors, the conventional dose (500 FTU kg^{-1}) of phytase cannot completely dephosphorylate the phytate[10]. Increasing the phytase level by more than 500 FTU kg^{-1} of diet could potentially maximize the phytase-induced benefits in poultry.

Phytase causes stepwise hydrolysis of dietary phytate (IP6) and produces intermediate esters (like IP5, IP4, IP3, IP2 and IP1) of inositol. Commercial or standard phytase dose breakdown the phytate (IP6) and subsequently releases IP4 and IP3 esters which are more soluble and suspectable to phytase-induced digestion than IP6 and IP5. As broiler lacks endogenous phytase, the concentration of these intermediate esters increases in the intestine unless an increased dose of exogenous phytase is added to the diet[11,12]. Previous study claimed that higher dose of phytase (1500 FTU phytase kg^{-1} of diet) could potentially hydrolyze the IP4 and IP3 and thereby ameliorate their anti-nutritive effect completely through the production of free inositol which subsequently plays an important role in improving the performance of broiler chickens[13].

The first work of phytase super-dosing was reported by Nelson *et al.*[14] where the effect of phytase super dose (950-7600 FTU kg^{-1}) was evaluated. The authors observed that the phytate-P disappearance increased by 55.5% when the phytase dose increased from 950-7600 FTU kg^{-1}. The weight gain and ash content of bone at 21 day were highest at 7,600 FTU kg^{-1} of diet[14]. Another study stated that supplementation of phytase between 1000 FTU kg^{-1} and 5000 FTU kg^{-1} of diet significantly improved length, width and mineral content of tibia bone compared to a diet with 500 FTU kg^{-1} of phytase[15]. According to Shirley and Edwards[16] supplementation of 12000 FTU phytase kg^{-1} of the diet effectively hydrolyzed 95% of phytate-P. This enhanced efficacy of phytase super dosing could be due to the complete hydrolysis of phytate and release of minerals (P, Ca, Zn, Fe, etc.) and other nutrients, like protein and energy[17,18].

Most of the studies stated that the benefits of phytase super dosing become more pronounce when supplemented to non-phytate P (NPP) deficient diet[2,15,19-22]. Although these studies reported the positive effect, the impact of phytase super dosing is still inconsistent as the phytase dose and NPP level of the diet varied over the literature. Moreover, the amount of literature on phytase super dosing is very limited in the Bangladesh context. Therefore, the present study was undertaken to evaluate the effect of phytase super dosing on the performance, bone quality and serum profile of broiler chickens.

MATERIALS AND METHODS

Study site and Ethical approval: The experiment was carried out at the Department of Dairy and Poultry Science, Chattogram Veterinary and Animal Sciences University (CVASU). The experimental procedures were approved by CVASU Ethics Committee (EC) and the EC Approval No. is CVASU/Dir(R&E) EC/2019/94(7).

Formulation of experimental diets: The birds were fed a commercial (Nahar TM) broiler starter diet (Table 1) up to 12 days of age. After that, experimental diets were prepared and fed the birds from day 13-28. Four different test diets (D0, D1, D2 and D3) were formulated with the locally available feed ingredients to fulfill or exceed the requirements of NRC[23] where diets were iso-caloric and iso-nitrogenous. All feedstuffs were used to formulate a control diet without phytase (D0),

Table 1: Nutrient composition of ready-made starter diet (Nahar starter feed™)

Nutrient components (%)	Proximate values
ME (kcal kg^{-1})	3035
Moisture	11
DM	89
CP	22
CF	3
EE	5.70
Ash	6.20
Ca	0.9
P	0.45
Lysine	1.32

Table 2: Composition of finisher diets for broiler chickens (13-28 days)

	Diets			
Ingredients (g kg^{-1})	D0	D1	D2	D3
Maize	618.0	618.8	618.9	619.8
Palm oil	39.5	39.5	39.5	39.5
Protein concentrate	39.0	39.0	39.0	39.0
Soybean meal	278.4	278.3	278.9	278.8
Limestone	11.3	11.4	11.4	12.0
DCP	4.0	3.0	2.2	0.4
NaCl	2.5	2.5	2.5	2.5
L-lysine	1.6	1.6	1.6	1.6
DL-methionine	2.5	2.5	2.5	2.5
Vitamin min premix	2.5	2.5	2.5	2.5
Toxin binder	0.3	0.3	0.3	0.3
Choline chloride	0.4	0.4	0.4	0.4
Phytase	0	0.25	0.375	0.75

Control diet (D0) with no Phytase, whereas D1, D2 and D3 diets are supplemented with 500 FTU, 1500 FTU and 2500 FTU Phytase kg^{-1} of ration respectively

Table 3: Calculated and analyzed value of the nutrient components (%) of finisher Diet

	Finisher diets			
Nutrients	D0	D1	D2	D3
Calculated value				
ME (kcal kg^{-1})	3121.021	3118.78	3118.42	3117.33
CP	20.10	20.13	20.07	20.09
Ca	0.91	0.91	0.91	0.91
P	0.52	0.52	0.52	0.52
Lysine	1.22	1.22	1.22	1.22
Methionine	0.34	0.34	0.34	0.33
CF	3.27	3.27	3.27	3.27
EE	3.51	3.48	3.48	3.48
Analyzed value				
DM	85.25	85.85	91.20	86.90
Moisture	14.75	14.15	8.80	13.10
CP	20.15	20.19	20.20	20.18
CF	3.20	3.50	3.50	3.20
EE	3.78	3.77	3.76	3.75
Ash	5.40	6.60	6.50	5.20

whereas D1, D2 and D3 experimental diets were prepared with the supplementation of phytase at the rate of 500 FTU, 1500 FTU and 2500 FTU, respectively. Phytase (Renaphytase®) was purchased from Renata Pharmaceuticals Ltd. The mineral matrix value of phytase (Ca = 80%, avP = 92%) were considered during formulation of experimental diets. The composition and nutritive values of formulated finisher test diets are shown in Table 2 and 3, respectively.

Management of birds: A total of 96 Cobb-500 day-old broiler chicks of both sexes were purchased from the local hatchery. The chicks were weighed on receiving day and then randomly assigned into four dietary treatment groups (D0, D1, D2 and D3), where each treatment was replicated 4 times with 6 birds per replicate in a completely randomized design (CRD). The birds were allocated to 16 equal-sized, clean and disinfected cages which were furnished with feeders and drinkers. Each pen (4.4 sq. ft.) was allotted for 6 birds. Therefore, floor space for each bird was 0.73 sq. ft. The birds were exposed to a temperature of 35°C for the first two days. Then the temperature was gradually reduced by 1 or 2°C after every 1 or 2 days until the chicks arrived at 10 days of age. Afterward, the poultry shed temperature was maintained at 25°C for the rest of the trial. All the birds had free access to diets and fresh, clean and cool drinking water during the entire trial period. Other standard management and vaccination programs were maintained according to the breeder manual.

Sample collection: On day 28, two birds were selected randomly from each replicate for sample collection. The birds were slaughtered humanely by cutting the jugular vein. Blood samples were collected in a falcon tube separately. After centrifugation at 5000 revolutions per minute, the serum samples were taken into the 2ml Eppendorf tube and stored at -20°C until further analysis. The tibia bones were also collected from the same birds and stored at -20°C for further processing and analysis. Different meat yield parameters such as carcass weight, dressed weight, weights of different meat cuts (neck, thigh, wings, breast, drumstick) and giblets weights (heart, lungs, liver, shank, proventriculus and gizzard and abdominal fat) were recorded. Besides, weights of other samples such as small intestine, pancreas, proventriculus, meat yields and cuts were also recorded from the same birds to evaluate carcass yields. Bodyweight, feed intake and remaining feeds were recorded weekly basis and the FCR was calculated accordingly. As there was no occurrence of death in the bird population during the trial period, so mortality was not recorded.

Sample processing and analyses: Feed samples were collected from formulated test diets before feeding the birds. The samples were processed by grinding with the help of mortar and pestle and then mixed thoroughly for lab analyses. About 500 g of each diet of finisher were taken for proximate analysis. The samples were tested for proximate analysis having dry matter (DM%), moisture%, crude protein (CP%), Crude Fiber (CF%) and ash using standard laboratory procedures[24]. Dry matter estimation was done by the oven-dry method. Crude protein estimation was accomplished by the Kjeldahl Method. Ash was measured by igniting the pre-ashing sample on a muffle furnace at a temperature of 600°C for four to six hours. The serum total protein (TP), Calcium (Ca), Phosphorus (P), alkaline phosphatase (AP), GPT (glutamic pyruvic transaminase), GOT (glutamic oxaloacetic

transaminase) levels were analyzed by using their respective standard assay kit (Randox Laboratories Ltd, UK) and semi-automated Humalyzer (Humalyzer 4000 Merck®, Germany).

Bone Sample: The left tibia from each sampled bird was collected and weighed. Length and width were also measured for each tibia after removing the flesh. The Seedor index (the ratio of bone weight and length) was also calculated. The seedor index was calculated by dividing the bone weight (mg) by its length (mm)[2,25]. The tibia bones were dried in a force draft oven (95°C) to reach a constant weight. The dried tibia bones were ashed at 650°C for 23 h. The samples were processed by grinding with the help of mortar and pestle and then mixed thoroughly. The bone ash for each tibia was then analyzed for Ca and P content using standard laboratory procedures[26].

Production cost: Cost of production was calculated considering the expense on chick, feed, medicine, labor, etc. Chick cost was calculated from the purchasing cost. Feed cost was considered from the sale price of the feed marketed through dealers.

Statistical analysis: All collected data were analyzed using one-way analysis of variance (ANOVA)followed by least significance difference (LSD) test using the SPSS software V.25 (SPSS, Inc., IBM, Chicago, Illinois, USA). Statistical significance was considered at $p \leq 0.05$.

RESULTS

Gross responses: The effect of the increased level of phytase supplementation on gross responses of broiler chickens is summarized in Table 4. There was no effect (p>0.05) of phytase on BWG and FI of broiler chickens from day 13-28. However, the highest weight gain was recorded for the diet supplemented with 1500 FTU phytase kg^{-1}. The FCR of broilers was significantly influenced by dietary treatment from day 13-28. Supplementation of 1500 FTU phytase kg^{-1} of diet showed better (p<0.05) FCR compared to the birds that received diets containing 2500 phytase kg^{-1}.

Tibia bone development: The effect of phytase supplementation on tibia bone development of broiler chickens is summarized in Table 5. There was no effect (p>0.05) of phytase inclusion on the weight of the tibia bone of broiler chickens from day 13-28. However, the length and width of the tibia bone were significantly influenced by phytase supplementation. Addition of 2500 FTU phytase kg^{-1} of diet reduced (p<0.001) the length and width of the tibia bone of broiler chickens. Birds supplemented 500 FTU phytase kg^{-1} of diet showed the highest (p<0.001) SI of the tibia bone. Diet supplemented with 1500 FTU phytase kg^{-1} of the diet increased (p<0.002) the Ca content of tibia bone compared to other diets. Supplementation of phytase did not affect (p>0.05) the P concentration of tibia bone of broiler chickens.

Serum biochemistry: The effect of phytase supplementation on serum contents of broiler chickens from day 13-28 is summarized in Table 6. Supplementation of 500 and 1500 FTU phytase kg^{-1} of the diet increased p<0.001) the serum TP and P levels of birds compared to those fed diets with 0 and 2500 FTU phytase kg^{-1}. Phytase supplementation had no significant effect on serum Ca, GPT and GOT levels in broiler chickens.

Carcass yield parameters: The effect of phytase supplementation on carcass yield and cuts of broiler chickens is summarized in Table 7. There was no significant effect of

Table 4: Effects of different levels of phytase on growth performance (day 13-28)

Dietary treatments	Phytase (FTU kg^{-1})	BWG	FI	FCR
Control	0	916.040	1640.830	1.790^{ab}
	500	937.040	1730.920	1.850^{ab}
	1500	966.210	1620.630	1.680^{a}
	2500	892.830	1899.460	2.010^{b}
SEM		22.110	67.570	0.110
p-value		0.275	0.056	0.034

$^{a-b}$Means with different letters within the same column differ significantly (p<0.05), SEM: Standard error mean

Table 5: Effects of dietary phytase level on tibia bone quality of birds at day 28

Dietary treatments	Phytase (FTU kg^{-1})	Weight (g)	Length (mm)	Width (mm)	Seedor index (mg mm^{-1})	Ca (%)	P (%)
Control	0	16.550	77.120^{a}	7.960^{a}	209.730^{c}	11.670^{b}	7.320
	500	17.950	77.810^{a}	8.110^{a}	234.640^{a}	12.200^{b}	7.290
	1500	17.760	77.990^{a}	8.100^{a}	227.700^{ab}	14.260^{a}	7.550
	2500	17.420	74.590^{b}	6.920^{b}	218.600^{bc}	11.890^{b}	6.830
SEM		0.240	0.400	0.130	2.750	0.320	0.120
p-value		0.185	0.001	0.001	0.001	0.002	0.206

$^{a-b}$Means with different letters within the same column differ significantly (p<0.05), SEM: Standard error mean

Table 6: Effects of different levels of phytase on blood parameters of broiler chicken (day 13-28)

Dietary treatments	Phytase (FTU kg^{-1})	TP (g dL^{-1})	P (mg dL^{-1})	Ca (mg dL^{-1})	GPT (U L^{-1})	GOT (U L^{-1})	AP (U L^{-1})
Control	0	3.220^{b}	4.980^{b}	9.070	56.010	204.020	1200.880
	500	4.080^{a}	5.550^{a}	11.280	51.750	201.470	1201.560
	1500	4.320^{a}	5.670^{a}	11.770	55.380	219.280	1229.310
	2500	3.290^{b}	4.080^{b}	10.420	56.380	202.280	1200.940
SEM		0.140	0.500	0.110	1.380	2.820	39.380
P-value		0.001	0.001	0.528	0.669	0.068	0.994

$^{a-b}$Means with different letters within the same column differ significantly (p<0.05), SEM: Standard error mean, TP: Total protein, P: Phosphorus, Ca: Calcium, GPT: Glutamic pyruvic transaminase, GOT: Glutamic oxaloacetic transaminase, AP: Alkaline phosphatase

Table 7: Effects of different levels of phytase on carcass characteristics of birds (day 13-28)

Dietary treatments	Phytase (FTU kg^{-1})	Dressing (%)	Breast (%)	Drumstick (%)	Thigh (%)	Neck (%)	Shank (%)	Wing (%)
Control	0	67.240	22.660	8.000^{b}	10.280	2.510	4.290	5.28
	500	65.610	22.470	8.850^{a}	10.240	2.680	4.150	5.30
	1500	66.630	20.880	8.800^{a}	10.440	10.500	4.110	4.79
	2500	65.670	22.730	8.020^{b}	10.360	10.360	4.200	5.23
SEM	0.55	0.490	0.120	0.150	0.090	0.070	0.110	
p-value	0.136	0.534	0.022	0.932	0.833	0.895	0.376	

$^{a-b}$Means with different letters within the same column differ significantly (p<0.05), SEM: Standard error mean

Table 8: Effects of different levels of phytase on visceral organ development (g/100 g of BW) of birds (day 13-28)

	Phytase (FTU kg^{-1})					
	0	500	1500	2500	SEM	p-value
SI	2.46	2.43	2.65	2.67	0.99	0.790
Proventriculus	0.58	0.64	0.58	0.71	0.05	0.844
Gizzard	2.95	3.33	3.37	3.30	0.12	0.637
Liver	2.10^{b}	2.72^{a}	2.52^{ab}	2.43^{ab}	0.08	0.019
Heart	0.58^{a}	0.45^{b}	0.48^{b}	0.63^{a}	0.02	0.001
Spleen	0.07^{b}	0.08^{b}	0.08^{b}	0.13^{a}	0.01	0.009
Pancreas	0.30	0.30	0.25	0.23	0.01	0.153
Bursa	0.05	0.05	0.06	0.05	0.003	0.812
Abdominal fat	2.09	2.54	2.24	2.66	0.13	0.405

$^{a-b}$Means with different letters within the same row differ significantly (p<0.05), SEM: Standard error mean, SI: Small intestine

phytase supplementation on dressing percent and meat yields except for drumstick. Diet supplemented with 500 and 1500 FTU phytase kg^{-1} had bigger (p<0.05) drumsticks than those on diets containing 0 and 2500 FTU phytase kg^{-1}.

Visceral organs development: Table 8 summarizes the effect of phytase supplementation on visceral organ development of broiler chickens. Supplementation of phytase influenced (p<0.05) the weight of the liver, heart and spleen. Birds that consumed a diet containing 500 FTU phytase kg^{-1} showed bigger liver (p<0.019) compared to those offered diets with 0, 1500 and 2500 FTU phytase kg^{-1}. Dietary supplementation of 500 and 1500 FTU phytase kg^{-1} reduced (p<0.001) the heart weight of birds. The weight of the spleen was increased (p<0.009) in birds consumed a diet supplemented with 2500 FTU phytase kg^{-1} than birds received diets with different levels of phytase. There was no significant effect of phytase supplementation on the weight of the small intestine, proventriculus, gizzard, pancreas, bursa and abdominal fat.

Cost-benefit analysis: Table 9 shows the cost-benefit analysis of broiler chickens fed diets supplemented with different levels of phytase. The cost-benefit parameters were significantly better in birds fed diets supplemented with 500 and 1500 FTU phytase kg^{-1} of diets than those received diets with 0 and 2500 FTU phytase kg^{-1}. These same group of birds also showed reduced (p<0.001) feed cost and production cost kg^{-1} live bird than those on other diets. The highest (p<0.001) profit kg^{-1} live bird was observed in birds fed the diet supplemented with 1500 FTU phytase kg^{-1}. Supplementation of 500 and 1500 FTU phytase kg^{-1} of diet also showed better (p<0.001) cost: benefit ratio compared to 0 and 2500 FTU phytase kg^{-1} of diet.

DISCUSSION

Gross response: In this study, dietary supplementation of phytase did not affect BWG of broiler chickens from day 13-28. This is consistent with the previous findings[9,27]. However, the

Table 9: Economics of broiler production supplemented with varying levels of phytase

Dietary treatment	Phytase (FTU kg^{-1})	Feed cost kg^{-1} live bird	Production cost kg^{-1} live birds	Profit (TK) kg^{-1} live bird	Cost benefit ratio
Control	0	67.170^{a}	117.170^{a}	12.830^{b}	9.180^{a}
	500	59.870^{b}	109.870^{b}	20.130^{a}	5.800^{b}
	1500	59.620^{b}	109.620^{b}	20.380^{a}	5.380^{b}
	2500	69.330^{a}	119.330^{a}	10.670^{b}	10.890^{a}
SEM		1.320	1.320	1.320	0.650
P-value		0.001	0.001	0.001	0.001

$^{a-b}$Means with different letters within the same row differ significantly ($p<0.05$), SEM: Standard error mean

overall BWG of broiler chickens of different diet groups at day 28 was below the standard weight of cobb broilers[28]. This could be due to the use of mash diets instead of pellet diets for birds[29]. Increasing the phytase level (2500 FTU kg^{-1}) in diet also tended ($p = 0.056$) to improve FI of broiler chickens which is partly consistent with a similar study by Pirgozliev *et al.*[19] who reported that FI increased by 22% when the phytase level was increased to 2500 FTU kg^{-1}. Besides, the lowest FI was observed in birds fed diets with 500 FTU phytase kg^{-1} which agrees with some previous studies[30,31]. This finding justifies the claims made by Cowieson *et al.*[3] who suggested that phytate can depress the bird's appetite due to deficiency of P which can be compensated by phytase supplementation. After the fulfillment of birds' requirement of P, FI of birds become stagnant until further increase of phytase level in diet. Phytase super dosing therefore further can improve the FI[3] through the release of more P and nutrient from the remaining lower ester phytates esters which were escaped from initial phytase dephosphorylation. The increased FI in birds fed diet supplemented with 2500 FTU kg^{-1} phytase is in line of this statement.

In the current study, dietary supplementation of 1500 FTU phytase kg^{-1} showed the better FCR of birds than those birds fed diet with 2500 FTU phytase kg^{-1}. Moreover, 1500 FTU phytase kg^{-1} of diet also non-significantly reduced the feed consumption but improved the weight gain. This improvement in growth therefore could be the result of the beneficial effect of phytase that releases phytate-bound nutrients and make them available for the utilization of the birds[21,22,32].

Tibia bone development: An increased Seedor Index (SI) signifies the better bone density, strength, weight and overall bone quality of broiler chickens[2]. The present study observed better SI of tibia bone in birds offered diets with 500 and 1500 FTU phytase kg^{-1}. These groups of birds also had the longest tibia bone with maximum Ca deposition which is consistent with previous studies[21,22]. The findings of the present study indicate that supplementation of 500 and 1500 FTU phytase kg^{-1} of diet improved the overall tibia bone quality. Interestingly, supplementation of 2500 FTU phytase kg^{-1} of diet negatively affected the Ca deposition, length and width of the tibia bone of broiler chickens. It has been claimed that a higher dose of phytase can cause complete hydrolysis of phytate, thus releases more Ca than P[29]. Therefore, there is a possibility of occurring imbalance in the Ca:P ratio when an increased level of phytase is added to the diet that already contained enough available P. The imbalance of the Ca and P ratio consequently leads to the formation of the Ca-phytate complex and reduce the phytase activity[9].

Serum biochemistry: Phytate forms a complex with dietary protein especially with free amino acids and limits their availability to the birds. Phytase breakdown this phytate-protein complex and improves the utilization of protein[1] and consequently can increase the serum TP level. In this study, supplementation of 500 and 1500 FTU phytase kg^{-1} of diet improved the serum TP level compared to the birds received diets with 2500 FTU phytase kg^{-1} of diet. The reason behind the low level of serum TP level in birds fed the diet with 2500 FTU phytase kg^{-1} of diet is not clear. However, it has been suggested that supplementation of a high dose of phytase without reducing the dietary Ca and NPP level may disrupt the Ca and P balance in the gut through the release of additional Ca and P and subsequently changes the gut pH balance. This condition further facilitates the formation of the phytate-mineral-protein complex in the poultry intestine which reduces the phytase activity and consequently limits the release of phytate-bound nutrients[13].

The concentration of serum P was increased in birds consumed diets supplemented with 500 and 1500 FTU phytase kg^{-1} of diet which is in line with the growth performance of these groups of birds. The improvement in serum P level could be the result of phytase-induced hydrolysis of phytate-mineral complex. Previous studies also reported increased concentration of serum P level by phytase supplementation to the diet[33-38]. The serum GOT, GPT and AP level act as an indicator for liver health. The non-significant effect of treatment on serum enzymes suggested that

the liver functions were not affected by phytase supplementation in this study. A similar finding was also reported by Ciurescu *et al.*[39].

Carcass yield and visceral organ development: In this study, the maximum weight of the drumstick was observed in birds offered diets containing 500 and 1500 FTU phytase kg^{-1}. Non-significant effects of phytase were recorded for breast, thigh, wing and neck development which is consistent with the previous study[40]. In this study, supplementation of 2500 FTU phytase kg^{-1} of the diet increased the weight of the spleen and this agrees with the findings of a previous study[40]. Birds fed phytase supplemented diets showed larger liver than those on diet without phytase. The increased weight of the heart in the birds fed diet without phytase in this trial can be due to the lack of available P. It has been reported that lack of available P supply causes hyperphosphatemia resulting in heart hypertrophy[41]. The lack of phytase effect on the relative weight of bursa and abdominal fat is also consistent with the previous studies[2,40].

Cost-benefit analysis: It was observed that the total profit kg^{-1} live bird was significantly better in broiler chickens fed diets supplemented with 500 and 1500 FTU kg^{-1}. These findings agree with Raut *et al.*[32]. The reduced total feed and production cost of the birds on the diets supports this finding. Notably, in this study, the amount of DCP was reduced during formulation of phytase supplemented diets. Phytase supplemented at 500 and 1500 FTU kg^{-1} had around 25 and 45% less DCP than control diets and this group of diets also showed better FCR despite of low FI, which may possibly explain the reason behind the low feed cost but high profit kg^{-1} live bird of these diet groups. Phytase supplementation not only releases P but also shows extra phosphoric effect by liberating other minerals (like Ca, Zn, Fe etc.) and nutrients (protein) which subsequently alleviates the anti-nutrient effects of phytate and consequently improves the FCR and thus reduces the feed cost. However, supplementation of 2500 FTU phytase kg^{-1} of diet showed the lowest total profit kg^{-1} live bird and highest cost: benefit ratio that is comparable to those on the phytase-free diet. This can be explained by the worst FCR of this diet group of birds.

CONCLUSION

Supplementation of 500 and 1500 FTU kg^{-1} of phytase improved the FCR, tibia bone characteristics (width and length and SI) serum parameters (P and TP level) of birds. However, there was no improvement in growth response and other variables when the highest level (2500 FTU kg^{-1}) of phytase was supplemented to diets. Diet supplemented with 500 and 1500 FTU kg^{-1} also reduced the total feed and production cost kg^{-1} live bird, thus improved the total profit kg^{-1} of bird. In conclusion, the study results indicate that 500 and 1500 FTU phytase kg^{-1} of diet could potentially improve the overall performance and consequently increase the total profit kg^{-1} live bird. Further research is needed to examine the effect of increase dose of phytase on a marginally P-deficient diet.

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