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Research Article
Effectiveness of Superdosing Phytase to Low Phosphorus and Calcium Diet on Growth Performance, Nutrient Digestibility, Blood Parameters of Broiler Chickens

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Abstract
Objective: This study was designed to investigate the Effects of superdosing phytase to low available phosphorus (avP) and calcium (Ca) diet on growth performance, serum biochemical, digestibility and phosphorus excretion in broiler chickens. **Materials and Methods:** A total of 375 one-day-old broiler chicks were randomly assigned to 5 treatments with 5 replicates. Treatments consisted of PC, positive control (adequate avP and Ca); NC, negative control (low avP and Ca) and the NC group supplemented with 3 levels of phytase (5000, 10000 and 15000 FTU/kg diet). Body weight and feed intake of birds were recorded at 11, 25 and 42 days of age. Blood samples of ten birds from each treatment were collected from wing venous at 21 and 42 days of age to evaluate serum biochemical indices and inositol in plasma. A digestibility trial was conducted to assess the apparent dry matter, crude protein, metabolizable energy and content of P excretion in feces at the end of the experimental period. **Results:** In all rearing period, phytase-treated NC diet group of birds showed improved body weight ($p<0.01$) and weight gain ($p<0.01$) similar to PC diet. However, birds fed high phytase doses gained more body weight and weight gain. The highest dose (15000 FTU/kg) increased serum albumin ($p<0.05$) at 42 days and serum IL-2 and IL-6 at a similar level, which was statistically equivalent to PC diet. On 42 days, serum protein and plasma inositol levels increased in response to all doses of phytase. On 42 days, serum Ca and P values differed by treatment, with NC diet having the lowest value. Birds fed NC or phytase treated diets had similar levels of P excreta. **Conclusion:** The super dose of phytase supplementation to a low avP and Ca diet can improve broiler performance and serum protein, calcium and phosphorus levels in the blood.

Key words: Blood, broiler chickens, digestibility, performance, phytase enzyme

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

INTRODUCTION

Phosphorus (P) is an important macro-mineral involved in growth and development of poultry. It is necessary for normal functioning of many biological processes and present in phospholipids, nucleic acids, ATP molecules, coenzymes and hormones. The deficiency of P can impede growth in birds and, with an additional lack of calcium (Ca) and vitamin D3, cause the onset of rickets. In modern poultry feeding industry, the lack of digestible P is a major problem due to the fact that in plant-based diets about two-thirds of total P is present in the form of insoluble and indigestible phytate, which leads to significant losses of this element[1]. Moreover, phytates have also a strong capacity to make chelate form with other nutrient components such Ca, Cu, Fe, Zn, Mg, starch, lipids and certain amino acids and making them poorly available[2,3]. Exogenous phytases are able to dephosphorylation of nutrient binding phytate, myo-inositol hexaphosphate, which thereby increases absorption of phytate-bound nutrients as well as can reduce environmental P pollution[4,5]. However, monogastric animals like poultry has an inadequate endogenous phytase activity in the gastrointestinal tract and thus cannot effectively digest phytate[6]. As a result, the supplementation of diet with inorganic P sources are commonly added more to meet the P requirement, which in turns leads to excess P excretion in the manure. Consequently, the cost of feed and the environmental adverse impact are increased. In line with this, it is important to find a way to reduce the P content in the poultry manure without reducing the amount of available P. The application of phytase in poultry diets is one of the extensive ways to decrease the need for supplementation with inorganic P, increase the availability of P and reduce its excretion in the environment[7]. The efficacy of supplemental phytase in improving the performance, bioavailability of phytate-bound minerals, energy metabolizability, amino acids digestibility and bone quality has been shown by several studies in poultry[8-11]. Pieniazek *et al.*[12] reported that phytase supplementation in the diet of broiler chicks improved utilization of phytate P, decreased its excretion and further phosphate pollution.

Up to date poultry industry is benefiting by the use of super-dosing phytase levels in diet, which is considered to provide extra-phosphoric effect. Phytate hydrolyze is currently practiced in poultry diet formulation according to conventionally recommended levels, in order to decrease the antinutritional effects of phytate. As a result, birds perform better due to the maximum utilization of minerals and other nutrients[13,14]. Beyond improving the performance of birds, increasing doses of phytase, can also provide economic advantages, as a more robust nutritional matrix can be incorporated (avP, Ca and Na), where the inorganic phosphate sources, such as monocalcium phosphate or dicalcium phosphate, can be replaced partially or completely in diets as an alternative or translating economic efficiency[15]. Therefore, the aim of this study was to investigate the impact of super-dosing phytase on growth performance, blood parameters, apparent nutrient digestibility in broiler chickens fed low avP and Ca diet.

MATERIALS AND METHODS

Birds and husbandry: This study was conducted at the Poultry Research Center Farm of Bangladesh Livestock Research Institute (BLRI). All experimental and animal management procedures were approved by the Institutional Animal Care and Use Committee of BLRI (approval number: SPRD-BLRI 2022-2023/014).

A total 375 broilers chicks (Cobb 500) were obtained from a commercial local hatchery with an average initial body weight of 42 ± 0.15 g. Birds were randomly allotted to five experimental groups with fifteen chicks were assigned to each of 25 floor pens (5 replicates/treatment), fresh rice hulls served as litter material on the floor pens. Each pen was equipped with hanging feeder and a nipple drinker line. The temperature was maintained in the room at 33°C during the first seven days of post hatching and then gradually reduced to 23 ± 2°C at the end of 21 days of age and it was maintained until rest of the trial period. Light was made available for 24 hrs during the whole experimental period. Birds were given free access to water and mash feed throughout the experimental trial. The diets for each rearing period were formulated to be isoenergetic and isonitrogenous and were fed to birds as pre-starter, starter and grower diets from 0 to 11, 12 to 25 and 26 to 42 days of age, respectively.

Diets and experimental treatments: The ingredient and nutrient composition of formulated diets are presented in Table 1. Treatments were consisted of a positive control diet (PC) and a negative control diet (NC). The PC group was fed a basal diet that was formulated to meet or exceed all nutrient concentrations for commercial broiler pre-starter, starter and grower chicks as recommended by NRC[16]. The NC group was fed with low avP and Ca content diet, which reduced by 0.15% avP and 0.17% Ca from the PC of pre-starter, starter and grower diets, respectively. Phytase enzyme was supplemented into the NC diet at the recommended level by the manufacturer (5000 FTU/kg) and super-doses levels at 10000 and 15000 FTU/kg (Sunphase, Sunhy Biology Co; Ltd).

Table 1: Ingredients and calculated nutrient composition of experimental diets

	Pre-starter (0-11 days)		Starter (12-25 days)		Grower (26-42 days)	
Ingredients (%)	PC	NC	PC	NC	PC	NC
Corn	43.171	44.060	49.079	50.100	55.577	56.750
Corn gluten meal	0.686	-	0.686	-	0.686	-
Wheat HRW	5.438	5.550	4.800	4.900	4.750	4.850
Wheat bran	7.447	7.600	6.074	6.200	3.966	4.050
Soy bean meal (44%)	37.762	38.540	34.003	34.710	29.086	29.700
Vegetable oil	1.411	1.000	1.636	1.200	2.546	2.100
Limestone (38.5%)	1.244	1.100	1.127	0.990	1.028	0.890
MonoCalP (Biofos: 17% avP and 21.1% Ca)	1.617	0.920	1.411	0.710	1.214	0.510
Iodized salt	0.294	0.300	0.294	0.300	0.294	0.300
Vitamin premix	0.098	0.100	0.098	0.100	0.098	0.100
Mineral premix	0.098	0.100	0.098	0.100	0.098	0.100
Lys-HCl (99%)	0.206	0.190	0.206	0.190	0.206	0.190
L-arginine (99%)	0.098	0.100	0.098	0.100	0.098	0.100
DL-methionine (99%)	0.284	0.290	0.245	0.250	0.206	0.210
Threonine (99%)	0.098	0.10	0.098	0.100	0.098	0.100
Valine (96.53%)	0.049	0.05	0.049	0.05	0.049	0.050
Total	100.000	100.000	100.000	100.000	100.000	100.000
Calculated analysis						
ME (kcal/kg)	3050.00		3100.00		3200.00	
CP (%)	23.0.0		21.50		19.50	
Ca (%)	0.97	0.80	0.88	0.71	0.79	0.62
avP (%)	0.45	0.30	0.40	0.25	0.35	0.20
Met (%)	0.63		0.57		0.51	
Lys (%)	1.46		1.36		1.22	
Thr (%)	0.95		0.89		0.82	
Arg (%)	1.60		1.48		1.32	

PC: Positive control, NC: Negative control, ME: Metabolizable energy, CP: Crude protein, avP: Available phosphorus, Ca: Calcium, Met: Methionine, Lys: Lysine, Thr: Thrionine arg: Arginine and Wheat HRW: Wheat hard red winter

Growth performance: The broilers were weighed and feed consumption was recorded on pen basis at the 11, 25 and 42 days of age. The cumulative live weight gain, feed consumption and feed conversion ratio were computed for 0-11, 12-25, 26-42 and 0-42 days. Body weight gain was determined as difference between initial weight and final weight, feed intake was estimated by the difference between feed and leftovers and feed conversion was calculated based on the data of feed intake to weight gain ratio.

Serum biochemical parameters: At 21 and 42 days of age, blood samples of ten birds from each treatment were collected from wing venous by sterile syringe (approximately 5 ml blood each bird). Collected blood samples were kept into heparinized (K2-EDTA, BD vacutainer®, Plymouth, Devon, UK) and non-heparinized tubes. Serum was separated by centrifugation at 3000 rpm for 15 min at 4°C, put into Eppendorf tubes and then kept at -20°C until being assayed. The concentration of Ca and P in serum and inositol in plasma were estimated at 21 and 42 days of experimental samples. Serum Aspartate Aminotransferase (AST), total protein (TP), albumin (ALB), cholesterol (CHOL), high density-lipoprotein (HDL) and triglycerides (TG), interleukin-2 (IL-2) and interleukin-6 (IL-6) concentration were measured at 42 days of samples by commercial diagnostic kits (Ratastie 2, FI-01620 Vantaa, Thermo Fisher Scientific Oy, Finland) using an automatic biochemistry analyzer (Konelab 20 analyzer, Thermo Fisher Scientific, Vantaa, Finland).

Nutrients digestibility trial: A digestibility trial was conducted at the end of the experiment to assess the apparent dry matter, crude protein, metabolizable energy and content of P excretion in feces. At the start of digestion trial, ten birds with uniform body weight (approximate) were selected from each treatment, kept in individual metabolic cages and fastened overnight. Then an equal measured amount of diet was given to birds daily with fresh water all the times. The duration of the digestion trial was total seven days, consisting of four days of adaptation and three days for excreta collection. The excreta were quantitatively collected for three days from trays of each cage in plastic bags and feed consumption data were also recorded. The excreta samples were dried in hot air oven at 60°C for 72 hrs, then ground and stored until being analysis.

Statistical analysis: Data were subjected to one-way analysis of variance (ANOVA) using GLM procedure of statistical analysis system (SAS, 9.1, 2009) in a completely randomized design. Significant differences among treatments were measured by Duncan's multiple range test at p<0.05 and tendency effect was considered at p<0.10.

RESULTS

Bird performance: The results of the growth performance of broilers at different rearing period are presented in Table 2 and 3. During the period from 0-11, 12-25, 26-42 and overall 0-42 days of age, the deficiency of avP and Ca in the NC diet decreased body weight and weight gain when compared to birds fed the PC diet with adequate supplies of avP and Ca. Phytase supplementation in the NC diet with all doses improved body weight (p<0.01) and weight gain (p<0.01) of birds and showed similar to those fed nutrient adequate control diet (PC group). However, no significant difference was observed due to increasing levels of phytase inclusion in the NC diet during the entire experimental period. Feed intake and feed conversion ratio were not influenced by lowering both avP and Ca level in the NC diet.

Serum biochemical parameters: The effects of dietary treatments on serum biochemical levels at 42 days are presented in Table 4. Reducing the dietary avP and Ca in the NC diet had significantly reduced serum ALB (p<0.05), IL-2 (p<0.01) and IL-6 (p<0.01) concentration of broilers compared to the PC diet. However, addition of phytase to the NC diet, especially at highest doses (up to 15000 FTU/kg) increased serum ALB concentration. The concentration of serum IL-2 and IL-6 was also increased by all phytase doses to a similar level and these levels were statistically equivalent to birds fed the PC diet. Comparing with the NC diet, the serum TP concentration had tended to higher (p<0.10) with the addition of phytase doses in the NC diet; however, greater value was obtained when birds fed the diet with phytase level up to 10000 FTU/kg. No differences were observed in AST, GLU, CHOL, HDL and TG among all the dietary groups.

Serum Ca, P and plasma inositol concentration: The effect of dietary treatments on Ca, P concentration in serum and inositol concentration in plasma at 21 and 42 days of age are summarized in Table 5. On 21 days of age, compared to the PC group, the broilers fed with the NC diet had not comparable plasma inositol level; however, addition of phytase, especially at levels greater than 5000 FTU/kg, significantly increased plasma inositol level (p<0.01). Serum P concentration had a decreasing tendency among the treatments (p<0.10) and least value was observed in the NC diet. During the lengthened growing period at 42 days of age, the serum Ca and P concentration significantly decreased in NC group compared to the PC group (p<0.05). However, addition of phytase to the

Table 2: Effects of experimental treatments on growth performance of broilers at pre-starter and starter period

	Pre-starter (0-11 days)				Starter (12-25 days)			
Treatments	BW (g)	BWG (g)	FI (g)	FCR	BW (g)	BWG (g)	FI (g)	FCR
PC	318.640^{a}	275.180^{a}	355.330	1.295	1144.700^{a}	826.060^{a}	1252.100	1.516
NC	281.770^{b}	237.890^{b}	322.610	1.361	1050.330^{b}	768.560^{b}	1152.020	1.503
NC 5000	309.000^{a}	265.750^{a}	352.670	1.333	1123.600^{a}	814.600^{a}	1232.590	1.513
NC 10000	311.690^{a}	268.020^{a}	351.720	1.317	1132.410^{a}	820.720^{a}	1206.170	1.471
NC 15000	306.270^{a}	262.360^{a}	343.130	1.309	1145.840^{a}	839.570^{a}	1244.370	1.481
SEM	3.860	3.870	4.860	0.010	8.750	6.700	12.800	0.012
P value	0.025	0.022	0.195	0.727	0.001	0.007	0.084	0.717

^{a,b}Means within column with no common superscripts differ significantly (p<0.05), SEM: Standard error of mean, BW: Body weight, BWG: Body weight gain, FI: Feed intake, FCR: Feed conversion ratio, PC: Positive control, NC: Negative control, NC 5000: NC +phytase at 5000 FTU/kg, NC 10000: NC +phytase at 10000 FTU/kg, NC 15000: NC +phytase at 15000 FTU/kg, The exogenous phytase was used Sunphase (Sunhy Biology Co; Ltd.) with recommended dose at 5000 FTU/kg

Table 3: Effects of experimental treatments on growth performance of broilers at grower and overall period

	Grower (26-42 day)				Overall (0-42 day)			
Treatments	BW (g)	BWG (g)	FI (g)	FCR	BW (g)	BWG (g)	FI (g)	FCR
PC	2572.58^{a}	1427.88^{a}	2541.55	1.780	2572.58^{a}	2529.13^{a}	4148.98	1.641
NC	2415.10^{b}	1364.77^{b}	2396.65	1.756	2415.10^{b}	2371.22^{b}	3871.28	1.632
NC 5000	2507.02^{a}	1383.42^{a}	2459.00	1.777	2507.02^{a}	2463.77^{a}	4044.26	1.641
NC10000	2516.18^{a}	1383.77^{a}	2461.65	1.779	2516.18^{a}	2472.52^{a}	4019.55	1.625
NC15000	2537.32^{a}	1391.48^{a}	2485.91	1.785	2537.32^{a}	2493.41^{a}	4073.42	1.633
SEM	12.98	6.78	20.0369	0.010	12.98	12.98	31.28	0.007
p-value	0.001	0.045	0.247	0.927	0.001	0.001	0.069	0.965

^{a,b}Means within column with no common superscripts differ significantly (p<0.05), SEM: standard error of mean; BW: body weight; BWG: body weight gain; FI: Feed intake; FCR: Feed conversion ratio; PC: Positive control, NC: Negative control; NC 5000: NC + phytase at 5000 FTU/kg; NC 10000: NC + phytase at 10000 FTU/kg; NC 15000: NC+phytase at 15000 FTU/kg, The exogenous phytase was used Sunphase (Sunhy Biology Co; Ltd.) with recommended dose at 5000 FTU/kg

Table 4: Effects of experimental treatments on serum biochemical parameters of broilers at 42 days of age

Treatments	ALB (g/dL)	AST (IU/L)	CHOL (mg/dL)	HDL (mg/dL)	GLU (mg/dL)	TP (g/dL)	TG (mg/dL)	IL-2 (pg/mL)	IL-6 (pg/mL)
PC	1.620[ab]	288.520	167.890	116.110	280.840	3.500	34.270	161.600[a]	170.460[a]
NC	1.590[b]	307.700	167.270	112.160	266.160	3.100	35.810	123.440[b]	127.710[b]
NC 5000	1.510[b]	274.870	175.160	111.240	275.720	3.370	39.420	162.730[a]	164.050[a]
NC 10000	1.650[ab]	281.590	169.390	109.260	281.590	3.500	36.270	162.550[a]	158.790[a]
NC 15000	1.770[a]	324.130	177.570	107.250	293.400	3.310	39.210	166.820[a]	166.380[a]
SEM	0.020	16.210	2.500	1.650	5.690	0.050	0.860	4.540	3.890
p-value	0.024	0.894	0.632	0.541	0.688	0.085	0.243	0.007	0.001

[a,b]Means within column with no common superscripts differ significantly (p<0.05), SEM: Standard error of mean, PC: Positive control, NC: Negative control, NC 5000: NC+phytase at 5000 FTU/kg, NC 10000: NC+phytase at 10000 FTU/kg, NC 15000: NC+phytase at 15000 FTU/kg, The exogenous phytase was used Sunphase (Sunhy Biology Co; Ltd.) with recommended dose at 5000 FTU/kg

Table 5: Effects of experimental treatments on serum Ca, P and plasma inositol level of broilers

	21 days of age			42 days of age		
Treatments	Ca (mg/dL)	P (mg/dL)	Inositol (mg/dL)	Ca (mg/dL)	P (mg/dL)	Inositol (mg/dL)
PC	169.230	259.610	55.6700[b]	125.700[ab]	219.520[a]	74.710
NC	152.170	225.380	67.6300[b]	117.200[b]	187.950[b]	67.280
NC 5000	170.800	259.840	74.2100[b]	123.470[ab]	202.510[ab]	98.070
NC 10000	168.260	241.360	102.3500[a]	130.840[a]	193.670[b]	111.650
NC15000	177.230	257.340	112.1000[a]	133.730[a]	208.160[ab]	92.170
SEM	3.720	4.670	4.8600	1.860	3.740	5.930
p-value	0.299	0.058	<0.0001	0.034	0.042	0.102

[a,b]Means within column with no common superscripts differ significantly (p<0.05), SEM: Standard error of mean, PC: Positive control, NC: Negative control, NC 5000: NC+phytase at 5000 FTU/kg, NC 10000: NC+phytase at 10000 FTU/kg, NC 15000: NC+phytase at 15000 FTU/kg, The exogenous phytase used was Sunphase (Sunhy Biology Co; Ltd.) with recommended dose at 5000 FTU/kg

Table 6: Effects of experimental treatments on apparent nutrient digestibility and phosphorus excretion in broilers

Treatments	Dry Matter (%)	Crude Protein (%)	Apparent ME (kcal/kg)	Total phosphorus Excretion (g)
PC	78.170	66.100	3218.900	1.62[a]
NC	79.770	67.740	3159.830	1.11[b]
NC 5000	78.240	67.060	3205.230	1.05[b]
NC 10000	77.780	66.520	3206.640	1.16[b]
NC 15000	80.660	68.540	3268.150	0.98[b]
SEM	0.490	1.180	13.980	0.05
p-value	0.298	0.973	0.187	<0.0001

[a,b]Means within column with no common superscripts differ significantly (p<0.05), SEM: Standard error of mean, PC: Positive control, NC: Negative control, NC 5000: NC+phytase at 5000 FTU/kg, NC 10000: NC+phytase at 10000 FTU/kg, NC 15000: NC+phytase at 15000 FTU/kg, The exogenous phytase was used Sunphase (Sunhy Biology Co; Ltd.) with recommended dose at 5000 FTU/kg

NC diet, especially at the highest dose (up to 15000 FTU/kg) increased blood serum Ca and P level. In phytase supplemented groups, increasing tendency was observed in plasma inositol concentration.

Digestibility trial: As shown in Table 6, NC diets supplemented with phytase did not improve apparent digestibility of dry matter, crude protein and metabolizable energy even was not comparable with the PC diet where adequate level of avP and Ca was provided. The P excretion differed significantly (p<0.01) among the treatments. It was observed that reducing dietary avP and Ca content in the NC diet; and NC diet supplemented with all inclusion levels of phytase had significantly reduced (p<0.10) P excretion when compared with the birds fed the PC diet. There was no consistent difference in P excretion for birds fed NC diet, regardless of whether they were fed phytase or not, however, least excretion was noticed in broilers fed NC with high level of phytase at 15000 FTU/kg.

DISCUSSION

It has been well documented in the literature that supplementation of phytase to the diet can improve bird performance[17-20]. Phytic acid in corn-soybean meal diets has a negative impact on the growth performance by making phytate-bound complexes with nutrients and reduce the availability of Ca, P and other nutrients in diet. The phytase enzyme was supplemented into the diets of nonruminants to improve the utilization of phytate-bound minerals and reduce the release of P into the environment. Phytase break-down phyate to release divalent cations such as Ca and P, which is used by the animals to meet their requirement. This study has shown that supplementing super-doses of phytase to low avP and Ca diets in the NC group had significantly similar effects on body weight and weight gain as supplementing nutrient-adequate PC diets. Walk *et al.*[21] reported that the addition of phytase to the NC diet with low Ca and available P had increased body weight gain and feed intake of broilers from 0 to 21 days of age. Farhad *et al.*[22] observed that birds fed a P deficient diet with higher doses of phytase, especially beyond 4000 FTU/kg, showed maximum body weight and weight gain, however, these improvements were not statistically significant when compared with standard dose of phytase and the adequate nutrient diet group. Some previous studies have reported that birds fed low P diets with increasing levels of phytase (i.e., 1500 FTU/kg and above) performed better than birds fed commercially recommended diets.[2,17,22-24], however, the results of the present study showed that broiler performance was not improved when high doses of phytase were included, even though the performance was similar to that of the PC group. Thus, the results of the present study suggest that those birds fed an avP and Ca deficient diet with commercially recommended levels of phytase at 5000 FTU phytase and increasing phytase dosage might be able to provide sufficient nutrients, including Ca and P, to meet the needs of birds for proper body weight across all rearing phases, including pre-starter, starter and grower diets.

ALB and TP serum levels decreased in birds fed the NC diet and these levels were increased with the addition of phytase which is in agreement with the findings of Viveros *et al.*[25] and Farhadi *et al.*[22]. Such a response may be due to phytate interfering with protein digestion and thus phytase releases this constraint and increases amino acid availability from the dietary protein[22]. According to Underwood and Suttle[26], serum protein may be affected by hepatic metabolism, which is sensitive to protein and P supply. The cytokine level in serum is an important index for humoral immunity in chicken. It is generally known that Th1 cells excrete pro-inflammatory cytokines, such as TNF-α, IL-2 IL-4, IL-5, IL-6, IL-10, IL-12; Th2 cells secrete anti-inflammatory cytokines[27]. Results of the current study showed that phytase supplementation increased the level of serum interleukins (IL-2 and IL-6) in birds receiving NC diet and statistically had the same level in birds receiving PC diet, suggesting that the birds' immune function was enhanced through phytase supplementation, which increased the availability of nutrients from the diet by reducing phytate anti-nutritive properties. According to Dersjant-Li and Dusel[28], direct-fed microbes and enzyme combinations increased cytokine concentrations in serum, suggesting that a portion of the nutritional benefits of enzymes may be mediated by immunocyte activity.

The nutritional status of poultry is determined by the concentrations of Ca and P in serum. To maintain normal Ca and P homeostasis in a low avP and Ca diet, the regulatory mechanism mobilizes bone Ca and P. In the current study, it has been shown that the phytase supplementation increased serum Ca and P concentrations at 42 days of age, indicating that it can increase Ca and P availability by hydrolyzing phytate bound molecules in poultry diet. These results are supported by Leyva-Jimenez *et al.*[8] and Babatunde *et al.*[29]. Similar results were reported by Liu *et al.*[30] who observed reduced mineral retention when birds fed reduced nutrient specification diet with Ca and P, which increased when phytase at the level of 500 and 1000 FTU/kg was added to the diet. In addition to its functions in cell signaling and cell growth, inositol plays an important role in maintaining phospholipid structures and lipid metabolism. In this study, serum inositol concentrations increased with phytase doses, indicating that inclusion of phytase enzyme may increase inositol concentration and improve growth performance. Similar to the present findings, Cowieson *et al.*[31] observed that the plasma inositol concentration was substantially increased by phytase addition to the diet. It was previously shown that phytase supplementation increased myo-inositol concentration in the blood plasma of broilers on 22 days of age[32] and in the gizzard, ileum and excreta of broilers on 21 days of age[18].

Previous studies[30,33,34] have shown that by supplementing phytase into down specified diets in fast-growing birds, P excretion might be reduced, resulting in improved mineral digestion and availability. It is probably related to the release of minerals from phytate-bound minerals, as well as the prevention of making phytate-bound minerals. Srikanthithansan *et al.*[20] observed that Phytase supplementation at 500 and 1000 FTU/kg in the diet reduced P excretion more than normal P diet (4.5 available P/kg), with the lowest fecal P content at high phytase dose. This result agrees with the findings of Srikanthithansan *et al.*[20]. Manobhavan *et al.*[17] have found that phytase supplementation at 2500 and 5000 FTU/kg in low-P diet reduced P excretion by 32 and 28% when compared to normal P-diet group. NC diets supplemented with phytase, particularly at higher doses, resulted in reduced Ca and P content in ileal digesta, indicating that phytase can enhance Ca and P bioavailability and retention[22]. These observations support the findings of the current study.

CONCLUSION

Supplementation of phytase up to 15000 FTU/kg to low avP and Ca diet can improve growth performance along with serum protein, Ca, P and interleukin level in the blood of broilers as well as reduce P content in the excreta. Increased phytase doses in broiler chickens fed avP and Ca deficient diets does not seem to have any adverse effects.

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