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Effect of phytase supplementation on performance, fecal excretion, and compost characteristics in broilers fed diets deficient in phosphorus and calcium

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Abstract

This study investigated the effect of dietary supplementation with phytase on growth performance, fecal excretion, and compost nutrition on broilers fed available phosphorus (avP)- and calcium (Ca)-deficient diets. A total of 750 one-day-old broiler chicks were randomly divided into five dietary groups having ten replications in a floor house. Diets of the groups were formulated with positive control (PC), negative control (NC; low avP and Ca), and NC supplemented with phytase levels; 500 (NC500), 1,000 (NC1000), and 1,500 FTU/kg (NC1500). A three-phase feeding program was used in the trial. Average daily gain (ADG) and average daily feed intake (ADFI) in the groups fed diets supplemented with phytase were significantly ($p < 0.05$) higher than those fed NC and the increase was equivalent to those fed PC. Serum levels of Ca and phosphorus (P) were higher ($p < 0.05$) in broilers fed NC1000 and NC1500 than in those fed NC. Interleukin (IL) level was the lowest in the group fed NC. Plasma *myo*-inositol (INS) concentrations in the NC1500 group were higher ($p < 0.05$) than PC, NC, and NC500 groups. Crude protein (CP) excretion was notably ($p < 0.05$) lower in the NC1500 group than in PC and NC groups. A lower ($p < 0.05$) concentration of P_2O_5 was observed in compost from the group fed NC1500 than the groups fed PC and NC. Accordingly, we suggest that phytase supplementation in lower avP and Ca levels of broiler diet can improve their productive performance and reduce environmental pollution.

Keywords: Broiler, Phytase, Performance, Fecal excretion, Compost

INTRODUCTION

Poultry industry is growing globally. The increased productivity induces an increase in generated poultry excreta [1]. Manure compost from poultry is used as an organic fertilizer for soils low in nitrogen (N), due to its N, phosphorus (P), and potassium (K) levels. The global market for chicken manure has

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Lim CI, Park JH.
Data curation: Choo HJ.
Formal analysis: Lim CI.
Validation: Park JH.
Investigation: Lim CI.
Writing - original draft: Lim CI, Choo HJ.
Writing - review & editing: Lim CI, Choo HJ, Park JH.

Ethics approval and consent to participate
The protocol for these experiments was approved, and animals were cared for according to the guidelines of the Animal Care and Use Committee of Jeonbuk National University, Jeonju, Korea (JBNU 2021-0168).

grown because of increased reliance on organic farming, agro-based industries, and poultry breeding [2]. However, excessive use of poultry-composts has caused adverse effects on air quality, water eutrophication, and soil acidification [3].

Poultry is unable to utilize P sufficiently from a grain-based diet due to their insufficient endogenous phytase production in the gastrointestinal tract. To achieve the P requirements of birds, inorganic phosphate supplementation is needed [4]. The inclusion of inorganic phosphate in their diet imposes a considerable cost; P is the third most expensive ingredient following energy and protein. Furthermore, anti-nutritional properties of phytate can cause the formation of an insoluble complex through mineral chelation and nutrient binding, as well as an increase in endogenous losses, which makes it harder for birds to digest and absorb nutrition, and negatively affect their performance [5]. The supplementation of exogenous phytase has been used in the poultry diet, especially lower level of P [4], calcium (Ca), and available P (avP) diet [6] to improve the productivity of broiler chickens. Another goal is to reduce the excretion of P and associated environmental pollution [7]. Improvement in the growth performance of broilers has been observed with phytase supplementation, which may increase the availability of nutrients by breaking down phytate, including inorganic phosphate, protein, and other minerals such as Ca, copper, iron, zinc, and sodium [8,9]. Furthermore, a good way to recycle organic P is composting animal feces. The compost from hens fed a diet supplemented with phytase (500 FTU/kg) is a major part of nutrient-balanced organic fertilizers [10]. The organic fertilizers can be used on crops without causing side effects [11]. Since poultry dietary management affects nutritional excretion, a research on the process among diet, feces, and compost could be needed to improve the usability of the poultry excretion in the fertilizer industry. To our knowledge, however most studies have confirmed the effect of phytase supplementation on nutritional excretion. there are no studies which have investigated nutritional values in composting using such excretions.

Therefore, this study aimed to evaluate the productive performance and excreted nutrients of broilers fed diets supplemented with various levels of phytase (500, 1,000, and 1,500 FTU/kg), and to confirm the nutritional value of mature compost from the excreted feces.

MATERIALS AND METHODS

Birds and experimental diets

This study was carried out at the Poultry Experimental Station of the Department of Animal Sciences at Jeonbuk National University in the Korea. The protocols for the experiment were approved by the Jeonbuk National University Institutional Animal Care and Use Committee (JBNU 2021-0168).

A total of 750 one-day-old broiler chicks (Ross 308) were obtained from a local hatchery and randomly allocated to five dietary treatments in an environmentally controlled house (12.4 birds/m^2), with each treatment replicated ten times with 15 birds. Each pen was covered with rice husk as a bedding material on the floor. During the first seven days post-hatching the temperature was maintained in the room at 33℃, after which it was gradually reduced until each bird was 21 days old to 23 ± 2℃, at which it was maintained throughout the remainder of the trial period. All diets were corn and soybean meal-based and were formulated in three phases; starter (0 to 11 d), grower (12 to 25 d), and finisher (26 to 42 d). The diets consisted of the positive control (PC) and the negative control (NC) in all phases. The PC was formulated to meet the nutrient requirements of the broilers as described in the National Institute of Animal Science [12] while the NC was formulated with 0.15% less avP and 0.17% less Ca than the PC (Table 1). The NC was provided to three dietary groups with diets supplemented with phytase (Ronozyme HiPhos GT, DSM

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Table 1. Ingredients and nutritional composition of experimental basal diets

Ingredients (g/kg)	Starter (0–11 d)		Grower (12–25 d)		Finisher (26–42 d)	
	PC	NC	PC	NC	PC	NC
Maize	432	441	491	501	556	568
Soybean meal (49%)	378	385	340	347	291	297
Wheat bran	74.5	76.0	60.7	62.0	39.7	40.5
Wheat	54.4	55.5	48.0	49.0	47.5	48.5
Soybean oil	14.1	10.0	16.4	12.0	25.5	21.0
Corn gluten meal	6.86	-	6.86	-	6.86	-
Limestone	12.4	11.0	11.3	9.90	10.3	8.90
Monocalcium phosphate	16.2	9.20	14.1	7.10	12.1	5.10
Iodized Salt	2.94	3.00	2.94	3.00	2.94	3.00
Vit-Min Premix[1)]	2.00	2.00	2.00	2.00	2.00	2.00
Lysine-HCl (99%)	2.06	1.90	2.06	1.90	2.06	1.90
L-Arginine (99%)	0.98	1.00	0.98	1.00	0.98	1.00
DL-Methionine (99%)	2.84	2.90	2.45	2.50	2.06	2.10
Threonine (99%)	0.98	1.00	0.98	1.00	0.98	1.00
Valine (96.5%)	0.49	0.50	0.49	0.50	0.49	0.50
Total	1,000	1,000	1,000	1,000	1,000	1,000
Calculated composition						
ME (kcal/kg)	3,050		3,100		3,200	
CP (%)	23.0		21.5		19.5	
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
Methionine (%)	0.630		0.570		0.510	
Lysine (%)	1.46		1.36		1.22	
Analyzed composition						
CP (%)	23.2	23.1	21.3	21.5	19.8	19.6
Ca (%)	1.001	0.813	0.896	0.730	0.799	0.642
P (%)	0.853	0.711	0.780	0.639	0.699	0.557

[1)]Contains per kg: Vit A, 12,000 IU; Vit D_3, 5,000 IU; Vit K_3, 3 mg, Vit B_1, 2 mg, Vit B_2, 6 mg; Vit B_6, 4 mg; Vit B_{12}, 25 mg; biotin, 0.2 mg; folic acid, 0.2 mg; niacin, 70 mg; pantothenic acid, 20 mg; Cu, 20 mg; Co, 0.5 mg; Fe, 50 mg; I, 1,300 mg; Mn, 120 mg; Se, 0.3 mg; Zn: 100 mg.

PC, positive control; NC, negative control; ME, metabolic energy; CP, crude protein; Ca, calcium; avP, available phosphorus; P, phosphorus.

Nutritional Products, Heerlen, Netherlands) levels of 500 (NC500), 1,000 (NC1000), and 1,500 FTU/kg (NC1500). All experimental diets were provided in mash form throughout the trial period. Birds were offered free access to feed via round feeders and fresh drinking water was served via a nipple drinker system.

Growth performances

Birds were randomly weighed from chick boxes on arrival to obtain information as to their average weight, thereby ensuring there were no statistically significant differences in starting pen weight between the treatment groups. Average daily gain (ADG) and average daily feed intake (ADFI) were later measured over the experimental period (42 d). Feed conversion ratio (FCR) was calculated by dividing the ADFI by the ADG.

Blood profiles

Approximately 3 ml of blood was collected from the wing vein of each bird (ten birds per treatment) by a sterile syringe needle at the end of the experimental period. The serum was separated by centrifugation at 3,000 rpm at 4℃ for 15 minutes. Separated serum was put into Eppendorf tubes and stored at −20℃ until analysis. Serum Ca and P concentrations were measured by commercial diagnostic kits using an automatic blood biochemistry analyzer (Konelab 20 analyzer, Thermo Fisher Scientific, Vantaa, Finland). Serum interleukin (IL)-2 and IL-6 concentrations were analyzed using ELISA kits (E-EL-Ch0120 and E-EL-Ch0228, respectively, Elabscience Biotechnology, Houston, TX, USA) according to the manufacturer's instructions. Absorbances were read at 450 nm using a microplate spectrophotometer (ELX 800, BioTek, Winooski, VT, USA). Plasma *myo*-inositol (INS) concentration was analyzed using the method described by Pirgozliev et al. [13]. Collected blood samples from ten birds per treatment were immediately kept into heparinized. The samples were mixed with 2 ml of ice-cold 5% w/v perchloric acid and maintained on ice for 20 min to precipitate protein. The samples were then centrifuged at 16,000×g for 15 min at 4℃, and the supernatant was diluted 50-fold in 18.2 MOhm.cm water. The samples (20 μL) were then injected into a 4 mm × 50 mm CarboPac PA1 column (Dionex, Cheshire, UK). INS was determined by high-performance liquid chromatography (HPLC) pulsed amperometry (HPLC-PAD) on a gold electrode at 30℃ after separation by 2-dimensional HPLC (Dionex DX-600 HPLC System, Dionex).

Fecal excretions

At the end of the experiment, a total of male thirty birds whose body weight was closest to the mean were selected, including six birds from each treatment group, and placed in an individual metabolic cage (the dimension of each cage was 0.35 m × 0.43 m) which was fastened for 24 hours. The digestive trial period lasted for 7 d and included 4 d of acclimation to diet and environment and 3 d of excreta sample collection. Excreta samples were collected into plastic bags from each cage and immediately frozen at −20℃. After raw excreta (RE), dry excreta (DE), and moisture (MS) contents were measured, the samples were finely ground and passed through a one-millimeter sieve and then analyzed for N and P excretions per feed intake (kg) by AOAC [14]. CP concentration was calculated by multiplying 6.25 by the N concentration.

Composts

At the end of the analysis of experimental fecal excretion, in total, we collected the feces from thirty birds (six birds per group), each held in an individual cage for 10 d. Each fecal sample (5 kg) was matured for one month in an independent composting facility with heating (40℃) and ventilation (using the compost maturity method of Walker [15]) until turning blackish-brown without the form and smell of feces. Briefly, water was supplied so that the moisture content of the broiler feces could be maintained at about 60%. Additionally, to prevent the solidification of the feces and supply fresh air, the feces were stirred 3 times daily. After hydrolyzing the water sample, the produced compost was analyzed using the Kjeldahl method for N, Vanadate method for P_2O_5, and inductively coupled plasma atomic absorption spectrophotometer (ICP-OES, GBC Scientific Equipment, Keysborough, Australia) for K_2O. For pH analysis, the ratio of the samples to distilled water was 1:5 (wt. %). The mixture was stirred for 1 hour and filtered through a glass fiber filter paper (GF/C Filter), and the filtrate was measured with a pH meter (HI-2222, HANA, Woonsocket, Rhode Island, USA).

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Statistical analysis

All data were analyzed using one-way analysis of variance (ANOVA) of SAS software (SAS 9.1, 2009, SAS Institute, Cary NC, USA) in a completely randomized design. The means of different dietary groups were compared with Duncan multiple range tests. Significant differences were determined at $p < 0.05$.

RESULTS

Productive performance

The productive performances of broilers fed experimental diets are presented in Table 2. The ADG of broilers fed NC500, NC1000, and NC1500 was significantly ($p < 0.05$) higher than those fed NC and the increase was equivalent to those fed PC. The higher ADFI was confirmed in birds fed NC1000, NC1500, and PC than in those fed NC. However, groups fed diets supplemented with phytase showed no changes FCR compared to groups fed PC and NC.

Blood profiles

In blood concentrations (Table 3), serum Ca level was increased ($p < 0.05$) in the NC1000- and NC1500-fed groups than in NC- and NC500-fed groups. In addition, the serum Ca level in broilers fed NC1500 was higher ($p < 0.05$) than those fed PC. A significant increase ($p < 0.05$) in serum P levels was observed in birds fed NC1000 and NC1500 compared to those fed NC. Both IL-2 and IL-6 levels were significantly ($p < 0.05$) lower in the group fed NC than in the other groups. Plasma INS concentration in broilers fed NC1500 was higher ($p < 0.05$) than those fed PC, NC, and NC500.

Fecal excretion

The fecal excretion per feed intake (1 kg) of broilers fed experimental diets is shown in Table 4. There were no significant differences in excretions of RE, DE, and MS among the dietary groups.

Table 2. Effects of phytase supplementation on the productive performance of broilers (n=10)

Variable	PC	NC	NC500	NC1000	NC1500	SEM	*p*-value
ADG (g/d)	60.2^{a}	56.5^{b}	60.1^{a}	60.3^{a}	61.6^{a}	0.41	0.001
ADFI (g/d)	99.0^{a}	93.0^{b}	96.6^{ab}	98.2^{a}	98.9^{a}	0.64	0.009
FCR	1.64	1.65	1.61	1.63	1.61	0.02	0.756

^{a,b}Means within the column with no common superscripts differ significantly ($p < 0.05$).

PC, positive control; NC, negative control; NC500, NC + phytase at 500 FTU/kg; NC1000, NC + phytase at 1,000 FTU/kg; NC1500, NC + phytase at 1,500 FTU/kg; ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.

Table 3. Effects of phytase supplementation on blood concentrations of Ca, P, IL, and INS in broilers (n=10)

Variable	PC	NC	NC500	NC1000	NC1500	SEM	*p*-value
Ca (mg/dL)	126^{bc}	117^{c}	121^{c}	134^{ab}	140^{a}	2.07	0.001
P (mg/dL)	220^{a}	188^{c}	195^{bc}	211^{ab}	204^{abc}	3.34	0.013
IL-2 (pg/mL)	162^{a}	123^{b}	162^{a}	157^{a}	159^{a}	4.10	0.007
IL-6 (pg/mL)	170^{a}	128^{b}	176^{a}	169^{a}	168^{a}	4.73	0.004
INS (mg/dL)	74.7^{b}	67.3^{b}	80.2^{b}	109.5^{ab}	122.6^{a}	6.86	0.032

$^{a-c}$Means within the column with no common superscripts differ significantly ($p < 0.05$).

PC, positive control; NC, negative control; NC500, NC + phytase at 500 FTU/kg; NC1000, NC + phytase at 1,000 FTU/kg; NC1500, NC + phytase at 1,500 FTU/kg; Ca, calcium; P, phosphorus; IL, interleukin; INS, *myo*-inositol.

Table 4. Effects of phytase supplementation on the fecal excretion of broilers (n=6)

Variable	PC	NC	NC500	NC1000	NC1500	SEM	*p*-value
RE (g)	983	977	962	949	968	16.5	0.978
DE (g)	185	185	182	177	170	2.87	0.390
MS (g)	798	792	780	773	798	16.5	0.987
CP (g)	68.6^{a}	62.3^{ab}	58.2^{bc}	58.0^{bc}	53.5^{c}	1.42	0.006
P (g)	3.01^{a}	2.21^{bc}	2.72^{ab}	2.39^{bc}	2.03^{c}	0.098	0.006

$^{a-c}$Means within the column with no common superscripts differ significantly ($p < 0.05$).

PC, positive control; NC, negative control; NC500, NC + phytase at 500 FTU/kg; NC1000, NC + phytase at 1,000 FTU/kg; NC1500, NC + phytase at 1,500 FTU/kg; RE, raw excreta; DE, dry excreta; MS, moisture; CP, crude protein; P, phosphorus.

However, the CP excretion of birds fed NC500, NC1000, and NC1500 was significantly ($p < 0.05$) lower than those fed PC diet. The excretion of P was significantly lower ($p < 0.05$) in broilers fed NC, NC1000, and NC1500 than in those fed PC.

Compost characteristics

Table 5 reflects that no differences were found regarding most compost characteristics (N, K_2O, MS, and pH). However, the P_2O_5 levels were significantly ($p < 0.05$) lower in the group fed NC1500 than in the groups fed PC and NC.

DISCUSSION

Phytase supplementation to a diet low in avP and Ca is well-documented as improving the growth performance of broilers [4,6,16]. The breakdown of phytate complexes by phytase provides a more available source of P and other dietary essential nutrients, which in turn increase productive performance (ADG, ADFI, and FCR) in birds with diets low in avP [17] or low in both Ca and avP [6]. The growth-improving effect of the phytase-supplemented NC diet in our study (Table 2) may therefore be attributable to the fact that phytase, at all levels, facilitates the utilization of nutrients (Ca and avP).

The serum Ca and P concentrations are major indicators of poultry nutritional status of Ca and P. In broilers fed a low avP and Ca diet, the regulatory mechanism orchestrates to maintain normal Ca and P levels by taking these away from the bones [18]. In our study, dietary supplementation with phytase in broilers fed a low avP and Ca diet increased serum Ca and P concentrations (Table 3), indicating that phytase enhances the availability of Ca and P by hydrolyzing phytate-bound molecules. These findings are supported by Liu et al. [19], who reported that the serum concentrations of Ca and P were decreased in birds fed a diet low in Ca and P, and then increased with the dietary supplement of phytase (500 and 1,000 FTU/kg).

Table 5. Effects of phytase supplementation on the nutritional characteristics of compost from broiler feces (n=6)

Variable	PC	NC	NC500	NC1000	NC1500	SEM	*p*-value
N (%)	2.92	3.13	2.82	2.81	2.54	0.127	0.721
P_2O_5 (%)	1.76^{a}	1.51^{b}	1.42^{bc}	1.35^{bc}	1.21^{c}	0.052	0.005
K_2O (%)	1.38	1.45	1.47	1.31	1.32	0.047	0.781
MS (%)	59.2	57.5	60.0	59.0	59.3	1.317	0.986
pH	7.09	7.08	6.95	6.92	6.97	0.062	0.882

$^{a-c}$Means within the column with no common superscripts differ significantly ($p < 0.05$).

PC, positive control; NC, negative control; NC500, NC + phytase at 500 FTU/kg; NC1000, NC + phytase at 1,000 FTU/kg; NC1500, NC + phytase at 1,500 FTU/kg; N, nitrogen; P_2O_5, phosphorus pentoxide; K_2O, potassium oxide; MS, moisture.

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INS, which is the final product of degradation produced by the binding of phytase to phytate, plays an important role in maintaining phospholipid structures, lipid metabolism, cell signaling, and cell growth [20]. We confirmed that plasma INS concentrations were improved in birds fed NC1500 diets compared to those fed PC and NC diets (Table 3), suggesting that phytase supplementation degraded the phytates. Cowieson et al. [21] also reported significantly elevated levels of INS in the plasma of birds by the addition of phytase to their diets. Similarly, Summerfeld et al. [22] showed that phytase supplementation can increase the concentration of INS in the blood of broilers, and Beeson et al. [23] showed that dietary phytase can also increase it in their gizzard, ileum, and excreta.

The expression of IL proteins plays an important role in the differentiation and proliferation of immune cells, which in turn are important indicators of humoral immunity in chickens [24]. Th1 cells are known to excrete pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, IL-2 and IL-12, while Th2 cells secrete anti-inflammatory cytokines, such as IL-4, IL-5, IL-6 and IL-10 [25]. We showed in this study that the concentration of serum ILs (IL-2 and IL-6) increased in birds fed NC diets enhanced with any level of phytase, and the increase is statistically similar to that of the PC diet (Table 3). Khan et al. [26] suggested that ILs enhanced some cell-mediated immune responses of broiler chickens by modulating macrophage activity in response to enzyme supplementation in a diet. We postulate that some of these effects are mediated by cytokines secreted from immune cells stimulated with the enzyme. To the best of our knowledge, no study has reported effects of phytase supplemtation as feed additives on interleukins of broilers. However, according to one relevant report [27], it demonstrated a higher serum cytokine activity in birds fed feed additives (direct-fed microbes and enzyme combination), suggesting that the nutritional improvements associated with enzymes may in part be mediated through immunocyte activity. Accordingly, we suggest that a diet supplemented with 500 to 1,500 FTU/kg phytase enhanced humoral immunity in chickens by releasing nutrients from phytate complexes and mitigating anti-nutritive properties of phytate.

Reducing the amount of CP and P in poultry manure is particularly important to lower the pollution of soil and water [28]. We confirmed that the CP and P excretions of broilers fed the NC1500 diet 22% and 32.6% lower, respectively than those fed the PC diet (Table 4). These results are supported by Srikanthithansan et al. [29] who observed that less P was excreted by broilers fed a low phosphorus diet (3.0% avP/kg) supplemented with 500 to 1000 FTU/kg phytase than those fed a normal P diet (4.5% avP/kg). Walk and Olukosi [30] also reported that broilers fed a phytase-supplemented diet (2,000 or 4,000 FTU/kg) showed a higher CP digestibility, together with less CP excreted.

The high level of P pollution in the poultry industry is a result of the intensity of poultry production; P inputs to diets and composts often exceed P outputs in crops. When the compost is applied to soil, the soil could contain excessive residual P. Since this would lead to soil and groundwater pollution, and disturb the entire ecosystem [28], the nutrition values of the compost from poultry feces should be considered for their application to soil. To our knowledge, only few studies have investigated the N, P, and K concentrations of compost matured from feces of the broilers supplemented with phytase. Nevertheless, according to one relevant report [31], dietary phytase supplementation (500 FTU/kg) improves ileal digestibility in broilers and significantly decreases N, P, and K concentrations in excreta. Our experiments likewise showed a decrease in P levels in the fecal excreta of the phytase-fed groups (Table 4). Furthermore, we confirmed a decrease in P_2O_5 levels in the compost from broilers fed a diet that included 1,500 FTU/kg phytase (Table 5). It is suggested that a compost based on the excreta of phytase-fed chickens reduces water-extractable P runoff [11]. Although our finding showed less CP excretions of the NC 1500 group

than those of the PC and NC groups ($p < 0.05$; Table 4), the N levels in the compost were not a significantly different among the group ($p > 0.05$; Table 5). The N contents in compost may be affected by the maturation process of compost caused in the huge variation of moisture content, N loss, and weight from feces of each group [32,33]. Since poultry compost which has rich N, P, and K concentrations is used for efficient crop growth, their NPK balance must be considered important in the soil [34,35]. The optimal NPK balance not only improves the growth of crops by providing nutrients that are lacking to crops, but also reduces environmental pollution by mitigating the excess of some nutrients in the soil [10,28,36]. In our findings, although the P content in compost from chickens fed dietary phytase is reduced, the compost may be used to maintain optimal NPK balance in soils with excess P.

CONCLUSION

The results from the current study showed the dietary supplementation of phytase in the broiler diet increase the productive performance, the nutritional digestibility and improve their immunity. Also, the phytase supplements to broiler could potentially could reduce environmental pollution through low CP and P excretions, as well as P_2O_5 levels in composts matured from the feces.

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