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Exogenous Phytase Improves Growth Performance, Nutrient Retention, Tibia Mineralization, and Breast Meat Quality in Ross-308 Broilers

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Abstract: To evaluate the effect of exogenous phytase (Ronozyme HiPhos-L, produced by the strain of *Aspergillus oryzae*), 1050 one-day-old Ross-308 broilers with an initial average body weight of 50.29 ± 0.98 g were randomly assigned to one of seven dietary treatments with 10 replicates and 15 chicks/cage. During starter (d 1–7), grower (d 8–21), and finisher (d 21–35) periods, broilers were allowed to feed: positive control (PC), negative control (NC), and NC diet supplemented with 250, 500, 1000, 1500, and 3000 U/kg of phytase. Broilers fed NC diet supplemented with graded levels of phytase (250 U/kg to 3000 U/kg) showed a linear increase ($p < 0.05$) in body weight gain and feed intake during the starter and the overall trial period with no adverse effect on feed conversion ratio. The nutrient utilization of broilers showed neither an increase nor a decrease with the PC and NC diet until d 35 but when the NC diet was supplemented with a graded dose of phytase, the broilers showed a linear increase ($p < 0.05$) in dry matter, gross energy, calcium (Ca), ash, and phosphorus (P) utilization and a tendency to increase ($p < 0.10$) nitrogen retention at the end of the starter phase. Unlike the starter phase, broilers fed the PC diet showed increased ($p < 0.05$) ash and Ca retention at the end of the grower and finisher phase compared to those fed the NC diet. In addition, the phytase group showed a linear increase ($p < 0.05$) in ash, Ca, and P utilization at the end of d 21 and 35. Moreover, the inclusion of graded levels of phytase in the NC diet showed a linear increase (<0.0001) in bone (ash, Ca, and P) mineralization in broilers. Also, they showed a linear increase ($p < 0.05$) in gizzard weight, breast muscle, color of lightness, redness, and pH and a decreased cooking loss and drip loss. Though the standard diets were able to compensate for the P deficiency and promote performance and bone mineralization, the elevated (1000 FTU/kg) levels of phytase in the NC diet showed a higher response. Thus, we suggest that incorporating the exogenous enzyme phytase into the broiler feed in excess of 1000 units (U)/kg would be more beneficial to achieve better productivity.

Keywords: phytase; broilers; growth performance; foot pad; bone mineralization

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1. Introduction

Poultry feed mainly consists of plant-based elements; specifically, corn and soybeans became staple feed ingredients in animal diets that possess 50–70% of the total phosphorous (P) in the form of phytate [1]. Hence, the cost of these kinds of feedstuff often hikes, especially during the off-season. In addition, the global demand for poultry products has been increasing over the past few decades [2]. According to the Food and Agriculture Organization of the United Nations, global poultry meat production has grown by four percent per year and was expected to reach 14.2 million metric tons in 2021 [3]. Concurrently, global egg production has increased from 57 million metric tons in 2000 to over 82 million metric tons in 2021 [4]. In 2006, the European Commission prohibited the use of certain antibiotic growth promoters (AGPs) (sodium, monensin, avilamycin, salinomycin sodium, and flavophospholipol) in livestock diets [5]. The mounting feed prices of raw materials, growing demand for poultry meat, and the prohibition of synthetic feed additives provoked the researchers to find other nutritious feed ingredients that could maintain health status and increase livestock productivity [6]. As a result, organic acids, enzymes, probiotics, prebiotics, phytobiotic stimulants, and some herbs were found as alternatives, and these additives were proven to be organic, antibiotic residue-free, and less hazardous for livestock [7].

Phytase, an enzyme that initiates the removal of phosphate from phytic acid [8], has been extensively used in animal diets. It is generally dispersed in microorganisms, plants, and animals and phytase is used in animal diets for its anti-nutritional effects on phytate in reducing environmental pollution and increasing the availability of starch, protein, amino acids, calcium, and phosphorous (P). Such P sources represent the third most expensive component in poultry diets after energy and amino acids [9]. Also, it becomes an essential mineral for the growth and development of livestock [10]. The anti-nutritional properties associated with phytate can interfere with nutrient digestion and adversely affect poultry performance by increasing endogenous losses, as well as forming insoluble complexes through mineral chelation and nutrient binding [11]. Previously, several studies demonstrated that dietary phytase had increased the growth performance of broilers. For instance, Yi et al. [12] reported that microbial phytase inclusion had significantly increased the P availability and decreased P excretion in broilers. Similarly, Woyengo et al. [13] stated that the inclusion of phytase supplements had increased bone mineralization and P retention in broilers. Likewise, Zyla et al. [14] noted that broilers fed a wheat-based diet that was supplemented with 750 phytase U/kg significantly increased their body weight gain, daily feed intake, and P retention. However, Cowieson et al. [15] reported that the use of high doses of phytase (>1000 U/kg of diet) could improve nutrient availability in poultry diets compared to the diets containing lower (<1000 U/kg) phytase activities. Abbasi et al. [16] showed that adding a low dose of phytase significantly increased animal performance and reduced environmental P pollution. Though this practice is common in livestock farming, Coweison et al. [17] stated that super-dosing, i.e., adding >1500 FTU/kg phytase could be a new opportunity for poultry producers to reduce raw ingredient costs. From this, we hypothesized that feeding broilers with phytase exceeding 1000 FTU/kg or greater might be beneficial for improving their growth performance, nutrient retention, and bone mineralization compared with a lower phytase supplement. Thus, we aimed to examine the supplemental effect of exogenous phytase on the performance of the broiler at doses from 250 to 3000 U/kg feed.

2. Materials and Methods

The animal care procedures implemented in this study were approved (Approval No. DK-1-2210) by the Institutional Animal Care and Use Committee (IACUC) of Dankook University (Republic of Korea).

2.1. Birds and Husbandry Management

Prior to the trial, rearing cages and equipment were sterilized with Roxycide™ powder. A total of 1050 one-day-old Ross-308 broilers (mixed sex) with an initial average weight of 50.29 ± 0.98 g were obtained from Cherry-Buro, a commercial hatchery (Cheonan, Republic of Korea), and raised at "Dankook University Poultry Experimental Farm" in thermostatically controlled three-layer cages with 20 h of fluorescent lighting. During the first week, the temperature of the cages was maintained at 28 to 30 °C, and slowly it was decreased to 26 to 27 °C. Sixty percent humidity was maintained until day 35. The elimination of noxious gases was aided by cross-ventilation, and hygiene management was implemented throughout the trial.

2.2. Experimental Diet

The feeding program consists of 3 phases: starter (1–7 days), grower (8–21 days), and finisher (22–35 days). The broilers were randomly allotted to one of seven dietary treatments with 10 replications/treatment and 15 chicks/cage, and the dietary treatments were as follows: (a) positive control (PC)—a basal diet prepared in a single batch with available P at 0.48% (starter), 0.44% (grower), and 0.41% (finisher); and (b) negative control (NC) diet prepared with reduced P at 0.33% (starter), 0.29% (grower), and 0.26% (finisher). Appropriate quantities of the NC diet were collected and stored in huge feeder bags for the other five groups and mixed with different concentrations (250, 500, 1000, 1500, and 3000 U/kg) of phytase (Ronozyme HiPhos-L, produced by the strain of *Aspergillus oryzae*), which was commercially prepared under the name of "Growin Act" and obtained from CJ Cheil-Jedang Corporation (Seoul, Republic of Korea). The composition and calculated nutrient provision of the basal diets (mash form) are illustrated in Table 1 [18]. The feeder trough and a suspended nipple drinker were fixed at the corners of each cage which allowed chicks to have free access to the feed and water until the end of the experiment.

Table 1. Feed composition of broiler (as fed-basis).

Item	Starter (1–7 Days)		Grower (8–21 Days)		Finisher (22–35 Days)	
	PC	NC	PC	NC	PC	NC
Ingredients (%)						
Corn	47.36	48.90	49.77	51.33	54.85	56.36
Soybean meal	29.36	29.10	25.91	25.65	23.05	22.80
Corn gluten meal	13.00	13.00	13.00	13.00	10.00	10.00
Wheat bran	3.00	3.00	3.00	3.00	3.00	3.00
Soy-Oil	3.39	2.86	4.57	4.04	5.57	5.05
MDCP [1]	1.65	0.84	1.50	0.65	1.37	0.55
Limestone	1.01	1.07	1.07	1.15	1.00	1.08
Salt	0.36	0.36	0.36	0.36	0.36	0.36
Methionine (99%)	0.19	0.19	0.19	0.19	0.19	0.19
Lysine	0.48	0.48	0.43	0.43	0.41	0.41
Mineral mix [2]	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin mix [3]	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutritional characteristic						
Crude protein, %	21.00	21.00	19.50	19.50	18.00	18.00
Calcium, %	0.90	0.75	0.88	0.73	0.82	0.67
Phosphorus, %	0.76	0.61	0.71	0.56	0.66	0.51
Available P, %	0.48	0.33	0.44	0.29	0.41	0.26
dig Lys, %	1.28	1.27	1.15	1.15	1.06	1.05
dig Met, %	0.50	0.50	0.48	0.48	0.46	0.47
dig Ile, %	0.80	0.80	0.74	0.73	0.67	0.67
dig Leu, %	1.68	1.69	1.59	1.59	1.48	1.49
dig Val, %	0.89	0.89	0.82	0.82	0.75	0.75
dig Arg, %	1.26	1.26	1.16	1.15	1.05	1.05
dig Thr, %	0.64	0.64	0.60	0.60	0.51	0.54
dig Trp, %	0.23	0.22	0.20	0.20	0.18	0.18
ME, kcal/kg	3000.00	3000.00	3100.00	3100.00	3200.00	3200.00

[1] MDCP-mono-dicalcium phosphate; [2] Provided per kg of complete diet: 37.5 mg zinc (as $ZnSO_4$); 37.5 mg manganese (as manganese dioxide); 37.5 mg iron (as ferrous sulfate); 3.75 mg Cu (as copper sulfate pentahydrate); 0.83 mg iodine (as potassium iodide); and 0.23 mg selenium (as sodium selenite pentahydrate). [3] Provided per kg of complete diet: 15,000 IU of vitamin A, 3750 IU of vitamin D_3, 37.5 IU of vitamin E, 2.55 mg of vitamin K_3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B_6, 24 ug of vitamin B_{12}, 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin and 13.5 mg of Ca-pantothenate.

2.3. *Sample Collection and Laboratory Analysis*

Growth Measurements

At the end of days 7, 21, 35, and the overall feeding period, all birds (cage basis) were weighed and feed offered, and those that remained in the feeders were recorded to determine body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR = FI/BWG). Overall, mortality was also calculated.

2.4. Nutrient Retention

Seven days prior to excreta collection (day 7, 21, and 35) chromic oxide (0.5%), as an indigestible marker, was added to the chicks' diet to measure nutrient digestibility (ND). Immediately after mixing the marker, representative feed samples were collected in sterilized plastic bags from each treatment group and stored for further analysis. On days 7, 21, and 35, approximately 100 g of fresh excreta samples were randomly collected from 5 replicate cages/treatment using a stainless-steel collection tray and mixed evenly. Then, excreta samples were transported to the laboratory and stored at −20 °C to examine the nutrient retention, nitrogen (N), gross energy (GE), Ca, P, and ash of the dry matter (DM). The excreta specimens were placed in a hot air-drying convection oven at 105 °C for 32 h. Then, the samples were ground to pass the 1 mm sieve mesh. The DM and chromium absorption, GE, and N analysis were carried out following the detailed method of Sampath et al. [19]. The apparent nutrient digestibility (AND) was calculated using: AND (%) = 100 − [(Nutrient concentration in the excreta sample/nutrient concentration in the diet × chromium concentration in the diet/chromium concentration in the excreta sample × 100]. Ca and P retention were determined following the methods of AOAC [20]. Ash retention was determined with the following equation: ash % = ash weight (wg) (g)/dried wg (g) × 100 [21].

2.5. Cecal Score

The cecal specimens were scraped from each replicate cage using the collection tray and kept on blank paper. Later, cecal assessment (visually) was performed by two independent trainees following the Hu et al. [22] scoring criteria: Score 1: dry and firm cecal; Score 2: mostly dried cecal; Score 3: moist cecal; Score 4: wet and loose cecal; and Score 5: extreme wet cecal. The daily average was summed to calculate the mean score for each treatment period (0 to 7 days, 8 to 21 days, and 22 to 35 days).

2.6. Meat Quality

On day 35, 10 birds/treatment were randomly picked and sacrificed by intravenous injection of pentobarbital with cervical dislocation. Then, the breast meat, liver, spleen, abdominal fat, kidney, bursa of Fabricius, and gizzard were removed carefully and weighed to calculate relative organ weight (g/kg) = organ wg (g)/live BW (kg). Within 20 min, the breast meat was taken to the laboratory for further analysis. Meat quality traits, like breast muscle color, pH, cooking loss, water-holding capacity (WHC), and drip loss, were analyzed following the methods described by Sampath et al. [19].

2.7. Footpad Lesion Score

Following meat quality, the incidence and severity of footpad lesion infection (left and right toe) were measured at the end of the experiment using the scoring method: (1) Score 0, no lesions; (2) Score 1, lesions cover < 25% of the footpad; (3) Score 2, lesions in wide areas covering 25% to 50% of the footpad; Score 3, >50% lesion on the footpads. This scoring evaluation was performed on both feet by two independent observers with the above-mentioned broiler specimens, and the average score was used for statistical analyses.

2.8. Tibia Bone Mineralization

The tibia (left) was separated from 10 broilers/treatment (slaughtered) and soaked in hexane for 48 h to remove the fat. Then, they were dried at 100 °C to reach constant weight and ashed in a muffle furnace at 550 °C. The percentage of ash in the defatted tibia and the Ca and P content were measured according to AOAC [20].

2.9. Statistical Analysis

Experimental data (growth performance, nutrient retention, and meat quality) were analyzed via the general linear model procedure of SAS Institute 2004 in a completely randomized design. For growth performance, nutrient digestibility, and cecal score, the cage served as an experimental unit, whereas for the meat quality, the tibia bone ash and foot pad lesion score for individual birds served as the experimental unit. Treatment means were separated using Duncan's multiple range test, and the impact of dietary phytase was calculated using an orthogonal polynomial contrast test for linear and quadratic effects. The tibia bone statistics were analyzed using the Shapiro–Wilk test. The significance was set as $p < 0.05$ and trends were set to <0.10, respectively.

3. Result and Discussion

The hypothesis for this study was aimed to investigate whether enhancing the broilers' diets with various levels of phytase (ranging from 250 to 3000 U/kg of feed) could lead to improvements in their growth performance, nutrient utilization, Ca and P retention, meat quality, and foot pad score when compared to those fed standard PC and NC diets. Our anticipation has been partially confirmed by the present outcome in which broilers fed the NC diet supplemented with graded levels of phytase (250 u/kg to 3000 u/kg) had a linear improvement ($p < 0.05$) in BWG and FI during the starter phase and overall trial period. Also, during the overall trial period, the daily feed intake of the broilers tended ($p < 0.10$) to increase linearly with phytase supplementation in the NC diet. However, there was no significant difference observed in the growth performance during the grower and finisher stages; in particular, the FCR remained constant throughout the trial (Table 2). The current finding agreed with Onyango et al. [23] and Bahadoran et al. [24] who noted no improvements in the feed efficacy of the broilers fed dietary phytase. Yet, Dos Santos et al. [25] found significant improvement in the feed efficacy of broilers with phytase supplementation. The reason for the increased BWG observed in chickens fed phytase, which is associated with improved feed intake, might be due to the utilization of P from the phytate-mineral complex [26] or due to the overall utilization of nutrients. Previously, Bavaresco et al. [27] stated that the inclusion of 1000 U/kg had significantly increased the FCR in birds, which is not in agreement with the present findings. We supposed that the reason for this contradiction in the FCR responses in birds might be due to their genetics, housing, environmental conditions, sources of phytase, or processing methods.

Table 2. The effect of phytase supplementation on the growth performance of broiler.

Items	PC	NC	NC + Phytase (U/kg) in Diet					SEM [1]	PC vs. NC	*p*-Value		
			250	500	1000	1500	3000			Linear	Quadratic	Cubic
d 1 to 7												
BWG, g	153 [ab]	147 [b]	151 [ab]	149 [ab]	159 [a]	158 [ab]	160 [a]	4	0.188	0.011	0.206	0.162
FI, g	182	179	181	181	188	186	189	3	0.556	0.023	0.475	0.345
FCR	1.190	1.219	1.202	1.218	1.182	1.186	1.180	0.021	0.388	0.303	0.367	0.405
d 8 to 21												
BWG, g	647	626	633	627	654	651	655	10	0.203	0.173	0.116	0.111
FI, g	874	851	857	853	882	880	883	14	0.179	0.143	0.219	0.176
FCR	1.354	1.364	1.360	1.362	1.350	1.353	1.349	0.024	0.806	0.724	0.774	0.780
d 22 to 35												
BWG, g	977	954	960	956	984	981	985	19	0.431	0.308	0.359	0.347
FI, g	1819	1786	1793	1790	1827	1825	1831	26	0.379	0.285	0.330	0.344
FCR	1.865	1.876	1.874	1.875	1.862	1.863	1.861	0.032	0.788	0.761	0.786	0.782
Overall												
BWG, g	1778 [ab]	1727 [b]	1744 [ab]	1732 [b]	1798 [a]	1790 [ab]	1801 [a]	20	0.138	0.024	0.166	0.161
FI, g	2876	2816	2832	2824	2896	2892	2903	31	0.188	0.078	0.154	0.140
FCR	1.618	1.633	1.625	1.632	1.613	1.617	1.613	0.018	0.627	0.547	0.618	0.629
Mortality (%)	7.33	8.00	7.33	8.00	5.33	6.00	5.33	-		-	-	-

Abbreviation: positive control (PC), negative control (NC), NC + phytase—NC supplemented with graded level of phytase. [1] Standard error of means. [a,b] means in the same row with different superscript denotes significant $p < 0.05$, and the trend was set to <0.10.

The nutrient utilization of broilers showed neither an increase nor a decrease with the PC and NC diet until d 35 but when the NC diet was supplemented with a graded dose of phytase, it showed a linear increase ($p < 0.05$) in the DM, GE, Ca, ash, and P digestibility, and a tendency to increase ($p < 0.10$) the N digestibility at the end of the starter phase. Unlike the starter phase, broilers fed the PC diet had significantly increased ($p < 0.05$) ash and Ca digestibility at the end of the grower and finisher phase compared to those fed the NC diet. In addition, broilers fed the NC diet supplemented with a graded level of phytase showed a linear increase ($p < 0.05$) in the ash, Ca, and P digestibility at the end of d 21 and 35. Furthermore, the nutrient digestibility of GE linearly increased at the end of d 35 (Table 3). The current finding was correlated with Silversides et al. [28] who noted an increased Ca digestibility in broilers when phytase was added to their diets that contained reduced non-phytase P and Ca concentrations. Previously, Ravindran et al. [29] reported that dietary phytase had positive effects on DM digestibility by releasing certain organic nutrients. A study by Cowieson et al. [17] demonstrated that supplemental phytase beyond 1200 FTU/kg had improved P retention and N and P digestibility in broilers compared to lower doses, which agrees with the current finding. The interaction of phytic acid with proteins forms phytate-protein complexes and is insoluble and less susceptible to attack by proteolytic enzymes than incomplete proteins [30]. It should be noted that the improvements seen here were obtained despite the fact that diets were formulated to be nutritionally identical to PC, except for the available P. Hence, if the NC diet had been designed to account for improvements in the nutritional status caused by phytase supplementation, it is reasonable to presume that improvements in nutrient utilization and growth performance would have been better with the inclusion of phytase concentration at above 500 U/kg.

Table 3. The effect of phytase supplementation on the nutrient utilization in broiler.

Items, %	PC	NC	NC + Phytase (U/kg) in Diet					SEM [1]	PC vs. NC	*p*-Value		
			250	500	1000	1500	3000			Linear	Quadratic	Cubic
d 7												
DM	70.76	69.01	70.62	70.58	72.39	71.80	72.54	1.18	0.333	0.054	0.646	0.357
N	68.83	67.22	68.53	68.42	69.86	69.84	70.50	1.17	0.372	0.072	0.479	0.438
GE	69.66	68.17	69.59	69.53	70.88	70.77	71.56	1.17	0.355	0.059	0.553	0.499
Ca	41.69 ^{a}	41.75 ^{b}	42.27 ^{a}	42.43 ^{a}	42.78 ^{a}	42.88 ^{a}	43.09 ^{a}	0.94	0.197	0.004	0.911	0.166
P	38.52 ^{a}	38.68 ^{b}	39.21 ^{a}	39.34 ^{a}	39.84 ^{a}	39.99 ^{a}	40.07 ^{a}	0.98	0.265	0.009	0.868	0.155
Ash	41.32 ^{a}	41.63 ^{b}	42.17 ^{a}	42.46 ^{a}	42.87 ^{a}	42.90 ^{a}	43.06 ^{a}	0.99	0.399	0.004	0.745	0.111
d 21												
DM	77.32 ^{a}	72.19 ^{b}	77.41 ^{a}	78.10 ^{a}	77.75 ^{a}	77.82 ^{a}	77.10 ^{a}	0.67	0.094	0.151	0.658	0.393
N	75.10 ^{ab}	71.03 ^{b}	75.98 a	75.46 ^{ab}	75.13 ^{ab}	75.86 ^{a}	75.89 ^{a}	0.71	0.178	0.156	0.988	0.393
GE	76.29 ^{ab}	72.35 ^{c}	76.40 ^{ab}	78.92 ^{a}	76.39 ^{ab}	76.83 ^{ab}	76.73 ^{ab}	0.68	0.601	0.108	0.414	0.171
Ca	43.05 ^{a}	37.80 ^{b}	41.23 ^{a}	42.24 ^{a}	42.63 ^{a}	42.45 ^{a}	43.5 ^{a}	0.70	0.020	0.051	0.190	0.107
P	39.96 ^{a}	35.77 ^{b}	35.99 ^{b}	36.79 ^{ab}	36.94 ^{ab}	37.88 ^{ab}	38.94 ^{ab}	0.79	0.187	0.006	0.715	0.131
Ash	41.32 ^{ab}	38.87 ^{b}	42.44 ^{a}	43.01 ^{a}	42.54 ^{a}	42.92 ^{a}	42.74 ^{a}	0.80	0.012	0.068	0.487	0.287
d 35												
DM	75.35 ^{a}	70.03 ^{b}	75.44 ^{a}	76.12 ^{a}	75.54 ^{a}	75.55 ^{a}	75.50 ^{a}	0.69	0.438	0.129	0.808	0.122
N	72.00	69.10	72.77	73.03	72.84	72.75	70.48	0.69	0.330	0.185	0.592	0.309
GE	75.20 ^{ab}	71.91 ^{c}	75.35 ^{ab}	76.90 ^{a}	75.85 ^{a}	76.54 ^{ab}	76.28 ^{ab}	0.66	0.688	0.024	0.701	0.124
Ca	42.80 ^{a}	37.78 ^{b}	41.23 ^{a}	43.28 ^{a}	42.48 ^{a}	42.04 ^{a}	42.63 ^{a}	1.17	0.041	0.031	0.665	0.185
P	39.45 ^{a}	36.05 ^{b}	39.84 ^{a}	40.12 ^{a}	39.81 ^{a}	39.15 ^{a}	40.47 ^{a}	1.27	0.078	0.008	0.980	0.396
Ash	41.57 ^{a}	36.59 ^{b}	41.03 ^{a}	42.05 ^{a}	41.53 ^{a}	41.99 ^{a}	41.73 ^{a}	1.25	0.025	0.044	0.953	0.555

Abbreviation: positive control (PC), negative control (NC), NC + phytase—NC supplemented with graded level of phytase. DM-dry matter, N—nitrogen, GE—gross energy, Ca—calcium, P—phosphorus. [1] Standard error of means ^{a,b,c} means in the same row with different superscript denotes significant $p < 0.05$ and trend was set to <0.10.

Chicken feet are edible in the Asian market; thus, the quality for export of the product always depends on the absence of lesions. Such a footpad infection is common in backyard flocks and in the poultry industry. It is a gateway for pathogens, such as *Staphylococcus* or *E. coli*, which can aggravate the injury and cause systemic infections that lead to limited access to feed and water that, in turn, affects productivity. Previously, Keutgen et al. [31] reported that the changes in poultry foot pads are related to the housing system, particularly hyperplasia occurs due to mechanical stimulation when perches and hens are used in the litter and is also observed as an alteration with cage-reared hens' toe and claw regions. A study by McIlroy [32] stated that farms with high population densities and complex management practices had a higher incidence of footpad dermatitis. Similarly, Berg [33] noted that the stocking density affects the relative humidity, causes poor ventilation, and reduces bird activity. On the other hand, Leonie [34] stated that higher body weight, diet, leaky waterers, and poor ventilation have indirectly affected the occurrence of lesions. Even pathological, poor husbandry practice, and diet may affect footpad infection in broilers. In the current study, there was no sign of diarrhea (Figure 1) which in turn causes footpad dermatitis (Figure 2), and we supposed that the healthy foot pad condition of the broilers could be due to the effect of dietary phytase, which enhanced nutrient utilization by breaking down phytic acid, leading to improved digestion that resulted with reduced wet litters [35] or due to proper sanitary conditions.

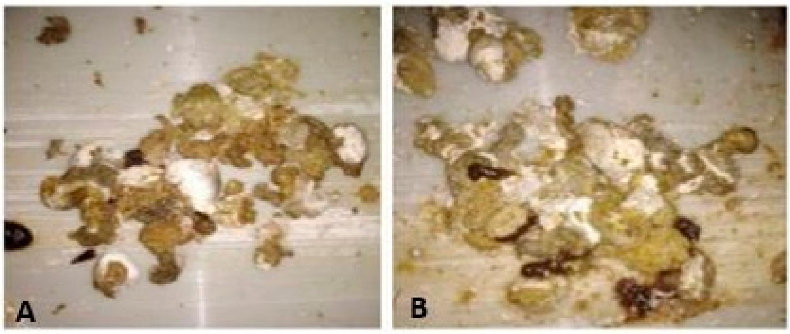

Figure 1. The effect of phytase supplementation on broiler cecal score. Broilers fed neither PC nor NC, and NC diet supplemented with graded levels of phytase (250 U/kg 3000 U/kg) showed no difference (table was not included) in their cecal score until d 35. (**A**) cecal score 1 and (**B**) cecal score 3.

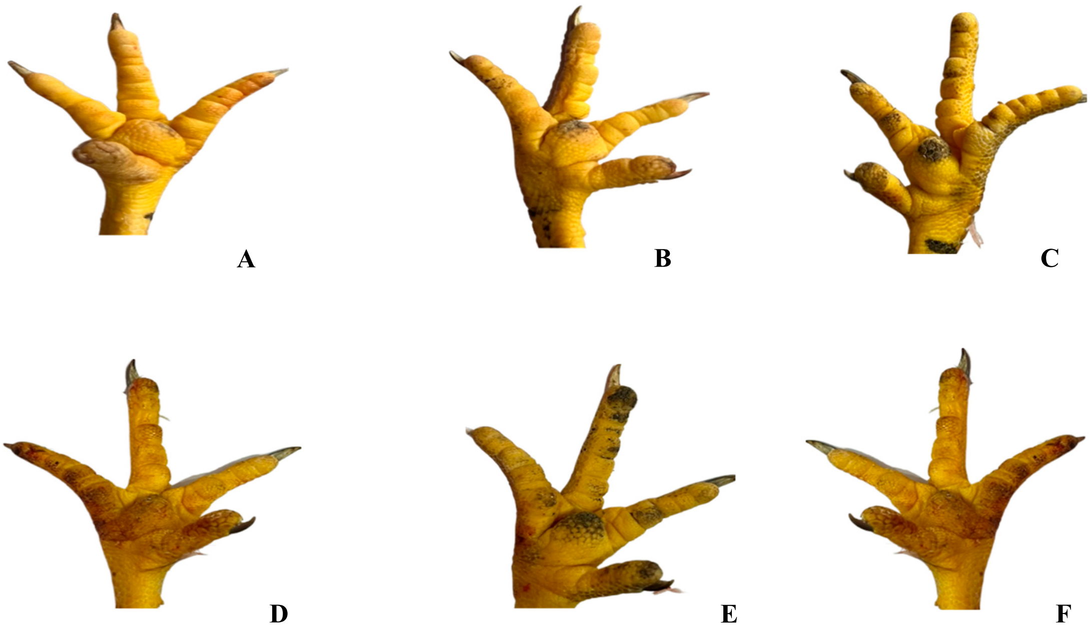

Figure 2. The effect of phytase supplement on broiler foot pad lesion score. Broilers fed neither PC (**A**) nor NC (**B**) and NC diet supplemented with graded level of phytase ((**C**–**F**); 250 U/kg to 3000 U/kg) showed no difference (table was not added) on their foot pad lesions until d 35.

Calcium and phosphorous play a substantial role in bone mineralization. P, a major component of the avian skeleton, plays a vital role in bone mineralization and in soft tissue growth [36]. Calcium helps bind phytate molecules and reduces the absorption of P in the intestine [37]. A study by Bavaresco et al. [27] noted a reduction in growth performance and bone quality when broilers were fed the NC diet, which contradicts the present study in which broilers fed neither the PC nor NC diet revealed no impact. Previously, Mitchell and Edward [38] stated that bone ash is a reliable technique to determine the animal's P needs. Phytate, being a strong acid, can form various salts with essential minerals and reduce their solubilities to increase the P absorption [39]. In the present study, broiler-fed NC diet supplemented with graded levels of phytase showed significantly increased (<0.0001) bone (ash, Ca, and P) mineralization at the end of d 35 (Figure 3) in agreement with Singh et al. [40] who found increased ash content of the tibia and Ca and P absorption in the broiler-fed diet supplemented with phytase. Moreover, Guo et al. [41] and Rousseau et al. [42] addressed the positive influence on the bone mineralization of the broiler chicks fed a low P diet supplemented with phytase. However, Lan et al. [43] noted that the addition of low phytases reduced tibia ash, Ca, and P content in the broilers. Hence it is reasonable to presume that improvements in tibia mineralization could be due to enhanced feed intake and nutrient utilization.

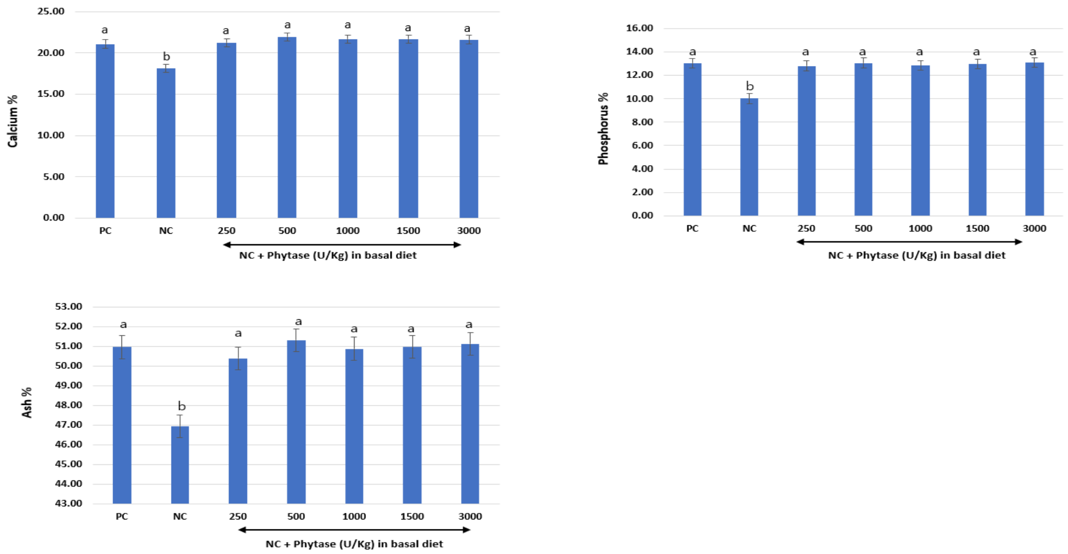

Figure 3. The effect of phytase supplement on tibia bone ash, Ca, and P mineralization in broiler on d 35. a, b in standard error bars denotes significant ($p < 0.05$).

The effect of dietary phytase on broiler meat quality is shown in Table 4. Broilers fed the NC diet supplemented with graded levels of phytase showed a linear increase ($p < 0.05$) in the gizzard weight, breast muscle color of lightness, redness, pH, and decreased cooking loss and drip loss (Table 4). The present result was not consistent with Singh et al. [40] and Broch et al. [44] who found no effect on the carcass yield of broilers in response to phytase supplementation. A study by Ristc and Dame [45] insists that good broiler meat must be in the 5.5–6.2 pH range after 45 min postmortem, which is constant with the present findings in which broilers fed the phytase diet showed a pH range above 5.5. We supposed that the breast muscle color of yellowness was found to negatively correlate with pH, while redness and lightness had a positive correlation. Concurrently, Nalunga et al. [46] stated that pH plays an important role in altering muscle meat and attributes that change meat color, texture, and shelf life; it can even influence cooking loss, which accounts for the potential loss of nutritional value of meat during cooking [47]. A low cooking loss of <35% will make good quality meat as they release fewer nutrients from meat during cooking [46]. According to this statement, we presume that adding graded levels of phytase to the broiler diet would be beneficial in reducing cooking loss, i.e., <35%, and enhance the shelf life and the quality of the breast meat for human consumption.

Table 4. The effect of phytase supplementation on organ weight and meat quality in broiler.

Items	PC	NC	NC + Phytase (U/kg) in Diet					SEM [1]	PC vs. NC	*p*-Value		
			250	500	1000	1500	3000			Linear	Quadratic	Cubic
Organ weight, % at d 35												
Breast muscle	10.13	9.98	10.07	10.00	10.36	10.22	10.43	0.20	0.542	0.122	0.393	0.630
Liver	2.55	2.52	2.54	2.53	2.57	2.56	2.58	0.04	0.726	0.394	0.652	0.676
Spleen	0.11	0.11	0.13	0.12	0.11	0.12	0.12	0.01	0.487	0.927	0.586	0.693
Abdominal fat	1.20	1.22	1.21	1.22	1.17	1.18	1.16	0.03	0.646	0.166	0.438	0.617
kidney	0.82	0.83	0.84	0.79	0.80	0.80	0.81	0.02	0.887	0.223	0.706	0.394
Bursa of Fabricius	0.17	0.17	0.20	0.22	0.19	0.18	0.20	0.02	0.857	0.217	0.129	0.381
Gizzard	1.56	1.52	1.62	1.55	1.60	1.69	1.69	0.06	0.653	0.019	0.454	0.897
Color												
Lightness (L)	57.07	57.11	54.55	55.04	56.21	56.59	57.42	0.88	0.977	0.016	0.718	0.712
Redness (a)	12.18	10.28	11.75	10.46	11.60	12.28	10.82	0.59	0.107	0.032	0.506	0.808
Yellowness (b)	16.83	16.73	14.78	14.60	15.49	15.50	16.65	0.73	0.926	0.561	0.109	0.853
pH value	5.56	5.44	5.51	5.47	5.62	5.58	5.66	0.06	0.290	0.051	0.172	0.352
Cooking loss, %	18.44	19.42	18.73	19.30	17.56	17.81	17.37	0.66	0.140	0.032	0.241	0.285
WHC, %	52.34	50.77	51.82	51.17	54.88	53.07	55.30	2.01	0.632	0.125	0.471	0.630
Drip loss, %												
d 1	1.30	1.39	1.33	1.32	1.28	1.24	1.34	0.09	0.541	0.646	0.965	0.324
d 3	3.63	3.79	3.69	3.62	3.60	3.56	3.74	0.13	0.423	0.766	0.675	0.186
d 5	6.89	7.02	6.61	6.00	6.17	5.96	6.83	0.34	0.794	0.034	0.186	0.169
d 7	8.11	8.14	7.80	7.23	7.59	7.24	8.08	0.34	0.948	0.068	0.253	0.200

Abbreviation: Positive control (PC), Negative control (NC), NC + Phytase—NC supplemented with graded level of phytase. [1] Standard error of means.

4. Conclusions

The inclusion of phytase (250–1000 FTU/kg) to the reduced nutrient corn–soybean basal diet (NC- available P-0.33%, 0.29%, 0.26%) reveals conclusive results on growth performance and better nutrient utilization. In addition, tibia bone mineralization, shelf life, and pH of broiler meat were increased. The standard phytase (500 FTU/kg) was able to compensate for the P deficiency and promote performance and bone mineralization; however, an elevated (1000 FTU/kg) level of phytase in the NC diet showed a higher response. Thus, we suggest that incorporating the exogenous enzyme phytase (Ronozyme HiPhos-L) into the broiler fed in excess of 1000 units (U)/kg would be beneficial to boost broiler productivity.

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Institutional Review Board Statement: The animal care procedures implemented in this study were approved (Approval No. DK-1-2210) by the Institutional Animal Care and Use Committee (IACUC) of Dankook University (Republic of Korea).

Data Availability Statement: Data can be available requiring via email to In Ho Kim, Inhokim@dankook.ac.kr.

Conflicts of Interest: The authors did not report any conflict of interest.

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