

Effects of phytase supplementation on bone development and growth in broilers fed nutritionally adequate and calcium, phosphorus, and sodium deficient diets

Fitase sobre o desenvolvimento e crescimento ósseo de frangos de corte alimentados com dietas nutricionalmente adequadas e deficientes em cálcio, fósforo e sódio

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Highlights

Phytase supplementation improves bone quality in broilers.

Dietary deficiencies of calcium, phosphorus, and sodium impair bone development.

Phytase superdosing restores bone quality in mineral-deficient diets.

Abstract

This study evaluated the effects of phytase supplementation on bone quality in broiler chickens fed corn-, soybean meal-, and soybean oil-based diets deficient in phosphorus (P), calcium (Ca) and sodium (Na). A total of 1,548 male broiler chicks were assigned to a completely randomized design comprising four dietary treatments, nine replicates per treatment, and 43 birds per replicate. Treatments consisted of a positive control (PC) formulated to meet recommended nutrient requirements, a negative control (NC) diet with reductions of 0.15% phosphorus, 0.16% calcium, and 0.03% sodium, and the NC diet supplemented with either 500 or 10000 FTU kg⁻¹ phytase. Phytase supplementation significantly

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improved ($p < 0.05$) tibial bone mineral density at 21 and 47 days compared with the unsupplemented NC diet. At 21 days, broilers fed the NC diet exhibited the lowest ($p < 0.05$) bone-to-cartilage ratio. Phytase supplementation also increased ($p < 0.05$) bone breaking strength throughout the experimental period, restoring values comparable to those observed in birds fed the nutritionally adequate diet. Supplementation with 10000 FTU kg^{-1} phytase reduced femoral bone elasticity at 21 and 47 days while increasing femur length at 7 and 21 days relative to the NC treatment. Overall, phytase supplementation effectively restored bone development in broilers fed diets deficient in P, Ca, and Na.

Key words: Bone mineral density. Bone quality. Bone breaking strength. Phytase superdosing.

Resumo

O objetivo do estudo foi avaliar a qualidade óssea de frangos de corte submetidos a dietas formuladas à base de milho, farelo de soja e óleo de soja, com redução nos níveis de P, Ca e Na suplementadas com 500 FTU kg^{-1} ou 1000 FTU kg^{-1} de fitase. Foram alojados 1548 pintos de corte, machos, distribuídos em um delineamento experimental inteiramente casualizado, com quatro dietas, nove repetições, com 43 aves por unidade experimental. As dietas experimentais foram compostas por: Controle Positivo com níveis nutricionais recomendados para cada fase de criação, sem adição de enzima; Controle Negativo (CN), com níveis nutricionais recomendados para cada fase de criação e com redução de fósforo (0,15%), cálcio (0,16%) e sódio (0,03%) sem adição de enzima; CN com adição de enzima fitase 500 FTU kg^{-1} de ração; CN com adição de enzima fitase 1000 FTU kg^{-1} de ração. A suplementação com fitase (500 e 1000 FTU kg^{-1}) em dietas deficientes resultou em melhores índices ($p < 0.05$) de densitometria óssea aos 21 e 47 dias, em comparação à dieta deficiente. A menor ($p < 0.05$) relação osso:cartilagem foi observada nas tíbias dos frangos de corte que receberam a dieta deficiente aos 21 dias de idade. As aves que consumiram dietas suplementadas com fitase apresentaram maior ($p < 0.05$) resistência óssea à quebra, em relação ao controle negativo em todo o período do experimento, porém semelhantes à observada para as aves que receberam dietas com níveis adequados. As aves suplementadas com superdosing de fitase apresentaram redução ($p < 0.05$) da elasticidade óssea de fêmur aos 21 e 47 dias, em comparação as aves do controle negativo. O consumo de dietas suplementadas com superdosing de fitase, em comparação com o controle negativo resultou em maior ($p < 0.05$) comprimento de fêmur aos 7 e 21 dias. A suplementação com fitase foi eficaz para o reestabelecimento dos parâmetros ósseos das aves alimentadas com dietas deficientes.

Palavras-chave: Densitometria óssea. Qualidade óssea. Resistência óssea. Superdosing de fitase.

Introduction

Phosphorus (P) is an essential macromineral involved in numerous biochemical processes and plays a fundamental role in the development and maintenance of the skeletal system in broilers (Tizziani et al., 2016; Li et al., 2017).

Given its nutritional importance, one of the most extensively studied aspects of P nutrition is the digestibility of phytate, the primary storage form of P in plant-based feed ingredients commonly used in poultry diets (Baloch et al., 2021; Kiarie et al., 2016; Cowieson et al., 2017). However, broilers have a limited ability to digest the phytate naturally present in plant-derived feed ingredients (Sommerfeld et al., 2018; Ingelmann et al., 2019; Lee & Kong, 2024). Consequently, the low digestibility of phytate-bound P reduces the availability of phosphorus, minerals, and proteins, increases P excretion into the environment, and raises feed costs (Cardoso et al., 2018).

In the broiler gastrointestinal tract, phytic acid (the free form of IP6), a myo-inositol molecule phosphorylated in all six hydroxyl groups, readily binds minerals and proteins through ionic interactions in aqueous media, thereby reducing nutrient availability. To improve P utilization and meet the nutritional requirements of broilers, phytase is routinely included in poultry diets. Phytase enzymes are phosphatases that catalyze the hydrolysis of phytate, releasing P in a form that can be absorbed by the birds (Sousa et al., 2015; Dersjant-Li et al., 2015; Martínez-Vallespín et al., 2022).

Dietary phytase supplementation enhances mineral absorption, reduces P excretion (An et al., 2020; Niño-Gómez et al., 2017; Selim et al., 2022), improves energy utilization and amino acid digestibility, and positively influences bone development and quality in broilers (An et al., 2020). However, the effects of phytase superdosing are not fully understood, and published findings are not always consistent, highlighting the need for further investigation. Much of the additional benefit attributed to phytase superdosing has been associated with the release of myoinositol during phytate hydrolysis, which is subsequently absorbed and participates in several biological functions in broilers (Fernandes et al., 2019; Martínez-Vallespín et al., 2022; Delezie et al., 2015).

Therefore, this study evaluated the effects of phytase superdosing on bone development and growth in broilers fed nutritionally adequate diets or diets deficient in calcium (Ca), phosphorus (P), and sodium (Na).

Material and Methods

The experiment was conducted at the Experimental Aviary of the Federal University of Paraná (UFPR), Palotina Campus, Brazil. All experimental procedures were approved by the Research Ethics Committee of the Federal University of Paraná, Palotina Campus, Brazil (Protocol No. 24/2017).

A total of 1,548 male Cobb 500® broiler chicks, obtained from breeder hens approximately 40 weeks of age, were allocated to a completely randomized design

comprising four dietary treatments, nine replicates per treatment, and 43 birds per experimental unit (13.33 chicks/m²). The four dietary treatments were as follows:

Positive Control (PC): diet formulated to meet the recommended nutritional requirements for each rearing phase without phytase supplementation.

Negative Control (NC): diet formulated to meet the recommended nutritional requirements for each rearing phase, except for reduced P, Ca, and Na, without phytase supplementation.

NC supplemented with 500 FTU kg⁻¹ phytase.

NC supplemented with 1,000 FTU kg⁻¹ phytase.

In the negative control diets, P, Ca, and Na concentrations were reduced according to the nutritional matrix provided by the phytase manufacturer. The enzyme used was 6-phytase, produced by fermentation of *Escherichia coli* modified with *Pichia pastoris* and marketed as Precizyon FIT TS 5000 by Quimtia® S.A., with a declared activity of 5,000 FTU kg⁻¹ (Quimtia S.A., 2017).

Experimental diets were formulated using corn, soybean meal, soybean oil, and a basal premix supplied by Quimtia® S.A. according to the manufacturer's specifications, following the nutritional recommendations of Rostagno et al. (2017) for low-performance broilers. Separate premixes were prepared for each treatment during the starter (1–18 days), grower (19–35), and finisher (36–47 days) phases (Tables 1, 2, and 3).

Table 1**Composition and nutritional levels of the diet in the initial phase (1 to 18 days)**

Ingredients (kg t ⁻¹)	Positive Control	Negative Control	Phytase 500 FTU kg ⁻¹	Phytase 1000 FTU kg ⁻¹
Corn	626.82	626.82	626.82	626.82
Soybean meal	317.00	317.00	317.00	317.00
Soybean oil	13.00	13.00	13.00	13.00
Limestone (34% Ca)	12.10	13.20	13.20	13.20
Dicalcium phosphate (18.5% P)	13.74	5.50	5.50	5.50
Sodium chloride	1.89	1.10	1.10	1.10
L-Lysine	2.24	2.24	2.24	2.24
DL-Methionine 99%	2.69	2.69	2.69	2.69
L-Threonine 98.5%	0.85	0.85	0.85	0.85
L-Valine 96.5%	0.89	0.89	0.89	0.89
Choline chloride (60%)	0.75	0.75	0.75	0.75
Sodium bicarbonate	4.00	4.00	4.00	4.00
Vitamin-mineral premix ¹	2.00	2.00	2.00	2.00
Anticoccidial	0.625	0.625	0.625	0.625
Phytase ²	-	-	0.100	0.200
Inert3	1.405	9.335	9.235	9.135
Calculated nutritional composition				
Metabolizable energy (kcal kg ⁻¹)	2983.97	2983.97	2983.97	2983.97
Crude protein (g kg ⁻¹)	200.01	200.01	200.01	200.01
Ether extract (g kg ⁻¹)	41.5	41.5	41.5	41.5
Crude fiber (g kg ⁻¹)	30.1	30.1	30.1	30.1
Calcium (g kg ⁻¹)	8.6	7.0	8.6	10.2
Available phosphorus (g kg ⁻¹)	6.3	4.8	4.8	4.8
Ash (g kg ⁻¹)	53.4	53.8	53.7	53.6
Digestible lysine (g kg ⁻¹)	11.4	11.4	11.4	11.4
Digestible methionine (g kg ⁻¹)	5.5	5.5	5.5	5.5
Digestible methionine + cystine (g kg ⁻¹)	8.2	8.2	8.2	8.2
Digestible tryptophan (g kg ⁻¹)	2.2	2.2	2.2	2.2
Digestible threonine (g kg ⁻¹)	7.4	7.4	7.4	7.4
Digestible arginine (g kg ⁻¹)	12.1	12.1	12.1	12.1
Digestible leucine (g kg ⁻¹)	15.3	15.3	15.3	15.3
Digestible isoleucine (g kg ⁻¹)	7.3	7.3	7.3	7.3
Digestible valine (g kg ⁻¹)	8.8	8.8	8.8	8.8

¹Initial vitamin-mineral premix: Vitamin A (9,500 IU kg⁻¹), Vitamin D3 (1,900 IU kg⁻¹), Vitamin E (30 mg kg⁻¹), Vitamin K3 (2 mg kg⁻¹), Vitamin B1 (2.51 mg kg⁻¹), Vitamin B2 (5.86 mg kg⁻¹), Vitamin B6 (3 mg kg⁻¹), Vitamin B12 (15 mg kg⁻¹), folic acid (1 mg kg⁻¹), pantothenic acid (12 mg kg⁻¹), niacin (40 mg kg⁻¹), biotin (0.1 mg kg⁻¹), choline (1,652.06 mg kg⁻¹), copper (8 mg kg⁻¹), iron (60 mg kg⁻¹), iodine (1 mg kg⁻¹), manganese (79.80 mg kg⁻¹), selenium (0.3 mg kg⁻¹), zinc (79 mg kg⁻¹).

²Each gram of phytase (Precizyon Fit TS) contained an activity of 5000 FTU.

³Kaolin was used as an inert ingredient to maintain the dietary formulation.

Table 2
Composition and nutritional levels of the diet in the growth phase (19 to 35 days)

Ingredients (kg t ⁻¹)	Positive Control	Negative Control	Phytase 500 FTU kg ⁻¹	Phytase 1000 FTU kg ⁻¹
Corn	650.12	650.12	650.12	650.12
Soybean meal	294.00	294.00	294.00	294.00
Soybean oil	18.20	18.20	18.20	18.20
Limestone (34% Ca)	10.50	11.50	11.50	11.50
Dicalcium phosphate (18.5% P)	11.70	3.50	3.50	3.50
Sodium chloride	1.88	1.20	1.20	1.20
L-Lysine	1.70	1.70	1.70	1.70
DL-Methionine (99%)	2.27	2.27	2.27	2.27
L-Threonine (98.5%)	0.48	0.48	0.48	0.48
L-Valine (96.5%)	0.56	0.56	0.56	0.56
Choline chloride (60%)	0.59	0.59	0.59	0.59
Sodium bicarbonate	3.66	3.66	3.66	3.66
Vitamin-mineral premix ¹	2.00	2.00	2.00	2.00
Anticoccidial	0.550	0.550	0.550	0.550
Phytase ²	-	-	0.100	0.200
Inert ³	1.79	9.67	9.57	9.47
Calculated nutritional composition				
Metabolizable energy (kcal kg ⁻¹)	3050.73	3050.73	3050.73	3050.73
Crude protein (g kg ⁻¹)	190.1	190.1	190.1	190.1
Ether extract (g kg ⁻¹)	47.1	47.1	47.1	47.1
Crude fiber (g kg ⁻¹)	29.1	29.1	29.1	29.1
Calcium (g kg ⁻¹)	7.5	5.9	7.5	9.1
Available phosphorus (g kg ⁻¹)	5.8	4.3	4.3	4.3
Ash (g kg ⁻¹)	49.2	49.6	49.5	49.4
Digestible lysine (g kg ⁻¹)	10.4	10.4	10.4	10.4
Digestible methionine (g kg ⁻¹)	5.0	5.0	5.0	5.0
Digestible methionine + cystine (g kg ⁻¹)	7.6	7.6	7.6	7.6
Digestible tryptophan (g kg ⁻¹)	2.0	2.0	2.0	2.0
Digestible threonine (g kg ⁻¹)	6.8	6.8	6.8	6.8
Digestible arginine (g kg ⁻¹)	11.4	11.4	11.4	11.4
Digestible leucine (g kg ⁻¹)	14.8	14.8	14.8	14.8
Digestible isoleucine (g kg ⁻¹)	6.9	6.9	6.9	6.9
Digestible valine (g kg ⁻¹)	8.1	8.1	8.1	8.1

¹Growth vitamin-mineral premix: Vitamin A (9,500 IU kg⁻¹), Vitamin D3 (1,900 IU kg⁻¹), Vitamin E (30 mg kg⁻¹), Vitamin K3 (2 mg kg⁻¹), Vitamin B1 (2.51 mg kg⁻¹), Vitamin B2 (5.86 mg kg⁻¹), Vitamin B6 (3 mg kg⁻¹), Vitamin B12 (15 mg kg⁻¹), folic acid (1 mg kg⁻¹), pantothenic acid (12 mg kg⁻¹), niacin (40 mg kg⁻¹), biotin (0.1 mg kg⁻¹), choline (1,652.06 mg kg⁻¹), copper (8 mg kg⁻¹), iron (60 mg kg⁻¹), iodine (1 mg kg⁻¹), manganese (79.80 mg kg⁻¹), selenium (0.3 mg kg⁻¹), zinc (79 mg kg⁻¹).

²Each gram of phytase (Precizyon Fit TS) contained an activity of 5000 FTU.

³Kaolin was used as an inert ingredient to maintain the dietary formulation.

Table 3

Composition and nutritional levels of the diet in the finisher phase (36 to 47 days)

Ingredients (kg t ⁻¹)	Positive Control	Negative Control	Phytase 500 FTU kg ⁻¹	Phytase 1000 FTU kg ⁻¹
Corn	688.10	688.10	688.10	688.10
Soybean meal	261.50	261.50	261.50	261.50
Soybean oil	17.50	17.50	17.50	17.50
Calcitic Limestone (34% Ca)	9.68	10.80	10.80	10.80
Dicalcium Phosphate (18.5% P)	9.14	1.00	1.00	1.00
Sodium chloride	1.65	1.00	1.00	1.00
L-Lysine	1.80	1.80	1.80	1.80
DL-Methionine (99%)	2.01	2.01	2.01	2.01
L-Threonine (98.5%)	0.42	0.42	0.42	0.42
L-Valine (96.5%)	0.49	0.49	0.49	0.49
Choline chloride (60%)	0.54	0.54	0.54	0.54
Sodium bicarbonate	3.65	3.65	3.65	3.65
Vitamin-mineral premix ¹	1.80	1.80	1.80	1.80
Phytase ²	-	-	0.100	0.200
Inert ³	1.72	9.39	9.29	9.19
Calculated nutritional composition				
Metabolizable energy (kcal kg ⁻¹)	3101.15	3101.15	3101.15	3101.15
Crude protein (g kg ⁻¹)	178.1	178.1	178.1	178.1
Ether extract (g kg ⁻¹)	47.2	47.2	47.2	47.2
Crude fiber (g kg ⁻¹)	27.8	27.8	27.8	27.8
Calcium (g kg ⁻¹)	6.5	4.9	6.5	8.1
Available phosphorus (g kg ⁻¹)	5.2	3.7	3.7	3.7
Ash (g kg ⁻¹)	44.1	44.5	44.4	44.3
Digestible lysine (g kg ⁻¹)	9.7	9.7	9.7	9.7
Digestible methionine (g kg ⁻¹)	4.6	4.6	4.6	4.6
Digestible methionine + cystine (g kg ⁻¹)	7.1	7.1	7.1	7.1
Digestible tryptophan (g kg ⁻¹)	1.9	1.9	1.9	1.9
Digestible threonine (g kg ⁻¹)	6.3	6.3	6.3	6.3
Digestible arginine (g kg ⁻¹)	10.5	10.5	10.5	10.5
Digestible leucine (g kg ⁻¹)	14.1	14.1	14.1	14.1
Digestible isoleucine (g kg ⁻¹)	6.4	6.4	6.4	6.4
Digestible valine (g kg ⁻¹)	7.6	7.6	7.6	7.6

¹Finisher vitamin-mineral premix: Vitamin A (7,600 IU kg⁻¹), Vitamin D3 (1,520 IU kg⁻¹), Vitamin E (24 mg kg⁻¹), Vitamin K3 (1.6 mg kg⁻¹), Vitamin B1 (2 mg kg⁻¹), Vitamin B2 (4.68 mg kg⁻¹), Vitamin B6 (2.4 mg kg⁻¹), Vitamin B12 (12 mg kg⁻¹), folic acid (0.8 mg kg⁻¹), pantothenic acid (9.6 mg kg⁻¹), niacin (32 mg kg⁻¹), biotin (0.08 mg kg⁻¹), choline (1,403.37 mg kg⁻¹), copper (8 mg kg⁻¹), iron (60 mg kg⁻¹), iodine (1 mg kg⁻¹), manganese (79.80 mg kg⁻¹), selenium (0.3 mg kg⁻¹), zinc (79 mg kg⁻¹).

²Each gram of phytase (Precizyon Fit TS) contained an activity of 5000 FTU.

³Kaolin was used as an inert ingredient to maintain the dietary formulation.

Broilers had ad libitum access to feed and water through tubular feeders and nipple drinkers, respectively. Ambient temperature was maintained within the birds' thermal comfort zone using infrared heat lamps, fans, exhaust systems, and evaporative cooling pads regulated by an automated system. Birds were exposed to continuous lighting (24 h light) until 14 days of age because the infrared heat lamps required for brooding remained on continuously during this period. Thereafter, the lighting program consisted of a 16-h light:8-h dark photoperiod from 15 to 21 days of age and a 14-h light:10-h dark photoperiod until the end of the experiment. Wood shavings were used as litter, and all birds were vaccinated against Marek's disease, Gumboro disease, and infectious bronchitis at the hatchery.

At 7, 21, and 47 days of age, two broilers per experimental unit (18 birds per treatment) were euthanized for bone analysis. Following the removal of all adhering soft tissue from the legs and wings, the tibia, femur, and humerus were weighed, and bone length and diameter were measured using a digital caliper (mm). The Seedor index (Seedor et al., 1991) was calculated as the ratio of bone weight (mg) to bone length (mm).

Bone mineral density was determined by radiographic bone densitometry. The tibiae were positioned in the anteroposterior projection on photographic film and radiographed using a PROCION® Ion 70x Mobile Column X-ray unit with a focal spot of 0.8 x 0.8 mm and an exposure time of 0.3 s. The voltage, current, and exposure settings were selected based on preliminary analyses. Radiographs were processed automatically, and bone mineral density was quantified using the histogram tool in Adobe Photoshop 8.0 software. A 10-step aluminum

wedge with 1-mm increments served as the radiographic reference. Measurements were collected from six points within the central region of each radiographic image and from each step of the aluminum scale. Gray-scale values were converted to equivalent aluminum thickness (mmAL), to serve as a proxy for bone mineral density, where higher mmAL values indicated greater bone radiopacity and bone mineral density.

The tibiae, femora, and humeri were subsequently subjected to a three-point bending test using a Brookfield CT3 universal testing machine equipped with a 500-kgf load cell and operated at a crosshead speed of 10 mm s⁻¹. The test determined bone breaking strength (maximum breaking force) and bone elasticity, defined as the traveled by the probe before reaching the maximum load).

The contralateral tibia was sectioned through the epiphyseal region and fixed in 10% buffered formalin. Samples were decalcified in a solution containing nitric acid, formic acid, and sodium citrate before paraffin embedding. Semi-serial 5 µm sections were stained with Weigert's Iron Hematoxylin and Safranin. Digital images were acquired using a slide device, and IMAGE PROPLUS 5.2 (Mídia Cibertecnicos) was used to quantify the proportions of cartilage and bone tissue relative to the total section area. The two tissue types were differentiated by color segmentation, allowing the software to calculate the percentage area occupied by each tissue (Figure 1).

Data from each experimental period and dietary treatment were subjected to analysis of variance (ANOVA) using the General Linear Model (GLM) procedure implemented in SAS (2002). When treatment effects were significant, means were compared using Tukey's test ($p < 0.05$).

Results and Discussion

A significant effect of dietary treatment ($p < 0.05$) was detected for bone mineral density at 21 and 47 days of age (Table 4). Broilers fed the mineral-deficient diet without phytase supplementation

exhibited the lowest bone mineral density values, whereas those receiving phytase achieved values comparable to those of birds fed nutritionally adequate diets. These findings likely reflect the reduced P, Ca, and Na availability required for normal bone mineralization in the absence of phytase.

Table 4

Bone densitometry, bone-to-cartilage ratio, bone breaking strength, and bone elasticity of broilers receiving diets supplemented or not with phytase

	Positive Control	Negative Control	NC + 500 FTU kg ⁻¹ hytase	NC + 1000 FTU kg ⁻¹ phytase	CV, %	p-value
Bone mineral density, mmAl						
Tibia, 7 days	1.72	1.64	1.76	1.67	12.09	0.3324
Tibia, 21 days	2.12 ^a	1.73 ^c	1.94 ^b	2.02 ^{ab}	8.86	0.0001
Tibia, 47 days	3.17 ^a	2.68 ^b	3.18 ^a	2.98 ^a	16.09	0.0001
Bone-to-cartilage ratio						
Tibia, 7 days	1.52	1.41	1.41	1.74	35.21	0.2854
Tibia, 21 days	1.76 ^a	1.24 ^b	1.67 ^a	1.68 ^a	30.39	0.0107
Tibia, 47 days	2.05 ^a	1.60 ^b	1.71 ^{ab}	1.74 ^{ab}	39.90	0.0011
Bone breaking strength, kgf						
Tibia, 7 days	3.67 ^a	2.33 ^c	3.05 ^b	3.53 ^{ab}	19.78	0.0001
Femur, 7 days	5.73 ^a	4.19 ^b	4.42 ^b	5.02 ^{ab}	21.82	0.0002
Tibia, 21 days	21.09 ^a	13.56 ^c	17.43 ^b	19.15 ^{ab}	17.79	0.0001
Femur, 21 days	23.07 ^a	11.84 ^c	19.00 ^b	22.46 ^{ab}	21.54	0.0001
Tibia, 47 days	38.48 ^a	27.49 ^b	36.19 ^a	37.01 ^a	21.07	0.0001
Femur, 47 days	32.37 ^a	18.17 ^b	31.19 ^a	32.05 ^a	17.14	0.0001
Humerus, 47 days	34.01 ^a	27.22 ^b	34.93 ^a	35.54 ^a	24.45	0.0001
Bone elasticity, mm						
Tibia, 7 days	2.39	2.15	2.23	2.22	24.54	0.6160
Femur, 7 days	2.03	2.06	2.01	1.85	37.64	0.8355
Tibia, 21 days	3.27 ^b	4.44 ^a	3.77 ^b	3.46 ^b	16.40	0.0001
Femur, 21 days	2.72 ^{ab}	3.22 ^a	3.01 ^{ab}	2.65 ^b	20.52	0.0196
Tibia, 47 days	4.05	4.21	4.25	4.24	18.80	0.5991
Femur, 47 days	3.33 ^{ab}	3.65 ^a	3.23 ^{ab}	3.12 ^b	26.26	0.0313
Humerus, 47 days	3.28	3.62	3.28	3.24	26.26	0.1339

CV%: Coefficient of variation. Means within the same row followed by different lowercase letters differ significantly according to Tukey's test ($p < 0.05$).

A similar pattern was observed for the bone-to-cartilage ratio. At 21 days of age, broilers receiving the mineral-deficient diet had the lowest ($p < 0.05$) bone-to-cartilage ratio. By 47 days of age, the negative control diet remained significantly lower than the phytase-supplemented treatments (Table 4). Because the bone-to-cartilage ratio reflects the progression of cartilage replacement by bone tissue, these results indicate that phytase supplementation accelerated skeletal maturation. This response is particularly important in modern broiler strains, whose rapid muscle accretion depends on efficient skeletal development.

Bone breaking strength followed the same trajectory as bone mineral density (Table 4). Supplementation with 500 or 1000 FTU kg^{-1} phytase significantly increased bone breaking strength at 7 and 21 days compared with the unsupplemented mineral-deficient diet. This effect persisted at 47 days and became more pronounced as ossification progressed toward skeletal maturity. Because avian bones undergo rapid maturation during growth, improvements in bone breaking strength provide strong evidence of enhanced bone quality resulting from increased P availability following phytase supplementation (Nuamah et al., 2024).

The humerus was also evaluated because, as a pneumatic bone, its medullary cavity is partially replaced by air spaces, reducing bone mass while maintaining mechanical strength. This anatomical feature reduces susceptibility to fractures during catching, transport, and pre-slaughter processing. Similar improvements in humeral breaking strength were observed in response to phytase supplementation, indicating that the beneficial effects extended across all major long bones evaluated.

Bone elasticity showed a different response. No treatment effect ($p < 0.05$) was detected at 7 days of age, likely because bone formation was still in the early stage of replacing the cartilaginous template. However, significant differences emerged at 21 days in both tibiae and femora and at 47 days in femora, with broilers receiving phytase superdosing exhibiting lower bone elasticity than those fed the negative control diet (Table 4).

According to Lu et al. (2019), phytase supplementation enhances tissue sensitivity to insulin, potentially mediated by myo-inositol released during phytate hydrolysis, which promotes growth through activation of insulin signaling pathways and increased cellular responsiveness. In addition, IGF-1 contributes to skeletal development by stimulating osteoblast proliferation and differentiation, osteocalcin production, type I collagen synthesis, and alkaline phosphatase activity (Selle et al., 2023).

Bone breaking strength and elasticity generally increase as broilers grow and receive nutritionally balanced diets (Fernandes et al., 2019; Selim et al., 2022). In the present study, greater bone breaking strength was associated with adequate mineral availability during growth, which promoted balanced mineral homeostasis and proper skeletal development in broilers receiving phytase supplementation. Optimal bone development is essential because muscle growth and productive performance depend on a robust skeletal framework.

The adverse effects of rapid growth on the locomotor system in broiler chickens are well documented and remain a major concern for the poultry industry. These impairments compromise animal welfare, increase mortality and culling rates, and consequently result in substantial economic losses (Kwon et al., 2024; Martins et al., 2020).

At 7 days of age, the only significant difference ($p < 0.05$) in bone measurements was femur length, where broilers supplemented with 1000 FTU kg^{-1} phytase exhibited longer femora than those fed the negative control diet (Table 5).

Table 5

Bone measurements of broilers receiving diets supplemented or not with phytase

	Positive Control	Negative Control	NC + 500 FTU kg ⁻¹ hytase	NC + 1000 FTU kg ⁻¹ phytase	CV, %	p-value
Bone measurements at 7 days						
Tibia weight, g	1.52	1.45	1.48	1.45	14.40	0.6953
Seedor index (tibia)	32.70	31.25	31.97	31.31	12.65	0.6612
Tibia length, mm	46.39	46.21	46.23	46.23	3.05	0.9784
Tibia diameter, mm	2.96	2.86	2.98	2.93	8.62	0.5405
Femur weight, g	0.82	0.75	0.85	0.76	18.92	0.1491
Seedor index (femur)	24.09	23.15	25.47	22.51	17.90	0.1988
Femur length, mm	33.78 ^a	32.33 ^b	33.30 ^{ab}	33.68 ^a	4.51	0.0197
Femur diameter, mm	3.29	3.23	3.20	3.25	9.46	0.8615
Bone measurements at 21 days						
Tibia weight, g	10.21	10.14	9.99	10.02	14.47	0.9656
Seedor index (tibia)	132.63	135.55	136.50	130.02	13.33	0.6933
Tibia length, mm	76.89 ^a	74.90 ^{ab}	73.04 ^b	77.03 ^a	5.59	0.0178
Tibia diameter, mm	5.94	6.12	6.04	5.97	10.28	0.8294
Femur weight, g	6.79	6.82	6.49	6.68	13.71	0.6967
Seedor index (femur)	125.64	129.63	122.93	120.57	12.85	0.3664
Femur length, mm	53.99 ^{ab}	52.71 ^b	52.82 ^b	55.36 ^a	5.03	0.0147
Femur diameter, mm	7.36 ^{ab}	7.83 ^a	6.81 ^b	7.03 ^b	9.83	0.0004
Bone measurements at 47 days						
Tibia weight, g	30.24 ^a	28.30 ^b	30.58 ^a	29.92 ^{ab}	10.78	0.0057
Seedor index (tibia)	259.23 ^a	246.17 ^b	258.28 ^a	253.15 ^a	9.65	0.0489
Tibia length, mm	116.94 ^{ab}	114.73 ^b	118.25 ^a	118.07 ^a	4.41	0.0053
Tibia diameter, mm	11.38	11.53	11.55	11.47	14.21	0.9316
Femur weight, g	20.34 ^a	18.61 ^b	19.65 ^{ab}	19.74 ^{ab}	11.19	0.0026
Seedor index (femur)	237.61 ^a	220.63 ^b	229.94 ^{ab}	230.99 ^{ab}	9.45	0.0037
Femur length, mm	85.50	84.32	85.41	85.34	4.11	0.3262
Femur diameter, mm	11.30	11.11	11.24	11.42	9.03	0.5369
Humerus weight, g	14.42	13.27	14.45	14.15	18.74	0.1313
Seedor index (humerus)	184.81	189.22	183.43	192.68	39.81	0.9420
Humerus length, mm	78.17	75.24	78.65	76.57	9.26	0.1365
Humerus diameter, mm	9.13	9.16	9.32	9.09	12.78	0.6558

CV: Coefficient of variation. Means within the same row followed by different lowercase letters differ significantly according to Tukey's test ($p < 0.05$).

At 21 days, supplementation with 1000 FTU kg⁻¹ phytase again increased femur length relative to the negative control. By contrast, broilers receiving 500 or 1000 FTU kg⁻¹ phytase exhibited smaller femur diameters ($p < 0.05$) than birds fed the negative control diet (Table 5).

By 47 days, broilers receiving 500 or 1000 FTU kg⁻¹ phytase showed greater tibia length and higher Seedor index values than those in the negative control treatment ($p < 0.05$). Although significant differences were also detected for femur weight and Seedor index, phytase-supplemented birds did not differ from those fed the mineral-deficient diet for these variables (Table 5).

The improvements in bone mineral density, breaking strength, elasticity, and morphometric measurements observed in the present study are likely attributable to phytase-mediated hydrolysis of phytic acid into lower myo-inositol phosphate esters (IP5 to IP1), thereby increasing phosphorus availability and reducing the antinutritional effects of phytate (Bradbury et al., 2016; Sommerfeld et al., 2018; Selle et al., 2023). Conversely, the reduction in bone elasticity observed with phytase superdosing may be associated with the extraphosphoric effects of phytate degradation and the consequent release of myo-inositol, which exerts vitamin-like and lipotropic functions involved in cell morphogenesis, histogenesis, cell membrane integrity, lipid synthesis, and cellular growth (Lee et al., 2017; Selle et al., 2023). Overall, phytase supplementation effectively compensated for reductions in dietary P, Na, and Ca by improving bone quality through enhanced phytate degradation while also contributing to lower feed costs.

Conclusion

Supplementation with 500 or 1000 FTU kg⁻¹ phytase effectively restored bone quality in broilers fed diets deficient in P, Ca, and Na by improving bone mineral density and bone breaking strength to values comparable to those observed in birds fed nutritionally adequate diets. In addition, phytase superdosing (1000 FTU kg⁻¹) reduced femur elasticity and increased femur length compared with the negative control treatment.

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