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Efficacy of different doses of phytase on performance, nutrient digestibility and tibia mineralization in broiler chickens*

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This study evaluated the effects of different phytase supplementation levels on performance, nutrient digestibility, bone mineralization, and carcass characteristics in broilers fed diets with reduced available phosphorus (P). A total of 840 one-day-old male Ross 308 broilers were randomly allocated to six dietary treatments: T1, positive control (PC; adequate P and Ca); T2, negative control (NC; low P and Ca); T3, NC + 167 FTU/kg phytase; T4, NC + 500 FTU/kg; T5, NC + 1,500 FTU/kg; and T6, NC + 4,500 FTU/kg (high dose). The trial lasted 35 days. Body weight (BW), average daily gain (ADG), feed intake, and feed conversion ratio (FCR) were recorded, while apparent ileal digestibility of dry matter (DM), Ca, and P, as well as tibia ash and carcass traits, were assessed. Phytase supplementation significantly improved BW, ADG, and FCR compared with the NC. The highest BW, ADG, and lowest FCR were observed in the T6 group, followed by T5, whereas the NC birds showed the poorest performance. Phytase enhanced P digestibility at all application rates, and DM digestibility at the high inclusion level. Tibia ash content increased linearly with phytase dose, indicating improved bone mineralization. The NC group exhibited elevated Footpad Dermatitis

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Index values and reduced carcass yield, which were mitigated by the addition of phytase. Overall, phytase supplementation improved performance and nutrient digestibility. Dosing phytase at 1,500 and 4,500 FTU/kg fully compensated for P deficiency, improved performance and skeletal integrity, and reduced environmental P impact, supporting its use as a nutritional strategy in broiler production.

KEY WORDS: broiler chicken/ phytase/ performance/ carcass characteristics / nutrient digestibility/ bone mineralization

Phosphorus (P) and calcium (Ca) are indispensable minerals in broiler nutrition, playing critical roles in skeletal development, metabolic processes, and growth performance. In conventional corn-soybean meal diets, a substantial portion of P is bound as phytate (inositol hexaphosphate), rendering it poorly available to chickens and contributing to mineral binding and impaired nutrient absorption [Kozłowski *et al.* 2009, Coudert *et al.* 2025]. At the same time, an elevated or imbalanced dietary Ca:available P (avP) ratio further complicates nutrient utilization, as excessive Ca relative to avP can precipitate Ca-phytate complexes, reduce phytase efficacy, impair P absorption and compromise bone mineralization [Bedford and Rousseau 2017]. Recent studies suggest that maintaining the Ca:avP ratio near 2:1 is essential for optimal bone integrity and growth in broilers, whereas wide ratios (e.g., 3:1) and P deficiencies impairs skeletal development, resulting in disorders such as rickets [Xu *et al.* 2021]. It is still not clear if the ratio or the Ca level and source are the most important parameter influencing phytase efficacy, but data confirms that excessive P and Ca reduces phytate degradation and therefore utilization of phytate P [Sommerfeld *et al.* 2018].

The exogenous addition of phytase to broiler diets has become a cornerstone of modern nutritional strategies. Phytase hydrolyses phytate, liberating inorganic P, by degradation to lower inositol phosphates and with intestinal phosphatases liberating myo-inositol, thereby enhancing P availability, improving mineral and amino acid digestibility, and boosting growth performance [Kozłowski *et al.* 2010, Cowieson *et al.* 2011]. More recently, the concept of dosing phytase at significantly higher levels (often >1500 FTU/kg feed) has gained attraction [Walk *et al.* 2013]. Such high doses not only enhance P release beyond conventional expectations, but also exhibit extra-phosphoric benefits: improved energy and amino acid digestibility, favorable gut microbiota shifts, lower gastric pH, and reduced endogenous nutrient losses [Lee *et al.* 2017, Wolfrum *et al.* 2025]. For instance, in broilers fed low-avP diets with imbalanced Ca:P ratios, high-dosing phytase has been shown to restore growth performance, elevate bone ash content, and reduce P excretion [Rana *et al.* 2024]. Despite such advances, optimal dosing strategies of phytase in low-avP, high-Ca:avP ratio contexts, and their cost-benefit balance in commercial production remain incompletely resolved.

The study aimed to evaluate the effects of graded dietary doses of phytase, including super-doses, added to a negative basal diet with reduced avP content, on performance parameters, carcass quality, tibia mineralization, and nutrient digestibility in broilers.

Material and methods

Ethical approval

The broiler experiment was conducted in compliance with Polish Law [2015] and the European Union Directive 2010/63/EU [OJEU 2010] on animal welfare regulations and did not require ethics committee approval. All procedures followed the guidelines for animal experimentation and animal care, and all efforts were made to enhance animal well-being status.

Birds, diets, and housing

The study was carried out on the experimental farm in Bałdy near Olsztyn, which belongs to University of Warmia and Mazury (UWM) in Olsztyn, Poland. In this experiment one-day-old male Ross 308 broilers from the commercial hatchery were used. After transporting to the farm they were randomly divided into treatments and replicates. The birds were kept in floor pens with netting walls avoiding migration. The experimental house was equipped with artificial, programmable lighting and climate control, automated electric heating, and forced ventilation. The heating program was conducted according to the recommendations of the breeder guidelines: 32ºC at the beginning of experiment and adjusted thereafter according to the age of birds: 18-21ºC from day 21 until the end. The birds were kept in pens – 60 pens in total, 0.7 m^2 each. Every treatment consisted of 10 pens (10 replicates), 14 birds each - resulting in 140 birds per treatment and 840 birds (140 × 6) in total. The length of trial period was 35 days. Drinking water was supplied *ad libitum* via nipple drinkers. The birds were divided into the following dietary treatments: T1 – Positive control (PC, P and Ca levels according to NRC recommendation); T2 – Negative control – (NC, with reduced in dietary P and Ca); T3 – NC diet+167 FTU/kg feed; T4 – NC+500 FTU/kg; T5 – NC+1,500 FTU/kg; T6 – NC+4,500 FTU/kg. The Quantum Blue product, an enhanced *E.coli* phytase, was used in the present study (AB Vista, UK). The supplier reported that the recommended dosage, based on the diet components, is 250-2,500 FTU/kg feed. Therefore, the T6 dietary treatment in the present study should be considered as a "higher" dose.

Mash feed mixtures were prepared by Agrocentrum Sp. z o.o. Feedmill in Kałęczyn (Poland). The composition and nutritional value of basal feed mixtures was shown in Tables 1 and 2. The basal diets were analyzed for their content of crude protein [AOAC 2006; 990.03], crude fiber [AOAC 2006; 978.10], crude fat [AOAC 2006; 920.39], dry matter [AOAC 2006; 930.15], ash [AOAC 2006; 942.05], phosphorus [AOAC 2006; 965.17], and Ca [AOAC 2006; 927.02] and phytase was analysed by the supplier following the procedure as in the recently published method (ISO 30024:2024).

Table 1. Composition of the starter basal diets (0-21 days), %

Compounds	PC - positive control	NC - negative control
Corn	54.461	56.242
Soybean meal	28.477	28.166
Rapeseed meal	8.000	8.000
Soybean oil	4.538	3.956
Na-Bicarbonate	0.150	0.150
Salt	0.212	0.134
Limestone	1.097	1.306
MCP	1.525	0.503
Choline chloride	0.090	0.090
DL-Methionine	0.289	0.287
L-Lysine	0.294	0.299
L-Threonine	0.116	0.117
TiO_2	0.500	0.500
Premix vit. - min.	0.250	0.250
Nutrient density (calculated)		
ME (kcal/kg)	3000	3000
Crude protein (g/kg)	215.00	215.00
Crude fibre (g/kg)	31.35	31.63
Crude fat (g/kg)	73.35	68.21
D. Lysine (g/kg)	12.00	12.00
D. Methionine (g/kg)	5.94	5.93
D. Met. + Cys (g/kg)	9.00	9.00
D. Threonine (g/kg)	8.00	8.00
Calcium (g/kg)	9.50	8.00
Total Phosphorus (g/kg)	7.28	5.01
Av. Phosphorus (g/kg)	4.50	2.20
Sodium (g/kg)	1.50	1.20
Nutrient density (analysed)		
Dry matter (g/kg)	896.90	899.60
Crude protein (g/kg)	220.60	218.80
Crude fibre (g/kg)	32.30	30.80
Crude fat (g/kg)	75.40	71.20
Crude ash (g/kg)	46.20	42.90
Calcium (g/kg)	9.27	8.43
Total Phosphorus (g/kg)	7.09	4.83

Table 2. Composition of the grower basal diets (22-35 days), %

Compounds	PC - positive control	NC - negative control
Corn	58.080	59.895
Soybean meal	24.723	24.406
Rapeseed meal	8.000	8.000
Soybean oil	5.298	4.704
Na-Bicarbonate	0.150	0.150
Salt	0.201	0.123
Limestone	1.028	1.265
MCP	1.467	0.400
Choline chloride	0.090	0.090
DL-Methionine	0.300	0.298
L-Lysine	0.277	0.282
L-Threonine	0.137	0.137
Premix vit. - min.	0.250	0.250
Nutrient density (calculated)		
ME (kcal/kg)	3100	3100
Crude protein (g/kg)	200.00	200.00
Crude fibre (g/kg)	30.80	31.08
Crude fat (g/kg)	81.71	76.48
D. Lysine (g/kg)	11.00	11.00
D. Methionine (g/kg)	5.89	5.88
D. Met. + Cys (g/kg)	8.88	8.80
D. Threonine (g/kg)	7.70	7.70
Calcium (g/kg)	9.00	7.50
Total Phosphorus (g/kg)	7.04	4.67
Av. Phosphorus (g/kg)	4.30	1.90
Sodium (g/kg)	1.45	1.15
Nutrient density (analysed)		
Dry matter (g/kg)	885.00	886.30
Crude protein (g/kg)	198.00	199.20
Crude fibre (g/kg)	29.30	30.50
Crude fat (g/kg)	79.20	75.60
Crude ash (g/kg)	49.30	48.40
Calcium (g/kg)	8.75	7.24
Total Phosphorus (g/kg)	6.81	4.52

Parameters measured

Body weight of birds (pen basis) was measured at day 1, 21, and 35. Feed intake was measured and calculated for following experimental periods: 1-21, 22-35 and 1-35 days. Mortalities were considered from chickens that died or were removed from the trial after weighing. On the basis of these results, FCR (feed conversion ratio) was calculated for all experimental periods (1-21, 22-35 and 1-35 days). For the determination of apparent ileal nutrient digestibility coefficients (DM, Ca, and P) TiO_2 was added to each of the experimental starter diets, as an indigestible dietary marker at a final concentration of 0.5% of a diet. At day 21 of the experiment, 5 birds per

pen were killed by cervical dislocation. The intestinal contents from the duodenum/jejunum and from the terminal ileum (last 2/3 between Meckel's diverticulum minus 2 cm before ileal-caecal junction) was collected. The samples were pooled on a per pen basis and immediately frozen at -20°C. In the samples, Ca, P, DM & Ti were analysed [AOAC, 2006]. From the birds chosen for digestibility assay also tibia bones were collected by dissection. The bones from each replicate (5 birds) were pooled and analyzed for bone ash (on fat free dry matter). Tibia samples were separated from muscles and cartilage, and they were freeze-stored (-25ºC) in sealed bags for 3 days until analysis. The bones were assayed for the average content of dry matter and ash. Tibia samples were weighed, and their volume was measured in distilled water. For acid digestion of the bones samples of 25 to 500 mg, were added with 4 mL H_2SO_4 (SUPRAPUR, Merck, Darmstadt, Germany) and 2 mL H_2O_2 (SUPRAPUR, Merck, Darmstadt, Germany) in closed 50-mL quartz vessels in a microwave Sample Preparation System (Anton Paar, Graz, Austria). After cooling, 5.0 mL internal standard reagent (Yttrium; 10 mg Y/L) was added and made up to 1000-mL volume with DI water. In order to avoid acid interference effects, the acid concentration of all solutions was identical to that in the digested samples.

At the end of the experiment, at 35th day of age, 15 birds representing the average BW per treatment (90 birds in total) were slaughtered in the experimental slaughterhouse of UWM. The birds were electrically stunned (150 mA; 350 Hz), hung on a shackle line and decapitated and immediately processed for sampling. After slaughter, the birds were plucked and eviscerated (non-edible viscera: intestines, proventriculus, gall bladder, spleen, esophagus, and crop). Head, legs, edible giblets (heart, gizzard, and liver), and the first joint to the wing tip were removed to obtain the eviscerated carcass. After 24-hour chilling, carcass, abdominal fat and breast meat were weighed. The weights of organs and muscles were calculated relative to live BW.

Following slaughter analysis (15 birds per treatment), the incidence and severity of footpad lesion/dermatities were measured at the end of the experiment on the same birds as taken for slaughter analysis using the scoring method: score 0 - no or very small superficial lesions, slight discoloration on a limited area of the footpad, healed lesions; score 1 – mild lesion, discoloration of the footpad, superficial lesions, dark papillae and hyperkeratosis; score 2 – severe lesion, epidermis is affected, ulcers or scabs, signs of hemorrhages or swollen footpads. This scoring evaluation was performed on both feet by three independent observers with the above-mentioned broiler specimens, and the average score was used for statistical analyses. The footpad lesion index was calculated with the aid of the following formula: Index = (score 0 × 0 + score 1 × 0.5 + score 2 × 2) × 100 (internal method of UWM Olsztyn).

Statistical analysis

Data were checked for normality before statistical analysis was performed (the Shapiro-Wilk test). The results were analyzed using one-way ANOVA followed by post-hoc Tukey's test, and differences were considered significant at $p \leq 0.05$.

All calculations were carried out using Statistica 13.3 software [TIBCO Software Inc. 2017]. For performance parameters, a pen served as the experimental unit. In the tables, results are presented as means±SD; additionally, the SEM was provided (standard error of the mean; SEM is calculated as SD for all birds (pens) divided by the square root of bird/pen number (n=10). For dry matter and ash content in tibia bones, and carcass quality, individual data of n=15 birds per treatment was analysed. Livability was transformed into arc sin for statistical evaluation, but values (mean± D) presented in % (Tab. 6).

Results and discussion

The positive control diet met the recommendation of breeding companies for dietary P and Ca level, but reduced in P and Ca in the negative control (NC) diet (Tab. 3). The addition of phytase to the diet was confirmed by the increase of the enzyme activity with application rate according to targets.

Table 3. Results of analysis of the experimental diets, %

Item	Ca	P	Ti	Phytase activity FTU/kg
Starter phase (0-21 days)				
T1 - Positive control (PC)	0.927	0.709	0.332	225
T2 - Negative control (NC)	0.843	0.483	0.324	134
T3 - NC + 167 FTU/kg	0.843	0.483	0.337	243
T4 - NC + 500 FTU/kg	0.843	0.483	0.340	391
T5 - NC + 1500 FTU/kg	0.843	0.483	0.334	1300
T6 - NC + 4500 FTU/kg	0.843	0.483	0.345	5610
Grower phase (22-35 days)				
T1 - Positive control (PC)	0.875	0.681	-	219
T2 - Negative control (NC)	0.724	0.452	-	57
T3 - NC + 167 FTU/kg	0.724	0.452	-	156
T4 - NC + 500 FTU/kg	0.724	0.452	-	457
T5 - NC + 1500 FTU/kg	0.724	0.452	-	1170
T6 - NC + 4500 FTU/kg	0.724	0.452	-	5210

FTU – The amount of enzyme that releases 1 μmol of inorganic phosphate from phytate per minute in acetate buffer at pH 5.5 and 37°C.

The highest body weight (BW) and average daily weight gain (ADG) on day 21 was noted in the T1 group (the positive control; $p<0.05$ vs. T2, T3, T6), while the lowest values for these parameters were observed in the T2 broilers (negative control without phytase supplementation; $p<0.05$ vs. all other groups; Tab. 4). The average daily feed intake (ADFI) for period 1-21 days was significantly lower in the T2, T3, T5, and T6 groups than in the T1 and T4 birds ($p<0.05$). The highest FCR value for this feeding period was observed in the T2 broilers ($p<0.05$ vs. all remaining groups except T3). The final BW on day 35 was the highest in the T6 birds (high phytase application; $p<0.05$ vs. T1, T2, T3; Tab. 5) and lowest in the NC birds ($p<0.05$ vs. all remaining treatments). Additionally, the low phytase application fed to the T3 group

Table 4. Growth performance from 1st to 21st day of age (mean±SD)

Item	BW, 1 days (kg)	BW, 21 days (kg)	ADG (g)	ADFI (g)	FCR (kg/kg)
Treatment					
T1	0.039±0.001	0.901^{a}±0.039	41.0^{a}±1.8	52.0^{a}±1.5	1.298^{b}±0.035
T2	0.040±0.001	0.781^{d}±0.035	35.3^{d}±1.7	47.9^{b}±2.5	1.376^{a}±0.076
T3	0.040±0.001	0.829^{c}±0.024	37.6^{c}±1.1	48.8^{b}±1.2	1.322^{ab}±0.051
T4	0.040±0.001	0.876^{ab}±0.038	39.8^{ab}±1.8	50.6^{a}±1.4	1.305^{b}±0.042
T5	0.040±0.001	0.869^{ab}±0.045	39.5^{ab}±2.2	48.4^{b}±1.4	1.278^{b}±0.088
T6	0.040±0.001	0.857^{bc}±0.033	38.9^{bc}±1.6	48.2^{b}±1.2	1.277^{b}±0.095
SEM	<0.001	0.007	0.320	0.287	0.010
p value	0.328	<0.001	<0.001	<0.001	0.022

nº replicates – 60 (10 replicates of 14 birds/treatment); SEM – standard error Mean; BW – body weight; ADG – average daily gain; ADFI – average daily feed intake; FCR – feed/gain.
Values in same columns with no common superscript (a-d) are significantly different ($p \leq 0.05$).

Table 5. Growth performance from 22nd to 35th day of age (mean±SD)

Item	BW, 21 days (kg)	BW, 35 days (kg)	ADG (g)	ADFI (g)	FCR (kg/kg)
Treatment					
T1	0.901^{a}±0.039	2.153^{bc}±0.063	90.0^{bc} ± 2.9	119.1^{ab} ± 5.8	1.486^{bc} ± 0.069
T2	0.781^{d}±0.035	1.980^{d}±0.078	85.7^{c} ± 4.0	116.5^{b} ± 7.4	1.538^{c} ± 0.100
T3	0.829^{c}±0.024	2.094^{c}±0.056	89.1^{bc} ± 5.0	119.4^{ab} ± 5.3	1.474^{abc} ± 0.048
T4	0.876^{ab}±0.038	2.176^{ab}±0.107	92.8^{ab} ± 5.9	121.5^{ab} ± 5.1	1.488^{bc} ± 0.047
T5	0.869^{ab}±0.045	2.172^{ab}±0.087	93.1^{ab} ± 4.0	123.7^{a} ± 5.5	1.442^{ab} ± 0.038
T6	0.857^{bc}±0.033	2.237^{a}±0.058	96.4^{a} ± 6.8	123.3^{a} ± 2.9	1.415^{a} ± 0.067
SEM	0.007	0.015	0.763	0.772	0.010
p value	<0.001	<0.001	<0.001	0.049	0.004

nº replicates – 60 (10 replicates of 14 birds/treatment); SEM – standard error Mean; BW – body weight; ADG – average daily gain; ADFI – average daily feed intake; FCR – feed/gain.
Values in same columns with no common superscript (a-d) are significantly different ($p \leq 0.05$).

Table 6. Growth performance from 1st to 35th day of age (mean±SD)

Item	BW, 1 days (kg)	BW, 35 days (kg)	ADG (g)	ADFI (g)	FCR (kg/kg)	Livability %
Treatment						
T1	0.039±0.001	2.153^{bc}±0.063	60.4^{bc}±1.8	94.9±6.7	1.384^{b}±0.034	97.1±5.0
T2	0.040±0.001	1.980^{d}±0.078	55.5^{d}±2.2	90.6±2.2	1.454^{a}±0.070	97.1±3.7
T3	0.040±0.001	2.094^{c}±0.056	58.7^{c}±1.6	88.4±5.0	1.401^{b}±0.039	95.7±3.7
T4	0.040±0.001	2.176^{ab}±0.107	61.0^{ab}±3.1	90.2±5.1	1.385^{b}±0.039	96.4±5.1
T5	0.040±0.001	2.172^{ab}±0.087	60.9^{ab}±2.5	90.7±4.7	1.363^{bc}±0.029	96.4±6.1
T6	0.040±0.001	2.237^{a}±0.058	62.8^{a}±1.7	92.8±4.2	1.340^{c}±0.033	99.3±2.3
SEM	<0.001	0.015	0.420	0.666	0.007	0.001
p value	0.328	<0.001	<0.001	0.073	<0.001	0.489

nº replicates – 60 (10 replicates of 14 birds/treatment); SEM – standard error mean; BW – body weight; ADG – average daily gain; ADFI – average daily feed intake; FCR – feed/gain.
Values in same columns with no common superscript (a-d) are significantly different ($p \leq 0.05$).

resulted in a lower final BW compared to groups T4, T5, and T6 (p<0.05). Similar differences were analyzed for the ADG over the entire feeding period 1-35 days. In the grower period (22-35 days) ADFI was lowest in the T2 group (p<0.05). For this treatment the highest FCR value in this period was noted (p<0.05 vs. T5, T6 groups), while the lowest FCR for that period was observed in the T6 group (p<0.05 vs. T1, T2, T4). Over the entire feeding period 1-35 days ADFI tended to be decreased in the T3 group vs. PC T1 (p=0.073) and the highest FCR was observed in the T2 group (p<0.05 vs. all other groups) while the best FCR for this period was noted in the T6 birds (p<0.05 vs. all groups except T5).

Apparent ileal digestibility in dry matter was improved in T6 treatment only and did not differ between other groups while apparent ileal Ca digestibility was increased only in birds fed the positive control diet (in both cases, p<0.05 vs. other groups; Tab. 7). The P apparent ileal digestibility was highest in T6 and lowest in T2 birds (in both cases, p<0.05 vs. all remaining treatments). Additionally, the T1 and T5 treatments were characterized by significantly higher phosphorus apparent ileal digestibility than respective digestibilities noted for T3 and T4 groups (p<0.05). The relation between the groups in terms of tibia ash content and statistical differences were as follows: T1, T5, T6^{a}>T4^{b}>T3^{c}>T2^{d} (p<0.05).

Table 7. Apparent ileal digestibility of nutrients and bone mineralization of broilers fed experimental diets (mean±SD), %

Item	Apparent ileal digestibility			Bone mineralization
treatment	dry matter	calcium	phosphorus	tibia ash
T1	67.83^{b}±3.23	55.87^{a}±3.01	55.08^{b}±2.80	52.1^{a}±0.6
T2	65.91^{b}±2.54	47.92^{b}±2.91	37.67^{d}±3.74	47.0^{d}±1.0
T3	67.18^{b}±1.86	48.51^{b}±6.27	43.48^{c}±2.97	48.4^{c}±1.3
T4	66.64^{b}±2.70	50.86^{b}±4.08	44.35^{c}±2.87	50.3^{b}±0.7
T5	65.01^{b}±3.65	49.75^{b}±3.00	57.69^{b}±4.24	51.4^{a}±0.7
T6	70.54^{a}±2.37	47.46^{b}±7.08	63.47^{a}±2.74	51.5^{a}±1.0
SEM	0.423	0.695	1.320	0.267
p value	0.001	0.012	<0.001	<0.001

Notes: nº replicates – 60 (10 replicates of 5 birds/treatment or 10 replicates (bones)/treatment); SEM – standard error mean.
Values in same columns with no common superscript (a-d) are significantly different (p≤0.05).

The carcass characteristics of broilers receiving experimental diets are given in Table 8. The highest carcass weight was observed in the T6 group, while the T2 group had the lowest carcass weight, with both results showing statistical significance (p<0.05) compared to all other treatments. Additionally, the dressing percentage in the negative control treatment was significantly lower than in all other treatments (p<0.05). Notably, the relative weight of the heart and liver was significantly elevated in the T2 group in comparison to all other groups, with the exception of the T3 group. The calculated Footpad Dermatitis Index showed the highest values in birds from

Table 8. Carcass characteristics of broilers fed experimental diets (mean±SD)

Item	Treatment						SEM	*p*-value
	T1	T2	T3	T4	T5	T6		
BWbs (kg)	2.167^{b}±0.039	1.991^{d}±0.048	2.105^{c}±0.052	2.189^{b}±0.025	2.195^{b}±0.039	2.250^{a}±0.044	0.011	<0.001
CW (kg)	1.570^{bc}±0.048	1.390^{d}±0.020	1.540^{c}±0.042	1.599^{b}±0.032	1.607^{b}±0.039	1.656^{a}±0.030	0.012	<0.001
DP (%)	72.4^{a}±1.2	69.8^{b}±1.4	73.2^{a}±0.9	73.0^{a}±0.8	73.2^{a}±0.7	73.6±0.5	0.202	<0.001
BM (%)	18.4±1.2	17.4±1.5	18.4±1.6	18.2±0.9	17.7±0.8	18.2±0.9	0.154	0.327
TM (%)	8.9±0.6	8.5±0.9	9.0±0.5	8.5 ±0.6	8.7±0.8	8.6±0.6	0.090	0.589
DM (%)	6.3±0.4	6.3±0.6	6.4±0.5	6.4±0.4	6.5±0.5	6.5±0.5	0.058	0.886
AF (%)	1.23±0.40	1.14±0.69	1.23±0.57	1.00±0.38	1.63±0.50	1.34±0.46	0.068	0.146
Heart (%)	0.50^{b}±0.06	0.58^{a}±0.06	0.54^{ab}±0.04	0.50^{b}±0.08	0.50^{b}±0.05	0.48^{b}±0.08	0.009	0.007
Gizzard (%)	1.39±0.17	1.35±0.18	1.27±0.09	1.31±0.17	1.33±1.13	1.29±0.14	0.019	0.521
Liver (%)	2.18^{b}±0.17	2.49^{a}±0.32	2.26^{ab}±0.12	2.04^{b}±0.16	2.10^{b}±0.13	2.01^{b}±0.16	0.032	<0.001

15 birds per treatment; BWbs – body weight before slaughter (100%), CW – carcass weight, DP – dressing percentage, BM – breast muscles, TM – thigh muscles, DM – drumstick muscles, AF – abdominal fat.
Values in the same row with no common superscript (a-c) are significantly different ($p \leq 0.05$).

Table 9. Frequency of footpad dermatitis (FPD)

Treatment	FPD (points) (mean±SD)	% of birds with changes according to the FPD rating scale[1]			Index
		0	1	2	
T1	0.47±0.52	53.33	46.67	0.00	23.33
T2	0.80±0.68	33.33	53.33	13.33	53.33
T3	0.67±0.49	33.33	66.67	0.00	33.33
T4	0.40±0.51	60.00	40.00	0.00	20.00
T5	0.73±0.46	26.67	73.33	0.00	36.67
T6	0.40±0.51	60.00	40.00	0.00	20.00
SEM	0.057				
p value	0.149				

15 birds per treatment; Values in same columns with no common superscript (a-d) are significantly different ($p \leq 0.05$).
[1]Score 0 – no or very small superficial lesions, slight discoloration on a limited area of the footpad, healed lesions; score 1 – mild lesion, discoloration of the footpad, superficial lesions, dark papillae and hyperkeratosis; score 2 – severe lesion, epidermis is affected, ulcers or scabs, signs of hemorrhages or swollen footpads.

group T2, which were 16.7-33.3 percent points higher than in the other groups (Tab. 9). It should be noted that the most severe changes (clearly damaged footpad, with ulcers, scabs, or necrosis covering a larger area (>0.8 cm diameter); with bleeding or swelling) were observed only in the group T2 without phytase supplementation.

The PC diet for broilers in respect of the total P, avP, and Ca content was formulated in accordance NRC [1994] representing an adequate Ca:avP ratio, i.e. 1.8-2.2:1. This was reduced in the negative control diet to the level of 3.6-3.9.

In the experiment conducted, various levels of phytase supplementation were utilized, with the highest level designated as a "high dose" even surpassing the manufacturer's recommended levels. A study of Smith *et al.* [2019] involving broilers tested different phytase doses of 500, 1,500, and 3,000 FTU/kg, with the latter being classified as a super dose. The results of that study indicated that a medium supplementation of 1,500 FTU/kg improved breast yield, while the 3,000 FTU/kg dosage markedly enhanced feed conversion ratio (FCR) over the entire study period. In the first nutritional phase (starter, 1-21 days) of our experiment, all doses of phytase tested improved ADG compared to the NC group; however, not all ADGs achieved those of the PC group like the lowest phytase dosage (167 FTU/kg) and the highest dosage (4,500 FTU/kg). In the second feeding phase (22-35 days) and throughout the entire feeding period (1-35 days), the highest dosage of 4,500 FTU/kg – proved to be the most effective in counteracting the negative effects of reduced avP levels in the diet on weight gain and feed conversion ratio (FCR). Application of 500 FTU/kg and above also improved growth and FCR to the level of the PC with slightly lower but still very positive benefits especially with a phytase dose of 1,500 FTU/kg vs. 4,500 FTU/kg. From the analysis of the above results, it can be concluded that in the first three weeks of rearing, when average daily gains fluctuated around 40 grams, the most effective phytase dosage was 1,500 FTU/kg. In the following two weeks, when average daily weight gains were twice as high, the most effective dose was the highest phytase dose of 4,500 FTU/kg. To select the most economically optimal dose of phytase, the benefits of improved bird growth and feed utilization should be weighed against the additional costs of higher phytase dosing and need to take the dietary phytate content of the diet into consideration.

Numerous studies in the field of poultry nutrition have revealed the beneficial effects of incorporating high doses of phytase into poultry diets [Leyva-Jimenez *et al.* 2019]. These findings highlight not only significant improvements in bone mineralization but also enhancements in overall performance. This improvement is attributed to increased digestibility of energy and amino acids, resulting in more efficient nutrient utilization and healthier, more productive birds [Pieniazek *et al.* 2017]. In the research conducted by Bassi *et al.* [2025] on broilers, raising the phytase dosage from 600 to 2,000 FTU/kg resulted in a lower feed conversion ratio (FCR) by reducing average feed intake (FI) while maintaining body weight gain (BWG) throughout the study period. Furthermore, this adjustment increased the mRNA expression of mTOR kinase in breast muscle, suggesting potential benefits for skeletal muscle development and breast meat yield. Paul *et al.* [2025] found that super-dosing phytase may act not only via phosphorus release but also via modulation of gut health/microbiome and might substitute for antibiotic growth promoters under certain conditions but did not affect body composition. The latter seems in line with our experiment, with phytase

not affecting the relative mass of breast, thigh, and drumstick muscles in broilers, but it is essential to note that the lack of the aforementioned impact concerned the relative mass, expressed as a percentage, of those muscles. However, the significant positive impact of the highest phytase dosage on the final body weight of broilers, and thus on carcass weight, should be taken into account. Kriseldi *et al.* [2021] showed in broilers that although breast meat weight showed a log-quadratic effect overall (p=0.004), the positive control and highest phytase (up to 40,500 FTU/kg) groups were not significantly different (p>0.05) in carcass/breast muscle yields. If differences in the efficiency of body composition are due to the phytase product, diet composition or bird performance level needs further investment.

In the current experiment, the highest dose of exogenous phytase added to broiler diets enhanced the apparent ileal digestibility of DM and P. However, the ileal digestibility of Ca was reduced in all groups that received diets with lower avP content, including those supplemented with phytase. It has been reported that phytase supplementation improves DM digestibility by breaking down phytate complexes in the gastrointestinal tract, thereby reducing nutrient-binding effects and improving overall feed utilization [Mulvenna *et al.* 2022]. Dietary phytase allows poultry to better access P bound in phytate in plant-based feed ingredients, thereby increasing P digestibility and reducing the need for high inorganic P supplementation [Venter *et al.* 2024]. Therefore, additional advantage of dietary phytase supplementation is to reduces P and N excreted in droppings as shown in our earlier study [Konieczka *et al*. 2020]. In our study, the applied lower phytase supplementation of 167 and 500 FTU/kg slightly improved the apparent ileal P digestibility vs. negative control group, but the observed improvement did not reach values noted in the control positive treatment. Such improvement was noted in broilers fed higher doses of supplemental phytase. However, it should be born in mind that the P content in the PC feed was beyond what is recommended for digestibility trials [WPSA 2013], why a direct comparison with the positive control is of limited significance. Martínez-Vallespín *et al.* [2022] analyzed in broilers fed low P diets an increase of the ileal digestibility of P, crude protein, and some amino acids increased with increasing levels of phytase (500, 1000, 3000 FTU/kg). Additionally, those authors found that crude ash, P, and the Ca content of tibia bones linearly increased with increasing levels of phytase (p<0.001). This is in line with our study, showing an increased bone mineralization, indicated by tibia ash content, with an increase of phytase application up to 1500 FTU/kg feed compared to the NC group and compared to the PC birds achieving comparable bone ash levels with 1,500 and 4,500 FTU/kg supplementation. This indicates the need for higher phytase dosages when feeding broilers a diet severely deficient in avP. In our study, the Footpad Dermatitis (FPD) Index was more than twice as high in the negative control broiler group compared to the positive control birds. High dietary calcium levels or an imbalanced Ca:P ratio are associated with an increased incidence and severity of FPD in broiler chickens [Zhang *et al.* 2025].

Conclusions

The obtained results shows that phytase improved growth performance, nutrient digestibility, bone mineralization, and carcass characteristics when added at graded levels to a diet low in P and Ca. Further improvements at high phytase inclusion levels (1,500 and 4,500 FTU/kg) on body weight gain, FCR, and bone mineralization throughout the feeding period, comparable to or exceeding those of the nutrient adequate diet shows that it is possible to feed broilers with diets deficient in P. Increasing phytase dosage enhanced P and DM digestibility, confirming the enzyme's role in hydrolyzing phytate complexes and improving nutrient utilization. Broilers fed the P deficient diet without phytase showed the poorest growth performance, weakest bone mineralization, and the highest Footpad Dermatitis Index, emphasizing the adverse effects of mineral imbalance. In addition, carcass yield and dressing percentage were significantly improved by phytase addition, especially at higher doses. Overall, high-dosing phytase at 1,500 to 4,500 FTU/kg effectively can mitigate the negative effect of P deficiency, supporting its use as a nutritional strategy to reduce the need for dietary inorganic phosphorus supplementation without compromising broiler performance, skeletal health, or feed efficiency even at a dietary Ca:P imbalance.

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