Effect of *Aspergillus oryzae* phytase on growth performance, nutrient digestibility, organs weight, and health status of broilers fed a corn soybean meal-based diet

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SUMMARY

Phytase is a key enzyme that degrades phytate, increases availability of phosphorus and other essential nutrients in poultry by breaking down this antinutritional plant compound. Phytase has been extensively used in poultry diets and can be derived from various microorganisms, including bacteria, fungi, and yeasts. This study specifically explored the effects of varying dietary levels of *Aspergillus oryzae* (*A. oryzae*) phytase on body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR), digestibility, organs weight, and health indicators including footpad lesion score, intestine lesion score, and fecal score in broilers. In total, 1512 one day old broiler chicks (ROSS 308) with average BW of 48 g were randomly allocated to four dietary treatments in a completely randomized design and fed a corn soybean meal-based diet supplemented with 0, 500, 1000, or 1500 FTU/kg of *A. oryzae* phytase for a period of 32 days. Each treatment consisted of 21 replicates and each replicate contained 18 birds. The data were collected throughout the experimental period, as well as at the end of trial and analyzed using General Linear Model (GLM) procedure in SAS. Results indicated that 1500 FTU/kg phytase supplementation significantly improved BWG during the starter (d 1 to 9), grower (d 10 to 21) and overall (d 1 to 32) period as well as the FI during the starter (d 1 to 9) and grower (d 10 to 21) period of the experiment. However, phytase supplementation at any level did not affect the FCR, mortality, nutrient digestibility, organs weight, or health related indicators such as foodpad or instestinal lesion scores, and fecal score. These findings suggest that *A. oryzae* phytase supplementation enhances BWG and FI of broilers by mitigating phytate's antinutritional effects, without altering digestibility and health status, thereby supporting its potential as a sustainable and environmental friendly nutritional strategy in the modern broiler production systems.

KEY WORDS

Aspergillus oryzae; digestibility; growth performance; phosphorus; phytase.

INTRODUCTION

Phosphorus (P) is an essential nutrient in poultry diet, required for bird's maintenance and growth. Bird's body requires P for multiple physiological processes and it must be provided in sufficient amounts in the diet. Poultry dites majorly contain plant-based ingredients, such as corn and soybean meal and they carry 50-70% of their total P in the form of phytate [1]. Phytate, also known as phytic acid or myo-inositol hexaphosphate, is a naturally occurring compound in many plant-based feed ingredients [2,3]. Phytate cannot be hydrolyzed by poultry because the birds are unable to produce phytase enzyme in their body and phytate-bound P remains unabsorbed [4]. Additionally, phytate is a strong chelating agent and has affinity for binding to essential minerals such as calcium, magnesium, iron, and zinc forming insoluble compounds that cannot be absorbed by birds [5]. It also decreases the digestibility of proteins, carbohydrates, and lipids by forming indigestible complexes, resulting in reduced growth performance in poultry [6]. The potential approach to resolve this problem is supplementing the exogenous phytase enzyme in poultry diets, which is commonly available from different microbial sources.

Supplementation of phytase enzyme in poultry diets increases the availability of P by breaking down the phytate molecule, ultemately reducing the inorganic P supplementation and also reduced excretion of bound P into the environment [7]. The dose of phytase required to be added in the poultry diets depend on mutliple factors, including the source and activity of phytase, P concentration in the feed, birds' age and performance, as well as the economic and environmental conditions [8]. Generally, the phytase supplementation in broiler diets ranges from 250 to 1500 FTU/kg of feed [9]. Previous studies exhibited that the phytase supplementation obtained from various sources can enhance the growth performance, nutrients digestibility, P retention, and bone mineralization in broilers

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by improving the availability of P bound to phytate [10-15]. An effecient phytase enzyme should be effective at the optimum pH in the gastro-intestinal tract environment (low pH), show stability over the wide pH range, and quickly hydrolyse the phytate resulting in less anti-nutritional effect on gut health [16]. Phytase is being used extensively in poultry diets and can be derived from different microbial sources, such as bacteria, fungi, and yeasts.

The phytase enzyme commercially used at industrial level was initially produced from the fungal strains [4]. In current study, we investigated the effects of phytase derived from *Aspergillus oryzae* (*A. oryzae*) on growth performance, nutrient digestiblity, organ weight, footpad and intestine lesion score, and fecal score in broilers.

MATERIALS AND METHODS

Ethical approval

The experiment protocols employed in this study were reviewed and approved by the Animal Care and Use Committee of Dankook University, Republic of Korea (Approval No. DK-1-2138).

Information about phytase

Citrobacter braakii was the source of microbial phytase (Ronozyme® Hiphos; DSM Nutritional Products, Parsippany, NJ), which was expressed in *A. oryzae* in fine granular form.

Birds and housing

In total, 1512 one-day-old mixed sexed broiler chicks (Ross 308) were obtained from a commercial hatchery, weighed on arrival, and housed in multi-layer battery cages for 32 days. The room temperature was kept at 33 ± 1 for first 3 days and then gradually decreased to 24 until the experiment was finished. The chicks had free access to water and feed.

Experimental design and diets

Chicks were randomly alotted to four different dietary treatments (21 replicates per treatment, 18 chickens per cage) and fed with a corn soybean meal-based basal diet with supplements containing 0, 500, 1000, and 1500 FTU/kg *A. oryzae* phytase. The basal starter (d 1-9), grower (d 10-21), and finisher (d 22-32) diets for broilers were formulated according to the nutrient requirements of Aviagen (Aviagen, 2014) as shown in Table 1.

Growth performance

The broilers were weighed at 1st, 9th, 21st, and 32nd days of age for BW. The FI was calculated by recording the quantity of feed consumed and residual each day. In the end, BWG, FI, and feed

Table 1 - Ingredient and analyzed nutrient compositions of the basal diets.

Ingredient (g/kg)	Starter (d 1-9)	Grower (d 10-21)	Finisher (d 22-32)
Corn	541.9	553.8	567.7
Soybean meal	338	261	182.3
Canola meal	50.0	100	150
Soybean oil	21.0	36.2	50.7
MDCP	-	12.8	11.2
DCP	17.0	-	-
Limestone	11.5	13.4	12.2
L-lysine	5.00	6.50	8.10
DL-methionine	4.60	4.70	5.20
L-threonine	2.00	2.50	3.20
L-tryptophan	-	0.10	0.40
$NaHCO_3$	1.00	1.00	1.00
Salt	3.00	3.00	3.00
Vitamin premix[1]	2.00	2.00	2.00
Mineral premix[2]	2.00	2.00	2.00
Choline	1.00	1.00	1.00
Nutrient composition			
ME, kcal/kg	3,000	3,100	3,200
Crude protein, %	23.0	21.5	20.0
Lysine, %	1.50	1.40	1.30
Methionine + Cysteine, %	1.08	0.99	0.94
Available phosphorus, %	0.48	0.44	0.41
Calcium, %	0.96	0.87	0.81

[1]Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D3; 44 IU vitamin E; 4.4 mg vitamin K; 8.3mg riboflavin; 50mg niacin; 4mg thiamine; 29mg d-pantothenic; 166mg choline; 33 g vitamin B12.

[2]Provided per kg of complete diet: 12mg Cu (as $CuSO_4 \cdot 5H_2O$); 85mg Zn (as $ZnSO_4$); 8mg Mn (as MnO_2); 0.28 mg I (as KI); 0.15mg Se (as $Na_2SeO_3 \cdot 5H_2O$).

MDCP, monodicalcium phosphate; DCP, dicalcium phosphate; $NaHCO_3$, Sodium bicarbonate; ME, metabolizable energy.

Table 2 - The effect of dietary supplementation of *Aspergillus oryzae* phytase on growth performance in broilers.

Parameters	Phytase, FTU/kg diet				SEM	*P*-value
	0	500	1000	1500		
Final BW	1778^{b}	1794^{ab}	1823^{ab}	1843^{a}	20.1	0.015
BWG (g)						
d 1 to 9	151^{b}	153^{ab}	156^{ab}	157^{a}	2.11	0.017
d 10 to 21	627^{b}	639^{ab}	655^{ab}	661^{a}	8.09	0.002
d 22 to 32	951	954	964	977	17.1	0.250
d 1 to 32	1730^{b}	1746^{ab}	1775^{ab}	1794^{a}	19.9	0.015
FI, g						
d 1 to 9	175^{b}	177^{ab}	180^{ab}	181^{a}	2.21	0.022
d 10 to 21	861^{b}	862^{ab}	880^{ab}	883^{a}	9.17	0.040
d 22 to 32	1833	1834	1843	1858	19.4	0.325
d 1 to 32	2868	2873	2903	2923	25.1	0.093
FCR						
d 1 to 9	1.163	1.163	1.159	1.158	0.020	0.838
d 10 to 21	1.374	1.354	1.349	1.341	0.021	0.277
d 22 to 32	1.940	1.941	1.925	1.912	0.041	0.586
d 1 to 32	1.662	1.652	1.642	1.632	0.022	0.307
Mortality, %	4.50	4.23	3.97	3.97	0.130	0.672

BW, body weight; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.
SEM = Standard error of means.
^{a,b}Means in the same row with different superscripts are significantly different ($P < 0.05$).

conversion ratios (FCR) were calculated. Dead birds (if any) were also identified, and the weights were noted daily to calculate mortality and the adjusted FCR.

Apparent total tract digestibility

The feeds were mixed with 0.2% chromium oxide (Cr_2O_3) as an indigestible marker from days 25 to 32 to evaluate the nutrient digestibility (ND) of dry matter (DM), nitrogen (N), and energy (E). After mixing of Cr_2O_3, the feed samples for each treatment were collected in sterilized plastic bags and stored until the further laboratory analysis. A stainless-steel collecting tray was used to collect fresh faecal samples (42 birds per treatment) on day 32 from a random sample set (2 birds per cage). The faecal samples were collected, transported to the lab, and kept at −20 until the further laboratory analysis for ND. For analysis, the excreta were kept in a hot air oven at 105°C for 24 hours for drying and then grounded well and sieved using 1 mm screen.

All the processed feed and excreta samples were examined for DM (method 930.15) and N (method 990.03) according to the guidelines of AOAC (2000). To determine gross energy, the samples were analyzed using an oxygen bomb calorimeter, Parr 6400 (Parr Instrument, Moline, IL, USA). The UV-1201 spectrophotometer (Shimadzu, Kyoto, Japan) was used to detect chromium levels in the samples.

Organ weight

On completion of experiment, 42 broilers were randomly selected from each treatment and sacrificed by cervical dislocation. The breast, liver, spleen, kidney, and bursa of Fabricius were manually disected to record their weight. The organ indexes were measured using the respective organ weights and the live BW of broilers.

Footpad lesion score, intestine lesion score, and faecal score

At the end of experiment, 42 birds per treatment were assessed for footpad dermatitis lesions. The lesions' severity was scored using the scoring categories described by Prescott [17]. For intestinal lesion scoring, the same number of birds were examined and the intestinal lesions were classified as described by Prescott [17]. At 1st, 9th, 20th, 30th, and 32nd day of experiment, fecal samples were collected from all replicate cages. Fecal scores were determined at morning (08:00) and evening (20:00) using the fecal scoring method. Scores were recorded on a cage basis following observations of individual birds and signs of stool consistency in the cage.

Statistical analysis

The data were analyzed as a completely randomized design using GLM procedure is SAS (SAS institute, Cary, NC, USA). The treatments differences were detected by Tukey's multiple range test. Probability values less than 0.05 were considered significant.

RESULTS

Growth performance

Table 2 shows the effects of different levels of *A. oryzae* phytase supplementation on final BW, BWG, FI, and FCR of broilers. Birds fed diet supplemented with 1500 FTU/kg phytase were havier than the CON group birds for the final BW. The BWG of broilers that consumed phytase in their diets was significantly increased ($P < 0.05$) linearly during starter (d 1 to 9), grower (d 10 to 21), and the overall period of experiment. Moreover, *A. oryzae* phytase supplementation improved FI linearly ($P <$

Table 3 - The effect of dietary supplementation of *Aspergillus oryzae* phytase on apparent total tract digestibility in broilers.

Nutrient digestibility, %	Phytase, FTU/kg diet				SEM	*P*-value
	0	500	1000	1500		
Dry matter	72.84	73.37	73.76	74.09	0.99	0.358
Nitrogen	70.61	71.21	71.55	71.79	1.02	0.407
Energy	71.82	72.32	72.62	72.74	0.97	0.487

SEM = Standard error of means.

0.05) compared to the CON group during starter and grower phases of experiment. Supplementation of *A. oryzae* phytase did not shown any significant effect on the FCR ($P > 0.05$) of broilers during different phases and overall experimental period, however, there is a numerical improvement in FCR with increasing the supplementation level of phytase from 0 to 1500 FTU/kg of diet. Mortality remained unaffected in between the dietary supplementation groups during the experimental period ($P > 0.05$).

Apparent total tract digestibility

Table 3 shows the effects of *A. oryzae* phytase supplementation on ATTD of DM, N, and E. The ATTD of DM, N, and E were not effected ($P > 0.05$) by supplementing broilers diets with varying levels of *A. oryzae* phytase in comparison to control.

Organ weight

Table 4 shows the effects of *A. oryzae* phytase supplementation on relative organ weight (%) of breast muslces, liver, spleen, kidney, and bursa of Fabricius. The relative organ weight of breast muscles, liver, spleen, kidney, and bursa of Fabricius were not effected ($P > 0.05$) by supplementing the broilers' diets with varying levels of *A. oryzae* phytase in comparison to control.

Footpad lesion score, intestine lesion score, and faecal score

Table 5 shows the effects of *A. oryzae* phytase supplementation on faecal score, footpad lesion score, and intestinal lesion score of broilers. The faecal score, footpad lesion score, and intestinal lesion score in broilers were not effected ($P > 0.05$) by supplementing the broilers' diets with varying levels of *A. oryzae* phytase in comparison to control.

DISCUSSION

Phytase is an enzyme responsible for breaking down phytic acid, a compound found in plant-based feed ingredients that binds phosphorus. Monogastric animals including poultry are not able to naturally breakdown the phytate and release P from it,

Table 4 - The effect of dietary supplementation of Aspergillus oryzae phytase on organ weight in broilers.

Relative organ weights, %	Phytase, FTU/kg diet				SEM	*P*-value
	0	500	1000	1500		
Breast muscle	9.89	10.17	10.50	10.65	0.43	0.226
Liver	3.1	3.14	3.29	3.38	0.20	0.320
Spleen	0.11	0.11	0.12	0.13	0.01	0.132
Kidney	0.83	0.75	0.79	0.86	0.04	0.423
Bursa of Fabricius	0.23	0.24	0.2	0.18	0.02	0.104

SEM = Standard error of means.

Table 5 - The effect of dietary supplementation of Aspergillus oryzae phytase on faecal score,footpad lesion score, and intestine lesion score in broilers.

Items	Phytase, FTU/kg diet				SEM	*P*-value
	0	500	1000	1500		
Faecal score						
d 1	1.69	1.69	1.67	1.67	0.13	0.871
d 9	1.67	1.64	1.62	1.60	0.12	0.669
d 20	1.64	1.62	1.62	1.60	0.11	0.772
d 30	1.60	1.62	1.64	1.60	0.12	0.965
d 32	1.64	1.62	1.60	1.57	0.13	0.686
Footpad lesion score	0.00	0.00	0.00	0.00	-	NA
Intestine lesion score	0.00	0.00	0.00	0.00	-	NA

SEM = Standard error of means.

so they require exogenous phytases added to their diets. Thus, their P utilization improves, and they require less inorganic P supplementation. In current study, the effects of *A. oryzae* phytase supplementation at different levels on growth performance, nutrient digestibility, organ weight, lesion score, and fecal score were evaluated in broilers. Our findings on growth performance demonstrated that *A. oryzae* phytase supplementation considerably increased the BWG and FI of broilers in a dose-dependent manner during starter, grower, and overall period of experiment. Similarly, Walters et al. [15] discovered that the addition of phytase derived from *A. niger* improved broiler BWG and FI at a supplementation level of 1500 FTU/kg. However, this study and our current one used two different species of fungi belonging to the same genus with different characteristics. *A. oryzae* and *A. niger* exhibit varied abilities to degrade various diets and produce different enzymes and metabolites [18].

Probably, the supplementation of microbial phytase in the diets could have degraded the phytate complexes in the birds' intestinal tract, which may increase the concentration of nutrients absorbed and ultimately improved growth performance. The phytase also have the potential to reduce the pH of the intestinal tract and increase activity of endogenous enzymes, resulting in modulated broilers' gut mictobiota and immune status, thus enhanced health and growth performance [19]. Furthermore, Moradi et al. [20] reported that the microbial phytase at a supplementation level of 500-1000 FTU/kg exhibited a significant effect on BWG of broilers, while in the study of Martinez-Vallespin et al. [21], *A. niger* phytase supplemented at 3000 FTU/kg resulted in improved BWG and FI in broilers. Willard et al. [22] reported the higher specific activity for *A. oryzae* phytase than that of *A. niger* phytase, which depicts that *A. oryzae* phytase can degrade more phytate per unit of enzyme and improve growth performance. However, Walk and Rao [23] reported that the phytase supplementation at 500-2000 FTU/kg in broilers' diet did not improved the BWG singificantly. This phytase was a modified *E. coli* 6-phytase expressed in *Trichoderma reesei*. They speculated that the higher doses of phytase may be required to alleviate the anti-nutritive effect, as the basal diets contained three phytate P concentrations (0.24, 0.345, or 0.45%). Possible reasons for these discrepancies between our research and previous ones could be the source and activity of the phytase used, as well as P levels and availability in the diet.

Broilers fed varying levels of *A. oryzae* phytase were used to measure the ATTD of DM, N, and E. No significant differences were found among groups in respect of DM, N, and E digestibility. Our findings indicate that the supplementation of *A. oryzae* phytase did not affect the nutrient utilization of broilers. However, the nutrient digestibility gradually increased numerically as *A. oryzae* phytase concentration increases. A mechanism for breaking the phytate-nutrient complexes could explain the effect of phytase in decreasing intestinal viscosity by allowing endogenous enzymes to interact with their substrates [24]. However, as phytase has specific mechanisms of action, its positive effects are due to substrate availability. The findings of current study are in agreement with the recent research conducted by Araujo et al. [25], who did not observed any significant effect of phytase supplementaion on DM, N, and E digestibility in broilers. It has been previously reported that an increased P digestibility can affect the digestibility of other nutrients [14]. It can be speculated that supplementing *A. oryzae* phytase in current study may have not significantly affected the P digestibility, which might have resulted in non significantly changed DM, N, and E digestibilities. However, some studies have reported the significantly improved DM, N, and E digesitibilites by phytase supplementation [2,20].

Relative internal organs weight is a key indicator of health status and physiological conditions of broilers. Several factors influence the organ development in broilers, including nutritional composition of diet, genetic potential of birds, as well as the environmental conditions. In current study, the supplementation of *A. oryzae* phytase in the diet did not significantly affected the weights of the breast muscle, liver, spleen, kidney, and bursa of Fabricius. These findings suggest that *A. oryzae* phytase supplementation does not adversly affect the morphological development of the considered organs in broilers. However, this finding is contrary with some previous reports who observed significant changes in organ weight following phytase supplementation in broiler diets [26,27]. These contrary findings indicate that the birds' body may respond differently to phytase supplemtation and can be influenced by mutliple factors, including enzyme source, dietary composition, and broiler strain.

The impact of different levels of *A. oryzae* phytase on three key health indicators was also evaluated, including fecal score, footpad lesion score, and intestinal lesion score. The fecal score is a good indicator of stool consistency and is often used to assess gastrointestinal health and potential enteric issues. One of the possible mechanisms by which the phytase can improve the fecal consistency is through its capacity to breaking down the phytate, thereby reducing its anti-nutritional effects. The phytate has a great affinity to bind minerals such as calcium and zinc, forming insoluble complexes and impair the mineral absorption and increase their excretion [28]. By hydrolyzing phytate, phytase could improve the bioavailability of mineral and enhance gut health and fecal consistency.

Footpad lesion score is commonly used to evaluate the prevalence and severity of footpad dermatitis, a welfare concern characterized by inflammation and ulceration of the footpad. Similarly, intestinal lesion score is a parameter to evaluate the gut integrity, reflecting damage such as erosion, necrosis, or ulceration of the intestinal mucosa [29]. Improved P availability due to phytase supplementation has been suggested to support intestinal health by maintaining epithelial structure and nutrient absorption [30]. Martinez et al. [31] reported that a high phytase dose (3000 FTU/kg) in P-deficient diets reduced both footpad and intestinal lesions in broilers. Contradicted to these expectations, current study found no significant differences in any of the measured health indicators in between the treatment groups, regardless to phytase supplementation levels. These findings suggest that *A. oryzae* phytase, under the conditions of this experiment, had no measurable impact-either positive or negative-on broiler fecal consistency, footpad condition, or intestinal morphology. It is possible that the selected health indicators were not sufficiently sensitive to detect subtle improvements in phytase degradation or P utilization, or that the baseline nutritional adequacy of the diets masked potential benefits of enzyme supplementation.

CONCLUSION

The findings of this study indicate that dietary supplementation with *A. oryzae* phytase at a level of 1500 FTU/kg significantly enhances BW and FI in broilers. This improvement in performance was achieved without any adverse effects on nutrient digestibility, fecal consistency, footpad conditions, and intestinal health. Thus, the inclusion of *A. oryzae* phytase at this dose appears to be a viable strategy for promoting growth in broiler production, while maintaining key indicators of digestive health and animal welfare.

Conflict of interest

All the authors declare no conflict of interst.

Authors contribution

Conceptualization, S.C., I.K.; Methodology, U.A., S.G; Formal analysis, S.C., U.A.; Investigation, I.K.; Supervision, S.C., U.A. and I.K. All authors have read and agreed to the published version of the manuscript.

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References

1. Sampath, V., Gao, S., Park, J. H., and Kim I. H. 2023. Exogenous phytase improves growth performance, nutrient retention, tibia mineralization, and breast meat quality in Ross-308 broilers. *Agriculture,* 13:1906. https://doi.org/10.3390/agriculture13101906
2. Humer, E., Schwarz, C. and Schedle, K. 2015. Phytate in pig and poultry nutrition. *J. Anim. Physiol. Anim. Nutr.,* 99(4):605-625. https://doi.org/10.1111/jpn.12258
3. Selle, P. H., Macelline, S. P., Chrystal, P. V., and Liu, S. Y. 2023. The contribution of phytate-degrading enzymes to chicken-meat production. *Animals,* 13(4):603. https://doi.org/10.3390/ani13040603
4. Puppala, K. R., Buddhiwant, P. G., and Agawane, S. B. 2021. Performance of *Aspergillus niger* (NCIM 563) phytase based feed supplement for broiler growth and phosphorus excretion. *Biocatal. Agric. Biotachnol.,* 31:101887. https://doi.org/10.1016/j.bcab.2020.101887
5. Balwani, I., Chakravarty, K., and Gaur, S. 2017. Role of phytase producing microorganisms towards agricultural sustainability. *Biocatal Agric Biotechnol.,* 12:23-29. https://doi.org/10.1016/j.bcab.2017.08.010
6. Woyengo, T. A., and Nyachoti, C. M. 2013. Anti-nutritional effects of phytic acid in diets for pigs and poultry-current knowledge and directions for future research. *Can. J. Anim. Sci.,* 93:9-21. https://doi.org/10.4141/cjas2012-017
7. Prabakar, S. 2023. Role of Phosphorus in Poultry. https://benisonmedia.com/role-of-phosphorus-in-poultry/2023.
8. Dersjant-Li, Y., Millán, C., Casabuena, O., Quiles, A., Romero, L. F., and Gracia, M. I. 2018. Supplementation of *Buttiauxella* sp. 6-phytase to commercial laying hen diets with reduced nutrient density on productive performance and egg quality. *J. Appl. Anim. Nut.,* 6. https://doi.org/10.1017/jan.2018.4
9. Broch, J., dos Santos, E. C., Damasceno, J. L., Nesello, P. D. O., de Souza, C., Eyng, C., Pesti, G. M., and Nunes, R.V. 2020. Phytase and phytate interactions on broilers' diet at 21 days of age. *J. Appl. Poult. Res.,* 29:240-250. https://doi.org/10.1016/j.japr.2019.10.010
10. Yi, Z., Kornegay, E. T., and Ravindran, V. 1996. Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase. *Poult. Sci.,* 75:240-249. https://doi.org/10.3382/ps.0750240
11. Zyla, K., Korelski, J., and Swiatkiewicz, S. 2000. Effect of phosphorylitic and cell wall-degrading enzymes on the performance of growing broilers fed wheat-based diet containing different calcium levels. *Poult. Sci.,* 79:66-76. https://doi.org/10.1093/ps/79.1.66
12. Woyengo, T. A., Slominski, B. A., and Jones, R. O. 2010. Growth performance and nutrient utilization of broiler chickens fed diets supplemented with phytase alone or in combination with citric acid and Multi carbohydrase. *Poult. Sci.,* 89:2221-2229. https://doi.org/10.3382/ps.2010-00832
13. Cowieson, A. J., Wilcock, P., and Bedford, M. R. 2011. Super-dosing effects of phytase in poultry and other monogastrics. *World Poult. Sci. J.,* 67:225-236. https://doi:10.1017/S0043933911000250
14. Abbasi, F., Fakhur-un-Nisa, T., Liu, J., Luo, X., and Abbasi, I. H. R. 2019. Low digestibility of phytate phosphorus, their impacts on the environment, and phytase opportunity in the poultry industry. *Environ. Sci. Poll. Res.,* 26. https://doi.org/10.1007/s11356-018-4000-0
15. Walters, H. G., Coelho, M., Coufal, C. D., and Lee, J. T. 2019. Effects of increasing phytase inclusion levels on broiler performance, nutrient digestibility, and bone mineralization in low-phosphorus diets. *J. Appl. Poult. Res.,* 28:1210-1225. https://doi.org/10.3382/japr/pfz087
16. Dersjant-Li, Y., Awati, A., and Schulze, H. 2015. Phytase in non-ruminant animal nutrition: a critical review on phytase activities in the gastrointestinal tract and influencing factors. *J. Sci. Food Agric.,* 95:878-896. https://doi.org/10.1002/jsfa.6998
17. Prescott, J. F. 1979. The prevention of experimentally induced necrotic enteritis in chickens by avoparcin. *Avian Diseases,* 1072-1074. https://doi.org/10.2307/1589625
18. Benoit-Gelber, I., Gruntjes, T., Vinck, A., Van Veluw, J. G., Wösten, H. A., Boeren, S., Vervoort, J. J. M., and De Vries, R. P. 2017. Mixed colonies of Aspergillus niger and Aspergillus oryzae cooperatively degrading wheat bran. *Fungal Genet. Biol.,* 102:31-37. https://doi.org/10.1016/j.fgb.2017.02.006
19. Moita, V. H. C., and Kim, S. W. 2022. Nutritional and functional roles of phytase and xylanase enhancing the intestinal health and growth of nursery pigs and broiler chickens. *Animals,* 12:3322. https://doi.org/10.3390/ani12233322
20. Moradi, S., Abdollahi, M. R., Moradi, A., and Jamshidi, L. 2023. Effect of bacterial phytase on growth performance, nutrient utilization, and bone mineralization in broilers fed pelleted diets. *Animals*, 13:1450. https://doi.org/10.3390/ani13091450
21. Martínez-Vallespín, B., Männer, K., Ader, P., and Zentek, J. 2022. Evaluation of high doses of phytase in a low-phosphorus diet in comparison to a phytate-free diet on performance, apparent ileal digestibility of nutrients, bone mineralization, intestinal morphology, and immune traits in 21-day-old broiler chickens. *Animals,* 12:1955. https://doi.org/10.3390/ani12151955
22. Willard, E. C., Sundquist, A., Glitsoe, V., Sorbara, J. O., Tamez-Hidalgo, P., Heine, C., Ricker, T., Lehmann, M., Bergman, A., Etheve, S. and Chatelle, C. V. A. 2022. New rapid and quantitative assay to determine the phytase activity of feed. *ACS omega* 7:5292-5299. https://doi.org/10.1021/acsomega.1c05917
23. Walk, C. L., and Rao, S. R. 2020. Dietary phytate has a greater anti-nutrient effect on feed conversion ratio compared to body weight gain and greater doses of phytase are required to alleviate this effect as evidenced by prediction equations on growth performance, bone ash and phytate degradation in broilers. *Poult. Sci.* 99:246-255. https://doi.org/10.3382/ps/pez469
24. Bedford, M., and Cowieson, A. J. 2012. Exogenous enzymes and their effects on intestinal microbiology. *Anim. Feed Sci. Tech.,* 173:76-85. https://doi.org/10.1016/j.anifeedsci.2011.12.018
25. Araujo, R. G. A. C., Vela, C. G., Sartori, J. R., and Neto, M. A. D. T. 2022. Impact of multicarbohydrase and phytase on apparent and standardized digestibility, energy, and nutrient balance in broilers fed sunflower meal. *Can. J. Anim. Sci.,* 102:571-578. https://doi.org/10.1139/cjas-2021-0099
26. Wang, W., Wang, Z., Yang, H., Cao, Y. and Zhu, X. 2013. Effects of phytase supplementation on growth performance, slaughter performance,

growth of internal organs and small intestine, and serum biochemical parameters of broilers. *Open J. Anim. Res.,* 3:236-241.
http://dx.doi.org/10.4236/ojas.2013.33035

27. Kumar, S. S. and Kim, I. H. 2022. Effect of dietary phytase supplementation on growth performance, organ weight and tibia ash of broilers. *K. J. Poult. Sci.,* 49:9-14.
https://doi.org/10.5536/KJPS.2022.49.1.9
28. Akter, S., Netzel, M., Tinggi, U., Fletcher, M., Osborne, S., and Sultanbawa, Y. 2020. Interactions between phytochemicals and minerals in *Terminalia ferdinandiana* and implications for mineral bioavailability. *Front. Nutr.,* 7:598219.
https://doi.org/10.3389/fnut.2020.598219
29. Teng, P. Y., Yadav, S., de Souza C. F. L., Tompkins, Y. H., Fuller, A. L. and Kim, W. K. 2020. Graded Eimeria challenge linearly regulated growth performance, dynamic change of gastrointestinal permeability, apparent ileal digestibility, intestinal morphology, and tight junctions of broiler chickens. *Poult. Sci.,* 99:4203-4216.
https://doi.org/10.1016/j.psj.2020.04.031
30. Ch'ng, H. Y., Ahmed, O. H. and Majid, N. M. A. 2016. Improving phosphorus availability, nutrient uptake and dry matter production of *Zea mays L.* on a tropical acid soil using poultry manure biochar and pineapple leaves compost. *Exp. Agric.,* 52:447-465.
https://doi.org/10.1017/S0014479715000204
31. Munezero, O. and Kim, I. H. 2022. Effects of protease enzyme supplementation in weanling pigs' diet with different crude protein levels on growth performance and nutrient digestibility. J. *Anim. Sci. Technol.,* 64:854-862.
https://doi.org/10.5187/jast.2022.e51