Research Article

Phytase in Feed Positively Affected the Phosphorus Digestibility and Growth Performance in Broilers

Muhammad Bilal Munawar[1], Saima Naveed[1], Shafqat Nawaz Qaisrani[1], Jibran Hussain[2], Shahzad Munir[3], Ayesha Ahmed[3], Mubbashir Hussain[4], Ahmad Kamran Khan[5], Abdullah Jalal[6] and Irfan Ahmed[7]*

[1]Department of Animal Nutrition, Faculty of Animal Production and Technology, University of Veterinary and Animal Sciences, Lahore-54000, Pakistan; [2]University of Veterinary and Animal Sciences, Lahore-54000, Pakistan; [3]Faculty of Plant Protection, Yunnan Agricultural University, Kunming 650201, Yunnan, PR China; [4]Kohat University of Science and Technology Kohat, Khyber Pakhtunkhwa 26000, Pakistan; [5]Department of Plant Protection, Ghazi University, Dera Ghazi Khan, Pakistan; [6]The University of Agriculture, Peshawar, 25000, Khyber Pakhtunkhwa, Pakistan; [7]Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan.

Abstract | The effect of post pelleting liquid phytase supplementation was evaluated in broilers on the basis of growth production, plasma and tibia phosphorus. The (n=504) day-old broiler chickens procured randomly allocated into 6 treatments dietary treatments were: B positive control (PC) diet (0.42% available phosphorus in grower feed, A negative control (NC) diet with (0.30% avail. P in starter and 0.26% avail. phosphorus in grower) while four diets C, D supplemented with NC+ (500 and 750 powder phytase units (FTU)/kg) and G, H supplemented with NC+ (500 and 750 liquid phytase units (FTU)/kg) respectively. Pelleting temperature was set at 85°C during feed processing production. Blood, ileal digesta, weight of visceral organs, left tibia were sampled for the quantity of phosphorus in plasma, its digestibility and tibial calcium and phosphorus. The present study reported that H group was found higher in FI, BWG and FCR as compared to other groups. Higher gizzard, liver and heart weight were recorded in group A and B while tibia ash, Ca and P in group H. Described results concluded that supplementing liquid phytase enhances breakdown of phytate phosphorus, phosphorus availability, digestibility, and growth performance.

Received | November 18, 2021; **Accepted** | June 28, 2022; **Published** | November 27, 2022
***Correspondence** | Irfan Ahmed, Department of Animal Nutrition, Faculty of Veterinary and Animal Sciences, The Islamia University of Bahawalpur, Pakistan; **Emails:** irfan.ahmed@iub.edu.pk, irfanahmad166@yahoo.com
Citation | Munawar, M.B., S. Naveed, S.N. Qaisrani, J. Hussain, S. Munir, A. Ahmed, M. Hussain, A.K. Khan, A. Jalal and I. Ahmed. 2022. Phytase in feed positively affected the phosphorus digestibility and growth performance in broilers. *Sarhad Journal of Agriculture,* 38(5): 121-129.
DOI | https://dx.doi.org/10.17582/journal.sja/2022/38.5.121.129
Keywords | Chickens, Liquid phytase, Growth performance, Tibia ash, Plasma phosphorus

Introduction

Corn-soy-based diet contains approximately 40-60% phosphorus (P) in phytate phosphorus form. The unabsorbed phytate P from the birds causes environmental pollution, therefore using phytase to control the excretion of phytate P for reduction of environmental pollution is necessary (Guo *et al.*, 2009; Attia *et al.*, 2019). Nowadays, exogenous enzymes like phytase are commercially used in poultry

diets because it makes the availability of nutrients in the feed. Fungi (*Aspergillus niger*) and bacteria (*Escherichia coli*) are main derivatives of the phytase formation (Saleh *et al*., 2021). Diet supplemented with phytase improves broiler growth performance, and increase phytase inclusion can reduces the nutrient specifications (Liu *et al*., 2015; Moss *et al*., 2017). Dietary addition of microbial phytase can increase the nutrients utilization including amino acids, energy and phosphorus availability in Broilers (Ahmed *et al*., 2017; Selle *et al*., 2000). Dietary phytase supplementation in broiler diet from 8 to 21 days can rise P availability in the diet (Jiang *et al*., 2013). Feed processing including, conditioning and pelleting are used to enhance the nutrient digestibility and to reduce the level of pathogenic bacteria such as *Salmonella* present in the feed (Boltz *et al*., 2019). The steam effect is an important factor contributing in enzymatic activity reduction due to the addition of steam, temperature, and moisture simultaneously but lead to enzyme inactivity (Perdana *et al*., 2012). So, nutritionists are in dire need to search alternative manipulations that can reduce phytase inactivity due to high pelleting temperature. Optimal temperature range for the best activity of plant phytase is 45°C - 60°C (Wodzinski and Ullah, 1995). That's why plant phytase became inactive at high steam pelleting temperature. One solution is the post-pelleting addition of liquid phytase to avoid losses in enzyme activity due to pelleting. Phytase activity reduces about 60% at 80°C, so at high pelleting temperature, phytase should be sprayed after pelleting (Eeckhout, 2000).

It is expected that liquid application on the surface of the pellet may give better results in terms of the nutritional value of diet and bird performance (Ziggers, 2003). Present trial was conducted to investigate the post pelleting effects supplementing liquid phytase to avoid phytase denaturation, enhance growth performance, economics and phosphorus utilization in broilers.

Materials and Methods

Animal experimentation ethical statement

Current study was conducted according to principals of Animal Ethics Committee of UVAS, Lahore. The guidance was followed for the animal care and use according to the National Research Council, USA (NRC, 2011).

Table 1: *Feed formulation (Positive control).*

Ingredients (%)	Starter	Grower
Maize	38.40	47.90
Rice tips	15.00	15.00
Canola meal	10.00	8.15
Rapeseed meal	3.00	3.00
Soybean meal	22.00	22.00
Sunflower meal	4.31	----
Marble chips	1.07	1.00
DCP	1.85	0.73
Lys. sulphate	0.53	0.43
Dig. methionine	0.16	0.16
L. threonine	0.08	0.13
Oil	2.49	0.77
Sodium bicarbonate	0.18	0.12
Salt	0.20	0.20
Premix	0.65	0.41
Total	100	100
Calculated nutrients		
CP	21.50	20.00
M.E kcal/kg	2900	3000
Dig. Lys	1.20	1.10
Dig. M+C	0.86	0.86
Dig. Threonine	0.80	0.78
Calcium	0.90	0.87
T. Phosphorus	0.80	0.70
Av. P	0.45	0.40

[1]*Supplied per kilogram of diet: vitamin A, 15,000 IU; vitamin D3, 3300 IU; vitamin E, 62.5 mg; vitamin K, 3.6 mg; vitamin B1, 3 mg; vitamin B2, 9 mg; vitamin B6, 6 mg; vitamin B12, 0.03 mg; niacin, 60 mg; calcium pantothenate, 18 mg; folic acid, 1.5 mg; biotin, 0.36 mg; choline chloride, 600 mg; Fe, 80 mg; Cu, 12 mg; Zn, 75 mg; Mn, 60 mg; I, 0.35 mg; Se, 0.15 mg; growth promoting agent, 30 mg; and antioxidant, 100 mg.*

Experimental site and feeding

This study was conducted at R and D farm of Sharif Mills (Pvt) Ltd, Okara for the period of 35 days. The study was conducted on 504 one-day-old (Hubbard) broilers purchased from a local commercial hatchery, individually weighed. Rice husk was used as a bedding material during the study. The broilers were exposed to 24 h light-period. For optimum growth of the chicken, 24 h light periods are preferred for stimulation of the different biological functions. During the experiment, water and feed were offered at ad-libitum. Vaccination was carried out according to the schedule. The data regarding slaughtered birds were recorded daily basis and their weights were used for feed conversion ratio (FCR) calculations. Pellet temperature during feed formulation was kept on 85°C. Liquid phytase was applied after pelleting on

the feed and powder form phytase was applied during pelleting with each batch of feed. Six iso-caloric (starter with 2900 kcal/kg and grower phase with 3000 kcal/kg) and iso-nitrogenous (21.5% in starter and 20% in grower phase) experimental diets; (A) a negative control (NC) corn-soy-based diet deficient in phosphorus levels 0.30%, 0.26% in starter and grower phase, respectively was formulated; (B) a positive control corn-soy based diet with prescribed level of available P and nutrient recommendations according to (NRC, 1994) was formulated; (C) the NC supplemented with 500 FTU/kg of powder phytase; (D) the NC supplemented with 750 FTU/kg of powder phytase; (G) the NC supplemented with 500 FTU/kg of liquid phytase; (H), NC supplemented with 750 FTU/kg of liquid phytase. All experimental diets were fed as crumbs in starter and grower phases. FTU (phytase activity) expresses the phytase activity. FTU, one phytase unit is described as the total enzyme proportion that liberates 1 micromole of inorganic phosphorus per minutes from sodium phytate (0.0051 mol/l) at pH 5.50 and 37 °C under experimental conditions (Dersjant-Li *et al.*, 2015).

Study parameters

Phytase activity determination was done by the AOAC methodology (AOAC 2000) (Loop *et al.* 2012). Production parameters (Feed intake (FI), body weight gain (BWG), and feed conversion ratio (FCR) were calculated on weekly basis. At d 35, 3 birds were selected from each replicate and slaughtered by cervical dislocation, blood was collected for plasma phosphorus and left tibia was removed to determine tibia ash, phosphorus and calcium. The adhering tissues were removed and the bones are dried at 105°C for 24 h in a hot air oven. The dried bones were ground and 5 g of sample was ashed in a muffle furnace at 600°C for 12 h. The ashed sample was used for determination of tibia ash, P and Ca contents in bone (Wilkinson *et al.*, 2013). Protein efficiency ratio (PER) was calculated on weekly basis by dividing weight gain from protein consumed. The organs indexes including liver, heart, empty gizzard and small intestine were measured by dividing the organ weight into whole bird weight and multiplying by 100. The digesta from Meckel's diverticulum to 2 cm anterior from the ileocecal junction, gently squeezed into a plastic container to avoid any contamination. The digesta sample, thereafter, was immediately stored at -20 °C until further analysis (Qaisrani *et al.*, 2015). Acid-insoluble ash from feed and digesta samples was analyzed (Helrich, 1990). Apparent ileal digestibility coefficient (AIDC) of phosphorus was estimated by collecting the digesta from Meckel's diverticulum to the ileocaecal junction from 3 birds/replicate. (Lalpanmawia *et al.*, 2014).

Table 2: *Feed formulation (Negative control).*

Ingredients (%)	Starter	Grower
Maize	38.48	47.28
Rice tips	15.00	15.00
Canola meal	10.00	8.15
Rapeseed meal	3.00	3.00
Soybean meal	22.00	22.00
Sunflower meal	5.16	---
Marble chips	1.07	1.00
DCP	1.00	1.50
Lys. sulphate	0.53	0.43
Dig. methionine	0.16	0.16
L. threonine	0.08	0.13
Oil	2.49	0.77
Sodium bicarbonate	0.18	0.12
Salt	0.20	0.20
Premix	0.65	0.26
Total	100	100
Calculated nutrients		
CP%	21.50	20.00
M.E kcal/kg	2900	3000
Dig. Lys%	1.20	1.10
Dig. M+C%	0.86	0.86
Dig. Threonine%	0.80	0.78
Calcium%	0.90	0.87
T. Phosphorus%	0.61	0.55
Av. P%	0.30	0.26

[2]*Supplied per kilogram of diet: vitamin A, 15,000 IU; vitamin D3, 3300 IU; vitamin E, 62.5 mg; vitamin K, 3.6 mg; vitamin B1, 3 mg; vitamin B2, 9 mg; vitamin B6, 6 mg; vitamin B12, 0.03 mg; niacin, 60 mg; calcium pantothenate, 18 mg; folic acid, 1.5 mg; biotin, 0.36 mg; choline chloride, 600 mg; Fe, 80 mg; Cu, 12 mg; Zn, 75 mg; Mn, 60 mg; I, 0.35 mg; Se, 0.15 mg; growth promoting agent, 30 mg; and antioxidant, 100 mg.*

Data analysis

The data collected were analyzed using analysis of variance (ANOVA) under Completely Randomized Block Design (CRBD) and SAS 9.1 version (SAS, 2001) was used to compare means through DMR test. Statistical significance was set at $p<0.05$. Data for all parameters were presented as mean ± SEM. There were non-significant differences with same numbers on mean value.

Table 3: *Experimental dietary treatments.*

Sr. No	Treatment	
A	Negative control (NC)	Treatments = 6 Replicates/ Treatment = 3 Birds/ Replicate = 28 6*3*28=504
B	Positive control (PC)	
C	Phytase 500FTU(Powder)+ (NC)(Pre-pelleting)	
D	Phytase750FTU (Powder)+(NC)(Pre-pelleting)	
G	Phytase500FTU (Liquid)+NC(Post Pelleting)	
H	Phytase750FTU (Liquid)+NC(Post Pelleting)	

Total 504 one-d-chicks were selected, divided into 6 treatments, 3 replicates and in one replicate 28 chickens. Six feeds were offered to chickens including A (Negative control (NC)), B (Positive control (PC), C (Phytase 500FTU(Powder)+ (NC)(Pre-pelleting), D (Phytase750FTU (Powder)+(NC)(Pre-pelleting), G (Phytase500FTU (Liquid)+NC(Post Pelleting)), H (Phytase750FTU (Liquid)+NC(Post Pelleting).

Table 4: *Phytase analysis in feed and retention in the chicken body instant phytase 10000FTU/ml.*

		%age		**%**	**Retention in the body (%)**	
Enzyme activity as such	10301/ml	103				
	Starter		Grower		P retention % starter	P retention in grower %
Recovery in feed target 500 FTU/kg	463 FTU/kg	93	481 FTU/kg	96	58	62
Recovery in feed target 750 FTU/kg	723 FTU/kg	96	703 FTU/kg	94	60	55
Quantam blue 5000FTU/g						
Enzyme activity as such	5309/g	106			P retention % in starter	P retention in grower %
Conditioning Temp. 80						
Recovery in feed target 500 FTU/kg	398 FTU/kg	80	413 FTU/kg	83	27	29
Recovery in feed target 750 FTU/kg	611 FTU/kg	81	595 FTU/kg	79	23	20

Results and Discussion

Production parameters

The experiment was conducted to evaluate phytase in feed effects on the phosphorus digestibility and growth performance in broilers. Two levels 500 and 750 FTU from each phytase source (liquid and powder) were added to low phosphorus diet. Phytase helps to breakdown phytic acid by releasing iron and zinc and available phosphorus. The results were compared with the negative control diet with low phosphorus than the normal. The enzyme retained activity was performed using the modified method for this specific phytase is presented in the Table 4. Decreased the enzyme activity in the powder form applied pre-pelleting showed the less the %age of activity as compared to the phytase activity of the liquid form applied post-pelleting in the feed formulating in started and grower feeds as shown in Table 4. Due to the increased phytase activity, P retention in the body was found higher in the liquid form phytase application after pelleting as compared to the powder form of phytase applying (Table 4). The average feed intake of broilers g/bird in starter phase showed significant differences ($p < 0.001$) among the groups A, C and D as shown in (Table 5). Feed intake in grower phase exhibited significant differences among A, C while non-significant among other groups. All groups exhibited the significantly difference ($p<0.001$) as comparing with group A that is deficient in available phosphorous (Table 5). Significant differences ($p < 0.001$) in BWG were noted among A, C, G and H groups (Table 6). Phytase supplementation in post-pelleting did influence on weight gain as shown in group H higher weight gain as compared with A negative control group. Significant ($p<0.001$) weight gain was observed in all groups when compared with the negative group (Table 6). Non-significant ($p > 0.05$) effects on FCR in starter between all the groups. In grower phase, FCR was improved linearly ($p < 0.12$) when NC supplemented with phytase. Better FCR was observed in group H that is more than NC, A (Table 7). In overall phase, best FCR observed in group H. Non-significant results were observed among groups B, C, D, and G.

Dressing percentages and organs weight

Highest dressing % was observed in group B (PC) that is more than A NC as shown in (Table 8). Phytase supplementation increased the dressing % and the results of group D and G were comparable with B group as shown in Table 8. Heart weight (g/100 g BW) was increased in low P diet. Group A showed significant differences ($p<0.02$) than all other

groups containing phytase. Liver weight improved linearly (p< 0.18) in group A NC when compared with groups B, C and G. Phytase addition decreased the liver weight when compared with NC. Gizzard weight was not influenced significantly (p<0.7879) by the exogenous phytase supplementation in all experimental diets.

Table 5: *Effect of phytase supplementation on feed intake in broilers.*

Treatments	Feed intake (g/bird)		
	Starter	Grower	Overall
A	0895±58.64^{d}	1406±117.33^{c}	2301±169.40^{c}
B	1264±31.59^{ab}	2119±15.54^{a}	3383±17.05^{a}
C	1102±10.03^{c}	1849±11.79^{b}	2951±07.25^{b}
D	1202±23.48^{b}	2067±75.18^{a}	3269±86.50^{a}
G	1262±28.30^{ab}	2111±49.11^{a}	3374±20.81^{a}
H	1310±04.48^{a}	2128±52.50^{a}	3439±56.64^{a}

Mean figure bearing different superscripts row wise differ significantly (p>0.05). Data for all parameters were presented as mean ± S.E.M. Means with the same letter are not significantly different.

Table 6: *Effect of phytase supplementation on body weight gain in broilers.*

Treatments	Body weight gain (g/bird)		
	Starter	Grower	Overall
A	605.52±29.15^{e}	645.10±33.11^{d}	1250.62±26.10^{e}
B	830.42±13.19^{b}	1078.96±23.89^{b}	1909.39±21.08^{c}
C	728.34±5.37^{d}	991.02±7.31^{c}	1719.37±3.57^{d}
D	776.25±2.21^{c}	1086.83±11.44^{b}	1863.08±10.07^{c}
G	861.45±1.62^{ab}	1111.55±23.15^{ab}	1973.00±22.32^{b}
H	887.78±1.81^{a}	1170.49±13.13^{a}	2058.28±14.89^{a}

Mean figure bearing different superscripts row wise differ significantly (p>0.05). Data for all parameters were presented as mean ± S.E.M. Means with the same letter are not significantly different.

Table 7: *Effect of phytase supplementation on feed conversion ratio in broilers.*

Treatments	Feed conversion ratio		
	Starter	Grower	Overall
A	1.44±0.03^{a}	2.10±0.13^{a}	1.83±0.09^{a}
B	1.46±0.04^{a}	1.96±0.05^{ab}	1.77±0.01^{ab}
C	1.45±0.01^{a}	1.82±0.02^{b}	1.71±0.00^{ab}
D	1.47±0.02^{a}	1.89±0.08^{ab}	1.75±0.05^{ab}
G	1.42±0.01^{a}	1.89±0.05^{ab}	1.71±0.01^{ab}
H	1.43±0.01^{a}	1.81±0.03^{b}	1.67±0.02^{b}

Mean figure bearing different superscripts row wise differ significantly (p>0.05). Data for all parameters were presented as mean ± S.E.M. Means with the same letter are not significantly different.

Table 8: *Effect of phytase supplementation on dressing%, liver, heart and gizzard weight in broilers.*

Treatments	Dressing %	Heart (g/100 g BW)	Liver (g/100 g BW)	Gizzard (g/100 g BW)
A	59.71±0.82^{b}	0.65±0.01^{a}	2.68±0.02^{a}	1.56±0.04^{a}
B	62.52±0.61^{a}	0.51±0.01^{b}	2.37±0.12^{ab}	1.69±0.11^{a}
C	61.62±0.60^{ab}	0.47±0.04^{b}	2.33±0.07^{ab}	1.53±0.04^{a}
D	62.26±0.66^{a}	0.50±0.03^{b}	2.28±0.11^{b}	1.63±0.06^{a}
G	62.10±0.82^{a}	0.50±0.02^{b}	2.36±0.15^{ab}	1.67±0.11^{a}
H	61.74±0.45^{ab}	0.48±0.05^{b}	2.29±0.11^{b}	1.58±0.13^{a}

Mean figure bearing different superscripts row wise differ significantly (p>0.05). Data for all parameters were presented as mean ± S.E.M. Means with the same letter are not significantly different.

Determination of Ca and P

Tibia ash % in groups H and G supplemented with NC+ 750 and 500 FTU liquid phytase were observed significantly higher (p<0.003) than all other groups but non-significant among each other (Table 8). Tibia P % was observed significantly higher (p<0.01) in group H than all other groups. Non-significant results were observed in A, B, C and G groups, but group D showed significantly higher results groups A, B, C and G. Tibia Ca % was observed significantly higher (p<0.001) in groups C, D, G, and H than A and B. Non-significant results were observed between C, D, G, H and between A and B groups (Table 9). Plasma P content was higher (p<0.001) in H group compared with all other groups followed by group D supplemented with NC+750 FTU powder phytase (Table 10). In group H higher plasma P observed than group A supplemented with NC. Non-significant differences (p>0.05) were noted between groups B, C, and G. Phosphorus digestibility in groups A, B and C showed significant differences (p < 0.05). Higher digestibility was observed in group H that was found more than A group. Non-significant differences (p > 0.05) were observed between groups D, G, and H (Table 10).

Table 9: *Effect of phytase supplementation on tibia parameters.*

Treatments	Tibia ash %	Tibia P %	Tibia Ca %
A	40.97±0.19^{b}	6.92±0.07^{c}	8.65±0.82^{b}
B	41.93±0.13^{b}	7.46±0.30^{c}	8.90±0.18^{b}
C	41.92±1.01^{b}	7.36±0.17^{c}	14.21±0.61^{a}
D	42.38±0.30^{b}	7.74±0.20^{b}	15.18±0.48^{a}
G	44.27±0.28^{a}	7.33±0.06^{c}	15.41±0.14^{a}
H	45.11±0.25^{a}	8.06±0.05^{a}	15.67±0.22^{a}

Mean figure bearing different superscripts row wise differ significantly (p>0.05). Data for all parameters were presented as mean ± S.E.M. Means with the same letter are not significantly different.

Table 10: *Effect of phytase supplementation on plasma phosphorous contents and phosphorous digestibility in broiler at 5 weeks of age.*

Treatments	Phosphorous (mg/dl)	Phosphorous digestibility
A	3.93±0.02^{d}	0.50±0.00^{c}
B	4.23±0.02^{c}	0.52±0.00^{bc}
C	4.25±0.02^{c}	0.54±0.00^{b}
D	4.43±0.06^{b}	0.57±0.00^{a}
G	4.25±0.04^{c}	0.57±0.00^{a}
H	4.96±0.03^{a}	0.60±0.00^{a}

Mean figure bearing different superscripts row wise differ significantly (p>0.05). Data for all parameters were presented as mean ± S.E.M. Means with the same letter are not significantly different.

Table 11: *Effect of phytase supplementation on protein efficiency ratio.*

Treatments	PER (0-21 days)	PER (22-35 days)
A	3.16±0.19^{a}	2.30±0.10^{b}
B	3.05±0.09^{a}	2.54±0.07^{ab}
C	3.07±0.01^{a}	2.67±0.01^{a}
D	3.00± 0.05^{a}	2.63±0.11^{a}
G	3.17±0.07^{a}	2.63±0.07^{a}
H	3.15±0.00^{a}	2.75±0.05^{a}

Mean figure bearing different superscripts row wise differ significantly (p>0.05). Data for all parameters were presented as mean ± S.E.M. Means with the same letter are not significantly different.

Protein efficiency ratio

Protein efficiency ratio in starter phase exhibited non-significant differences (p>0.05) among all groups as shown in (Table 11). In grower phase, all phytase supplemented diets C, D, G, H showed non-significant differences (p<0.05) when compared with NC. Group H in grower phase was recorded highest protein efficiency ratio than other groups (Table 11).

The effect of liquid phytase supplementation was recorded for growth performance and P utilization in broilers. The 2 levels of phytase enzymes (500 FTU and 750 FTU) in the forms of liquid and powder were supplemented to broilers. This study revealed that phytase supplementation in low phosphorus diet increased the feed intake owes (Watson *et al.*, 2006; Ravindran *et al.*, 2008) findings. The increase in feed intake might be due to faster transit time in birds given phytase. More feed intake and more supplementation of phytase in low phosphorus diet improve FI that results in increase BWG (Bozkurt *et al.*, 2006). Further, the increase in feed intake increases the weight gain, contradicting the study of (Ravindran *et al.*, 2008). Liu *et al.* (2010), reported that low P diet decreased the body weight gain but this is enriched by phytase supplementation. Singh *et al.* (2013) highlight that broilers supplemented with phytase observed improved weight gain. Our study owes to the one conducted by Wilkinson *et al.* (2013) and Bozkurt *et al.* (2006) who reported that supplementation of phytase in diet improved feed conversion ratio but contradicting the Powell *et al.* (2011) who displayed no effect on FCR when given phytase supplemented diet. These differences can be related to the different sources of phytase available in the market (Elkhalil *et al.*, 2007). Kiarie *et al.* (2015) reported 7.4% increase in FCR as the same showed by (dos Santos, 2013; Walk *et al.*, 2013). The release of nutrients and trace minerals from phytate by phytase addition can improve the FCR. Higher tibia ash contents in broilers fed low P diet supplemented with phytase because the phytase in feed ingredients increase the release of P and other minerals. The improvement in tibia ash was noted with the addition of phytase in broilers diet (Wilkinson *et al.*, 2013). Higher tibia ash % in P deficient diet supplemented with phytase was observed. The increase in heart relative weight decreases the P concentration (Ciurescu *et al.*, 2020; Lalpanmawia *et al.*, 2014; Baradaran *et al.*, 2014; Viveros *et al.*, 2002). The liver weight was reduced by phytase powder supplementation. NC showed the maximum liver weight as compared to other dietary treatments (Baradaran *et al.*, 2014; Viveros *et al.*, 2002).

The increase in hydrostatic pressure within the liver can make the change to this weight (Huchzermeyer and De Ruyck, 1986). The gizzard weight showed that phytase had no significant effect (p<0.3326) among all the dietary treatments on gizzard weight same as Liu *et al.* (2014) who reported no significant effect (p<0.440) of enzyme supplementation at 1000 FTU/kg and 500 FTU/kg (Viveros *et al.*, 2002). The higher plasma P concentration in birds was noted with fed phytase supplemented diet (Naves *et al.*, 2015). Mondal *et al.* (2007) highlight phytase supplementation also improves plasma P up to 4.24% than low P diet. The present study findings owe the Powell *et al.* (2011) who observed increased digestibility of phosphorus by phytase supplementation in low calcium and P diet. Phytase improved phosphorus digestibility at all Ca and available phosphorus levels (Amerah *et al.*, 2011). The present work results are not in accordance

with the Adebiyi and Olukosi (2015) findings. These differences rely upon diet type, the low of improvement in dietary phosphorus digestibility as observed in their study and the characteristics of the wheat-DDG because it contained lower levels of phytate-bound phosphorus. Similar improvements in PER with supplementation of phytase were observed by Kong and Adeola (2011). Diets used with different phytate levels supplemented with powder phytase that improves PER might be due to the breakdown of the bond between phytate and basic amino acids (Ravindran *et al*., 2006).

Conclusions and Recommendations

In this work, broilers were given two levels of phytase from liquid and powder source and we observed that by giving 750 FTU liquid post-pelleting phytase, the performance of broilers was best. The decreased activity of phytase is due to high temperature, pressure and moisture related to steam conditioning and pelleting. This trial will help the farmers for economical meat production. Dietary inclusion of liquid post-pelleting phytase generates the bioavailable phosphorus and reduces the phosphorus load in the environment. Further work should be done to replace the expensive phosphorous source with the appropriate level of liquid post-pelleting application of phytase.

Novelty Statement

Liquid post-pelleting application produce more available phosphorus for the poultry and improve overall performance and reduce environmental pollution.

Author's Contribution

Data curation was made by MBM, SN, IA. Formal analysis was made by SNQ and JH. Methodology was made by MBM,SM, AA, MH and AJ. Software was assigned to AKK and IA.Writing-original draft was assigned to SN, SNQ and JH.Writing-review and editing was assigned to IA, MBM, AKK, SM, and AA.

Conflict of interest

The authors have declared no conflict of interest.

References

Adebiyi, A.O., and O.A. Olukosi. 2015. Metabolizable energy content of wheat distillers' dried grains with solubles supplemented with or without a mixture of carbohydrases and protease for broilers and turkeys. J. Poult. Sci., 94(6): 1270–1276. https://doi.org/10.3382/ps/pev089

Ahmed, I., S. Munir, A.M. Jamal, T.N. Pasha, Y.A. Ditta, A. Mahmud, A.K. Khan, M. Saima, Z. Talpur and J. Jia. 2017. Effect of enzyme complex at different wheat-based diets on growth performance of broilers. J. Entomol. Zool. Stud., 5(3): 525-531.

Amerah, A.M., C. Gilbert, P.H. Simmins and V. Ravindran. 2011. Influence of feed processing on the efficacy of exogenous enzymes in broiler diets. World Poult. Sci. J., 67(01): 29-46. https://doi.org/10.1017/S0043933911000031

AOAC, 2000. International Official Methods of Analysis of the AOAC International (17th ed.), AOAC International, Gaithersburg, MD.

Attia, Y.A., F.A. Nicola, E.A. Al-Hamid, A. Al-Hamid and F. Bovera. 2019. Effects of phytase supplementation to diets with or without zinc addition on growth performance and zinc utilization of white Pekin ducks. Animals, 9: 280. https://doi.org/10.3390/ani9050280

Baradaran, N., M. Shahir, K.Z. Asadi, P. Waldroup and M. Sirjani. 2014. Effects of high non-phytate phosphorus starter diet on subsequent growth performance and carcass characteristics of broiler chickens. J. Anim. Phys. Anim. Nutr., 98(4): 643-650. https://doi.org/10.1111/jpn.12106

Boltz, T.P., J.W. Boney, C. Shen, J. Jaczynski and J.S. Moritz. 2019. The effect of standard pelleting and more thermally aggressive pelleting utilizing a hygieniser on feed manufacture and reduction of *Enterococcus faecium*, a *Salmonella* Surrogate. J. Appl. Poult. Res., 28(4): 1226-1233. https://doi.org/10.3382/japr/pfz088

Bozkurt, M., M. Çabuk and A. Alçiçek. 2006. The effect of microbial phytase in broiler grower diets containing low phosphorus, energy and protein. J. Poult. Sci., 43(1): 29-34. https://doi.org/10.2141/jpsa.43.29

Ciurescu, G., A. Vasilachi and H. Grosu. 2020. Efficacy of microbial phytase on growth performance, carcass traits, bone mineralization, and blood biochemistry parameters in broiler turkeys fed raw chickpea (*Cicer arietinum* L., cv. Burnas) diets. J. Appl. Poult. Res., 29: 171–184.

https://doi.org/10.1016/j.japr.2019.10.004

Dersjant-Li, Y., A. Ajay, S. Hagen and P. Gary. 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(5): 878–896. https://doi.org/10.1002/jsfa.6998

Eeckhout, I.M., 2000. Phytase quality suffers from steam pelleting and storage temperature. Feed Technol., 4(2): 18-21.

Elkhalil, E., K. Männer, R. Borriss and O. Simon. 2007. *In-vitro* and *in-vivo* characteristics of bacterial phytases and their efficacy in broiler chickens. Br. Poult. Sci., 48: 64-70. https://doi.org/10.1080/00071660601148195

Guo, Y., Y. Shi, F. Li, J. Chen, C. Zhen and Z. Hao. 2009. Effects of sodium gluconate and phytase on performance and bone characteristics in broiler chickens. Anim. Feed Sci. Tech., 150: 270-282. https://doi.org/10.1016/j.anifeedsci.2008.09.006

Helrich, K., 1990. Official methods of Analysis of the AOAC. Volume 2. Association of Official Analytical Chemists Inc. pp. 2.

Huchzermeyer, F., and A. De-Ruyck. 1986. Pulmonary hypertension syndrome associated with ascites in broilers. Vet. Rec., 119(4): 94-94. https://doi.org/10.1136/vr.119.4.94

Jiang, X.R., F.H. Luo, M.R. Qu, V. Bontempo, S.G. Wu, H.J. Zhang, H.Y. Yue and G.H. Qi. 2013. Effect of non-phytate phosphorus levels and phytase sources on the growth performance, serum biochemical and tibial parameters of broiler chickens. Ital. J. Anim. Sci., 12(3): e60. https://doi.org/10.4081/ijas.2013.e60

Kiarie, E., T. Woyengo and C. Nyachoti. 2015. Efficacy of new 6-phytase from *Buttiauxella* spp. on growth performance and nutrient retention in broiler chickens fed corn soybean meal-based diets. Asian Austral. J. Anim., 28(10): 1479. https://doi.org/10.5713/ajas.15.0059

Kong, C., and O. Adeola. 2011. Protein utilization and amino acid digestibility of canola meal in response to phytase in broiler chickens. Poult. Sci., 90(7): 1508-1515. https://doi.org/10.3382/ps.2011-01363

Lalpanmawia, H., A. Elangovan, M. Sridhar, D. Shet, S. Ajith and D. Pal. 2014. Efficacy of phytase on growth performance, nutrient utilization and bone mineralization in broiler chicken. Anim. Feed Sci. Tech., 192: 81-89. https://doi.org/10.1016/j.anifeedsci.2014.03.004

Liu, N., Y. Ru, J. Wang and T. Xu. 2010. Effect of dietary sodium phytate and microbial phytase on the lipase activity and lipid metabolism of broiler chickens. Br. J. Nutr., 103(06): 862-868. https://doi.org/10.1017/S0007114509992558

Liu, S., R. Bold, P. Plumstead and P. Selle. 2015. Effects of 500 and 1000 FTU/kg phytase supplementation of maize-based diets with two tiers of nutrient specifications on performance of broiler chickens. Anim. Feed Sci. Tech., 207: 159-167. https://doi.org/10.1016/j.anifeedsci.2015.06.002

Liu, S., D. Cadogan, A. Péron, H. Truong and P. Selle. 2014. Effects of phytase supplementation on growth performance, nutrient utilization and digestive dynamics of starch and protein in broiler chickens offered maize, sorghum- and wheat-based diets. Anim. Feed Sci. Tech., 197: 164-175. https://doi.org/10.1016/j.anifeedsci.2014.08.005

Loop, S.A., K.G.S. Lilly, L.K. Shires, C.K. Gehring, K.R. Beaman, M.E. Persia and J.S. Moritz. 2012. The phytase analytical activity of pelleted diets may not adequately describe efficacy in the bird. J. Appl. Poult. Res., 21: 492–501. https://doi.org/10.3382/japr.2011-00384

Mondal, M., S. Panda and P. Biswas. 2007. Effect of microbial phytase in soybean meal based broiler diets containing low phosphorus. Int. J. Poult. Sci., 6(3): 201-206. https://doi.org/10.3923/ijps.2007.201.206

Moss, A.F., P.V. Chrystal, H.H. Truong, S.Y. Liu and P.H. Selle. 2017. Effects of phytase inclusions in diets containing ground wheat or 12.5% whole wheat (pre- and post-pellet) and phytase and protease additions, individually and in combination, to diets containing 12.5% pre-pellet whole wheat on the performance of broiler chickens. Anim. Feed Sci. Tech., pp. 139-150. https://doi.org/10.1016/j.anifeedsci.2017.09.007

National Research Council, 2011. The guide for the care and use of laboratory animals. Washington, DC, 2011. https://doi.org/10.1258/la.2010.010031

Naves, L.P., P. Rodrigues, L.D.V. Teixeira, E. Oliveira, M. Saldanha, R. Alvarenga, A. Corrêa and R. Lima. 2015. Efficiency of microbial phytase supplementation in diets formulated with different calcium: Phosphorus ratios,

supplied to broilers from 22 to 33 days old. J. Anim. Phys. Anim. Nutr., 99(1): 139-149. https://doi.org/10.1111/jpn.12186

National Research Council, 1994. Nutrient requirements of poultry, 9th edn. National Academy Press, Washington, DC.

Perdana, J., M. Fox, M. Schutyser and R. Boom. 2012. Enzyme inactivation kinetics: Coupled effects of temperature and moisture content. Food Chem., 133(1): 116-123. https://doi.org/10.1016/j.foodchem.2011.12.080

Powell, S., T. Bidner and L. Southern. 2011. Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium. Poult. Sci., 90(3): 604-608. https://doi.org/10.3382/ps.2010-01000

Qaisrani, S.N., M.M. van Krimpen, R.P. Kwakkel, M.W.A. Verstegen and W.H. Hendriks. 2015. Diet structure, butyric acid, and fermentable carbohydrates influence growth performance, gut morphology, and cecal fermentation characteristics in broilers. Poult. Sci., 94(9): 2152-2164. https://doi.org/10.3382/ps/pev003

Ravindran, V., A. Cowieson and P. Selle. 2008. Influence of dietary electrolyte balance and microbial phytase on growth performance, nutrient utilization, and excreta quality of broiler chickens. Poult. Sci., 87(4): 677-688. https://doi.org/10.3382/ps.2007-00247

Ravindran, V., P. Morel, G. Partridge, M. Hruby and J. Sands. 2006. Influence of an *Escherichia coli* derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poult. Sci., 85(1): 82-89. https://doi.org/10.1093/ps/85.1.82

Saleh, A.A., M. Elsawee, M.M. Soliman, R.Y.N. Elkon, M.H. Alzawqari, M. Shukry, A. Abdel-Moneim, and M.E. Eltahan. 2021. Effect of bacterial or fungal phytase supplementation on the performance, egg quality, plasma biochemical parameters, and reproductive morphology of laying hens. Animals, 11: 540. https://doi.org/10.3390/ani11020540

SASS, 2001. System for Windows, Release 8.2 (TS2MO) SAS Inst. Inc., Cary, NC, USA.

Selle, P., V. Ravindran, A. Caldwell and W. Bryden. 2000. Phytate and phytase: Consequences for proteinutilization.Nutr.Res.R.,13(02):255-278. https://doi.org/10.1079/095442200108729098

Singh, A., C. Walk, T. Ghosh, M. Bedford and S. Haldar. 2013. Effect of a novel microbial phytase on production performance and tibia mineral concentration in broiler chickens given low calcium diets. Br. Poult. Sci., 54(2): 206-215. https://doi.org/10.1080/00071668.2013.775403

dos Santos, T.T., S.S.M.R. Bedford and C.L. Walk. 2013. Effect of high phytase inclusion rates on performance of broilers fed diets not severely limited in available phosphorus. Asian-Austral. J. Anim. Sci., 26(2): 227-232. https://doi.org/10.5713/ajas.2012.12445

Viveros, A., A. Brenes, I. Arija and C. Centeno. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. Poult. Sci., 81(8): 1172-1183. https://doi.org/10.1093/ps/81.8.1172

Walk, C., M. Bedford, T. Santos, D. Paiva, J. Bradley, H. Wladecki, C. Honaker and A. McElroy. 2013. Extra-phosphoric effects of superdoses of a novel microbial phytase. Poult. Sci., 92(3): 719-725. https://doi.org/10.3382/ps.2012-02727

Watson, B.C., J.O. Matthews, L.L. Southern and J.L. Shelton. 2006. The effects of phytase on growth performance and intestinal transit time of broilers fed nutritionally adequate diets and diets deficient in calcium and phosphorus. Poult Sci., 85(3): 493-497. https://doi.org/10.1093/ps/85.3.493

Wilkinson, S.J., C.L. Walk, M.R. Bedford and A.J. Cowieson. 2013. Influence of conditioning temperature on the post-pellet recovery and efficacy of 2 microbial phytases for broiler chicks. J. Appl. Poult. Res., 22(2): 308-313. https://doi.org/10.3382/japr.2012-00681

Wodzinski, R.J., and A. Ullah. 1995. Phytase. In: Adv. Appl. Microbiol., 42: 263-302. https://doi.org/10.1016/S0065-2164(08)70375-7

Ziggers, D., 2003. Die determines pellet production. Feed Technol., 7: 17-19.