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The potential Enhancing Effect of both Phytase and β-xylanase Enzymes on Performance, Bone Mineralization and Nutrient Absorption in Broiler Chicken

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Abstract

The response to dietary fortification of both phytase and β-xylanase enzymes was investigated in broilers (Ross 308, No.=300) that were allocated into 3 different treatments; control (G1) was fed the basal diet only, the second group (G2) was fed the basal diet supplemented with 100 g/ton feed phytase 5000 FTU in combination with 250 g /ton feed β-xylanase, while the third group (G3) was fed the basal diet fortified with 50 g/ton feed phytase 10000 FTU plus 250 g /ton feed β-xylanase. When compared to the control diet, our results exhibited that co-feeding either Phytase 5000 or 10000 FTU with xylanase improved both growth performance and feed utilization. Moreover, tibia Ca and P% were significantly higher ($P \leq 0.05$) when diets were supplemented with phytase 5000 and phytase 10000 FTU in comparison to control group indicating that each product could improve bioavailability of these minerals. Serum phosphorus (P) was greater with phytase inclusions in both doses, when compared to the control group ($P \leq 0.05$). Microscopical examination revealed a significant increase in the villus height of duodenum and jejunum of phytase and xylanase combination treated groups in a concentration dependent manner, when compared with the control group. Chemical examination of litter disclosed an improvement in litter moisture, reduced total nitrogen (N), and phosphorus percent in groups supplemented with phytase and xylanase enzymes. In conclusion, the use of combined supplementation of xylanase and phytase (5000 or 10000 FTU) could improve growth performance, P metabolism, bone mineralization and the intestinal nutrient absorption of broiler chicken.

KEYWORDS
Broiler chicken, Phytase, Growth performance, Tibia, Intestinal absorption

INTRODUCTION

Phytate phosphorus is considered as one of the anti-nutritional factors that have a detrimental influence on nutrient utilization and productive performance in poultry (Sebastian *et al.*, 1998; Woyengo and Nyachoti, 2013). The phytate and its associated nutrients, particularly minerals and amino acids, are poorly utilized by poultry, probably because of the insufficient endogenous phytase activity in poultry intestine (Ravindran *et al.*, 1995; Humer *et al.*, 2015). Most of the total phosphorus (P) in plants is found as phytate P (Ravindran *et al.*, 1994), which is discharged into the excreta with implications of environmental pollution. Supplementing higher levels of inorganic P (Mono- and- di-calcium phosphates) to meet the birds' requirements is not always cost-effective and further contributes to environmental pollution (Sharpley *et al.*, 2007).

Exogenous phytases are one of the most used feed enzymes in poultry diets not only to reduce inorganic P supplementation, but also to improve nutrient utilization and to reduce nutrient excretion into the environment. Phytase degrades the phytate and subsequently releases the phytate-associated nutrients, particularly minerals (P, zinc, iron) in addition to amino acids and carbohydrates (Selle and Ravindran, 2007; Romano and Kumar, 2018). Traditionally, exogenous phytases have been used to be added to poultry diets at a dose of 500 phytase units (FTU)/kg diet as a standard inclusion rate. Recently, higher doses of phytases have proven additional improvements in broiler performance and health (Karadas *et al.*, 2010; Pirgozliev *et al.*, 2011). However, poultry growth performance and nutrient utilization may not always benefit from higher phytase concentrations (Kim *et al.*, 2017; Romano and Kumar, 2018).

Moreover, Non-starch polysaccharides (NSP), also known as anti-nutritional factors, are abundant in corn-soybean meal-based poultry diets with levels ranging from 100 g/kg in cereals to 220 g/kg in soybean meal (Knudsen, 1997). Dietary carbohydrases reduce the viscosity and increase the nutritional value of diets rich in NSP. Carbohydrases may not directly benefit feedstuffs with less viscosity, such as maize and SBM (Olukosi *et al.*, 2007). However, other nutrients, particularly protein, fat, and starch, are trapped by the insoluble components of NSP found in various cell wall structures. Xylanases make these nutrients more available to digestive enzymes (Wu *et al.*, 2004). Additionally, the exogenous xylanases may also improve the effectiveness of phytase. The cell wall of corn and soybean meal entraps phytate P,

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making phytate inaccessible to phytase, so the absence of carbohydrases in poultry diets may reduce phytase enzyme efficiency (Zeller *et al*., 2015). Similar to this, carbohydrases won't be able to liberate any other nutrients that might be bound with the phytate molecule if phytase is insufficient (Olukosi *et al*., 2007). There is a synergistic effect on growth performance between phytase and carbohydrases (Cowieson and Adeola, 2005; Juanpere *et al*., 2005). Furthermore, when phytase and xylanase were fed together to broilers, growth performance was superior to that of phytase fed alone (Selle *et al*., 2009). On contrary, other reports have indicated that xylanase enzyme showed some limited potential to enhance phytase efficacy (Karimi *et al*., 2013).

In addition, chickens' heath and performance are greatly affected by gastrointestinal tract that is constantly exposed to different agents including the dietary factors. These dietary factors have impact on the healthy environment of the intestine including enterocytes, mucous secretion and immune response that affect the intestinal absorption of nutrients. Moreover, sound and proper gut morphology plays an essential and critical role in its absorptive activity and affects the activity of digestive enzymes, in addition to the nutrients transport through the epithelium. Moreover, villus height can indicate the gut health and function (Swatson *et al*., 2002). Confined studies have shown the influence of phytase supplementation on gut morphology. In the current study, microscopical examination and histomorphometeric analysis had been performed to assess the influence of combined feeding of phytase and xylanase on the intestinal morphology.

Based on the above context, the incorporation of different concentrations of phytase in poultry diets still deserves more investigation to fully understand whether it drives further improvements in bird performance and mineral metabolism. Moreover, potential synergism between phytase and xylanase under various dietary characteristics should be further elucidated. Therefore, the main goal of this experiment was to evaluate the influence of dietary combined supplementations of phytase 5000 or 10000 FTU plus xylanase on growth performance, bone mineralization, dressing percentage, gut morphology and certain blood indices in broilers.

MATERIALS AND METHODS

Birds and experimental diets

The institutional Animal Care and Use Committee of Cairo University authorized the protocol for this experimental study, which was conducted at the Poultry and Animal Research Center of the Faculty of Veterinary Medicine, Cairo University (Vet-CU03162023683).

Three hundred one-day-old, unsexed Ross-308 broiler chicks were randomly allocated into 3 dietary groups. Each group consisted of 100 broiler chicks which were allocated to 4 replicates of 25 chicks. Broiler chicks were housed in a floor pen bedded

Table 1. Formulations and chemical analysis of broiler diets throughout the experimental period.

Ingredients %	Experimental diets*								
	Control (G1)			Group 2 (G2)			Group 3 (G3)		
	Starter diet	Grower diet	Finisher diet	Starter diet	Grower diet	Finisher diet	Starter diet	Grower diet	Finisher Diet
Yellow corn	54.25	59.4	65	55.53	60.8	66.02	55.35	60.8	66.02
High fat soybean meal	12	23	24.45	8	14	19.5	8	14	19.5
Soybean meal (46% CP)	29	13	6	32.5	21.17	10.5	32.5	21.17	10.5
Monocalcium phosphate	1.6	1.5	1.5	1.1	1	1	1.1	1	1
Limestone	1.8	1.7	1.6	1.7	1.6	1.5	1.7	1.6	1.5
Common salt	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28	0.28
Sod bicarbonate	0.22	0.2	0.2	0.22	0.2	0.2	0.22	0.2	0.2
DL-Methionine	0.25	0.22	0.22	0.25	0.22	0.22	0.25	0.22	0.22
L-Lysine HCL	0.15	0.25	0.3	0.12	0.25	0.3	0.12	0.25	0.3
Toxin binder	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
LincoMix[1]	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Biostrong[2]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Maxiban[3]	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Phytase SPV	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01
Enziver	0	0	0.01	0.03	0.03	0.03	0.03	0.03	0.03
Broiler premix[4]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total (%)	100	100	100	100	100	100	100	100	100
Chemical analysis									
ME (Kcal/kg)	2973.5	3112.8	3203.84	3008.29	3112.03	3219.6	3008.29	3112.03	3219.6
Crude protein (%)	23.1	21.17	19.08	23.08	21.08	19.19	23.08	21.08	19.19
Crude fat (%)	3.14	3.9	4.12	2.94	3.42	3.87	2.94	3.42	3.87
Crude fiber (%)	2.72	2.96	2.93	2.62	2.72	2.8	2.62	2.72	2.8
Calcium (%)	1.01	0.94	0.89	1.02	0.95	0.89	1.02	0.95	0.89
Available phosphorus (%)	0.49	0.45	0.44	0.48	0.45	0.43	0.48	0.45	0.43

*G1, Control - basal diet; G2, basal diet + xylanase 250g/Ton of feed+ phytase 100g/Ton of feed; G3, basal diet + xylanase 250g/Ton of feed+ phytase 50g/Ton of feed.
[1]Anticlostridian (Zoeits); [2]Phytobiotics (Delacon); [3]Anticoccidian (Elanco).
4Per Kg Vitamin-mineral premix: 1200000 IU Vit A; 350000 IU Vit. D3; 4000 mg Vit. E; 250 mg Vit. B; 1,800 mg Vit. B; 2,600 mg Vit. B6; 3.2 mg Vit. B12; 450 mg Vit. K; 3,4.5g Nicotinic acid; 1.5 g, Ca pantothenate; 120 mg Folic acid; 5mg Biotin; 55 mg Choline chloride; 3g Fe; 2 g Cu; 10 g Mn; 8 g Zn; 120 mg I; 40 mg Co.

with a wheat straw litter of 10 cm depth, with a bird density at 10 birds/m^2. Before the feeding trial began, the house was cleaned and disinfected. Throughout the whole trial, light was available 24 hours a day, including natural light during the day and artificial light at night. Birds were vaccinated against Avian Influenza, Newcastle, Infectious Bronchitis and Gumboro diseases. Three experimental diets were formulated to be iso-caloric and iso-nitrogenous to meet the nutrient recommendations according to the Manual Standards of the broiler Ross-308 breed (Aviagen, 2022) as follows: control (G1) fed the basal diet with 100% of Ca and available P (AvP) requirements; (G2), the second group, received a basal diet with 100 g phytase 5000 FTU /ton feed plus 250 g /ton feed β-xylanase and the third group (G3) was given a reformulated basal diet with 50 g phytase 10000 FTU /ton feed in combination with 250 g/ton feed β-xylanase. The formulations and chemical analysis of the experimental diets are given in Table 1. The experimental diets were crumbled in the starter stage, pelleted in grower and finisher stages and were offered ad libitum together with freshwater over the 35 days of the study. The commercially available microbial phytase enzyme (Phytase SPV®, SPV Company, Egypt) and xylanase enzyme (Enziver®, Zoetis, USA) were used in this study. The phytase enzyme originated from a pathogenic *Escherichia coli* and *Aspergillus Niger*. The Enziver® originated from *Aspergillus, Trichoderma, Bacillus* and a pathogenic *E. Coli* manufactured by submerged fermentation and acting synergistically on non-starch polysaccharides for aiding in liberation of in-available energy. The nutrient releasing ability of phytase and xylanase (matrix values) were considered when formulating the experimental diets (Shelton *et al*., 2004). Extra nutrients which became available after adding phytase (500 FTU) to the diet included Ca (0.10%), AvP (0.12%), ME (55 kcal/kg diet), protein (0.25%), threonine (0.013%), methionine (0.001%), and lysine (0.012%). For Enziver ®, extra nutrients included Ca (0.05%), AvP (0.05%), ME (80 kcal/kg diet), protein (0.50%), threonine (0.014%), methionine (0.011%), and lysine (0.029%).

Sampling and measurements

Growth performance

Body weight changes, average body weight gain and feed consumption were recorded weekly and feed conversion ratio (FCR) was calculated in accordance with these measurements.

Blood profile, Carcass quality and Bone mineral assay

Five birds from each replicate (20 birds for each treatment) were randomly chosen at the end of the experiment to be used for blood sampling, carcass traits, and tibia mineral assay. The birds were slaughtered, and the draining blood was collected from the wing vein directly into heparinized sterile tubes. For the plasma analysis, samples were centrifuged at 15000 rpm for 3 min to isolate the plasma. The resultant plasma was transferred to eppendorf tubes and stored at -20°C until further analyses. The tests were determined with an automated biochemical analyser (EMCLAB UV/VIS Spectrophotometer, German) using the respective Diagnostic Kits. The plasma was analyzed for total cholesterol (Richmond, 1973) ; triglycerides (Fassati, 1982); high-density lipoproteins cholesterol (Seguchi *et al*., 1995); total protein (Gornall *et al*., 1949); albumin (Doumas *et al*., 1971); total bilirubin (Jacobs *et al*., 1996); creatinine (Gerald *et al*., 1975); urea (Machado and Horizonte, 1958); uric acid (Prætorius, 1949); calcium (Gindler and King, 1972) and inorganic phosphorus (El-Merzabani *et al*., 1977). Also, the activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were measured according to the method of Smith and Taylor (1973). The carcass traits (carcass weight, dressing percentage, breast yield, drumstick yield and thigh yield) were measured according to North (1984). Tibia samples were removed from the soft tissues and extracted with ether for 6 hours. They were then dried overnight at 100°C and ashed in a muffle furnace for 15 hours at 540°C (Taheri and Taherkhani, 2015). The percentage of tibia ash was calculated according to AOAC (2000). In addition, total Ca and P were determined according to Gindler and King (1972).

Histomorphometry of small intestine and bursa

Intestinal samples including duodenum, jejunum, and ileum, in addition to hepatic tissue and Bursa of Fabricius from all groups were prepared following the routine tissue processing for paraffin sections that includes rinsing in tap water, fixation in 10% neutral buffered formalin (10% NBF), clearance in xylene, embedding in paraffin wax, staining at 3- 5 µm thickness, deparaffinization and staining with hematoxylin and eosin (H& E) stain. The stained sections were examined via a light microscope (LEICA DM500). Moreover, attached camera (LEICA ICC50 HD) was used for imaging, and examination of the captured images was performed by image analysis software (Leica microsystems, LAS version 3.8.0 [build: 878] Leica Ltd image analyzer computer system) at Veterinary Medicine College, Cairo University, Egypt.

Chemical Examination of Litter

For chemical analysis of the litter, 500g of litter samples were gathered and mixed from four sites of each group. 10g of litter was heated for 24 hours at 105°C in a hot air oven to assess the percentage of litter moisture (Tran *et al*., 2015). Kjeldahl method was used to determine the total nitrogen (Jackson, 1973). The technique outlined by Houba *et al*. (1995) was used to calculate the total phosphorous. Calcium was determined using the method described by Nisar *et al*. (2014).

Statistical analysis

The obtained data were statistically analyzed using SAS Software by the one-way ANOVA. The comparison of means was done by Tukey test; Significance was pre-set at $P \leq 0.05$. Data are presented as means ± standard error of means (SEM).

RESULTS

Growth performance

The overall results of combination of phytase and xylanase enzymes on broiler performance are illustrated in Table 2. Our results revealed that dietary supplementation of β-xylanase and Phytase 10000 FTU (G3) significantly increased the final body weight (FBW), body weight gain (BWG) and improved the feed conversion ratio (FCR) compared to the control one, but there was no significant difference in feed intake (FI). The xylanase plus phytase 5000 FTU (G2) increased the FBW, BWG, and improved significantly FCR compared to the control group.

Serum profile parameters

The effect of dietary phytase-xylanase inclusion on broiler plasma metabolic profile is summarized in Table 3. Date showed a non-significance change in both energy and enzymatic profiles

in phytase-xylanase supplement groups and control. While phytase-xylanase inclusions in both doses (50 and 100 g/Ton diet) increased total plasma protein and phosphorus significantly when compared with control.

Carcass traits

The results of dietary supplementation of phytase 5000 and 10000 FTU plus β-xylanase on carcass characteristics are shown in Table 4. The data of the current study demonstrated that the dietary supplementation of phytase 5000 or 10000 FTU attained no significant impact on the dressing percentage, breast yield, drumstick yield as well as thigh yield compared to control group. However, there are numerical improvements associated with applying phytase and xylanase in combinations on processing yields in this study.

Tibia mineral content, P and Ca

The effect of dietary phytase-xylanase inclusion either 5000 or 10000 FTU plus β-xylanase on broiler tibia ash, P and Ca concentrations is illustrated in Table 5. The current findings revealed that the tibia ash, P and Ca contents were significantly higher ($P \leq 0.05$) when broiler chickens diets reformulated with phytase 5000 at 100 g/ton diet or phytase 10000 at 50g/ton diet.

Table 2. Influence of dietary fortification of phytase and xylanase on final performance indices and feed utilization of broilers.

Groups*	Body weight (g)	Weight gain (g)	Feed intake (g)	FCR
G1	2133[b]	2091[b]	3386	1.62[a]
G2	2258[ab]	2216[ab]	3289	1.49[b]
G3	2363[a]	2321[a]	3390	1.46[b]
SEM[1]	33.38	33.38	21.18	0.02
p- value	0.00	0.00	NS[2]	0.00

a and b denote separate superscript points in the same column to significant differences ($P \leq 0.05$). *G1: Control - basal diet; G2: basal diet +xylanase 250g/Ton of feed + phytase 100g/Ton of feed; G3: basal diet +xylanase 250g/Ton of feed +phytase 50g/Ton of feed. FCR, feed conversion ratio.
[1]SEM: Standard error of mean. [2] NS: Not significant.

Table 3. Influence of dietary supplementation of phytase and xylanase on blood biochemical profile of broiler chickens for 35 days.

Groups*	Energy profile			Protein profile						Mineral profile		Enzyme profile	
	T-Cho (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)	T-Pro (g/dL)	Alb (g/dL)	T-Bil (mg/dL)	Cre (mg/dL)	UA (mg/dL)	BUN (mg/dL)	Ca (mg/dL)	IP (mg/dL)	ALT (IU/L)	AST (IU/L)
G1	135.2	97.8	55.2	3.34[b]	1.78	0.12	0.56	4.62	3.33	12.74	4.6[b]	9.44	278.4
G2	108.4	89.8	35.8	4.09[a]	1.76	0.14	0.64	4.88	3.07	12.04	5.24[a]	8.61	236.6
G3	119.7	85.6	42.6	4.15[a]	1.74	0.16	0.67	5.02	3.24	11.93	5.11[a]	8.79	301.4
SEM[1]	5.56	8.57	13.89	0.11	0.08	0.03	0.06	0.26	0.24	0.44	0.3	1.45	57.98
p- value	NS	NS	NS	<0.0001	NS	NS	<0.0001	NS	NS	NS	0.01	NS	NS

a and b denote separate superscript points in the same column to significant differences ($P \leq 0.05$).*G1, Control - basal diet; G2, basal diet +xylanase 250g/Ton of feed + phytase 100g/Ton of feed; G3, basal diet +xylanase 250g/Ton of feed +phytase 50g/Ton of feed.T-Cho: total cholesterol; HDL-C: high-density lipoproteins cholesterol; TG: triglycerides; T-Pro: total protein; Alb: albumin; T-Bil: total bilirubin; Cre: creatinine; UA: uric acid; BUN: urea; Ca: calcium; IP: inorganic phosphorus; ALT/GPT: alanine aminotransferase; AST/GOT: aspartate aminotransferase
[1] SEM: Standard error of mean [2] NS: Not significant.

Table 4. Influence of dietary supplementation of phytase and xylanase on carcass traits of broiler chickens.

Groups*	Dressing (%)	Breast (%)	Thigh (%)	Drumstick (%)	Tibia (%)
G1	73.82	30.76	28.12	13.01	2.44
G2	74.83	32.22	28.11	13.28	2.76
G3	75.96	31.49	27.36	13.13	2.57
SEM[1]	0.88	0.49	0.36	0.12	0.1
p- value	NS[2]	NS	NS	NS	NS

Significance was indicated at $P \leq 0.05$. *G1: Control - basal diet; G2: basal diet +xylanase 100g/Ton of feed + phytase 100g/Ton of feed; G3: basal diet +xylanase 100g/Ton of feed +phytase 50g/Ton of feed.
1 SEM: Standard error of mean. 2 NS: Not significant.

Table 5. Effect of dietary supplementation of phytase and xylanase on bone mineral assay of broiler chickens at the end of the experiment.

Groups*	Tibia Ash (%)	Tibia Ca (%)	Tibia P (%)
G1	40.86[c]	21.00[c]	7.18[c]
G2	41.66[b]	22.10[b]	7.66[b]
G3	42.24[a]	22.84[a]	8.64[a]
SEM[1]	0.17	0.2	0.17
p- value	0.00	<0.0001	<0.0001

a, b and c denote separate superscript points in the same column to significant differences ($P \leq 0.05$). *G1: Control - basal diet; G2: basal diet +xylanase 250g/Ton of feed + phytase 100g/Ton of feed; G3: basal diet +xylanase 250g/Ton of feed +phytase 50g/Ton of feed.
[1]SEM: Standard error of mean[2]NS: Not significant.

Histomorphometry of small intestine

Morphometric evaluation of the small intestine was performed for the 3 treated groups. The intestinal villus length (height) was measured for duodenum, jejunum and ileum from the tip of the villus till the apical part of the intestinal crypt. Our finding revealed a clear difference in the intestinal villus height of the duodenum between the control group and both treatment (*, P. Value <0.005) in a concentration dependent manner. The villi were higher in the second group, while the highest villi were seen in the third group (Fig. 1). This difference was also observed in the samples obtained from the jejunum (Fig. 2). There was a slight difference between the control (G1) and the lower concentration treated group (G2), while a significant difference (*, P. Value <0.005) was noticed between the control group and the higher concentration of phytase and xylanase combination treated group (G3). However, no markable difference was detected between the 3 groups in the villi height of ileum (Fig. 3). Moreover, villus width of duodenum, jejunum and ileum showed no obvious difference between the control and the treated groups. In the current study, no significance was recorded regarding the villus width; however, the villus width in group (3) was slightly less than those of group (1) and group (2). Moreover, no change was observed in the hepatic tissue and bursa of Fabrious. The hepatic tissue histological architecture appeared normal showing the hepatic cords of hepatocytes radiating from the central vein

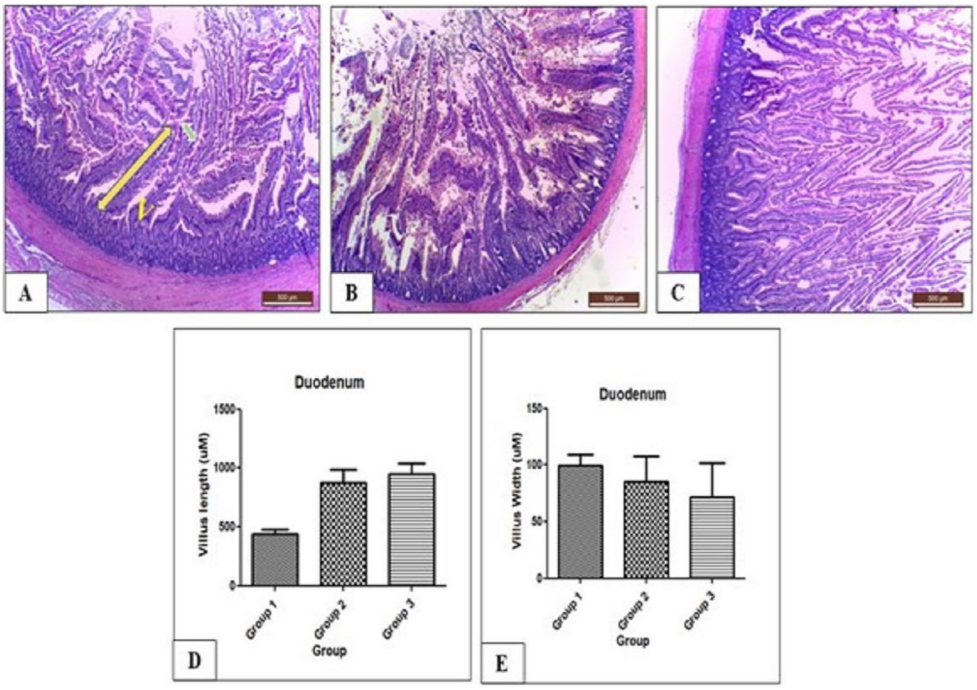

Fig. 1. A Photomicrograph of the duodenum (H& E stain, X40). (A, B, C) showing the duodenum of G1, G2 and G3, respectively. (D) shows the difference in the villus length between G1, G2 and G3, while (E) shows the difference in the villus width between G1, G2 and G3. The villus length is measured from the tip of the villus till the apical part of the intestinal gland (yellow arrow), while the width of the villus is measured as shown by the green arrow.

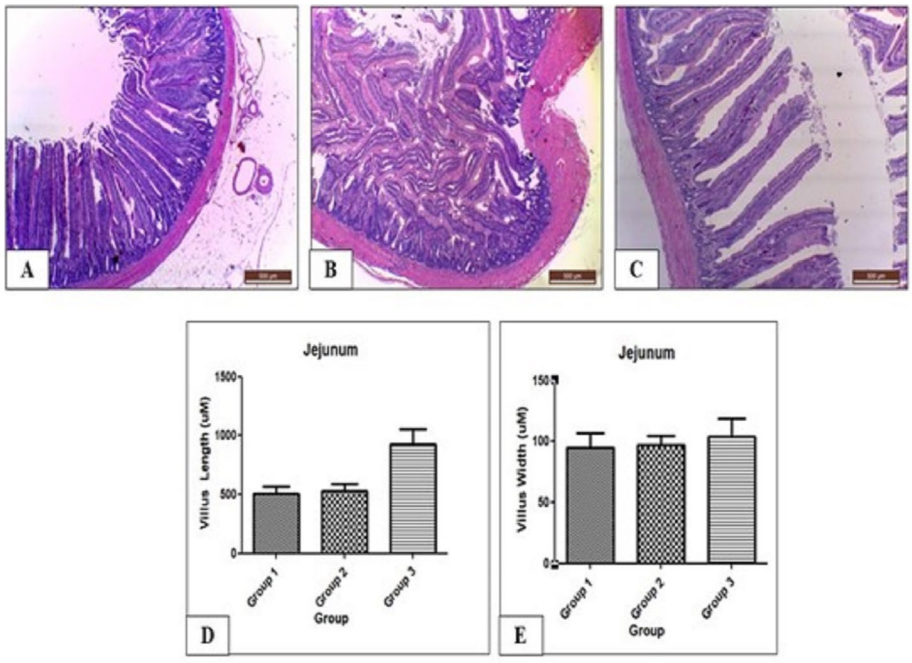

Fig. 2. A Photomicrograph of the jejunum (H& E stain, X40). (A, B, C) showing the jejunum of G1, G2 and G3, respectively. (D) shows the difference in the villus length between G1, G2 and G3, while (E) shows the difference in the villus width between G1, G2 and G3.

that appeared engorged with blood in both groups treated with phytase. On the other hand, the bursa of Fabricius as a primary lymphatic organ was examined, and our results exhibited the normal histological structure in all treated groups. The wall of the bursa appeared formed of mucosa, propria submucosa, muscularis and serosa. The mucosa formed the plicae (folds) at different length, and the propria of each plica was filled with follicles that had a darker cortex because of densely packed lymphocytes and a lighter less dense medulla (Fig. 4A, B, C). In addition, the microscopical examination of the hepatic tissue from all groups revealed a normal microscopical liver architecture; however, blood vessels and sinusoids appeared engorged with blood in both phytase xylanase combination treated groups (Fig. 4D, E, F).

Chemical Examination of Litter

At day 15, litter moisture was significantly reduced in G2 compared to G1 and G3. At day 35, moisture content was lower in G2 compared to G1 and G3; however, the difference was not significant (Table 6). The results showed that phytase enzyme supplementation at inclusion rate of 50 g/ton reduced the total nitrogen (N) content of litter at the end of the cycle compared to birds fed the basal diet (Fig. 5). When compared to the control group, (N) and (P) contents of litter were positively influenced by the addition of the phytase enzyme, but calcium ratios were not affected.

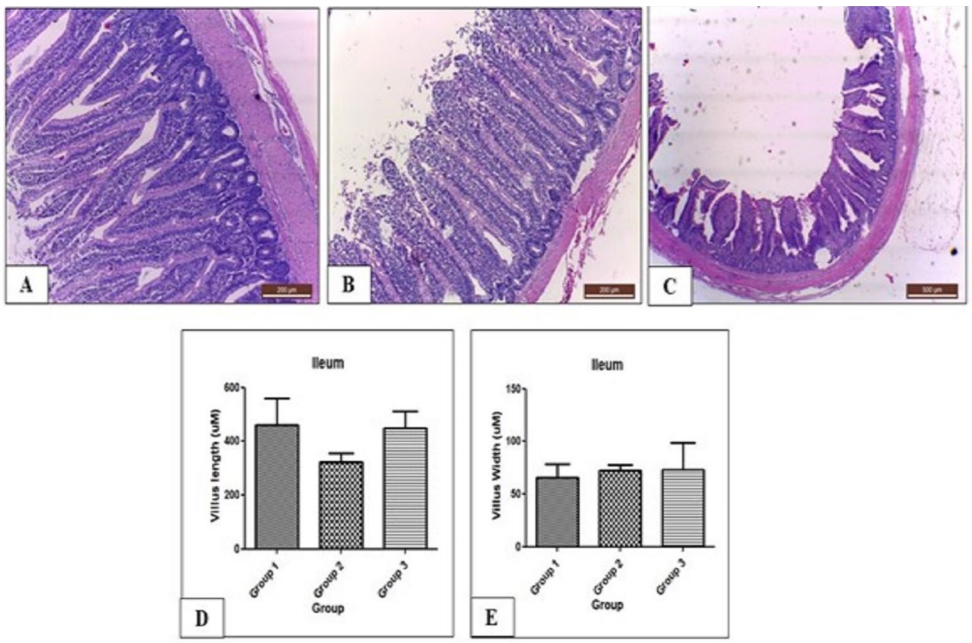

Fig. 3. A Photomicrograph of the ileum (H& E stain, X40). (A, B, C) showing the ileum of G1, G2 and G3, respectively. (D) shows the difference in the villus length between G1, G2 and G3, while (E) shows the difference in the villus width between G1, G2 and G3.

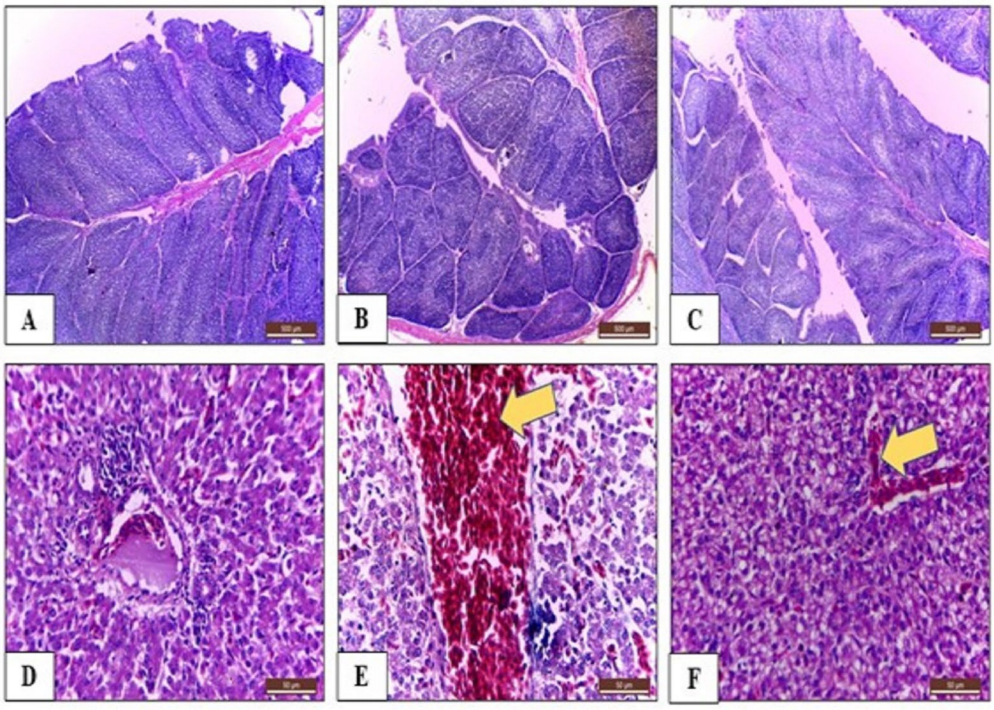

Fig. 4. A Photomicrograph showing (A, B, C) the Bursa of Fabricious (H& E stain, X40) that exhibits the normal histological structure in all groups, respectively, and (D, E, F) the hepatic tissue (H& E stain, X400) obtained from all groups showing the normal hepatic architecture, while the 2 groups treated with phytase reveal blood vessels and hepatic sinusoids engorged with blood.

Table 6. Moisture content per 100 gram poultry litter at 15 & 35 days.

Groups*	Moisture %	
	15 days	35 days
G1	34.37[a]	24.35
G2	17.14[b]	18.85
G3	33.42[a]	28.84
SEM[1]	3.22	1.89
P value	0.03	0.10

a and b denote separate superscript points in the same column to significant differences ($P \leq 0.05$). *G1: Control - basal diet; G2: basal diet +xylanase 250g/Ton of feed + phytase 100g/Ton of feed; G3: basal diet +xylanase 250g/Ton of feed +phytase 50g/Ton of feed.
[1]SEM: pooled standard error of the mean. [2]NS: Not significant.

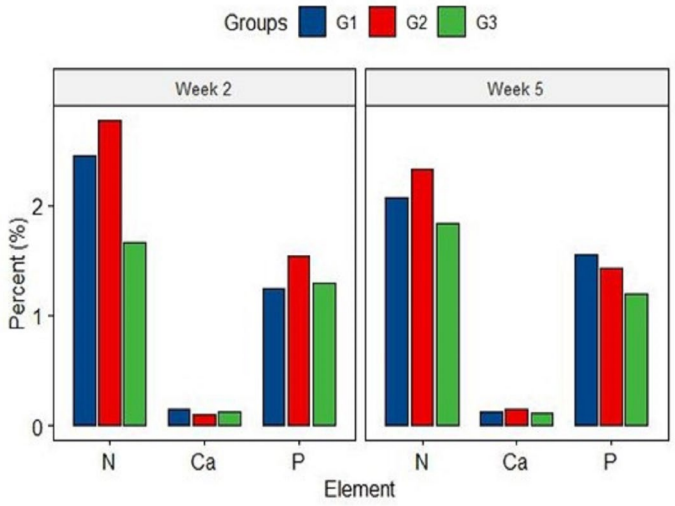

Fig. 5. shows the amount of total nitrogen (N), phosphorus (P) and calcium (Ca) per 100 grams of litter of each experimental group.

DISCUSSION

The study's findings revealed that the overall growth performance parameters were improved by supplementing the diet with either phytase 5000 FTU or 10000 FTU in addition to β-xylanase. The enhanced growth induced by the co-feeding of xylanase and phytase highlights the role of phytase in improving nutrient utilization resulting probably from phytate P hydrolysis and ameliorating the anti-nutritional effects of phytate (Selle and Ravindran, 2007; Dersjant-Li *et al*., 2015; Song *et al*., 2019). Previously, Tang *et al*. (2012) reported that using phytase at level of 500 phytase units/kg of diet improved growth performance parameters more than the control group. Further, the co-feeding of xylanase plus either the phytase 5000 or high concentration of phytase 10000 FTU synergistically improved the feed utilization efficiency as indicated by an improvement in FCR in the current research as compared to the control one. Additionally, it has been shown that xylanase can improve the efficiency of P utilization (Kim *et al*., 2005). This is most likely because it can release the available P that has been trapped within the arabinoxylan-complex (Frolich and Asp, 1985). It has been suggested that the two enzymes, xylanase and phytase, worked better together than they did separately (Cowieson and Adeola, 2005). Moreover, the reason for the enhanced performance of birds fed diets combining xylanase and phytase could be explained by the improvement in P and Ca digestibility caused by phytase. Lee *et al*. (2010) demonstrated that both phytase and xylanase enzymes can improve the gut health in term of decreasing the intestinal viscosity which enhances the performance of broilers.

Blood biochemical data is a reliable sign that reflects the changes that occur to the animal due to dietary supplementation. The present findings are in part consistent with those of Abd El-Hack *et al*. (2018) who reported increased protein utilization and retention by phytase supplementation. These findings could be linked to its effect on reducing nitrogen-phytate complex, which limited protein digestibility. Likewise, Manobhavan *et al*. (2016) recorded an increase in blood phosphorus level when phytase enzymes were added to the diet. It can be indicated that birds have a significant capacity to keep more phosphorus from the diet, reflecting this higher phosphorus in their plasma. In contrast, Deepa *et al*. (2011) indicated that the addition of phytase may enhance phosphorus retention due to its positive effect on increasing phosphorus molecules from the phosphorus-phytate-complex. In this study, there were no significant differences in ALT and AST activity between treatments implying that these additives had no negative effects on liver function. Thus, adding phytase had no adverse impact on liver health. Previously, Viveros *et al*. (2002) observed that phytase addition (Natuphos 500) in broiler reduced serum AST and ALT activities.

Dietary fortification with either phytase 5000 or 10000 FTU plus β-xylanase attained no significant increase on carcass parameters. These results were in accordance with earlier research by Poernama *et al*. (2021) which stated that carcass traits and ratio of breast muscle are not significantly affected by combined addition of phytase and xylanase. Broiler carcass yield and dressing percentage were found to be unaffected by the combination of phytase and xylanase (Bin Baraik, 2010). They also noted that the percentage of commercial cuts (drumstick, breast, and thigh) didn't differ statistically from one another. Additionally, these outcomes matched those recently attained by Younis (2013). However, there are numerical improvements associated with applying phytase and xylanase in combinations on processing yields in this study. This improving impact of the co-feeding of xylanase plus the phytase 5000 FTU or a concentrated phytase 10000 FTU on processing yields was supported by other researchers (Selle *et al*., 2003). The improvement in carcass traits could stem from phytase and β-xylanase synergistic effect on the decrease of the anti-nutritional properties of phytate and NSPS leads to optimal nutrient utilization and better growth performance.

In comparison to the control, dietary supplementation with phytase 5000 at 100 g/ton diet or phytase 10000 at 50g /ton diet plus β-xylanase enzyme significantly increased the tibia ash, P and Ca contents. Our findings supported those of Karimi *et al*. (2013), who found that bone mineralization was enhanced in birds fed diet supplemented with 500 FTU phytase per kg of feed. Phytase supplementation improved the metabolism of P from the phytic-acid complex, which was a previous explanation for this enhancement (Simons *et al*., 1990). Furthermore, using higher concentration of phytase might augment the Ca and P release from the phytate molecule and contribute to improving tibia mineralization (Olukosi *et al*., 2013). Additionally, Avila *et al*. (2012) claimed that supplementation of broiler diets with both phytase and β-xylanase enzymes improved mineralization. Feeding phytase in combination with xylanase may augment its effectiveness as carbohydrases may improve the efficiency of phytase by increasing the availability of phytate (Juanpere *et al*., 2005). Nonetheless, the increase of broilers tibia mineralization by dietary addition of phytase and xylanase was comparable to the results obtained by other studies (Cowieson *et al*., 2006; Olukosi *et al*., 2008). So, this positive impact might be due to the synergism between phytase and β-xylanase, which leads to an increase in the availability of both P and Ca and consequently deposit in bones resulting in improvement in tibia Ca and P contents, which agrees with other studies (Olukosi *et al*., 2007; Bedford and Masey O'Neill *et al*., 2012).

The intestinal morphology plays an important role as indicator for the intestinal absorption activity and function. The effectiveness of digestion and absorption is correlated with the height of the intestinal villi (Swatson *et al*., 2002). Cowieson *et al*. (2009) noticed that supplementation of exogenous enzymes such xylanase and phytase improved digestion and nutrient absorption. Taking all together, our findings revealed that phytase-xylanase combination improved the villus height in both duodenum and jejunum samples in a concentration dependent manner. This re-

sult is consistent with what has been reported by Kim *et al.* (2021) who noticed the improvement in villi height and crypts depth in the group fed on the dietary supplemental exogenous multi-enzymes containing phytase, when compared with the basal diet. Pekel *et al*. (2017) also recorded the increase of the height of the jejunum villi ($P<0.05$) after phytase supplementation. Moreover, jejunum and ileum villi were longer in chickens fed diets with higher dietary phytase level, when compared to those fed diet with lower phytase level (Smulikowska *et al*., 2010). Although Smulikowska *et al*. (2010) recorded the increase of villi width in jejunum after phytase supplementation, Chen *et al*. (2015) assumed that widening of intestinal villi may indicate insufficient absorption area and more proliferation and accumulation of gut-associated immune tissue that may indicate the compromised gut health. Taking all intestinal findings together, the microscopical examination and histomorphometric analysis of the current study reveals the improving effect of phytase-xylanase combination on the height of the intestinal villi, particularly in duodenum and jejunum, which subsequently would have a positive impact on improving nutrients absorption in those intestinal parts.

In this study, dietary supplementation of phytase enzyme reduced litter moisture contents, total nitrogen and phosphorus. This finding comes in the same context with Sharma *et al*. (2016) who used phytase enzyme at the inclusion rate of 500, 1000, and 1500 FTU, and noticed no significant difference in litter moisture but recorded an improvement in litter score. Because microbial enzymes may break down urea and uric acid in poultry litter, 80% of the total nitrogen (N) is lost as ammonia (NH_3) (Ritz *et al*. 2004). The longer the age of the litter the more ammonia emission occurs (Parker *et al*, 1959). Reduced environmental ammonia emissions might lessen ammonia's detrimental effects on poultry. Also, the result showed that supplementation of phytase 10000 FTU has decreased (p) excretion in broiler litter and enhanced phosphorus absorption, which reduced environmental pollution and feed costs at the end of the cycle. Because poultry digestive tract lacks the phytase enzyme, only 10 to 30 percent of the phosphorus (P) in corn and soybean meal is available to chickens (Sims and Vadas, 1997). In order to fulfill dietary needs, forms of inorganic phosphorus are frequently added to the feed. Litter P concentrations will always rise as a result of the addition of inorganic (P) and unavailable P from grain sources (Guo *et al*., 2009). Spreading litter over agricultural lands for a long time may therefore help phosphorus build up in soils. There is growing worry that excessively phosphorus-rich soils could lose phosphorus to surface and subsurface water through drainage, runoff, and erosion which may cause eutrophication, water quality problems like algal blooms, fish mortality, smells and increased turbidity (Sharpley *et al*., 1994).

CONCLUSION

The dietary supplementation of phytase 5000 or 10000 FTU at the level of 500 FTU/kg diet plus a 250g /ton diet of β-xylanase result in a positive impact on the growth performance, tibia mineralization, gut morphology, dressing percentage and serum phosphorus level in broiler chickens. More research is warranted to better understand the beneficial effects of the underlying mechanism of phytase and xylanase combination.

CONFLICT OF INTEREST

The authors declare that they have no conflict.

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