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RESEARCH ARTICLE

The Effect of Xylanase and Phytase Supplementation Alone or in Combination in a Wheat-based Diet on Intestinal Morphology and Blood Profile in Broilers

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ABSTRACT

The study aimed to evaluate the effect of supplementation of xylanase and phytase individually or in combination on the intestinal morphology and blood metabolites of broiler chickens fed a stored wheat-based diet. For this purpose, a total of 640, day-old broiler chicks were used with 8 replicates per treatment and 10 birds per replicate in a completely randomized design. Wheat was used as a major energy ingredient, therefore, was designated as a 100% replacement of corn with wheat in the experimental treatments which was 57.52 kg/100 kg feed. Eight isocaloric and isonitrogenous basal diets were prepared based on 1.5-year and 2.5-year-old stored wheat and diets were supplemented with or without exogenous enzymes. Exogenous enzymes supplemented diets were either supplemented with phytase 500FTU or xylanase 500XU alone or in combination. At the end of the trial, the blood metabolites were measured as well as intestinal morphology was evaluated through routine histological examinations. The results showed that combined supplementation of xylanase and phytase in diets T4 and T8 significantly lowered the blood cholesterol, and blood urea nitrogen ($P<0.05$) as compared to treatments without enzymes or supplemented with xylanase or phytase alone. Low blood glucose was observed in the T1 diet ($P<0.05$). Better villus height was observed in birds fed with T4 and T8 diets ($P<0.05$). A decrease in villus height and villus surface area was observed in birds-fed diets without the supplementation of xylanase and phytase enzymes ($P<0.05$). In conclusion, the inclusion of xylanase and phytase in combination in wheat-based diets in broilers improves intestinal morphology and blood metabolites.

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INTRODUCTION

In poultry nutrition, grains are the major portion of the diet that contributes to the main portion of dietary energy. It has been reported that plant grains contain starch polysaccharides and non-starch polysaccharides (Englyst and Cummings, 1988). Non-Starch polysaccharides are the portion of plant grains that is not degraded and digested by the endogenous enzymes of the poultry birds (Olukosi *et al.,* 2008; Choct, 2015). These non-digestible plant grains increase the digesta viscosity in the gut, lower feed intake, reduce the activity of digestive enzymes, and compromise nutrient digestibility (Adeola and Cowieson, 2011). Similar to NSPs, phosphorus is also stored in the form of phytate in plant material and it is not available to poultry birds due to its bound form (Slominski, 2011).

It is well documented that supplementation of xylanase lowers the negative effects of non-starch polysaccharides and minimizes the variation in apparent metabolizable energy and performance of poultry birds fed on a wheat-based diet (Kiarie *et al.,* 2014). While, phytase is being used in the diet of poultry birds to release phytate-bound

phosphorus to meet the phosphorus needs of poultry birds (Woyengo and Nyachoti 2011). A previous study has explored that the intestinal health of birds is affected by ingredients (Jia *et al.,* 2009). It has been reported that feeding of a diet containing 60% barley results in short, and thick jejunum and atrophy of the villi in birds as compared to birds fed a maize-soy diet (Viveros *et al.,* 1994). However, supplementation of NSPases overcomes the negative effects of NSP contents present in different ingredients on the intestinal health of the birds. For example, the study of Iji *et al.* (2001b) reported that the inclusion of xylanase in diets based on wheat had no impact on villus height, crypt depth, or villus surface area in the duodenum, jejunum, and ileum of birds. On the other side, the positive effects of exogenous carbohydrases such as xylanase and glucanase on the broiler gut morphology are well-known in several previous reports (Wang *et al.,* 2005; Sun *et al.,* 2015). According to our knowledge, no data is available on the effect of feeding stored wheat (1.5 and 2.5-year-old wheat) based diet with and without the supplementation of xylanase and phytase individually or in combination on intestinal morphology, and blood metabolites of broiler chickens. Therefore, a study was mandatory to explore the impact of supplementation of exogenous phytase and xylanase enzymes individually or in combination in feed on intestinal morphology, and blood metabolites of broilers. Thus, the objective of the current study was to check the influence of supplementation of exogenous phytase and xylanase enzymes individually or in combination in feed on intestinal morphology, and blood metabolites of broilers.

MATERIALS AND METHODS

For the experimental trial, a total of 640 broiler chicks (day old, Ross-308) were procured from Arslan Chicks (Pvt Ltd) Islamabad. A total of eight dietary treatments were used in this experiment. Experimental diets were formulated as, T1=100%-1.5 YOW; T2=100%-1.5 YOW+ Xylanase (500XU); T3=100%-1.5 YOW+ phytase (500FTU); T4=100%-1.5 YOW+ xylanase and phytase (500XU+500FTU); T5= 100%-2.5 YOW; T6=100%-2.5 YOW+ xylanase (500XU), T7= 100%-2.5 YOW+ phytase (500FTU) and T8=100%-2.5 YOW+ xylanase and phytase (500XU+500FTU) (Table 1). Ingredients data used in the diet formulation was followed from Brazilian Tables for Poultry and Swine (Rostagno *et al.,* 2011). Each dietary treatment was randomly assigned to the 64 pens of 8 replicates, and each replicate had 10 birds. The pen floor was covered with three-inch wood shavings for bedding. Chicks were reared for 35 days keeping the environmental conditions the same for all treatments. Fresh and clean water was offered around the clock. Birds were vaccinated according to the local vaccination program. A circular bottom feeder was provided for each pen, and the nipple drinking system allowed for continuous water availability. The experimental diet consisted of 1.5- or 2.5-year-old wheat making 57.52 %, soybean meal 29.89 %, canola meal 3.49 %, poultry y-product meal 3.20 %, limestone 1 %, rice polishing 3.57 %, mono calcium phosphate 0.34% lysine-sulfate 0.05%, methionine 0.21%, sodium chloride 0.17 %, sodium bicarbonate 0.09 %, premix 0.44 %, and threonine 0.03 %.

Intestinal Histo-morphometry: On day 35, three birds from each experimental treatment were randomly selected and slaughtered for collecting ileum specimens. By using image analysis software (Top View 3.7) villus height (VH), Villus Width (VW), crypt depth (CD), the ratio of Villus height to villus width (VH/VW), and the ratio of villus height to crypt depth (VH/CD) were measured. The Ilium specimens were washed with normal saline, fixed in 10% neutral buffered formalin solution for approximately 24 h, embedded in paraffin, and sectioned at a thickness of 4 μm. The sections were further stained with routine Hematoxylin and Eosin stains, examined under a microscope, and photographed for analysis using image analysis software (ToupView 3.7). The parameters measured were: villus height (VH), villus width (VW), and crypt depth (CD) (Govil *et al.,* 2017). Besides, ratios of VH/VW, ratios of VH/CD, and villus surface area (mm2) were calculated by multiplying 2π x VH x VW/2 (Sakamoto 2000).

Blood serum analysis: Blood serum glucose, urea nitrogen, and cholesterol were determined by using commercial kits BioMed- Urea, BioMed Glucose LS, and BioMed cholesterols kits (Sharif *et al.,* 2020). Briefly, on the 35th day of the experiment, three birds from each replicate were slaughtered. Blood was collected in Bolton gel & Clot tubes after slaughtering. The serum was aspirated by centrifuge and analyzed for blood metabolites by using manufacturer instructions.

Statistical analysis: Collected data were analyzed using the General Linear Model of Minitab Statistical Software 17 (Minitab Inc. 2010) under a completely randomized design. Significant means were tested by using Tukey's multiple comparison tests ($P<0.05$).

RESULTS

Intestinal morphology: The data on intestinal morphology is presented in Fig. 1. Increase in villus height was observed in birds fed T4 and T8 diets ($P<0.05$). Villus height and surface area were decreased in birds-fed T1 and T5 diets with no exogenous enzymes supplementation ($P<0.05$). Villus height to villus width ratios was not affected with the inclusion of xylanase and phytase alone or in combination in stored wheat-based diets fed to broiler birds from day 1 to 35 ($P>0.05$). Villus width was decreased in birds-fed T1 and T5 diets without supplementation of xylanase and phytase ($P<0.05$). The ratio of villus height to crypt depth was increased in birds fed T4 and T8 diets ($P<0.05$).

Blood metabolites: The impact of xylanase and phytase supplementation individually or in combination on certain blood metabolites of broiler birds from day 1 to 35 is presented in Table 2. High blood cholesterol and urea nitrogen were observed in T1 and T5 dietary treatments without the supplementation of xylanase and phytase ($P<0.05$). Results also explored that a 1.5-YOW and 2.5-YOW diets with the supplementation of xylanase and phytase resulted in low blood cholesterol and blood urea-nitrogen as compared to other experimental diets ($P<0.05$). Low blood glucose was observed in T1 and T5 diets (without the supplementation of xylanase and phytase) ($P<0.05$).

Table 1: Experimental design and treatments.

Treatments (T)	Description
T1	Control, 100% wheat (1.5YOW)
T2	100% wheat (1.5YOW) supplemented with Xylanase (500XU)
T3	100% wheat (1.5YOW) supplemented with Phytase (500FTU)
T4	100% wheat (1.5YOW) supplemented with Xylanase (500XU) + Phytase (500FTU)
T5	Control, 100% wheat (2.5YOW)
T6	100% wheat (2.5YOW) supplemented with Xylanase (500XU)
T7	100% wheat (2.5YOW) supplemented with Phytase (500FTU)
T8	100% wheat (2.5YOW) supplemented with Xylanase(500XU) + Phytase (500FTU)

*YOW=year old wheat, XU=Xylanase unit, FTU=Phytase unit

Table 2: The effects of stored wheat supplementation with and without xylanase and phytase on blood metabolites of broiler birds fed on a wheat-based diet from 1-35 days.

	Dietary Treatments									
	T1	T2	T3	T4	T5	T6	T7	T8	SEM	P-value
Cholesterol	156.8^{a}	150.1^{b}	152.7^{b}	149.2^{c}	159.6^{a}	152.9^{b}	154.2^{b}	146.4^{c}	1.72	0.022
Glucose	152.1^{b}	161.3^{ab}	160.1^{ab}	168.7^{a}	154.6^{b}	162.1^{ab}	160.6^{ab}	165.8^{a}	4.75	0.021
Urea-nitrogen	4.81^{a}	4.29^{ab}	4.59^{ab}	3.64^{b}	4.72^{a}	4.48^{ab}	4.56^{ab}	3.39^{b}	0.91	0.011

*Within rows, values with different superscripts differ statistically at **P<**0.05. The data is presented as mean ± standard error of mean (SEM). **T1**= Control, 100% wheat (1.5YOW), **T2**= 100% wheat (1.5YOW) supplemented with Xylanase (500XU), **T3**= 100% wheat (1.5YOW) supplemented with Phytase (500FTU), **T4**= 100% wheat (1.5YOW) supplemented with Xylanase (500XU) + Phytase (500FTU), **T5**= Control, 100% wheat (2.5YOW), **T6**= 100% wheat (2.5YOW) supplemented with Xylanase (500XU), **T7**= 100% wheat (2.5YOW) supplemented with Phytase (500FTU), **T8**= 100% wheat (2.5YOW) supplemented with Xylanase (500XU) + Phytase (500FTU).

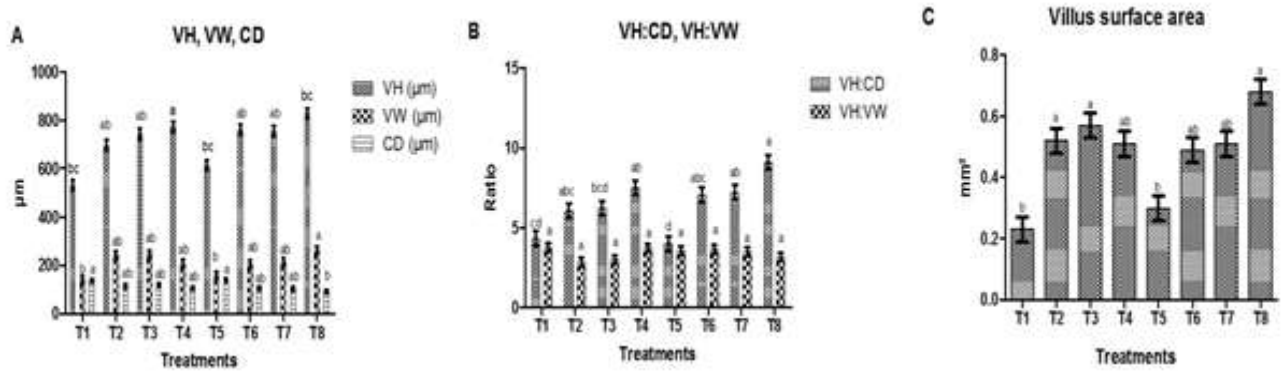

Fig. 1: Graphical representation of intestinal morphology: A= Graph representing villus height, width and crypt depth (µm). B= Graph representing the ratio of villus height to villus width and crypt depth. C= Graph representing the surface area of villi in the duodenum ($mm^{2)}$: **T1**= Control, 100% wheat (1.5YOW), **T2**= 100% wheat (1.5YOW) supplemented with Xylanase (500XU), **T3**= 100% wheat (1.5YOW) supplemented with Phytase (500FTU), **T4**= 100% wheat (1.5YOW) supplemented with Xylanase (500XU) + Phytase (500FTU), **T5**= Control, 100% wheat (2.5YOW), **T6**= 100% wheat (2.5YOW) supplemented with Xylanase (500XU), **T7**= 100% wheat (2.5YOW) supplemented with Phytase (500FTU), **T8**= 100% wheat (2.5YOW) supplemented with Xylanase (500XU) + Phytase (500FTU): *VH= Villus Height, VW= Villus Width, CD= Crypts depth, YOW= Year old wheat, XU=Xylanase unit, FTU= Phytase unit, Bars within the graphs with different superscripts^{abc} differ statistically (**P<**0.05). The data are presented as mean ± standard error of the mean (SEM).

DISCUSSION

New season grains are problematic for broiler production due to the high contents of soluble NSPs that are responsible for increasing the digesta viscosity (Fuente *et al.,* 1998; Pirgozliev *et al.,* 2006). However, storing grains for three to four months improves nutritive value and has a positive impact on broiler chicken production (Ravindran *et al.,* 2001; Anwar *et al.,* 2023). Storage conditions are considered important for the activation of the endogenous phytase and NSPases in barley and wheat (Anwar *et al.,* 2023). Based on these findings stored wheat diets were used (1.5-YOW and 2.5-YOW) with phytase and xylanase supplementation individually or in combination to check the effect on intestinal morphology and blood metabolites of broilers. Less viscosity was observed in the birds fed T8 and T4 diets (P<0.05).

Nutrient absorption is reflected by the intestinal histomorphology (villi length, crypt depth, and their ratio). Better absorption of nutrients and reduced mucosal tissue turnover rate are represented by the tall length of the villi and reduced crypt depth (Apperson and Cherian, 2017). The short length of the villi reduced the surface area for the absorption of nutrients because the villus height affects the enterocytes' ability to nutrients absorption (Parsaie *et al.,* 2007). In our study, better villus height and surface area were observed in birds fed a T8 diet. Like our results, as noted by Wu *et al.* (2004), the villus length of the ileum and crypt depth of the jejunum and ileum raised by the inclusion of phytase and xylanase in combination. The height of the villus and surface area was less in birds fed diets without xylanase and phytase or diets supplemented with xylanase and phytase alone. Wu *et al.* (2004) found similar results that phytase supplementation enhanced the height of villi in the duodenum in comparison with another basal experimental diet that had no inclusion of enzymes. The depth of the crypts in the jejunum tended to decrease after xylanase administration. These findings support a study by Iji *et al.* (2001a) that found no difference in villus height, crypt depth, and villus surface area of the duodenum, jejunum, or ileum of broilers fed diets that had wheat as a major ingredient when xylanase alone was added to the diet. Conversely, Jaroni *et al.* (1999) discovered that the inclusion of xylanase prevented the

shortening, thickness, and atrophy of jejunal villi of laying hens fed on diets with wheat middling as a major ingredient. Our results are in line with the results of (Kim *et al.,* 2003; Upadhaya *et al.,* 2021) who concluded that exogenous carbohydrase addition with phytase considerably improved the villus height and lowered the crypt depth in the intestinal segments and ultimately results in better growth performance of broiler chickens. Our results demonstrate that shorter villus height is induced by diet without the supplementation of xylanase and phytase as compared to the other dietary treatments that were supplemented with xylanase and phytase alone or in combination. A shorter villus height is associated with less absorption potential throughout the intestinal parts and leads to reduced growth efficiency.

Supplementation of phytase lowers the content of phytate that cannot be digested by poultry birds and negatively affects the growth performance of birds by binding several nutrients and minerals (Woyengo and Nyachoti, 2011). Supplementation of phytase leads to an increase in the level of blood glucose that can be justified by lesser contents of phytate in diets supplemented with phytase as compared to the diets that were not supplemented with phytase. These results are also supported by the findings of previous researchers Johnston *et al.* (2004) who reported that the destruction of phytic acid by exogenous phytase has resulted in an increase in circulating blood glucose levels in pigs. A previous study by Cowieson *et al.* (2004) has reported that higher phytate intake resulted in a higher loss of endogenous Na in the broiler gut, which is thought to be associated with a physiological cascade beginning with phytate-induced protein precipitation and cross-linking during the gastric stage of digestion and ending with increased $NaHCO_3$ in the small intestinal secretion and loss.

Therefore, it could be speculated that higher phytate contents in fresh wheat could obstruct Na partitioning in the small intestine and ion balance in general, and reduction of phytate contents by storing wheat before adding it into the diet of the broiler could enhance Na-dependent transport systems. It has been reported that 80% of glucose in meals, is absorbed through sodium-dependent active transport systems (Fuller and Reeds, 1998), and it could be assumed that lower phytate contents due to storage may increase blood glucose levels in the current study indirectly by effects on sodium. Our results are by the study of Khadem *et al.* (2016). In broiler birds, proteins are degraded to nitrogen, and blood urea nitrogen may indicate the protein metabolism regulation in broilers. Previous studies have reported an inverse correlation between plasma urea nitrogen levels and protein retention efficiency (Swennen *et al*., 2005). In the current experiment, blood metabolites results explored the inclusion of xylanase and phytase alone or in combination reduced the level of blood urea nitrogen and results suggest that the inclusion of a combination of xylanase and phytase in stored wheat enhanced nutrient metabolism, especially protein anabolism in broilers, therefore, could be responsible for better growth performance in broilers.

Conclusions: Based on the findings of the current study, it is concluded that the inclusion of xylanase and phytase (500XU and 500FTU) in combination or individually with stored wheat in the diet of the broiler has a positive impact on intestinal morphology and blood metabolites. Stored wheat had a good impact on the gut viscosity i.e., lowered as compared to the new/fresh wheat.

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Authors contribution: UA, MQB, MR, and MAR conceived and designed the experiment. UA, RM, MFK, UF, and MA carried out experiments and lab analyses. MA, MH, MH, FGE and AUR performed the statistical analysis. FGE and SS reviewed the manuscript and finalized the manuscript. All authors wrote, revised, and reviewed the manuscript.

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