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Effect of Xylanase Supplementation in Wheat-Based Feed on Growth, Metabolism and Gut Microbiota Function in Broiler Chicken

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Key words
Enzyme, Gut, Broiler chicken, Broiler health, Gut microbiota, Growth, Xylanase

ABSTRACT

The poultry industry has largely increased production and efficiency over the last few decades, due to advancements in genetics, nutrition, and management practice. At the same time, feeding is the main control point of profitability and making progress through understanding the physiology of digestion and metabolism. In a randomized control study, the effect of xylanase enzyme supplementation of wheat-based feed was investigated on growth rate, metabolic performance, and gut microbial functionality in broiler chickens. A significant increase in growth rate (P<0.0001) was seen in treatment groups compared to the control group. The xylanase supplementation did not alter the physiological range of liver function enzymes as manifested by a significant increase in aspartate aminotransferase (P<0.0001) and alanine aminotransferase (P<0.0001) in treatment groups. The total antioxidant capacity and oxidative status increased (P<0.05) in the treatment groups compared to the control group. Gut microbiota was analysed by quantitative real-time PCR. It showed a significant upregulation in the expression level of bacterial strains of *Firmicutes* (P<0.0001) and *Bifidobacteria* (P<0.0001) indicating a favorable shift in beneficial strains. Likewise, a significant downregulation in *Escherichia coli* (P<0.0001) indicated a decrease in pathogenic strain. Overall, the findings of the current study suggest the safety of xylanase supplementation in wheat-based feed as manifested by improved growth performance, and healthier serological and physiological attributes.

INTRODUCTION

Wheat is a popular cereal crop for human consumption and animal feed. The grain hardness, composition, and protein content of wheat determine its functional qualities. Wheat also has anti-nutritive properties. Thus, exogenous enzymes and enzyme mixtures in broiler feeds have been intensively studied in the last 20 years (Kogut, 2019). Enzymes have been shown to improve broiler chicken performance and feed conversion ratio (FCR) in nutritionally marginal diets, as well as reduce antinutrient effects and increase nutrient availability (Walk *et al*., 2011). The full and effective hydrolysis of xylan, a major NSP present in wheat, into its constituent sugars necessitates the synergistic activity of multiple enzymes that target xylan bonds. The gastrointestinal tract (GIT) in poultry is vital for nutrient absorption and digestion of feedstuff (Scanes and Pierzchala-Koziec, 2014). Specifically, the

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GIT corresponds to 1.5% of the total body weight in poultry, whereas the metabolized energy can go as high as 8% (Miao *et al.*, 2017). Microbial populations tend to proliferate when there is a substantially high amount of undigested nutrients in the hindgut (Zhao *et al.*, 2001). As a result, the nutrient balance and availability between the gut microbiota and the host is disrupted, resulting in pathogenic, metabolic, or sterile inflammation (Aruwa *et al.*, 2021).

Xylanase holds its efficacy in the lysis of the arabinoxylan content of feedstuff. The enzyme activity is observed as hydrolysis of arabinoxylans mainly by the action of endo-1,4-β-xylanase, which is specific for breaking down the internal xylan polymers (Grecco *et al.*, 2019). The present study thus clarifies the effectiveness of exogenous xylanase enzyme supplementation along with the dietary modulation and replacement of wheat from the conventional corn-soybean diet. The effect of the xylanase enzyme on the molecular level yields the catabolic products of non-starch polysaccharides present in the wheat, which allow the chickens to digest the feed ingredients better and absorb the readily available nutrients, eventually ensuring better growth performance and rapid increase in body weight in a smaller time window.

MATERIALS AND METHODS

Experimental design

A total of sixty broiler chicks (ROSS-308 strain) were obtained from the local hatchery and divided equally into three groups, with 20 birds in each group. Group 1 (G1) denoted the control group fed with standard corn and soybean feed, Group 2 (G2) denoted the wheat-fed group, and Group 3 (G3) denoted the xylanase supplemented wheat-fed group (Table I). The duration of the trial was 35 days. The vaccination plan was followed for Newcastle disease and infectious bronchitis for all the birds in both groups (Table II). Birds from G1, G2, and G3 were slaughtered at grower (day 14 and day 21), finisher (day 28 and day 35) ages. Blood was centrifuged and serum was collected for serological testing. The tissue samples of liver, thymus, and pancreas were collected for histological analyses and microbiota samples from the cecum and colon were collected for organic extraction of nucleic acids for genomic studies at the end of trial period.

Growth indices

The live body weight gain was measured on days 14, 21, 28, and 35 using an electric weighing balance. The FCR was calculated using the formula:

$$FCR = \frac{\text{Feed intake}}{\text{Body weight gain}}$$

Serological testing

Liver function tests were performed using standardized kit methods to check the levels of aspartate aminotransferase (AST), and alanine aminotransferase (ALT) following the principle of colorimetry (Bioclin® Kinetic diagnostic kit). The total oxidative stress (TOS) and total antioxidant capacity (TAC) were also checked by kit method.

Genomic studies

Organic extraction and purification steps were employed for the isolation of genomic DNA and RNA for microbiota analysis in the current study (Walk *et al.*, 2011). The forward and reverse primers were designed for the bacterial strains and the expression levels of the given genes were tracked using quantitative real time PCR data for up or down-regulation (Table III).

Histological examination

The tissue samples were fixed in 10% neutral buffered formalin, dehydrated through a graded series of ethanol, cleared in xylene, and embedded in paraffin wax. Paraffin blocks were sectioned at 4–5 µm thickness using a microtome, and the sections were stained with hematoxylin and eosin (H&E). The stained sections were examined under a light microscope to evaluate histopathological alterations.

Table I. Vaccination plans against viral infections administered through different routes.

Age (Days)	Vaccine for disease	Vaccine Type	Route of administration
5	Newcastle disease	Killed (full dose)	Subcutaneous infusion
15	Newcastle disease	Live (Lasota)	Drinking water
15	Infectious bronchitis	Live	Drinking water
22	Newcastle disease + Infectious bronchitis	Live	Drinking water

Table II. Nutrient composition of starter, grower, and finisher diets.

Feed Ingredients %	Group 1 (Control)			Group 2 (Treatment)		
	Starter	Grower	Finisher	Starter	Grower	Finisher
Corn	57.03	59.79	58.87	0.00	-	31.02
Wheat	-	-	-	61.18	64.06	31.02
Soybean meal 46%	30.63	30.34	26.64	26.34	25.86	23.19
Canola meal	5.00	0.00	-	5.00	0.00	-
Poultry by-product meal	3.00	4.00	4.00	3.00	4.00	4.00
Limestone	1.08	0.99	0.67	1.12	1.09	0.75
Rice polish	0.00	0.00	3.99	0.00	0.00	0.00
Poultry oil	0.64	1.99	3.24	0.79	2.24	3.27
Monocalcium phosphate	0.87	-	-	0.29	-	-
Lysine sulphate 70%	0.45	0.43	0.39	0.38	0.50	0.45
Methionine 99%	0.33	0.33	0.29	0.23	0.28	0.24
Sodium chloride	0.27	0.27	0.23	0.18	0.23	0.23
Sodium bicarbonate	0.16	0.23	0.14	0.07	0.29	0.22
Vitamin premix[1]	0.24	0.3	0.16	0.44	0.3	0.23
$_L$-Threonine 98%	0.21	0.21	0.27	0.09	0.31	0.23
DCP	-	1.04	-	-	0.78	0.81
Choline Chl. 70%	-	0.05	-	-	0.05	-

Vitamin Premix1, each kg of premix provided the following per kg of diet: 10,000 IU Vit A, 11.0 IU Vit E, 1.1 mg Vit K, 1100 IU Vit D3, Riboflavin 5mg, Calcium Pantothenate 12mg, 12.1µg Vit B12, 2.2mg Vit B6, 2.2mg Thiamine, 44mg Nicotinic acid, 250mg, Choline Chloride, 1.55mg Folic Acid, 0.11mg d-biotin, 60mg Manganese, 50mg Zinc, 0.3mg Iodine, 0.1mg Cobalt, 30mg Iron, 5mg Copper, 1mg Selenium.

Table III. Primers for bacterial strains.

Genes	Sequence (5' – 3')
Bifidobacterium	F= GTTAATACCTTTGCTCATTGA
	R= CCACATCCAGCATACCAC
*V2**	F= AGAGTTTGAGCCCGATCAG
	R= TGCTGCCTCCCGTAGGAGT
Firmicutes	F= GGCAGCAGTAGGGAATCTTC
	R= ACACTTAGAAACTCATCGTTT
Lactobacillus	F= AGCAGTAGGGAATCTTTCCA
	R= CACCGCTACACATGGAG
Escherichia coli	F= GTTAATACCTTTGCTCATTGA
	R= ACCAAGGGTATCTAATCCTGTT

*V2: Hypervariable region served as a housekeeping gene for bacterial functionality due to its expression pattern in all bacterial phyla studied in this experiment.

Statistical analysis

All the obtained numeric data was grouped and statistically tested using two-way analysis of variance (ANOVA) with row mean analysis (RM analysis). Two-way ANOVA was followed by Tukey's Post hoc test for those datasets that showed significance. The obtained results were considered significant at α = 0.05.

RESULTS

Physical and physiological parameters

The live body weight gain was calculated for the control and treatment groups at the grower and finisher phases of the trial.

The FCR is inversely proportional to body weight gain. The FCR increased during the initial phases of enzyme supplementation in the treatment groups, but due to increase in body weight gain explained by higher digestibility and metabolism, the FCR substantially decreased in the finisher phases.

Liver function tests

The serum biomarkers are positively influenced by enzyme supplementations (Ravindran and Abdollahi, 2021), and the present study investigated the effects of xylanase addition on liver function markers. In Figure 1C, the linearly significant (P<0.05) trend in treatment groups G2 compared to control group suggests the upsurge of AST levels upon xylanase enzyme supplementation.

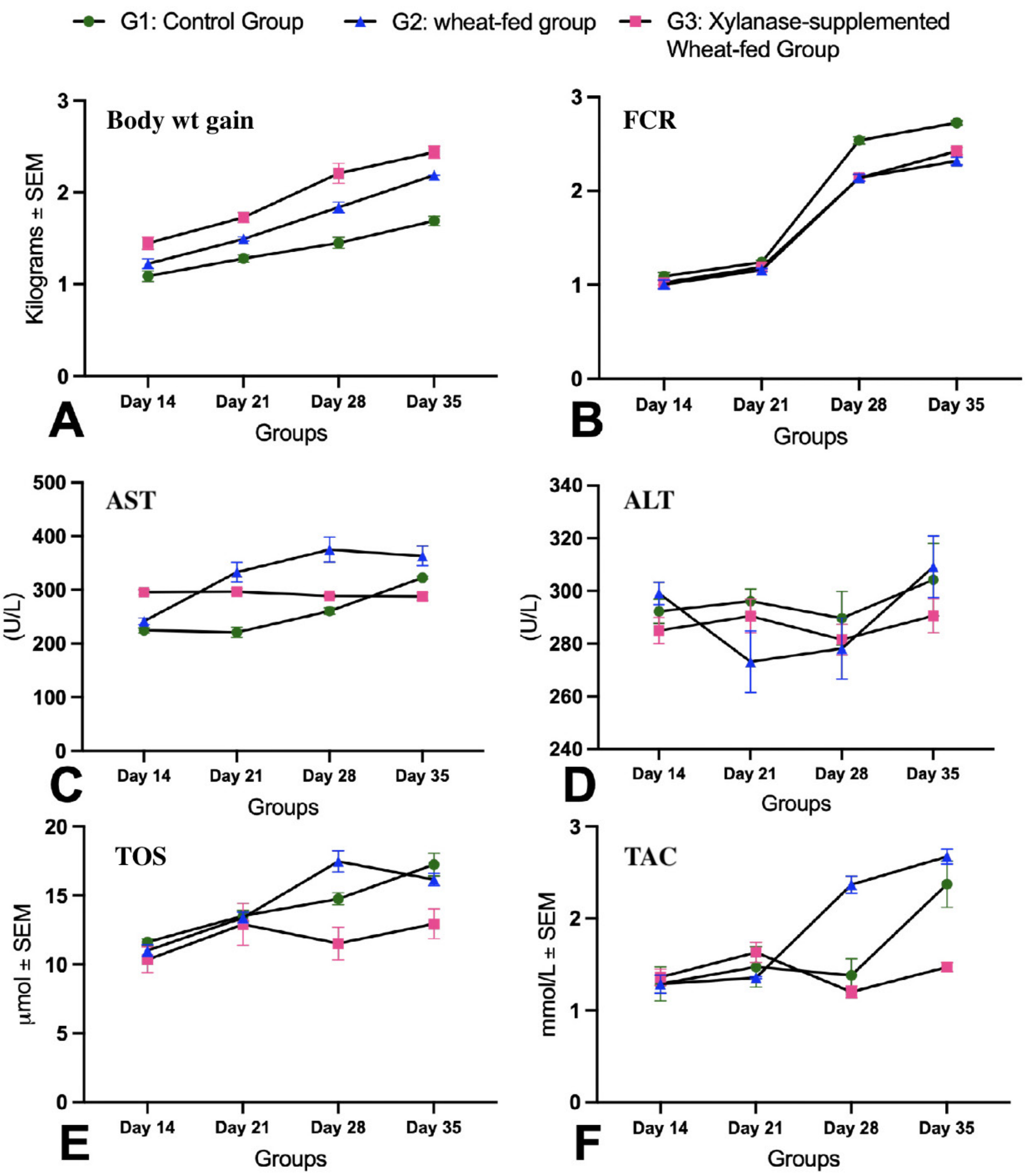

Fig. 1. Effect of xylanase supplemental wheat based feed on live body weight gain (A), feed conversion ratio (FCR) (B), level of aspartate aminotransferase (AST) (C). Level of alanine aminotransferase (ALT) (D), total oxidative stress (TOS). (E), total anti-oxidant capacity (TAC) (F) in broiler chicken.

In Figure 1D, the linear increase in the ALT levels in both G1 and G3 group can be explained by the effect of feed formulation and enzyme supplementation in control and treated groups as the initial physiological response to exogenous administration of NSP rich diet, whereas a significant decrease in ALT levels in G2 in grower phase suggest the compatibility of wheat as a feed ingredient for broiler birds.

Oxidative stress markers

The production of reactive oxygen species (ROS) is mediated by the addition of enzymes and changes in feed formulation which can cause changes in physiological processes of the body and overwhelm the cellular radical scavenging pathways (Rubio and Cerón, 2021). The current study is thus aimed at evaluating the potential of xylanase supplementation in mediating the production of ROS and enhancing the scavenging ability of cells to overcome the free radical pool that damages the intestinal epithelium. Figure 1E, F explains the TOS in control group and treatment group throughout the trial period. The statistical significance (P<0.05) between G1, G2, and G3 groups at days 28 and 35 can be explained by the initiation of digestive pathways with byproducts of reactive oxygen radicals that help in digestibility of non-starch polysaccharides in control feed (Ravindran *et al*., 2006). In G3, the linear shift in TOS levels explain the physiologically confined ROS production and adaptability of cellular systems with exogenous enzyme supplementation with treatment diet. A radical increase in TOS levels of G2 compared to G3 can be explained by subsequent ROS production in G3 by xylanase supplementation that could overwhelm the physiological antioxidant system. In Figure 3B, the antioxidant capacity linearly increases in G1, G2, as well as G3 groups, where the individual changes at grower stages are non-significant (P>0.05) but changes at finisher stages are significant (P<0.001).

Gene expression analysis of bacterial strains

The current study employed different bacterial strains, both beneficial and harmful strains, from the vast array of gut microbial populations which are cited as most affected bacterial strains by enzyme supplementation (Oviedo-Rondón, 2019) and changes in feed formulations. The beneficial bacterial strains chosen were *Bifidobacteria,* lactobacilli, and *Firmicutes,* whereas the pathogenic strain was *Escherichia coli.* The hypervariable region V2 was chosen as the housekeeping gene in this study for the select bacterial strains. Figure 2 explains the results obtained after running quantitative Real time PCR (qRT-PCR) on designated primer sequences for the bacterial strains. The statistical inference shows a significant upregulation (P<0.05) in the expression level of *Bifidobacteria, Firmicutes,* and *E. coli* whereas the expression level of lactobacilli was non-significant (P>0.05).

Histological analysis

The hematoxylin and eosin stained tissue sections were examined under a light microscope to assess histopathological changes. Microscopic evaluation focused on the structural integrity of the tissues, including evidence of inflammatory cell infiltration, cellular degeneration, and other morphological alterations among the control and treatment groups. In Figure 3A, liver tissue from G1 showed a compact cellular structure with intact tissue compartmentalization and no immune cell infiltration. Figure 3B shows thymus tissue from G1 where microcellular entities are condensed without any tears in the tissue integrity. Figure 3C shows the pancreatic tissue from G1 with similar integral structures and compact cellular compartmentalization. Figure 3D shows the liver tissue from G2 where the cellular integrity is compromised, and tissue structure shows interstitial gaps in the lumen. Immune infiltration is also evident in the tissue lumen, suggesting the influence of wheat replacement in exciting the immune response from microbiota as well as circulating immune bodies. Figure 3E shows thymus tissue from G2 where immune cell infiltration can be seen in the tissue lumen. Figure 3F shows the pancreatic tissue from G2, where larger gaps in the interstitial lumen are seen with intact cellular integrity and minute immune infiltration in the tissue. Figure 3G shows the liver tissue from G3, where necrotic tissue mass is seen as replaced with repair tissue and lesser immune body infiltration. Figure 3H shows thymus tissue from G3 where hyperactivity of immune system and myelopoietic activity ensuing in the thymus after enzyme supplementation is evident from immune infiltration. In Figure 3I, the pancreatic tissue from G3 shows a better cellular integrity and tissue morphological profile.

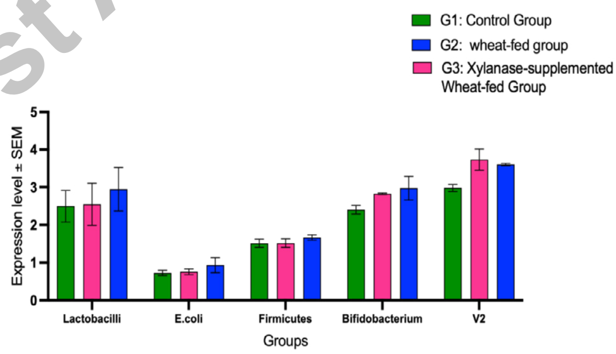

Fig. 2. Effect of xylanase supplemented wheat-based fed on gene expression using qRT-PCR.

The intestinal villi in G1 (Fig. 4A) appear densely packed and exhibit a well-defined structure. The villi are relatively thick, indicating good intestinal health and absorption surface area. The intestinal villi in G2 (Fig. 4B) present a longer and more spaced-out surface area with fenestration in between compared to Figure 4A. The villi are thinner, which suggests slight changes in the intestinal morphology due to the wheat as a basic dietary element for this group (Aleksandrova *et al*., 2021). The mild alterations in the structure compared to the soybean-corn meal potentially influence nutrient absorption. Figure 4C presents a clear difference, with villi that are more fragmented or disrupted in appearance. The inclusion of xylanase modified the structure of the villi significantly, making them appear less compact and more fragmented

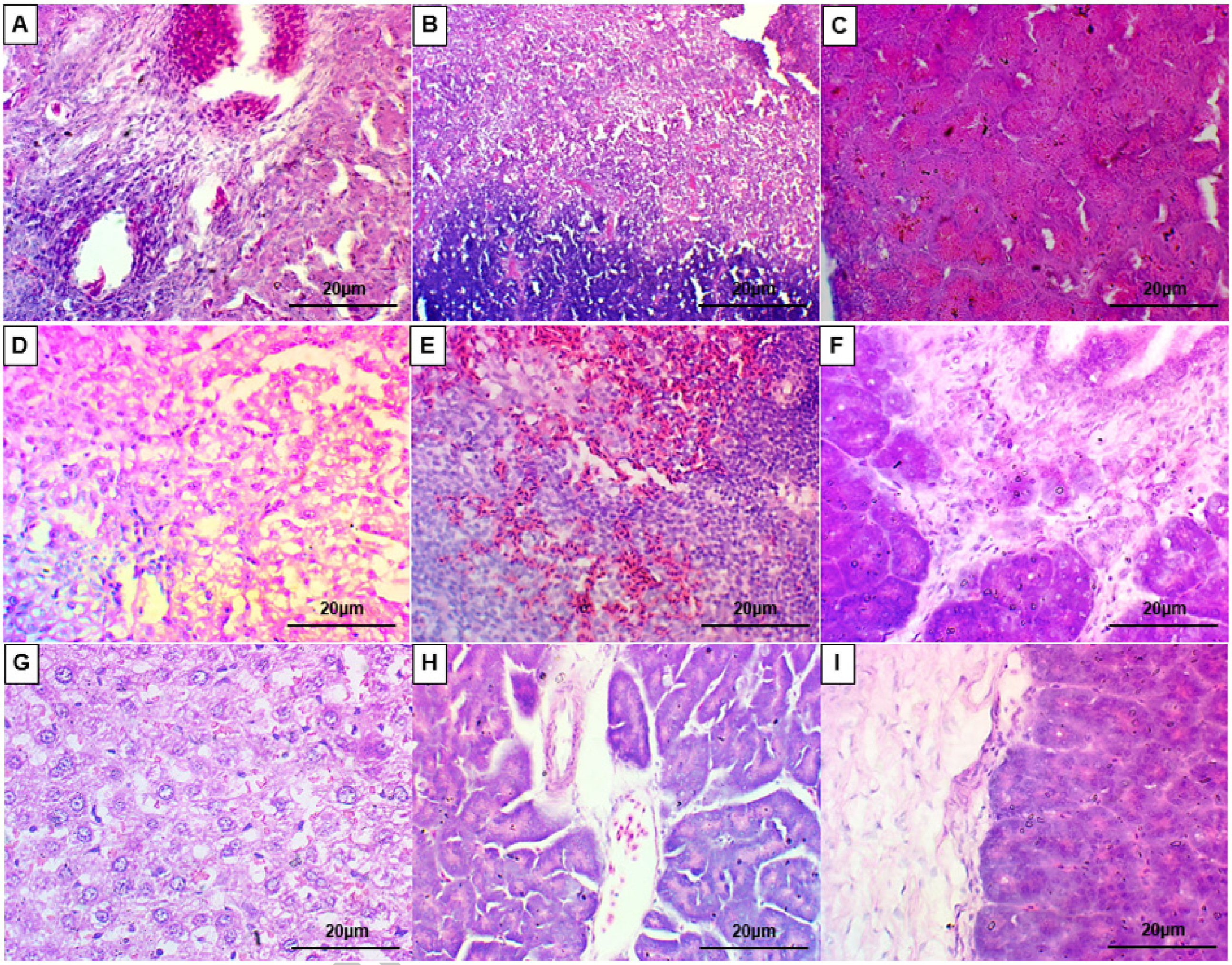

Fig. 3. Photomicrographs of organ tissue histology. A, Liver tissue from G1; B, Thymus tissue from G1; C, Pancreas tissue from G1; D, Liver tissue from G2; E, Thymus tissue from G2; F, Pancreas tissue from G2; G, Liver tissue from G3; H, Thymus tissue from G3; I, Pancreas tissue from G3. Magnified at 400X, stained with Hematoxylin and Eosin.

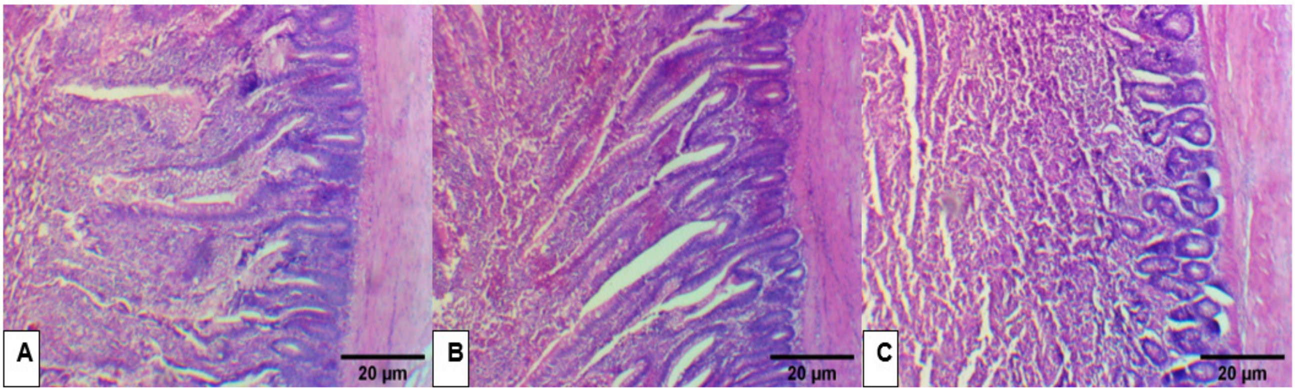

Fig. 4. Histological structure of small intestine of G1 (A), G2 (B), G3 (C). Magnified at 400×, stained with hematoxylin and eosin.

compared to images Figure 4A and B. This could indicate an enzymatic effect on the digestion process, potentially enhancing the breakdown of fibrous wheat components and enhanced absorption of nutrients (Qureshi *et al*., 2016).

DISCUSSION

Digestibility of non-starch polysaccharide (NSPs)

The substantial deviation of the poultry industry from using the conventional dietary base of corn and soybean and replacing these ingredients with wheat in broiler diets has marked expansion in research on the benefits of using an ingredient rich for its nutritional components but also controversial for its non-nutritious factors (Esmaeilipour *et al*., 2012; Scanes, 2020). The NSPs content in wheat makes it a rather questionable ingredient for use in poultry diets because these NSPs increase the overall viscidity of intestinal digesta which eventually influences the bird's feed intake and nutrient utilization capacity (Noy and Sklan, 1995; Singh *et al*., 2014). The depression and nutritional stress caused by such dietary modification can alter the bird's performance and growth patterns. A relatively viscous environment in the gut of poultry hinders the production of digestive enzymes and supportive secretions, which further disturb the normality of digestion and absorption of nutrients from the gut (Kiarie *et al*., 2014; Moftakharzadeh *et al*., 2019).

The ability of broilers to degrade such anti-nutritive elements in their diets is limited since chicks at an early age do not possess such digestive enzymes that can digest the NSPs of wheat and other NSP-rich dietary options (Chakdar *et al*., 2016; Scanes and Pierzchala-Koziec, 2014). Along with the use of a wheat-based diet for its economic availability, the addition of an NSP-degrading enzyme becomes necessary to increase the nutritive potential of wheat gained by the birds (Marcotuli *et al*., 2020; Nourmohammadi *et al*., 2018). The use of such enzymes allows the broiler to digest its feed properly and provide a better FCR, increasing the utilization and absorption of nutrients which ultimately results in better growth and body weight gain (Kiarie *et al*., 2014). The results of the current study corroborate the findings of (Esmaeilipour *et al*., 2012) where the addition of xylanase enzyme to a dietary formulation with overall compromised nutrient composition and non-digestible elements increased the digestibility of such diet with increased potential of gut morphological structures in absorbing and providing readily metabolizable nutrient elements in the intestinal lumen (Esmaeilipour *et al*., 2012).

Growth indices

In poultry, the gut morphological structures are responsible for the distribution of nutrients after proper absorptive mechanisms that ensue after feed consumption (Tellez *et al*., 2006). Wheat is a standard feed grain used for most animal feed formulations for its positive economic aspects as well as nutritious value, but the presence of NSPs renders the digestibility and absorption of accessory nutrients inadequate. The carbohydrase/glycosidases class of enzymes are responsible for proper digestion of NSPs, mainly targeting the heavy polysaccharide chains of xylan and arabinoxylans in wheat (Gorenz *et al*., 2022; Papadopoulos *et al*., 2022). The proper digestion of feedstuff leads to better absorption of nutrients, resulting in bird's growth and performance parameters. The results of the current study where live body weight gain were recorded at different time points throughout the trial showed a positive increase in the weight gain of control group birds, and an even significant increase ($P<0.0001$) was seen in the xylanase treated groups by the end of the trial. The overall carcass weight was significantly higher compared to the carcass weight of birds from control group, and these results are in accordance with the study conducted by Gitoee *et al*. (2015) suggesting the positive influence of xylanase enzyme on digestibility of wheat in the treated group.

The digestibility of gut and its influence in increasing body weight relate inversely with the feed intake, thus defining the concept of FCR (Dänicke *et al*., 1997). The FCR explains that feed intake decreases when the body weight gain increases, posing a direct influence on the overall physical growth of the body (Nourmohammadi *et al*., 2018). The current study investigated and compared the effects of standard poultry feed with enzyme-supplemented diet and wheat-based diet on the FCR of growing broilers. The results showed that in control group, the birds underwent a linear increase in body weight. A significant trend ($P<0.001$) was observed in G3 compared with G2 at days 21 and day 28, whereas a linear consistency was seen at day 35, explained by the subsequent increasing body weight gain. The significant ($P<0.05$) change in the initial grower phases can be explained by the lower body weights and higher feed intake for growth and development, and the higher the feed intake, the higher the body weight gain and subsequently lower FCR. The linearity in results at the finisher phases in G2 in the current study reflected a similar phenomenon (Liu and Kim, 2017).

Liver function tests

The serum biomarkers are positively influenced by enzyme supplementations, and the present study investigated the effects of xylanase addition on liver function tests. Serum levels of liver function markers such as ALT and AST demonstrate the overall activity of liver when an exogenous biochemical is administered and is metabolized by the liver (Ahmad *et al*., 2013). A physiological range is defined for liver function markers that circulate in the blood, and a sudden acute rise in these markers is not always evident of liver injury. However, a constant rise in serum levels of these enzymes verifies the incidence of liver injury and liver cirrhosis (Mirzaie *et al*., 2012). The results of the current study showed a linear increase in the ALT and AST levels (P<0.05) in the G1 group towards the finisher phases, whereas in G2 and G3 groups, an initial significant increase (P<0.05) in ALT levels remained consistent at a physiological setpoint (non-significance at P>0.05) with the progression of trial period into finisher phase. The pronounced variance in initial results for G3 group can be explained by the fact that new biochemical entities, when metabolized by the liver, result in rise in serum levels of liver function biomarkers within a physiological range. The administration of wheat-based di*et al*so contributed to the rise in LFT levels in G2 because a higher portion of calories in the carbohydrate fraction (xylan and arabinoxylans) in the diet contribute to higher metabolic functionality of the liver and increased digestive enzyme release as an accessory activity to enzyme supplementation (Gorenz *et al*., 2022; Purkins *et al*., 2004). The body's adaptability to the liver function markers during the finisher phases can be described by the effective influence of xylanase enzyme supplementation that supported the role of metabolic pathways of liver involved in emulsification of carbohydrate-rich dietary intake (Bader *et al*., 2018).

Oxidative stress markers

The cellular oxidative stress and antioxidant capacity of cells is explained by the production of reactive oxygen and reactive nitrogen species upon exposure to exogenous biochemical drugs and feedstuff. The current study employed serum TOS and TAC activity to explain the influence of enzyme supplementation on physiological cellular functionality in grower and finisher phases of the experimental trial (Bai *et al*., 2017; Niazi *et al*., 2017; Naseem *et al*., 2023). TOS is directly proportional to the generation of free radicals in the tissue, mainly as a by-product of cellular pathways that are involved in metabolism of exogenous elements interacting with the cells (Moftakharzadeh *et al*., 2017). The significant increase (P<0.05) in TOS in G2 and G3 towards the finisher phases can be explained by the presence of high-carbohydrate diets, the digestion of which through non-enzymatic glycation pathways in the cells can cause the generation of free radicals (Cancemi *et al*., 2023).

The antioxidant capacity of the cells is a reciprocal of TOS and is a diagnostic measure of free-radical scavenging capability of the cell (Aleksandrova *et al*., 2021). The results after TAC analysis for G1, G2, and G3 groups in the current study suggest that physiological antioxidant capacity of the cells is compromised in the initial stages of the trial, but a significant increase (P<0.05) in the antioxidant activity is seen towards the end of trial in both G2 and G3 groups. In G3 group, the increased antioxidant capacity is explained by the potential of xylanase enzyme as a detoxifying enzyme (Cancemi *et al*., 2023), where nutrition-mediated oxidative stress is managed by xylanase supplementation.

Dysbiosis studies

qPTPCR was employed to study the gene expression of dominant gut bacterial phyla, beneficial and pathogenic, that are reported in literature to fluctuate first in nutrition-induced dysbiosis (Pan and Yu, 2014). Hypervariable regions range from V1 to V9 and are specie-specific sequences (Chakravorty *et al*., 2007) and hypervariable region V2 was chosen as a housekeeping gene for the current genomic study. According to the results obtained, the expression level of beneficial bacteria such as *Bifidobacteria* and *Firmicutes* showed significant upregulation (P<0.001) in G2 and G3 groups compared to G1 group, whereas the levels of lactobacilli remained non-significant (P>0.05) for all groups. The current understanding on dysbiosis and the consistency in the expression level of Lactobacilli can be explained by the activity of enzyme supplementation and carbohydrate-rich diet which served as a potential prebiotic for the bacterial phyla (Yin *et al*., 2010). The significant (P<0.05) downregulatory genetic patterns of *E. coli* in G2 and G3 groups explain the reciprocal expression of beneficial bacteria against pathogenic strains. The evidence suggests that enzyme supplementation along with wheat-based diet acted as a prebiotic nutrition for the gut microbiota and potential risk of dysbiosis was culminated by downregulatory patterns of *E. coli.* These results are in coherence with Bai *et al*. (2017), where the use of probiotics substantially increased the expression level of lactobacilli and *Firmicutes.*

Histological changes

The tissue samples of liver, pancreas, and thymus were employed for microscopic analysis in the current study. According to cited literature, these organs are the most affected by enzyme supplementation and changes in nutritional composition of feedstuff (Esmaeilipour *et al*., 2012). The liver, pancreas and thymus tissue observed in the control group (G1) showed intact cellular structure with little to no immune infiltration and no signs of cellular hyperplasia. In wheat-fed treatment group (G2), the liver histology showed interstitial gaps with mild signs of inflammatory cell infiltration with minor cellular hyperplasia. This hyperplasia can be explained by the increased activity of hepatic Kupffer cells in metabolizing the enzyme and increased bile products that favor the liver functionality (Qureshi *et al*., 2016). In xylanase-supplemented wheat-fed group (G3), the tissue histology of pancreas and liver showed mild hyperplasia with overall intact cellular integrity of tissue, whereas the tissue histology of thymus showed mild degree of inflammatory cell infiltration and overall beneficial cellular architecture in the tissue. This immune infiltration in the thymus can be explained by the hyperactivity of immune system and influence of cytokines on circulating immune bodies, eventually affecting the myelopoiesis of immune cells against enzyme supplementation in the treated groups (Qureshi *et al*., 2016). The overall morphological presentation of organs and histological findings of the current study suggest the safe usage of xylanase enzyme as a carbohydrate-rich feed supplement for its potential in digesting complex NSPs of wheat.

In slide 4A, representing the G1 group (soybean-corn meal diet), the villi, which serve to increase the absorptive surface area of the small intestine, are tightly packed and well-structured. This observation indicates that the diet promotes intestinal health by maintaining an optimal villus architecture conducive to effective nutrient absorption. The compactness and thickness of the villi suggest efficient nutrient processing and digestion within the intestinal tract (Bader *et al*., 2018).

4B, corresponding to the G2 group (wheat diet), exhibits villi with a more typical structure, characterized by slightly elongated and thinner projections. While still relatively healthy, the villi appear less densely packed compared to those in slide A. This elongation may reflect adaptive changes in response to the wheat-based diet, potentially due to the more fibrous and complex nature of wheat, which could be more challenging to digest compared to soybean-corn meal. Despite these morphological changes, the villi likely remain functional in supporting nutrient absorption, though with some modifications to digestive efficiency.

In slide C, representing group G3 (wheat + xylanase enzyme treatment), the villi exhibit a more fragmented and less compact appearance compared to the previous slides. However, this alteration should not necessarily be viewed as detrimental. Xylanase is known to facilitate the breakdown of complex polysaccharides, such as those found in wheat, improving digestibility (Moftakharzadeh *et al*., 2017). The observed structural changes in the villi likely reflect the enhanced digestive processes facilitated by the enzyme, allowing the intestine to adapt for more efficient nutrient absorption. The fragmentation of the villi may indicate an increase in nutrient processing capacity as the enzyme reduces the fibrous content of the diet, thereby optimizing the functional efficiency of the intestinal mucosa (Awika, 2011; Bader *et al*., 2018; Zhang *et al*., 2014).

CONCLUSION

The present study was conducted to evaluate and compare the effectiveness of exogenous xylanase enzyme supplementation along with the dietary modulation and replacement of wheat from the conventional corn-soybean diet. The results of physical parameters, serological biomarkers, and gene expression analyses certify the safe use of xylanase enzyme in broiler feed to obtain better growth performance results. The effect of xylanase enzyme at molecular level yields the catabolic products of NSPs present in wheat, which allow the broilers to digest the feed ingredients better and absorb readily available nutrients ensuring rapid increase in live body weight in a smaller time window. The results of the present study can be used to economically stabilize the use of wheat as the main feed ingredient along with enzyme supplements in broiler diets to attain better animal produce than conventional means in a relatively shorter period. The study is also relevant to validate the safety of enzyme supplementation from the perspective of dysbiosis, in that the gut microflora is negligibly affected in a negative way by enzyme supplementation and only produces a prebiotic effect. Health concerns and risks associated with enzyme overload can also be determined using the documented data of current study. The two dietary modulations of using wheat as the main feed ingredient and using xylanase as an enzyme supplement can be separately or adjunctly evaluated for their benefits in nutritional aspects and can be replaced in food items having a major portion of NSPs to improve their shelf life.

DECLARATIONS

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IRB approval

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Ethical statement

All animal experiments were performed in the accredited animal research facility and comply with Bioethics Protocols issued by the Institutional Biosafety and Bioethics Committee of the Office of Research, Innovation, and Commercialization, University of Agriculture, Faisalabad.

Generative AI and AI assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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