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RESEARCH ARTICLE

Supplementation of Xylanase Levels in Lower Energy Diets on Digesta Viscosity, Blood Metabolites and Gut Health of Broiler

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A B S T R A C T

Current study was planned to evaluate supplementation of two different xylanase levels on digesta viscosity, blood metabolites and intestinal morphology of broiler chickens fed low energy wheat-based diet. A total of 480-day old broiler chicks were used in the current study with 8 replicates per treatment, and each replicate had ten birds. Six iso-caloric and iso-nitrogenous diets were prepared according to strain recommendations. Wheat was used in all dietary treatments except control treatment in which corn-soybean was used. Experimental diets were formulated as, 1) =control, corn-soybean based diet 2) = wheat-based diet, 100% wheat, no corn, 3) = 100% wheats, ME lowered 75 Kcal with 1000XU/kg of feed, 4) = 100% wheats, ME lowered 75 Kcal with 1500XU/kg of feed, 5) = 100% wheats, ME lowered 150 Kcal with 1000XU/kg of feed, 6) = 100% wheats, ME lowered 150 Kcal with 1500XU/kg of feed. Less viscosity, cholesterol and urea nitrogen noted in birds fed control diet and 100% wheat-based diet without xylanase supplementation ($P<0.05$). Low blood glucose was observed in diets without supplementation of xylanase and without reduced energy levels ($P<0.05$). Better villus height, villus width and villus surface area were in birds fed control diet and wheat-based diet with no lowered energy level and without xylanase supplementation ($P>0.05$). Based on the findings of current study, the inclusion of xylanase in diets with 75 Kcal lowered energy perform better than diets with 150 Kcal lowered energy.

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INTRODUCTION

Wheat and maize grains are being used as an energy source in the diet of broiler birds in many parts of the world (Arshad *et al.*, 2020; Kamran *et al.*, 2020). In Pakistan, surplus stored wheat is also being used in the diet of poultry (Anwar *et al.*, 2023a; 2023b). The nutritive value of wheat is lower because of soluble non-starch polysaccharides (NSPs). Wheat contains arabinoxylans as soluble NSPs (Beg *et al.*, 2001). Non starch polysaccharides are divided into two groups, insoluble and soluble NSPs (Castanon *et al.*, 1997). Soluble NSPs are those that can hold water up-to ten times of their own weight resulting in highly viscous condition in the intestine (Classen, 1996), lower the passage rate of digesta that leads to the lowered bird's performance (Choct, 1997). Poultry birds cannot produce enzymes that can hydrolyze structural carbohydrates (Khattak *et al.*, 2006). To degrade structural carbohydrates, exogenous enzymes are used in the diets of chickens high in NSPs (Gao *et al.*, 2007). The response of young poultry birds to exogenous enzymes is better as compared to the older birds because you birds are more sensitive to highly viscous cereals (Boros, 1998).

Xylanases have power to hydrolyze either soluble or non-soluble arabinoxylans. Commercial xylanases can degrade both soluble as well as non-soluble arabinoxylans present in the feed (Ravindran and Amerah, 2009). Supplementation of xylanases resulted in improved digestion of nutrients and utilization of energy by the degradation of NSPs present in the cell wall matrix of wheat-based diets (Cowieson *et al.*, 2006). Xylanases release encapsulated protein and starch, lower the viscosity of digesta and improve the access of nutrients to the endogenous enzymes. Xylanases also help in maintaining the passage rate of digesta and intestinal motility (Choct, 2006). Better nutrient absorption in broiler could easily be explained by small intestine histomorphology (villi length, crypt depth and their ratio) and it has previously been reported that higher length of villi and reduced crypt depth are major indicators for better absorption of nutrients. Crypt is known to be the major factory responsible for the synthesis of villus (Apperson and Cherian, 2017).

Supplementation of xylanase in the diet of broiler birds lower the variation that is due to the differences in wheat's chemical and physical structures (Choct, 2006). Luo *et al.* (2009) reported that supplementation of xylanase at the level of 500 U/kg and 1000 U/kg increased height of villus at duodenum, jejunum and ileum, however, supplementation of xylanase 1000 U/kg in diet results in improves the villus height/crypt depth ratio of all the segments while supplementation of xylanase 500 U/kg only results in improvement of the villus height to crypt depth.

The present study was designed to evaluate the effect of two different levels of xylanase on the wheat-based diet with lower energy levels, on digesta viscosity, intestinal morphology and blood metabolites of broilers. At level 1, xylanase was used 1000 XU/kg while at level 2, xylanase was used 1500 XU/kg of diet.

MATERIALS AND METHODS

Experimental treatments and birds' management: One month before the arrival of chicks, shed was cleaned thoroughly and fumigated. A total of 480-day old broiler chicks (Ross-308) were procured from Arslan Chicks (Pvt Ltd) Islamabad. A total of six dietary treatments were used in this experiment. Experimental diets were formulated as, , 1) =control, corn-soybean based diet 2) = wheat-based diet, 100% wheat, no corn, 3) = 100% wheat, ME lowered 75 Kcal with 1000XU/kg of feed, 4) = 100% wheat, ME lowered 75 Kcal with 1500XU/kg of feed, 5)= 100% wheat, ME lowered 150 Kcal with 1000XU/kg of feed, 6)= 100% wheat, ME lowered 150 Kcal with 1500XU/kg of feed. Experiment birds were randomly divided into six dietary treatments in such a way that each treatment had 8 replicates and each replicate had 10 birds. The ingredient data used in the diet formulation was taken from Brazilian Tables for Poultry and Swine (Rostagno *et al.*, 2011). Floor of the pens were covered with three-inch wood shavings for chicks bedding. Birds was vaccinated according to local vaccination program. A circular bottom feeder was provided for each pen, and a nipple drinking system allowed for continuous water availability.

Measurements, sampling and analyses

Intestinal morphology: On day 35, three birds were randomly selected and slaughtered from each experimental treatment for collecting ileum specimens. By using an image analysis software (Top View 3.7) the following parameters were measured:

a) VH
b) VW
c) CD
d) VH/VW
e) Ratio of villus height to crypt depth (VH/CD)

Ilium specimens (after slaughtering of birds) were obtained from the experimental birds. After collecting of samples, Ilium specimens were fixed in 10% neutral buffered formalin solution for approximately 24 h, then embedded in paraffin and sectioned at 4 µm. By using an image analysis software (Top View 3.7), the parameters measured were: VH, VW, CD (Govil *et al.,*), ratios of VH/VW, ratios of VH/CD, villus Surface Area (mm2) which was calculated by multiplying 2π x VH x VW/2 (Sakamoto *et al.,* 2000).

Determination of viscosity: Two broiler birds were slaughtered at day 30 for collecting digesta in order to determine viscosity of sample. The digestive contents of the gastrointestinal tract (except for the proventriculus) were collected gently by finger, stripping each gastrointestinal tract segment and finally frozen at -20°C for further analysis. Sample of digesta sample were pooled in a falcon tube per replicate. Viscosity of digesta samples were determined by using a Brookfield DV-E viscometer (Brookfield Eng., Middleboro, Mass., USA). Tubes were centrifuged for 5 min at 3000rpm and then supernatant was centrifuged for 5 min at 12,500rpm. The viscometer was preheated at 41°C then digesta supernatant was introduced in viscometer. The average shear rate used for measuring viscosity ranged between 45.0 s−1 and 22.5 s−1.

Blood metabolites parameters: Blood serology was done on 35th day of experiment. For collection of blood, a total of two birds were slaughtered from each replicate and blood was collected in Bolton gel & clot tube. Prior to determination of blood metabolites, blood cells and serum were separated by centrifugation. After centrifugation, serum from blood was collected and preserved in Eppendorf tubes. Blood serum glucose, urea nitrogen and cholesterol were determined by commercial kits BioMed-Urea, Bio-Med Glucose LS and Bio-Med Cholesterol kits (Sharif *et al.*, 2020).

Statistical analysis: The collected data was analyzed using General Linear Model of Minitab Statistical software 17 (Minitab Inc. 2010) under completely randomized design. Significant means will be tested by using Tukey's test.

RESULTS

Blood metabolites: Cholesterol content was observed high in birds fed 100% wheat based diet with 150 Kcal lowered energy level and with supplementation of xylanase at two levels (P<0.05). Blood glucose level was high in birds fed diets without lowered energy levels and without inclusion of xylanase (P<0.05). Urea nitrogen was less in birds fed control diet and 100% wheat based diet without supplementation of xylanase and without lowered energy levels (P<0.05).

Viscosity: Results explored that 100% wheat based diet with xylanase supplementation level 2 and with 150 Kcal lowered energy level significantly enhanced digesta viscosity (P<0.05), while corn-soybean based diet and 100% wheat based diet without xylanase supplementation and with no reduced energy level significantly reduced digesta viscosity as compared to other wheat based diets (P<0.05).

Table 1: Effect of two xylanase levels on wheat-based diet with lower energy levels on blood metabolites at day 35

	Dietary Treatments							
	Control	100%W	100%W+E<75kcal+Xy-1	100%W+E<75kcal+Xy-2	100%W+E<150kcal+Xy-1	100%W+E<150kcal+Xy-2	SEM	P-value
Cholesterol	152.1^{c}	148.5^{d}	160.6^{ab}	158.2^{ab}	168.2^{a}	162.5^{a}	1.77	0.021
Glucose	168.7^{a}	164.5^{a}	162.2^{ab}	160.4^{ab}	154.4^{b}	150.1^{c}	2.13	0.033
Urea-nitrogen	2.99^{c}	3.17^{b}	3.68^{ab}	3.89^{ab}	4.11^{a}	4.39^{a}	0.73	0.017

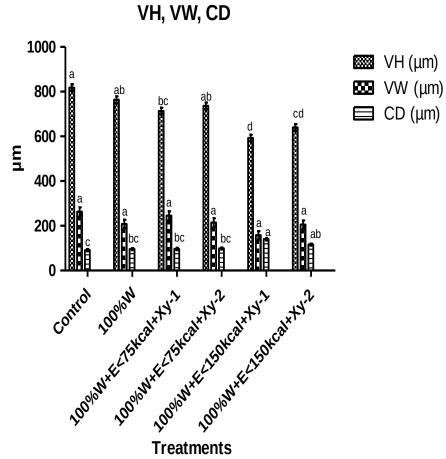
Control=Corn-soybean based diet, no wheat, 100%W=100% Wheat, no corn, 100%W+E<75kcal+Xy-1=100% Wheat, ME lowered 75Kcal with xylanase level 1, 100%W+E<75kcal+Xy-2=100% Wheat, ME lowered 75Kcal with xylanase level 2, 100%W+E<150kcal+Xy-1=100% Wheat, ME lowered 150Kcal with xylanase level 1, 100%W+E<150kcal+Xy-2=100% Wheat, ME lowered 150Kcal with xylanase level 2.

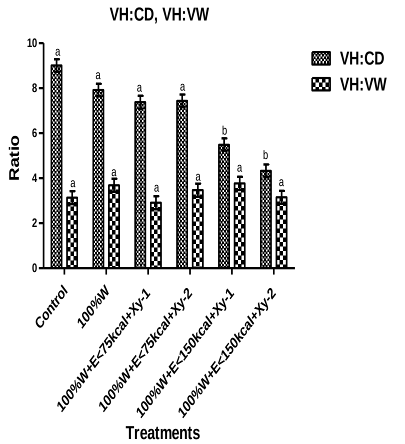
Table 2: Effect of two xylanase levels on wheat-based diet with lower energy levels on digesta viscosity at day 35

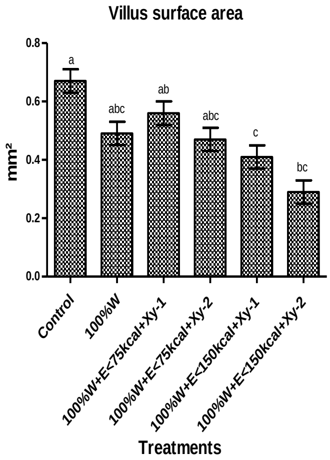
	Dietary Treatments							
Parameters	Control	100%W	100%W+E<75kcal+Xy-1	100%W+E<75kcal+Xy-2	100%W+E<150kcal+Xy-1	100%W+E<150kcal+Xy-2	SEM	P-Value
Viscosity (cps)	7.78^{c}	8.36^{c}	11.37^{ab}	9.73^{bc}	10.68^{ab}	12.52^{a}	2.37	0.005

Control=Corn-soybean based diet, no wheat, 100%W=100% Wheat, no corn, 100%W+E<75kcal+Xy-1=100% Wheat, ME lowered 75Kcal with xylanase level 1, 100%W+E<75kcal+Xy-2=100% Wheat, ME lowered 75Kcal with xylanase level 2, 100%W+E<150kcal+Xy-1=100% Wheat, ME lowered 150Kcal with xylanase level 1, 100%W+E<150kcal+Xy-2=100% Wheat, ME lowered 150Kcal with xylanase level 2.

Graphs (Intestinal Morphology)

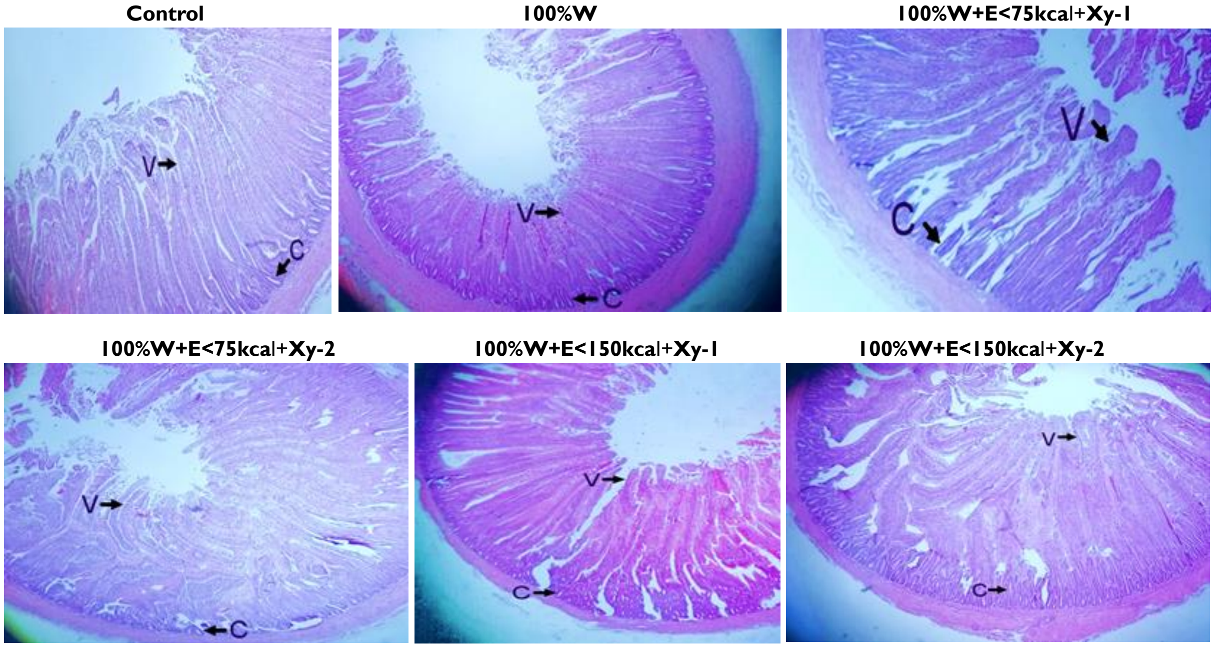






VH= Villus height, **VW**= Villus width, **CD**= Crypt depth
Control=Corn-soybean based diet, no wheat, **100%W**=100% Wheat, no corn, **100%W+E<75kcal+Xy-1**=100% Wheat, ME lowered 75Kcal with xylanase level 1, **100%W+E<75kcal+Xy-2**=100% Wheat, ME lowered 75Kcal with xylanase level 2, **100%W+E<150kcal+Xy-1**=100% Wheat, ME lowered 150Kcal with xylanase level 1, **100%W+E<150kcal+Xy-2**=100% Wheat, ME lowered 150Kcal with xylanase level 2.

Intestinal Morphology



Control=Corn-soybean based diet, no wheat, **100%W**=100% Wheat, no corn, **100%W+E<75kcal+Xy-1**=100% Wheat, ME lowered 75Kcal with xylanase level 1, **100%W+E<75kcal+Xy-2**=100% Wheat, ME lowered 75Kcal with xylanase level 2, **100%W+E<150kcal+Xy-1**=100% Wheat, ME lowered 150Kcal with xylanase level 1, **100%W+E<150kcal+Xy-2**=100% Wheat, ME lowered 150Kcal with xylanase level 2.

Intestinal morphology: Effect of different xylanase levels on wheat-based diets with lower energy levels on intestinal morphology at day 35 is presented in graphs and figures given below. Villus height (VH) was better (P<0.05) in birds fed control diet and then in wheat-based diet had no lowered energy level and not supplemented with xylanase. Villus width was numerically better (P>0.05) in birds fed corn-soybean based (control) diet and 100% wheat-based diet with no xylanase supplementation and with no lowered energy in comparison with other experimental diets. Crypt depth was high (P<0.05) in birds fed 100% wheat-based diet with xylanase supplementation level 1 and 2 with 150 kcal lowered energy level (P<0.05). VH:CD ratio was high (P<0.05) in birds fed control diet and diets with xylanase supplementation level 1 and 2 with 75 kcal lowered energy level (P<0.05) as compared to other dietary treatments. No significant effect was noticed on villus width (P>0.05). Villus surface area was high (P<0.05) in birds fed control and diet with no xylanase supplementation and with no lowered energy level (P<0.05) as compared to other dietary treatments.

DISCUSSION

Reduction of dietary energy negatively affects the growth performance of broiler birds. However, supplementation of xylanase overcomes these negative effects by maintaining the energy levels of broiler birds (Williams *et al.,* 2014). The present study tested 6 different dietary treatments, two of them had energy according to the recommendations of strain while 4 dietary treatments had lowered energy levels. Energy was reduced by 75 Kcal and 150 Kcal with wheat-based diets and these diets were further supplemented with 2 different levels of xylanase.

Less viscosity was observed in the birds fed control diet and 100% wheat-based diet without xylanase supplementation. Diets with 100% wheat with 75 Kcal lowered energy level with supplementation of xylanase had less digesta viscosity as compared to the diets with 100% wheat with 150 Kcal lowered energy value and supplemented with xylanase. (Choct *et al.*, 2004) reported that addition of enzymes decreased ileal viscosity and increased apparent metabolizable energy with diets that had lower apparent metabolizable energy.

In this study, results explored that cholesterol content was high in birds fed 100% wheat based diet with 150 Kcal lowered energy level and with supplementation of xylanase at two levels. Blood glucose level was high in birds fed diets without lowered energy levels and without inclusion of xylanase. Nutritional factors like quantity of feed and composition of feed affect an intermediary metabolism of broiler birds, resulting in the alteration of plasma metabolite levels (Buyse *et al.,* 2002; Swennen *et al.,* 2005).

Urea nitrogen was less in birds fed control diet and 100% wheat based diet without the supplementation of xylanase and without lowered energy levels (P<0.05). 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).

Intestinal histomorphology (villi length, crypt depth and their ratio) can be used as indicators of nutrient absorption. Higher length of villi and lower crypt depth are indicators of better nutrient absorption and lower mucosal tissue turnover rate (Apperson and Cherian, 2017). The height of villi is related to the absorptive capacity of the intestinal epithelial cells, and the presence of short villi reduces the surface area for nutrient absorption (Parsaie *et al.,* 2007). In our study, villus height, villus width and villus surface area were enhanced in birds fed control diet and the wheat-based diet with no lowered energy level and without supplementation of xylanase. Diets with 100% wheat with 75 Kcal lowered energy level with supplementation of xylanase had better villus height, villus width and surface area as compared to the diets with 100% wheat with 150 Kcal lowered energy value. The increasing villi height increases the surface area and contact of nutrients for maximum absorption (Silva *et al.,* 2009). These results are in good agreement with previous studies done by Luo *et al*. (2009). The active action of xylanase in birds appears to reduce the intestinal viscosity. It is hypothesized that when broilers are fed a wheat based diet, the reduced villi length is due to the increased digesta viscosity as the viscous environment can encapsulate nutrients thus inhibiting effective villi and nutrients contact, leading to an impaired structure and function of intestine (Dworkin *et al.,* 1976). Therefore, NSPases can reduce the negative effects of NSP on gut morphology by reducing digesta viscosity in diets that are not low in energy values. Furthermore, the positive impact of NSPases on gut morphology may improve the gut functions and ileal nutrients digestibility.

Diets with 100% wheat with 150 Kcal lowered energy value and supplemented with xylanase at 1000 and 1500 xylanase units had no effect on gut morphology. Like our results (Wang *et al.,* 2015), did not observe that reducing dietary energy by 100 kcal/kg had a significant effect on intestinal histomorphology of broiler chickens (Wickramasuriya *et al.*, 2019), nor observed reducing dietary energy or adding enzymes to intestinal gut morphology was significantly affected. On the other hand, Karimi and Zhandi (2015) reported that the addition of B-glucanase and/or B-mannanase significantly improved intestinal histomorphological parameters in broilers. They also reported improvements in body weight gain and FCR due to enzyme supplementation.

Conclusions: The results of this study showed that feeding wheat based with 75 Kcal lowered energy levels and with the supplementation of xylanase enzyme at 1000 and 1500XU have positive impact on digesta viscosity, blood metabolites and intestinal morphology. Based on the finding of the current study, it is recommended that broiler diet could be prepared with wheat at 75 Kcal lowered energy with supplementation of xylanase enzyme at 1000 XU without compromising broiler gut health, digesta viscosity and blood metabolites.

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Authors contribution: UA, AFE, MQB, MR, MFAC and MAR conceived and designed the experiment. UA, RM, MFK, UF and MA carried out experiment and lab analysis. MA, AFE, UF, MR and AUR performed statistcal analysis. MQB reviewed the manuscript and finalized the manuscript. All authors write, revised and reviewed the manuscript.

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