

EFFECT OF XYLO-OLIGOSACCHARIDE AND XYLANASE SUPPLEMENTATION ON GROWTH PERFORMANCE, CARCASS CHARACTERISTICS, NUTRIENT DIGESTIBILITY, AND INTESTINAL HISTOLOGY OF BROILERS

S. A. Afzal¹, U. Farooq¹, M. F. Khalid¹, S. L. Butt and G. Abbas²

¹Department of Poultry Science, Sub-Campus Toba Tek Singh, University of Agriculture Faisalabad

²Department of Animal Production, Riphah College of Veterinary Sciences, Riphah International University, Pakistan.

Corresponding Author: ghulamabbas_hashmi@gmail.com

ABSTRACT: The present study was conducted to investigate the effect of xylo-oligosaccharide and xylanase supplementation on growth performance, carcass characteristics, nutrient digestibility, and intestinal histology of broilers. Six hundred day-old chicks (ROSS-308) were divided into four experimental groups (i.e. T₁, T₂, T₃, and T₄). Each experimental unit had six replicates of 25 chicks/replicate. Following treatments were offered: T₁= positive control (PC, commercial diet); T₂= negative control (NC, basal diet); T₃= NC+Signis[®]; T₄= NC+XOS+Xylanase. The experiment was conducted from 1 to 35 days of broiler age and during this trial data regarding growth performance (feed intake, weight gain) was recorded and the feed conversion ratio was calculated. At the end of the experiment, 2 birds from every replicate were randomly selected and slaughtered to get data on carcass characteristics and gilet's weight. Intestinal tissues (duodenum, jejunum, and ileum) were also collected for histological analysis. Digestibility data was collected toward the end of the experiment to estimate nutrient digestibility. The data so generated were analyzed using the General Linear Model procedure of SPSS, 18.0, and the mean was compared using Tukey's test. Results showed that during the starter phase, the effect of the treatments was non-significant for mortality and feed conversion ratio whereas a significant effect was observed for body weight gain and feed intake. Treatment C had significantly higher feed intake whereas for body weight treatments C and D had significantly higher body weight than positive and negative control groups. During the grower phase, the effect of the treatment was significant for mortality where treatments C had significantly higher mortality compared with all other treatments. The effect of the treatments was significant on body weight gain during the grower phase where treatment D had a significantly higher body weight compared with treatment The effect of the treatments was non-significant on the feed intake and feed conversion ratio. During the finisher phase, the effect of the treatments was significant on mortality where treatment D had the highest mortality followed by treatment A. Treatments C B and C showed no mortality during the finisher phase of the study. The effect of the treatments was significant on FCR during the finisher phase. Treatments B and C had significantly poor FCR compared with treatment A while treatment D had non-significant differences with all other treatments. The effect of the treatments was non-significant for body weight and feed intake during the finisher phase. For carcass characteristics and intestinal histology, none of the treatments showed any significant difference. Keeping in view the data on growth performance we concluded that the supplementation of these enzyme energy contents of the feed can be decreased without affecting the performance of the broilers.

Key words: xylo-oligosaccharide; xylanase; ROSS-308; performance; nutrient digestibility; intestinal histology.

(Received 15.10.2022

Accepted 17.12.2022)

INTRODUCTION

There is a pressing need for a well-balanced diet for good health, vigor, and productivity of people. In Pakistan, peoples usually take unbalanced diets rich in carbohydrates and deficient in protein. Animals and plants are two major protein sources. Pakistan takes diets poor in animal proteins that are highly biologically available forms of proteins; approximately 66% are deficient in proteins. Due to a lack of sufficient energy

and protein in the food or because of insufficient food accessibility, malnutrition and stunting growth are dominant in Pakistan. Pakistan's Food Security and Nutrition Strategy Review estimate that the overall prevalence of undernourishment in the country is 18% which is moderately high compared with the global hunger map threshold. Protein plays an indispensable role in forming a stable diet for human consumption (Maqbool, 2002). In Pakistan, the existing per capita daily available protein from animal sources such as beef, lamb, poultry, and fish is only 17 grams. According to the

World Health Organization, this supply is well below the recommended intake of 26 grams of animal-derived dietary protein (anonymous, 2012). To provide animal protein at an affordable price to overcome the gap between protein supply and demand, poultry meat contributes a large part. By controlling the production losses of intestinal-related intestinal pathogens, the performance of chickens can be improved, thereby further increasing this share. By adopting the lowest-cost feed production strategies to prevent and control intestinal pathogens, thereby reducing the overall production cost.

In poultry production systems, feed is vital to nutritionists because it accounts for more than 70% of total production costs. Protein and energy are generally considered the main expensive components of poultry diets (Firman and Boling, 1998). The main share of the protein portion (up to 50%) comes from soybean meal (SBM), which is imported and is now the most expensive. When formulating broiler diets, the key focus is on crude protein (CP), adjusting the protein level in chicken diets significantly affects the growth, feed cost, and profitability of broiler producers (Eits *et al.*, 2004).

Pakistan has become the 11th largest poultry producer in the world. However, the industry still faces many problems, such as high feed costs, salmonellosis, coccidiosis, intestinal-related problems, emerging diseases, etc., as well as the massive loss of food contaminated by pathogens such as *Salmonella typhi* and its impact on birds and consumers. The effects of slow weight gain and even increased mortality are the main obstacles to its progress (Saima *et al.*, 2010). This sector is constantly facing the challenges of ever-increasing prices of feed ingredients. Broilers are offered diets containing certain levels of non-starch polysaccharides (NSPs) which result in the increase of the viscosity of intestinal digesta and cause problems in achieving production goals (Jia *et al.*, 2009; Saima *et al.*, 2010; Hafez *et al.*, 2020).

This problem, however, can be overcome in various ways and one of the recommended solutions is the use of enzymes. Enzymes are beneficial for the health, growth, and production of broilers but should be used at recommended levels for better results (Acamovic, 2001). Supplemented enzymes reduce the anti-nutritional factors and make the nutrients available to the broilers which are otherwise blocked by non-starch polysaccharides (NSP). Enzymes also reduce the viscosity of digesta, thus improving the availability of nutrients to the birds and ultimately enhancing their growth performance (Bedford and Classen, 1992). NSPase breaks down the NSPs, into smaller oligosaccharides to release blocked nutrients and lower the digesta viscosity. Another benefit of using NSPase is that during the breakdown of NSPs, small

oligosaccharides are produced which are prebiotic and help in intestinal health (Mushtaq *et al.*, 2007; Courtin *et al.*, 2008; Knudsen, 2014; Morgan *et al.*, 2020).

Keeping in view the state of affairs the present field of study has not been explored. Therefore; the present study was planned to explore the effect of xylo-oligosaccharide and xylanase on broilers in our local environment with the following objectives;

To see the effect on growth performance, carcass characteristics, nutrient digestibility, and intestinal histology.

MATERIALS AND METHODS

This study was conducted at the Research and Development unit of Sadiq Feeds (Pvt.) Ltd Mandra, Rawalpindi, Pakistan. For this, six hundred (600) Day-old ROSS-308 broiler chicks were procured from Sadiq Poultry's Salman hatchery Chakri and reared at Sadiq Feed's Research and Development unit, Mandra. Chicks were randomly assigned into four treatment groups A, B, C and D respectively. Six replicates per treatment were prepared with 25 chicks in each replicate. The trial duration was day 1 to day 35th of broiler age.

The chicks were raised in a (75'x25') environmentally controlled house having 3 tunnel fans of 54" for tunnel ventilation and 1 side fan of 42" for minimum ventilation with a 225 square feet pad area. The pen size was 5'x4.5' for each replicate of a treatment. The whole house was whitewashed and disinfected 2 weeks before the arrival of the chicks. Fumigation of the shed was carried out by the use of $KMnO_4$ and formalin at a ratio of 1:2. For fumigation, the house was closed tightly, temperature was brought to 70 °F and then fumigants were discharged. After fumigation house was locked for 24 hours. After 24 hours air was exhausted out thoroughly. The pens were allotted at random to different experimental units. As a litter material, a 3 inches layer of rice husk was used in each pen. The birds were allotted to respective pens at day 1 of age. To maintain the brooding temperature gas brooder was used. Biosecurity measures were strictly followed during the research trial. The brooding temperature was maintained at the standard recommended by the producer's guidelines. The birds in all replicates were reared under the same environment and management conditions. Broilers were vaccinated against Newcastle Disease (ND) and Infectious Bursal Disease (IBD) according to the recommended schedule. Treatment Plan: Four experimental diets were formulated (Figure-1) to feed in a four-phase feeding program, namely S1 (Pre-Starter), S2 (Starter), S3 (Grower), and S4 (Finisher) designated as Treatment A positive control (PC basal diet) B negative control (NC, 100 Kcal and 5% Amino acids level reduction), C NC+Signis® and D (NC+ Xylanase® + XOS at 0.01%).

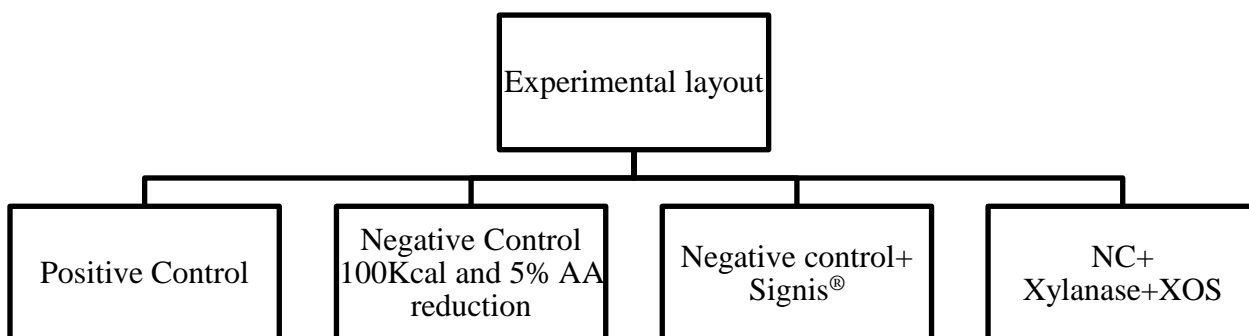


Figure 1: Experimental layout

Table 1: Treatment Plan and composition of pre-starter, starter, grower, and finisher diets.

Phase	T ₁ (PC*)		T ₂ (NC*)		T ₃ (NC+ Signis®)		T ₄ (NC+XOS+Xylanase)	
	CP ¹	ME ²	CP	ME	CP	ME	CP	ME
S ₁	22.5	2720	20.9	2620	20.9	2620	20.9	2620
S ₂	22.1	2900	20.7	2800	20.7	2800	20.7	2800
S ₃	21.7	2989	20.3	2889	20.3	2889	20.3	2889
S ₄	21.5	3000	20.1	2900	20.1	2900	20.1	2900

PC*= Positive Control, NC*= Negative Control, CP¹= Crude Protein%, ME²=Metabolizable Energy Kcal/Kg
 Experimental diets were analyzed by AOAC (2005) for dry matter, organic matter, crude protein, crude fiber, ether extract, total ash, and nitrogen-free extract. The birds were fed ad libitum according to the following plan.

Table 2 Composition of pre-starter (S1) feed.

S1	PC	NC	NC+SIGNIS	NC+XOS+XYL
Corn	43.80	45.05	45.05	45.05
SBM	22.58	19.07	19.07	19.07
SFM	3.00	12.00	12.00	12.00
Canola meal	6.00	6.00	6.00	6.00
Rice polish	15.00	10.00	10.00	10.00
PBM	4.50	3.00	3.00	3.00
Fish meal	0.00	--	--	--
MBM	2.70	--	--	--
molasses	0.04	1.06	1.06	1.06
mcp		0.77	0.77	0.77
Chips	0.68	1.29	1.29	1.29
Chemical Composition of pre-starter (S1) feed				
Salt	0.33	0.35	0.35	0.35
Sodium Bi-Carb	0.10	0.10	0.10	0.10
Lysine sulfate	0.52	0.58	0.58	0.58
DLM	0.29	0.25	0.25	0.25
Threonine	0.18	0.18	0.18	0.18
Valine	0.01	0.01	0.01	0.01
CC 70 %	0.02	0.02	0.02	0.02
Vit premix	0.10	0.10	0.10	0.10
Min premix	0.10	0.10	0.10	0.10
Antioxidants	0.01	0.01	0.01	0.01
Diclazuril	0.02	0.02	0.02	0.02
Enra	0.01	0.01	0.01	0.01
Phytase	0.02	0.02	0.02	0.02

PC=Positive control, NC=Negative control

Table 43 Composition of starter (S2) feed.

S2	PC	NC	NC+SIGNIS	NC+XOS+XYL
Corn	57.00	54.00	54.00	54.00
SBM	25.71	18.05	18.05	18.05
SFM	0.00	10.00	10.00	10.00
Canola meal	6.00	6.00	6.00	6.00
Rice polish	3.00	3.61	3.61	3.61
PBM	3.64	5.00	5.00	5.00
Fish meal	0.00	--	--	--
MBM	2.39	--	--	--
MCP		0.55	0.55	0.55
Chips	0.63	1.09	1.09	1.09
Chemical Composition of pre-starter (S1) feed				
Salt	0.35	0.34	0.34	0.34
Sodium Bi-Carb	0.10	0.10	0.10	0.10
Lysine sulfate	0.46	0.58	0.58	0.58
DLM	0.28	0.22	0.22	0.22
Threonine	0.17	0.16	0.16	0.16
Valine	0.01			
CC 70 %	0.01	0.01	0.01	0.01
Vit premix	0.10	0.10	0.10	0.10
Min premix	0.10	0.10	0.10	0.10
Antioxidant	0.01	0.01	0.01	0.01
Diclazuril	0.02	0.02	0.02	0.02
Enra	0.01	0.01	0.01	0.01
Phytase	0.02	0.02	0.02	0.02

PC=Positive control, NC=Negative control

Table 4 Composition of Grower (S3) feed.

S3	PC	NC	NC+SIGNIS	NC+XOS+XYL
Corn	60.96	58.93	58.93	58.93
SBM	21.82	14.32	14.32	14.32
SFM	0.00	10.00	10.00	10.00
Canola meal	7.00	7.00	7.00	7.00
Fish meal	0.00			
PBM	6.00	6.00	6.00	6.00
MBM	2.14	1.17	1.17	1.17
MCP		0.22	0.22	0.22
Chips	0.56	0.76	0.76	0.76
Chemical Composition of pre starter (S1) feed				
Salt	0.32	0.25	0.25	0.25
Sodium Bi Carb	0.10	0.16	0.16	0.16
Lysine sulfate	0.44	0.56	0.56	0.56
DLM	0.22	0.18	0.18	0.18
Threonine	0.12	0.13	0.13	0.13
Valine	0.03	0.03	0.03	0.03
Vit premix	0.10	0.10	0.10	0.10
Min premix	0.10	0.10	0.10	0.10
Antioxidant	0.01	0.01	0.01	0.01
Salinomycine	0.05	0.05	0.05	0.05
Enra	0.01	0.01	0.01	0.01
Phytase	0.02	0.02	0.02	0.02

PC=Positive control, NC=Negative control

Table 5 Composition of Grower (S4) feed.

S4	PC	NC	NC+SIGNIS	NC+XOS+XYL
Corn	61.47	59.76	59.76	59.76
SBM	19.92	12.89	12.89	12.89
Canola meal	8.00	8.00	8.00	8.00
SFM		10.00	10.00	10.00
Fish meal	1.81			
PBM	6.00	6.00	6.00	6.00
MBM	0.90	1.18	1.18	1.18
MCP		0.02	0.02	0.02
Chips	0.57	0.63	0.63	0.63
Chemical Composition of pre starter (S1) feed				
Salt	0.26	0.20	0.20	0.20
Sodium Bi Carb	0.11	0.19	0.19	0.19
Lysine sulfate	0.37	0.54	0.54	0.54
DLM	0.19	0.17	0.17	0.17
VALINE		0.01	0.01	0.01
Threonine	0.10	0.11	0.11	0.11
Vit premix	0.10	0.10	0.10	0.10
Min premix	0.10	0.10	0.10	0.10
Antioxidant	0.01	0.01	0.01	0.01
Salinomycine	0.05	0.05	0.05	0.05
Enra	0.01	0.01	0.01	0.01
Phytase	0.02	0.02	0.02	0.02

PC=Positive control, NC=Negative control

Data regarding the performance (weekly body weight gain, weekly feed consumed, FCR) and slaughter characteristics were recorded (days 7th to 35th). A complete record of mortality in each replicate was recorded throughout the experimental period.

A separate digestibility trial was also conducted. For this indirect marker method was used to determine nutrient digestibility. For this purpose, acid-insoluble ash (Celite®) was included in the experimental diet @ 1%. Feces samples were collected on day 35th of the trial followed by an adaptation period of three days. The polythene sheets were placed under each pen and droppings were collected twice a day. Feces samples were stored at -10°C. Nutrient digestibility was determined by the following relationship:

$$\text{Digestibility coefficient} = 100 - \left(100 \times \frac{\text{Marker in feed (\%)}}{\text{Nutrient in feed (\%)}} \times \frac{\text{Nutrient in feed (\%)}}{\text{Marker in feed (\%)}} \right)$$

For the intestinal histological study, samples from the distal portion of the duodenum, jejunum, and ileum were collected from slaughtered birds and fixed in a 10 % buffered formalin solution. These intestinal segments were dehydrated by immersing through a series of alcohols of increasing concentrations (from 70% to absolute), infiltrated with xylene, and embedded in

paraffin wax. A microtome was used to make cuts of 5µm which were mounted on glass slides and stained with hematoxylin-eosin. The values were measured using a light microscope (Bancroft and Stevens, 1990). Data collected for each parameter were analyzed using PROC GLM procedure of Statistical Analysis System (SAS, 2009). The means were compared using Turkey's test and the differences were checked for statistical significance (P<0.05).

RESULTS AND DISCUSSION

The study was divided into four phases as per ROSS-308 guidelines i.e. pre-starter, starter, grower, and finisher phases. The data for body weight, feed intake, mortality, and FCR were recorded weekly during each phase and described below under the section on growth performance

Growth performance

Week 1-2 (1-14 days)

The data for mortality, feed intake, body weight, and FCR for weeks 1-2 (1-14 days) is given in Table 6

Table 6: Comparison of mortality, feed intake, body weight, and FCR fed on different treatments A (Positive control), B (Negative control), C (Negative control +SIGNIS®), and D (Negative control+XOS+XYL).

Treatment	Mortality (%)	Feed Intake (g)	Body Weight (g)	FCR
A	0.00	661±16	410±14.6	1.61±0.025
B	0.167±0.19	699±17	432±9.92	1.61±0.025
C	0.167±0.19	714±19	454±15.1	1.57±0.025
D	0.00	703±13	446.2±10.0	1.57±0.025

A = Positive Control (PC)
 B = Negative control (NC)
 C = NC + SIGNIS®
 D = NC + XOS + XYL

Effect on Production performance during starter phase i.e. Week 1-2 (1-14 days)

The mortality was lowest in treatments A and D while the highest mortality was observed in treatments B and C during weeks 1-2 (1-14 days). The effect of the treatments was non-significant on mortality. The highest feed intake was observed in treatments C and D followed by treatments B and A. The control group had the lowest feed intake during weeks 1-2 (1-14 days). The effect of the treatments was significant on feed intake where C treatment had significantly higher feed intake than treatment A. The body weight was highest for treatment C followed by treatments D, B, and A. So the negative control diet had the lowest body weight. The effect of the

treatments was significant where treatments C and D had significantly better weight gain compared with A (Table 6). The graphical presentation of the data is given in figure 4.4. The poorest FCR was obtained in treatments A and B while treatments C and D had comparatively better FR compared with the other treatments. The effect of the treatments was non-significant on the treatments.

Effect on Production performance during weeks 3-4 (15-28 days)

The period of weeks 3-4 started on day 15th and ended on the 28th day. The data on mortality, feed intake, body weight, and feed conversion ratio is described in table 7.

Table 7: Mean values for the growth-related parameters i.e. the body weight gain, feed intake FCR, and mortality fed on different treatments A (Positive control), B (Negative control), C (Negative control +SIGNIS®), and D (Negative control+XOS+XYL) from week 3-4(15 to 28days) the day of broiler age.

Groups	Mortality (%)	Feed Intake (g)	Body Weight (g)	FCR
A	0.00	2438±110	1481±51.4	1.64±0.20
B	0.00	2551±50	1504±54.0	1.69±0.19
C	0.333	2589±72	1536±53.4	1.68±0.19
D	0.00	2557±40	1545±69.0	1.65±0.19

A = Positive Control (PC)
 B = Negative control (NC)
 C = NC + SIGNIS®
 D = NC + XOS + XYL

During weeks 3-4(15-28 days) treatment C had the highest mortality compared with all other treatments whereas no mortality was observed in any of the other treatment groups. The effect of the treatment was significant for mortality during weeks 3-4(15-28 days) where treatments C had significantly higher mortality compared with all other treatments. The data for mean feed intake during weeks 3-4(15-28 days) is presented in table 7. The effect of the treatments was non-significant on the feed intake during weeks 3-4(15-28 days). However, treatment C had the highest feed intake followed by treatments D, B, and A. The effect of the treatments was significant on body weight gain during

weeks 3-4(15-28 days) where treatment D had a significantly higher body weight compared with treatment A. whereas no significant differences were observed between other treatments for body weight gain during the growing phase. The effect of the treatments was non-significant for the FCR during weeks 3-4(15-28 days). The poorest FCR was observed in treatments C and D while the better FCR was observed for treatments D and A.

Effect on Production performance during Finisher phase on Week 5 (29-35 days): The data for the finisher phase is presented in this section. The finisher phase lasted for one week and the period was 28th to 35th days

of broiler age. The data on mortality, feed intake, body weight, and feed conversion ratio is described in table 8

Table 8: Mean values for the growth-related parameters i.e. the body weight gain, feed intake FCR and mortality from 29 to 35 the day of broiler age fed on different treatments A (Positive control), B (Negative control), C (Negative control +SIGNIS[®]), and D (Negative control+XOS+XYL) from 15 to 28 the day of broiler age.

Groups	Mortality (%)	Feed Intake (g)	Body Weight (g)	FCR
A	0.167±0.12	3426±108	2035±97.3	1.68±0.20
B	0.00±0.00	3552±162	1967±131	1.81±0.19
C	0.00±0.00	3589±161	1967±93.7	1.82±0.18
D	0.667±0.89	3589±73	2036±120	1.77±0.18

A = Positive Control (PC)
 B = Negative control (NC)
 C = NC + SIGNIS[®]
 D = NC + XOS + XYL

The effect of the treatments on mortality is presented in table 8. the data comparison between the treatments is given in figure 4.9. The effect of the treatments was significant on mortality where treatment D had the highest mortality followed by treatment A. Treatments C B and C showed no mortality during the finisher phase of the study.

Feed intake (FI)
 Week 5 (29-35 days)

The mean feed intake for the broilers fed on different treatments is given in table 4.3 and the comparison between the treatments is shown in figure 4.10. The effect of the treatments was non-significant on feed intake during the finisher phase. Treatments C and D had similar but highest feed intake followed by

treatments B and A. The effect of the treatments was non-significant between the treatments whereas treatment D had the highest body weight followed by treatments A, C, and D. The effect of the treatments was significant on FCR during the finisher phase. Treatments B and C had significantly poor FCR compared with treatment A while treatment D had non-significant differences with all other treatments.

Digestibility Trial:

DM digestibility

The digestibility trial was conducted at the end of the experiment i.e. days 34th and 35th and the result for the experiment is described in table 9.

Table 9: Mean values for the dry matter, crude protein, Fats, Ash, AIA, and Fiber broilers fed on different treatments i.e. A (Positive control), B (Negative control), C (Negative control +SIGNIS[®]), and D (Negative control+XOS+XYL).

Fecel Tag	DM	Cp (Leco)	Oil	Ash	AIA	Fiber
A	14.8	22.5	1.2	18.7	6.6	22.0
B	18.4	20.6	1.2	16.0	5.4	26.8
C	19.8	26.3	1.1	14.8	5.5	24.0
D	18.4	23.1	1.3	15.0	5.1	22.1

A = Positive Control (PC)
 B = Negative control (NC)
 C = NC + SIGNIS[®]
 D = NC + XOS + XYL

The highest moisture was observed in Treatment A followed by treatments B, D and C. The effect of the treatments was non-significant for the trial. The dry matter contents were highest in treatments C followed by treatments D, B, and A. The effect of the treatments were non-significant for the trial. The CP digestibility was highest for treatment C followed by treatment D. The effect of the treatments was non-significant for the trial. The oil digestibility was highest for treatments A, B, and D and the lowest value was observed for treatment C.

The effect of the treatments was nonsignificant for the trial. The comparison of the treatments for the digestibility of the fiber contents is given in Figure 4.17. The fiber digestibility was highest for treatments B followed by C, A, and D. The effect of the treatments were non-significant for the trial

Carcass Characteristics: The data for the carcass characteristics are given in table 10. The data showed a

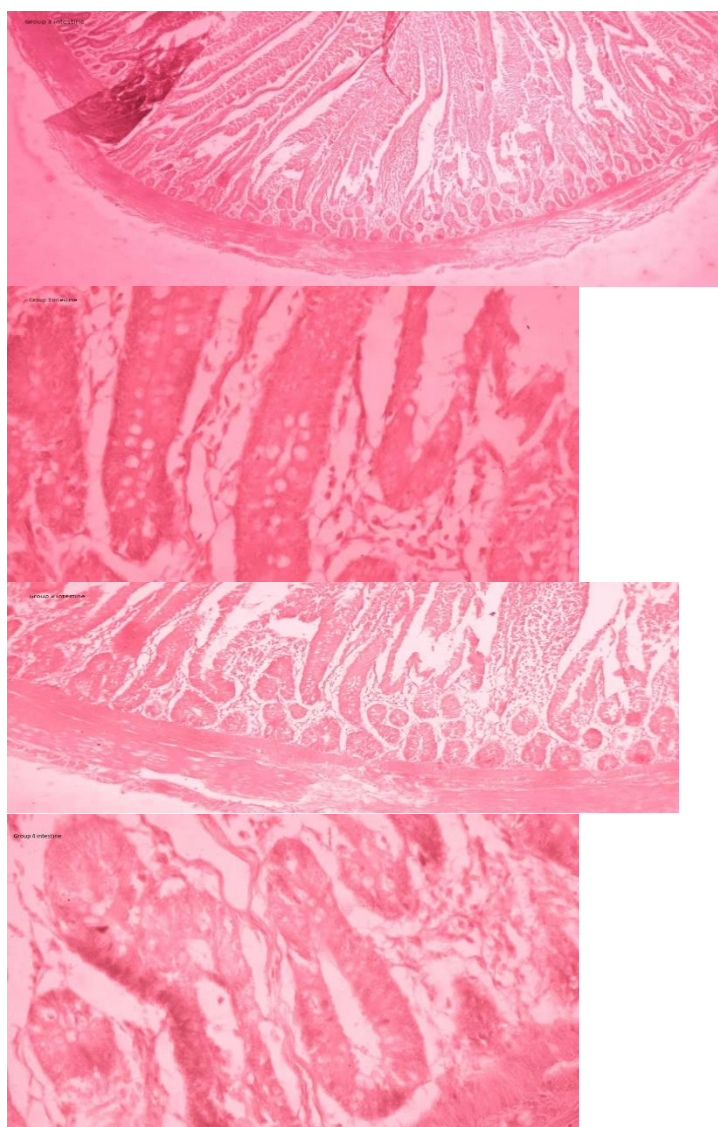
non-significant effect of treatments on carcass characteristics.

Table 10: Mean values for the eviscerated carcass, breast meat, drumstick, and abdominal fat for broiler fed on different treatments i.e. A (Positive control), B (Negative control), C (Negative control +SIGNIS[®]), and D (Negative control+XOS+XYL) from 15 to 28 the day of broiler age.

Parameters	A	B	C	D
Eviscerated carcass	71.0	70.1	71.5	71.8
Breast	19.6	19.5	19.1	19.2
Drumstick	15.6	15.4	15.5	16.1
Abdominal fat	1.8	1.8	2.1	1.9

Intestinal Histology: The intestinal tissues for the duodenum, jejunum, and ileum were analyzed for any histological changes, however normal histology was

observed and there was no significant difference in villi height and crypt depth for all the treatments



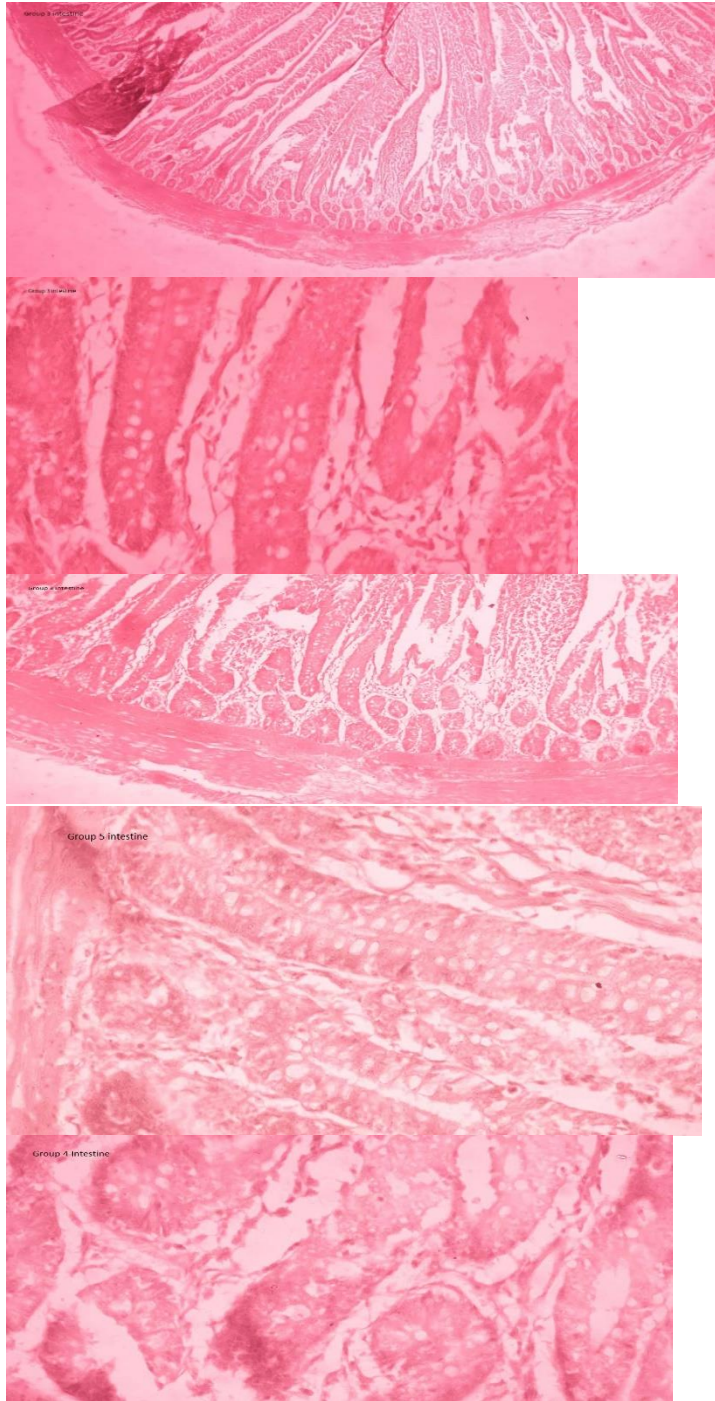


Figure 2: Effect of the treatments on intestinal histology for broilers fed on different treatments A (Positive control), B (Negative control), C (Negative control +SIGNIS[®]), and D (Negative control+XOS+XYL)

During the starter phase, the effect of the treatments was non-significant for mortality and feed conversion ratio whereas a significant effect was observed for body weight gain and feed intake. Treatment C had significantly higher feed intake while the treatments c and D where enzymes were supplemented

had significantly higher body weights than positive and negative control groups

During the grower phase, the effect of the treatment was significant for mortality where treatments C had significantly higher mortality compared with all other treatments. The effect of the treatments was significant on body weight gain during the grower phase

where treatment D had a significantly higher body weight compared with treatment The effect of the treatments was non-significant on the feed intake and feed conversion ratio

During the finisher phase, the effect of the treatments was significant on mortality where treatment D had the highest mortality followed by treatment A. Treatments C B and C showed no mortality during the finisher phase of the study. The effect of the treatments was significant on FCR during the finisher phase. Treatments B and C had significantly poor FCR compared with treatment A while treatment D had non-significant differences with all other treatments. The effect of the treatments was non-significant for body weight and feed intake during the finisher phase.

Our results are in line with Al-Qahtani *et al.* (2021) who evaluated the effect of supplementation of varying levels of xylanase, β -glucanase and phytase on intestinal enzyme activities and tibia bone development in broiler chickens fed wheat-based diets. Our results are also in line with Chaves *et al.* (2020) conducted a study to examine the association between dietary phytase and xylanase by determining the morphology of the intestinal tract of broilers, Similarly, Bautil *et al.* (2020) conducted a trial to evaluate the effects of dietary supplementation of arabinoxylan-oligosaccharides on the rate of arabinoxylans digestion in the gastrointestinal tract of broilers and reported an increase in body weight gain and feed intake.

Our results are also in line with Abdallah *et al.* (2020) conducted a study to replace soybean meal (SBM) with cottonseed meal (CSM) in a wheat/sorghum/SBM-based diet. CSM-enzyme interaction had a significant effect on feed intake and weight gain at the initial stage. In contrast to our results, however, the addition of enzymes significantly improved the feed conversion ratio and body weight during the growing and finisher phases. The experimental diets had a positive effect on gizzard and intestinal weights. CSM improved the yield of thighs and breast meat. Supplementation of enzymes improved the ileum digestibility. So supplementation of enzymes in CSM-based feed replaced SBM in broiler diets by up to 90 % without affecting their performance..

Similarly, Craig *et al.* (2020) studied the effect of xylanase or xylo-oligosaccharide supplementation on growth performance, the caecal concentration of non-starch polysaccharide hydrolysis products, and the concentration of short-chain fatty acids of broiler birds.. Supplementation showed improved caecal acetic acid, iso butyric acid, iso valeric acid, n valeric acid, and total short-chain fatty acid concentrations on day 14 of age as compared to xylanase. High levels of xylanase supplementation showed improved ileal concentration of arabinose, galactose, and glucuronic acid (GlucA2) in the insoluble non-starch polysaccharides fraction as compared to the control treatment. A high level of

xylanase or low level of xylooligosaccharides supplementation resulted in an improved ileal concentration of fructose in the water-soluble non-starch polysaccharides as compared to the control treatment.

Contrary to our results Feng *et al.* (2020) studied *Bacillus cereus* xylanase produced by the fermentation of wheat bran and its impact on growth performance and intestinal microflora of broilers and reported solid-state fermentation by xylanase-producing *Bacillus cereus* a feasible approach to pre-treat wheat bran for feedstuff industry. They found no significant differences in growth performance among treatments, although the improved activity of amylase in the duodenum of the fermented wheat bran group than the control group.

Similarly, Govil *et al.* (2017) conducted a study to evaluate the performance of broiler chicks fed low-energy corn-soya diets with multicarbohydrase supplementation. Multicarbohydrases (xylanase at 50 g/ton+mannanase at 50 g/ton+amylase at 40 g/ton) supplementation showed a significant improvement in total weight gain, feed conversion efficiency, and performance index. Dietary supplementation of multicarbohydrase resulted in increased retention of crude protein and ether extract significantly. However, the retention of dry matter, crude fiber, and nitrogen-free extract were comparable in all three groups. Multicarbohydrases supplementation resulted in the highest dressed weight, eviscerated weight, and drawn weight (% of live body weight) significantly.

Conclusion: The birds that were fed diets supplemented with enzymes had significantly higher body weight and good digestibility of feed than the positive and negative control groups. For carcass characteristics and intestinal histology, none of the treatments showed any significant difference. Keeping in view the data on growth performance and digestibility, it can be concluded that by the supplementation of these enzymes energy contents of the feed can be decreased without affecting the performance of the broilers.

REFERENCES

- Abdallah, M.E., S. Musigwa, E.U. Ahiwe, E.P. Chang'a, M. Al-Qahtani, M. Bhuiyan and P.A. 2020. Replacement value of cottonseed meal for soybean meal in broiler chicken diets with or without microbial enzymes. *J. Anim. Sci. and Tech.* 62(2): 159–173.
- Adhikari, P., A. Kiess, R. Adhikari, and R. Jha. 2020. An approach to alternative strategies to control avian coccidiosis and necrotic enteritis. *J. Appl. Poult. Res.* 29(2): 515–534.

- Acamovic, T. 2001. Commercial application of enzyme technology for poultry production. *World's Poult. Sci.* 57: 225-242.
- AOAC. 2005. Official Methods of Analytical Chemists. 18th ed. The Association of Official Analytical Chemists. Arlington, USA.
- Al-Qahtani, M., E.U. Ahiwe, M.E. Abdallah, E.P. Chang'a, H. Gausi, M.R. Bedford and P.A. Iji. 2021. Endogenous enzyme activities and tibia bone development of broiler chickens fed wheat-based diets supplemented with xylanase, β -glucanase and phytase. *Anim. Biosci.* 34(6): 1049–1060.
- Amerah, A., L. Romero, A. Awati and V. Ravindran. 2017. Effect of exogenous xylanase, amylase, and protease as single or combined activities on nutrient digestibility and growth performance of broilers fed corn/soy diets. *Poult. Sci.* 96(4): 807–816.
- Amerah, A., K. Van de Belt and J.D. Van der Klis. 2015. Effect of different levels of rapeseed meal and sunflower meal and enzyme combination on the performance, digesta viscosity and carcass traits of broiler chickens fed wheat-based diets. *Anim.* 9(7): 1131–1137.
- Anonymous. 2012. Modernization of poultry farming. [<http://pakagri.blogspot.com/2012/01/modernization-of-poultry-farming.html>].
- Arczewska-Wlosek, A., S. Swiatkiewicz, D. Bederska-Lojewska, S. Orczewska-Dudek, W. Szczurek, D. Boros, A. Frasz, E. Tomaszewska, P. Dobrowolski, S. Muszynski, M. Kwiecien and T. Schwarz. 2019. The Efficiency of Xylanase in Broiler Chickens Fed with Increasing Dietary Levels of Rye. *Anim.* 9(2): 46.
- Baffoni, L., F. Gaggia, G. Garofolo, G. di Serafino, E. Buglione, E. di Giannatale and D. di Gioia. 2017. Evidence of *Campylobacter jejuni* reduction in broilers with early synbiotic administration. *Int. J. Food Microbiol.* 251: 41–47.
- Bancroft, J.D., and A. Stevens. 1990. *Theory and Practice of Histological Techniques* Churchill Livingstone, Edinburgh, London. pp. 21-119.
- Bautil, A., J. Verspreet, J. Buyse, P. Goos, M. Bedford, and C. Courtin. 2020. Arabinoxylan-oligosaccharides kick-start arabinoxylan digestion in the aging broiler. *Poult. Sci. J.* 99(5): 2555–2565.
- Bedford, M.R. and H.L. Classen. 1992. Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate composition of the intestinal aqueous phase and results in improved growth rate and food conversion efficiency of broiler chicks. *J. Nut.* 122(5): 60-569.
- Bhuiyan, M. M. and P.A. Iji. 2015. Energy Value of Cassava Products in Broiler Chicken Diets with or without Enzyme Supplementation. *Asian-Austral. J. Anim. Sci.* 28(9): 1317–1326.
- Chaves, N.R., K.M. Nascimento, C. Kiefer, M.S. Rosa, H.B. Feitas, L.L. Paiva, T.R. Silva, L.A. Silva, V.A. Macie, C.R. Leal and A.I. Souza. 2020. Phytase and xylanase in diets with nutritional adjustments and their effects on serum biochemistry, morphometry and intestinal health of broilers. *Ana. Acad. Bras. Cienc.* 92.
- Choct, M., A. Kocher, D. L. E. Waters, D. Pettersson and G. Ross. 2004. A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. *Br. J. Nut.* 92(1): 53–61.
- Chuang, W.Y., L.J. Lin, Y.C. Hsieh, S.C. Chang and T.T. Lee. 2021. Effects of *Saccharomyces cerevisiae* and phytase co-fermentation of wheat bran on growth, antioxidation, immunity and intestinal morphology in broilers. *Anim. Biosci.* 34(7): 1157–1168.
- Cowieson, A.J., M. Toghyani, S.K. Kheravii, S.B. Wu, L.F. Romero and M. Choct. 2019. A mono-component microbial protease improves performance, net energy, and digestibility of amino acids and starch, and upregulates jejunal expression of genes responsible for peptide transport in broilers fed corn/wheat-based diets supplemented with xylanase and phytase. *Poult. Sci. J.* 98(3): 1321–1332.
- Courtin, C.M., W.F. Broekaert, K. Swennen, O. Lescroart, O. Onagbesan, J. Buyse and J.A. Delcour. 2008. Dietary inclusion of wheat bran arabinoxyloligosaccharides induces beneficial nutritional effects in chickens. *Cer. Chem.* 85: 607-613.
- Craig, A.D., M.R. Bedford, P. Hastie, F. Khattak and O.A. Olukosi. 2019. The effect of carbohydrases or prebiotic oligosaccharides on growth performance, nutrient utilisation and development of small intestine and immune organs in broilers fed nutrient-adequate diets based on either wheat or barley. *J. Sci. Food Agric.* 99(7): 3246–3254.
- Craig, A.D., F. Khattak, P. Hastie, M.R. Bedford and O.A. Olukosi. 2020. The similarity of the effect of carbohydrase or prebiotic supplementation in broilers aged 21 days, fed mixed cereal diets and challenged with coccidiosis infection. *PLoS One.* 15(2): e0229281.
- Dänicke, S., W. Böttcher, H. Jeroch, J. Thielebein and O. Simon. 2000. Replacement of Soybean Oil with Tallow in Rye-Based Diets without Xylanase

- Increases Protein Synthesis in Small Intestine of Broilers. *J. Nutr.* 130(4): 827–834.
- De Maesschalck, C., V. Eeckhaut, L. Maertens, L. De Lange, L. Marchal, G. Daube, J. Dewulf, F. Haesebrouck, R. Ducatelle and B. Taminau. 2019. Amorphous cellulose feed supplement alters the broiler caecal microbiome. *Poult. Sci.* 98(9): 3811–3817.
- Dinani, O.P., P.K. Tyagi, J.S. Tyagi, S.K. Bhanja and J.J. Rokade. 2020. Effect of feeding rice gluten meal with and without enzymes on hematobiochemical profile of broiler chickens. *Veterinary World*, 13(10): 2062–2069. Abstract.
- Disetlhe, A.R.P., U. Marume, V. Mlambo and A. Hugo. 2019. Effects of dietary humic acid and enzymes on meat quality and fatty acid profiles of broiler chickens fed canola-based diets. *Asian-Austral. J. Anim. Sci.* 32(5): 711–720.
- Eits, R.M., R. Meijerhof and G. Santoma. 2004. Economics determine optimal protein levels in broiler nutrition. *World Poult. J.* 20: 21-22.
- Esmailipour, O., H. Moravej, M. Shivazad, M. Rezaian, S. Aminzadeh and M.M. Krimpen. 2012. Effects of diet acidification and xylanase supplementation on performance, nutrient digestibility, duodenal histology and gut microflora of broilers fed wheat based diet. *Br. Poult. Sci.* 53: 235-244.
- Feng, Y., L. Wang, A. Khan, R. Zhao, S. Wei and X. Jing. 2020. Fermented wheat bran by xylanase-producing *Bacillus cereus* boosts the intestinal microflora of broiler chickens. *Poult. Sci. J.* 99(1): 263–271.
- Gallardo, C., J.C. Dadalt and M.A. Trindade Neto. 2018. Nitrogen retention, energy, and amino acid digestibility of wheat bran, without or with multicarbohydase and phytase supplementation, fed to broiler chickens. *J. Anim. Sci.* 96(6): 2371–2379.
- Govil, K., S. Nayak, R.P.S. Baghel, A.K. Patil, C.D. Malapure and D. Thakur. 2017. Performance of broiler chicken fed multicarbohydases supplemented low energy diet. *Vet. World.* 10(7): 727–731.
- Hafez, H.M. and Y.A. Attia. 2020. Challenges to the poultry industry: current perspectives and strategic future after the COVID-19 outbreak. *Front. Vet. Sci.* 7: 516.
- Haiqing, 2015. Hosseini, S. M., M. Manafi and H. Nazarizadeh. 2017. Effects of Xylanase Supplementation and Citric Acid on Performance, Ileal Nutrients Digestibility, and Gene Expression of Intestinal Nutrient Transporters in Broilers Challenged with *Clostridium perfringens*. *J. Poul. Sci.* 54(2): 149–156.
- Hu, H., S. Dai, A. Wen and X. Bai. 2019. Efficient Expression of Xylanase by Codon Optimization and Its Effects on the Growth Performance and Carcass Characteristics of Broiler. *Animals.* 9(2): 65.
- Isikhuemhen, O.S. and N.A. Mikiashvilli. 2009. Lignocellulolytic enzyme activity, substrate utilization, and mushroom yield by *Pleurotus ostreatus* cultivated on substrate containing anaerobic digester solids. *J. Ind. Microbiol. Biotechnol.* 36(11): 1353–1362.
- Islam, M.R., D. Lepp, D.V. Godfrey, S. Orban, K. Ross, P. Delaquis and M.S. Diarra. 2019. Effects of wild blueberry (*Vaccinium angustifolium*) pomace feeding on gut microbiota and blood metabolites in free-range pastured broiler chickens. *Poult. Sci. J.* 98(9): 3739–3755.
- Jia, W., B.A. Slominski, H.L. Bruce, G. Blank, G. Crow and O. Jones. 2009. Effects of diet type and enzyme addition on growth performance and gut health of broiler chickens during subclinical *Clostridium perfringens* challenge. *Poult. Sci.* 88(1): 132-140.
- Jung, S., R. Houde, B. Baurhoo, X. Zhao and B. Lee. 2008. Effects of Galacto-Oligosaccharides and a *Bifidobacteria lactis*-Based Probiotic Strain on the Growth Performance and Fecal Microflora of Broiler Chickens. *Poult. Sci. J.* 87(9): 1694–1699.
- Kareem, K., T. Loh, H. Foo, S. Asmara and H. Akit. 2017. Influence of postbiotic RG14 and inulin combination on cecal microbiota, organic acid concentration, and cytokine expression in broiler chickens. *Poult. Sci. J.* 96(4): 966–975.
- Khadem, A., M. Lourenço, E. Delezie, L. Maertens, A. Goderis, R. Mombaerts, M. Höfte, V. Eeckhaut, F. van Immerseel and G. Janssens. 2016. Does release of encapsulated nutrients have an important role in the efficacy of xylanase in broilers? *Poult. Sci. J.* 95(5): 1066–1076.
- Kiarie, E., L. Romero and V. Ravindran. 2014. Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. *Poult. Sci. J.* 93(5): 1186–1196.
- Knudsen, K.E.B. 2014. Fiber and nonstarch polysaccharide content and variation in common crops used in broiler diets. *Poult. Sci.* 93(9): 2380-2393.
- Lu, P.Y., J. Wang, S.G. Wu, J. Gao, Y. Dong, H.J. Zhang, G.H. Qi. 2020. Standardized ileal digestible amino acid and metabolizable energy content of wheat from different origins and the effect of exogenous xylanase on their

- determination in broilers. *Poult. Sci.* 99: 992-1000.
- Maqbool, A. 2002. Marketing of commercial poultry, poultry meat and eggs in Faisalabad City. M.Sc. Thesis, Univ. Agri. Faisalabad, Pakistan.
- Melo-Durán, D., J.F. Pérez, G. González-Ortiz, S. Villagómez-Estrada, M.R. Bedford, H. Graham and D. Sola-Oriol. 2021. Maize nutrient composition and the influence of xylanase addition. *J. Cereal Sci.* 97: 103155.
- Morgan, N.K., A. Wallace, M.R. Bedford, K.L. Hawking, I. Rodrigues, M. Hilliar and M. Choct. 2020. In vitro versus in situ evaluation of xylan hydrolysis into xylo-oligosaccharides in broiler chicken gastrointestinal tract. *Carbohydr. Polym.* 230: 115645.
- Mottet, A., C. de Haan, A. Falcucci, G. Tempio, C. Opio and P. Gerber. 2017. Livestock: On our plates or eating at our table? A new analysis of the feed/food debate. *Glob. Food Sec.* 14: 1–8.
- Mushtaq, T., M. Sarwar, G. Ahmad, M.A. Mirza, H. Nawaz, M.M. Mushtaq and U. Noreen .2007. Influence of canola meal-based diets supplemented with exogenous enzyme and digestible lysine on performance, digestibility, carcass, and immunity responses of broiler chickens. *Poult. Sci.* 86: 2144-2151.
- Owens, B., Tucker, L., Collins, M. A. and Mccracken, K. J. (2008) Effects of different feed additives alone or in combination on broiler performance, gut microflora and ileal histology. *Br Poult Sci* 49: 202-212.
- Pirgozliev, V., S.P. Rose, T. Pellny, A.M. Amerah, M. Wickramasinghe, M. Ulker, M. Rakszegi, Z. Bedo, P.R. Shewry and A. Lovegrove. 2015. Energy utilization and growth performance of chickens fed novel wheat inbred lines selected for different pentosan levels with and without xylanase supplementation. *Poult. Sci.* 94: 232-239.
- Saima, M.Z., U. Khan, M.A. Jabbar, A. Mehmud, M.M. Abbas and A. Mahmood. 2010. Effect of lysine supplementation in low protein diets on the performance of growing broilers. *Pak. Vet. J.* 30: 17-20.
- Saleh, A.A., A.A. Kirrella, S.E. Abdo, M. Mousa, N.A. Badwi, T.A. Ebeid, A.L. Nada and M.A. Mohamed. 2019. Effects of dietary xylanase and arabinofuranosidase combination on the growth performance, lipid peroxidation, blood constituents, and immune response of broilers fed low-energy diets. *Animals.* 111: 525-531
- Sanchez, J., A.Thanabalan, T. Khanal, R. Patterson, B.A. Slominski, E. Kiarie. 2019. Growth performance, gastrointestinal weight, microbial metabolites and apparent retention of components in broiler chickens fed up to 11% rice bran in a corn-soybean meal diet without or with a multi-enzyme supplement. *Anim Nutr* 5: 41-48.
- Schramm, V., J. Durau, L. Barrilli, J. Sorbara, A. Cowieson, A. Félix and A. Maiorka. 2017. Interaction between xylanase and phytase on the digestibility of corn and a corn/soy diet for broiler chickens. *Poult. Sci. J.* 96(5): 1204–1211.
- Sheng, Q. K., L. Yang, H.B. Zhao, X.L. Wang and K. Wang. 2013. Effects of low level water-soluble pentosans, alkaline-extractable pentosans, and xylanase on the growth and development of broiler chicks. *Asian-Australas J. Anim. Sci.* 26: 1313-1319.
- Stefanello, C., S. Vieira, P. Carvalho, J. Sorbara and A. Cowieson. 2016. Energy and nutrient utilization of broiler chickens fed corn-soybean meal and corn-based diets supplemented with xylanase. *Poult. Sci. J.* 95(8): 1881–1887.
- flavonoid on immune function, antioxidative ability and intestinal microbiota of broilers. *J. Integr. Agric.* 18(9): 2123–2132.
- Wang, Q., X.F. Wang, T. Xing, J.L. Li, X.D. Zhu, L. Zhang and F. Gao. 2021. The combined impact of xylo-oligosaccharides and gamma-irradiated astragalus polysaccharides on growth performance and intestinal mucosal barrier function of broilers. *Poult. Sci.* 26: 13-19.
- Yang, Y. F., Zhao, L. L., Shao, Y. X., Liao, X. D., Zhang, L. Y., Lu, L., & Luo, X. G .2019. Effects of dietary graded levels of cinnamon essential oil and its combination with bamboo leaf flavonoid on immune function, antioxidative ability and intestinal microbiota of broilers. *J. Integr. Agric.* 18 (9), 2123–2132.