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Research Article

Effect of Dietary *Aspergillus* Xylanase on Nutrient Digestibility and Utilization, Growth Performance and Size of Internal Organs in Broiler Chickens Offered Maize-Soybean Meal Based-Diets

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Abstract

Background and Objective: Recently, the term "resistant starch" has been increasingly used in the literature to describe starch that escapes digestion in the small intestine together with non-starch polysaccharides. Exogenous enzymes have been employed to ameliorate these challenges. Hence, the optimum performance of *Aspergillus* xylanase on maize-soy bean meal has not been fully investigated. This study was designed to test the effects of *Aspergillus* xylanase on apparent nutrient digestibility, protein utilization efficiency, growth performance and size of visceral organs on broilers. **Materials and Methods:** Three-hundred-day-old mixed sex Cobb 500® chicks were randomly allocated to five dietary treatments with five replicates of 12 birds each. Dietary treatments include, xylanase (XYL) 0 (0 g kg^{-1}), XYL10 (1 g kg^{-1}), XYL15 (1.5 g kg^{-1}), XYL20 (2 g kg^{-1}) and XYL25 (2.5 g kg^{-1}). **Results:** Results showed that birds fed XYL20 and 25 had higher ($p<0.05$) crude fiber and dry matter digestibility. Dietary treatment XYL20 promoted the highest ($p<0.05$) body weight gain (BWG) in the final week. Birds fed XYL20 recorded the best ($p<0.05$) feed conversion ratio during all phases of the feeding trial and the highest ($p<0.05$) BWG during the starter phase. Birds fed XYL20 had the highest ($p<0.05$) values for thigh, breast, wing and carcass yields. Both protein and energy efficiency ratios (PER and EER, respectively) were improved ($p<0.05$) for birds fed XYL20 during all phases. The small intestine lengths decreased ($p<0.05$) but spleen weights increased ($p<0.05$) as *Aspergillus* xylanase enzyme levels increased. **Conclusion:** The optimum *Aspergillus* xylanase inclusion levels that caused the greatest response for all measured parameters was 2 g kg^{-1}.

Key words: *Aspergillus* xylanase, crude protein, digestibility, growth traits, optimum performance

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Conventional broiler production is dependent on maize as a source of energy and soybean meal as a source of protein. Nutrient digestibility of plant-based feeds in poultry is limited by the proportion of their components for which there are no corresponding endogenous enzyme secretions. These components include non-starch polysaccharides (NSPs) that are present within the cell walls of maize and soybean meal[1,2]. The NSPs are either indigestible or of very low digestibility when fed to poultry[3,4]. The low energy contribution from NSPs is due to lower organic combustion as a result of cell walls that restrict enzyme access to substrate such as starch[5,6].

Non-starch polysaccharides increase digesta viscosity and have been implicated in the reduction of starch, fat and protein digestibility[4,7]. However, corn-soybean diets, including diets containing wheat, rye and barley[8], may contain sufficient concentrations of high-molecular weight soluble polysaccharides that increase intestinal viscosity to the detriment of nutrient utilization in poultry[9,10]. Recently, the term "resistant starch" has been increasingly used in the literature to describe starch that escapes digestion in the small intestine[4]. The amount of "resistant starch" is variable in corn and other plant seeds and can significantly influence the apparent metabolizable energy content of these feedstuffs[11].

Studies with supplemental enzymes targeting the degradation of energy-releasing substrates for poultry are ubiquitous. Exogenous carbohydrases (such as xylanases, amylases and glucanases) have been reported to improve energy utilization and broiler chicken performances[12]. Exogenous xylanase enzyme supplementation can change the nutritional status, which may regulate the metabolism and functioning of the growth-related endocrine system that will improve broiler chicken carcass yields[13]. These enzymes may improve the activities of endogenous enzymes for the hydrolysis of cell wall arabinoxylans[14] in addition to supporting or augmenting endogenous amylase activity in young animals[9]. Exogenous xylanase enzyme supplementation reduces endogenous amino acid losses, which may result from a reduction in the anti-nutritional effect of some polysaccharides[15]. Again, exogenous xylanase enzyme supplementation may reduce the need for endogenous enzyme secretion through feedback mechanisms[16]. Most authors have reported the effects of exogenous xylanase supplementation on rye-, barley- and wheat-based diets[17-20]. These exogenous xylanase enzymes are also used to supplement poultry diets that contain non-conventional feed ingredients with high fiber contents such as sunflower meal[21], bambara nut offal meal[2,6] and brewer's dried grain[22,23]. There is little information that exists on the effect of *Aspergillus* xylanase supplementation in maize-soybean meal diets on nutrient utilization and digestibility in broiler chickens. Therefore, the present study investigated the effects of graded levels of *Aspergillus* xylanase enzyme on nutrient utilization and digestibility, growth traits and internal organ sizes in broiler chickens offered maize-soybean meal based-diets.

MATERIALS AND METHODS

Ethical consideration: Ethical principles were taken into consideration during the study to adhere to the national and international standards governing research of this nature with regards to the use of research animals. Ethical approval was obtained from the Ethical Clearance Committee of University of Fort Hare, Alice, South Africa "MUC271SOYE01".

Study site: This study was conducted at the poultry unit of North-West University experimental farm (Molelwane), in the Northwest province of South Africa. The study area is located at 25.80°S and 25.50°E and experiences summer climate from August to March with temperatures ranging from 22-35°C and average annual rainfall ranging from 200-450 mm per annum. The study site experiences winter from May to July with sunny dry days and cool nights with average minimum and maximum temperatures of 2 and 20°C, respectively. The study lasted for six weeks within the months of April and May.

Enzyme characteristics: The tested *Aspergillus* xylanase enzyme [RONOZYME® WX (CT), DSM Nutritional Products Johannesburg South Africa] is a granulated heat stable endo-xylanase. The active substance in the enzyme is endo-1, 4-β-xylanase (IUB No. 3.2.1.8), which is produced by a genetically modified strain of *Aspergillus oryzae* micro-organism[24]. This strain is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ) with the accession number DSM 26372[25]. According to the manufacturer, the bulk density is approximately 1.1 g mL^{-1} and the average particle size is approximately 600 µm with an enzyme activity of 1000 FXU t^{-1} (1000 g t^{-1})[26].

Experimental diets: The feeding strategy consisted of starting (0-21 days) and finishing (22-42 days) basal diets (BD) (Table 1 and 2), which were formulated to meet the birds' dietary nutritional requirements[27]. At each feeding phase (starting and finishing), five dietary treatments were formulated through the addition of *Aspergillus* xylanase enzyme at five iso-nitrogenous and iso-caloric different levels.

Table 1: Ingredient (%) and chemical composition (g kg^{-1} DM unless otherwise stated) of experimental diets for broiler chicks at the starter phase (0-3 weeks)

Ingredients	XYL0	XYL10	XYL15	XYL20	XYL25
Yellow maize	62.981	62.981	62.981	62.981	62.981
Soybean meal	27.380	27.380	27.380	27.380	27.380
Sunflower meal	4.000	4.000	4.000	4.000	4.000
Fish meal	2.500	2.500	2.500	2.500	2.500
Canola oil	0.113	0.113	0.113	0.113	0.113
Limestone	1.317	1.317	1.317	1.317	1.317
MonoCaP	0.441	0.441	0.441	0.441	0.441
Salt	0.277	0.277	0.277	0.277	0.277
Methionine	0.230	0.230	0.230	0.230	0.230
Threonine	0.051	0.051	0.051	0.051	0.051
Lysine	0.321	0.321	0.321	0.321	0.321
Choline Cl	0.096	0.096	0.096	0.096	0.096
[1]VMP	0.200	0.200	0.200	0.200	0.200
[2]Maxiban	0.050	0.050	0.050	0.050	0.050
[3]Surmax	0.040	0.040	0.040	0.040	0.040
Xylanase	0.000	0.100	0.150	0.200	0.250
Total	100.000	100.000	100.000	100.000	100.000
Chemical composition					
Moisture	11.110	10.890	11.000	11.080	11.010
ME (MJ kg^{-1})	12.790	12.710	12.790	12.680	12.760
Crude protein	23.830	23.900	23.790	23.850	23.880
Crude fat	4.010	4.030	3.990	4.110	4.080
NDF	15.320	15.270	15.390	15.280	15.300
ADF	3.850	3.950	3.970	4.000	4.020
Calcium	0.930	0.940	0.920	0.930	0.940
Phosphorus	0.660	0.680	0.650	0.690	0.670

MonoCaP: Monocalcium phosphate, Choline Cl: Choline chloride, VMP: Vitamine mineral premix, ME: Metabolizable energy, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, XYL0: Basal diet: BD (without *Aspergillus* xylanase; XYL), XYL10: BD+1 g XYL kg^{-1} feed, XYL15: BD+1.5 g XYL kg^{-1} feed, XYL20: BD+2 g XYL kg^{-1} feed, XYL25: BD+2.5 g XYL kg^{-1} feed. [1a]2.5 kg of vitamin premix contained: 2700 mg retinal, 400 mg calcidiol, 18 g tocopheryl acetate, 2000 mg menadione, 1800 mg thiamine, 6600 mg riboflavin, 10 g niacin, 30 g calcium pantothenate, 3 g pyridoxine, 1 g folic acid, 15 mg cobalamin, 250 g choline chloride, 100 mg biotin. b2.5 kg of trace mineral premix contained: 100 g Mn, 50 g Fe, 100 g Zn, 10 g Cu, 1 g I, 200 mg Se. 21000 g of Maxiban contained: 80 g kg^{-1} narasin, 80 g kg^{-1} nicarbazin 31000 g of Surmax contained: 100 g kg^{-1} avilamycin

The five experimental diets formulated consisted of different combinations of basal diet (BD) and xylanase feed (XYL): (1) XYL0 (only BD), (2) XYL10 (BD+1 g *Aspergillus* XYL kg^{-1} feed), (3) XYL15 (BD+1.5 g *Aspergillus* XYL kg^{-1} feed), (4) XYL20 (BD+2 g *Aspergillus* XYL kg^{-1} feed) and (5) XYL25 (BD+2.5 g *Aspergillus* XYL kg^{-1} feed) for both starting and finishing phases. The ingredient and chemical composition of the five experimental diets for starting and finishing phases are presented in Table 1 and 2, respectively. The chemical (proximate) composition of the experimental diets was analyzed according to the Association of Official Agricultural Chemists (AOAC)[28] methods.

Experimental birds and management: A total of 300, one-day-old, non-sexed broiler birds (Cobb 500®) were used in this study. Sixty birds were assigned randomly to one of the five experimental diets (XYL0, 10, 15, 20 and 25). Each experimental diet was replicated in five experimental pens measuring 2.5 m length×2.5 m width×2.5 m height with twelve birds each. The birds were housed in cages with wood shavings as litter. General flock prophylactic management and routine vaccinations were administered: (1) Day 1-intra ocular New castle disease vaccine, (2) Week 2-Gumboro disease vaccine, (3) Week 3-Lasota (New castle disease vaccine), (4) Week 4-Gumboro disease vaccine and (5) Week 5-fowl pox vaccine. A stress pack was administered to the birds via drinking water at 100 g/50 L (according to manufacturer's recommendation) to boost appetite and energy supply. Dietary treatments and clean water were provided *ad libitum* during a six-week feeding trial.

Growth performance: Average daily feed intake (ADFI) per bird was measured from day 1-42 of age by subtracting the weight of the refused feed from the offered feed and dividing the difference by the total number of birds in the pen. The average live-weight was measured weekly by weighing all of the birds in each pen using a 10.1 kg capacity precision weighing balance with models A and D Weighing GF-10K industrial balance that was made in Japan. These live-weights were used to calculate the average weekly weight gain (AWG) per bird according to the equation as outlined by Mnisi *et al.*[29]:

Table 2: Ingredient (%) and chemical composition (g kg^{-1} DM unless otherwise stated) of experimental diets for broilers at the finisher phase (4-6 weeks)

Ingredients	XYL0	XYL10	XYL15	XYL20	XYL25
Yellow maize	72.360	72.360	72.360	72.360	72.360
Soybean meal	24.552	24.552	24.552	24.552	24.552
Canola oil	0.241	0.241	0.241	0.241	0.241
Limestone	1.249	1.249	1.249	1.249	1.249
MonoCaP	0.174	0.174	0.174	0.174	0.174
Salt	0.392	0.392	0.392	0.392	0.392
Methionine	0.209	0.209	0.209	0.209	0.209
Tryptophan	0.050	0.050	0.050	0.050	0.050
Threonine	0.048	0.048	0.048	0.048	0.048
Lysine	0.335	0.335	0.335	0.335	0.335
Choline Cl	0.096	0.096	0.096	0.096	0.096
[1]VMP	0.200	0.200	0.200	0.200	0.200
[2]Maxiban	0.050	0.050	0.050	0.050	0.050
[3]Surmax	0.040	0.040	0.040	0.040	0.040
Xylanase	0.000	0.100	0.150	0.200	0.250
Total	100.000	100.000	100.000	100.000	100.000
Chemical composition					
Moisture	11.010	11.030	10.970	11.100	11.130
ME (MJ kg^{-1})	13.080	13.040	13.010	13.030	13.050
Crude protein	19.750	19.910	19.700	19.830	19.850
Crude fat	4.080	4.120	4.120	4.150	4.090
NDF	15.930	18.320	18.280	18.210	18.020
ADF	4.850	4.970	5.020	4.950	5.000
Calcium	0.940	0.920	0.940	0.930	0.930
Phosphorus	0.610	0.620	0.610	0.610	0.610

MonoCaP: Monocalcium Phosphate, Choline Cl: Choline Cloride, VMP: Vitamine mineral premix, ME: Metabolizable energy, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, XYL0: Basal diet: BD (without *Aspergillus* xylanase; XYL). XYL10: BD+1 g XYL kg^{-1} feed, XYL15: BD+1.5 g XYL kg^{-1} feed, XYL20: BD+2 g XYL kg^{-1} feed, XYL25: BD+2.5 g XYL kg^{-1} feed.[1a]2.5 kg of vitamin premix contained: 2700 mg retinal, 400 mg calcidiol, 18 g tocopheryl acetate, 2000 mg menadione, 1800 mg thiamine, 6600 mg riboflavin, 10 g niacin, 30 g calcium pantothenate, 3 g pyridoxine, 1 g folic acid, 15 mg cobalamin, 250 g choline chloride, 100 mg biotin. b2.5 kg of trace mineral premix contained: 100 g Mn, 50 g Fe, 100 g Zn, 10 g Cu, 1 g I, 200 mg Se. 21000 g of Maxiban contained: 80 g kg^{-1} narasin, 80 g kg^{-1} nicarbazin 31000 g of Surmax contained: 100g kg^{-1} avilamycin

$$4WG\ (t_0,T) = W(T)-W(t_0)$$

Where

t_0 = Initial time (days)

T = Final time

W (T) = Final body weight/bird (g)

W (t_0) = Initial body weight/bird (g)

Weekly feed conversion efficiency was calculated as average weekly weight gained divided by average weekly feed consumption per bird.

Nutrient utilization efficiency

Protein and energy utilization efficiency: Daily protein intake (g) was determined as the percentage crude protein of the diet multiplied by the daily feed intake. Total protein intake (TPI) was calculated for each bird by multiplying feed intake by crude protein content of the diet. Protein efficiency ratio (PER) was determined as the ratio of weight gained to TPI.

Total metabolizable energy intake (TMEI) was calculated for each growth phase as metabolizable energy (ME) of a diet in kcal multiplied by its intake during the whole experimental period.

Energy efficiency ratio (EER) was calculated as the ratio of weight gain to TMEI.

Nutrient digestibility trial: On the 35th day of the feeding trial, a bird was randomly selected from each replicate (n = 5 per treatment) and moved to cleaned and disinfected metabolic cages. A 3-day adaptation period was allowed prior to the four-day collection period. Feed intake was measured and droppings were collected per bird on daily basis. The collected droppings were air dried at room temperature before being ground for proximate analysis according to AOAC[28] methods. Apparent nutrient digestibility of crude fiber, crude protein, crude fat and dry matter was computed according to the following equation as outlined by Mnisi *et al.*[29]:

$$\text{Nutrient digestibility} = \frac{\text{Nutrient in feed} - \text{nutrient in faeces}}{\text{Nutrient in feed}} \times 100$$

Slaughter procedure: At 42 days of age, all chickens were taken to Rooigrond Poultry Abattoir (North-West province, South Africa) for slaughter.

Visceral organ size: On day 42, five birds per replicate were randomly selected from the slaughtered birds in order to assess and measure the weight of visceral organs (weights of liver, heart, gizzard, spleen, small intestine length, small intestine weight and proventriculus) and the length of intestine.

Carcass characteristics: Immediately after slaughter, the feathers were plucked and the gastrointestinal tract (GIT) was removed. The carcasses were then weighed to obtain the carcass weight of the birds. Five birds per replicate pen were randomly selected for determination of carcass characteristics and meat quality. For the measurement of carcass cuts, head and shanks were removed close to the scull and at the hock joint, respectively. Wings were removed by cutting at the humeroscapular joint, the cuts were made through the rib head to the shoulder girdle and the vertebrae was then removed intact by pulling outwardly. The breast muscle, neck, wings, shank, thighs, drumsticks and vertebrae (back) were each weighed separately using a 10.1 kg capacity precision weighing balance with models A and D Weighing GF-10K industrial balance (Japan).

Experimental design and statistical analysis: All of the reported parameters were tested for normality using the NORMAL option in the Proc Univariate statement before being subject to analysis of variance. Average weekly feed intake, average weekly weight gain and weekly feed conversion efficiency data were analyzed using the repeated measures analysis procedure of SAS[30]. The following statistical linear model was employed:

$$Y_{ijk} = \mu + D_i + W_j + (D \times W)_{ij} + E_{ijk}$$

where

Y_{ijk} = Dependent variable
μ = Population mean
D_i = Effect of dietary treatments
$W_{j=}$ = Effect of week
$(D \times W)_{ij}$ = Effect of interaction between diets and week
E_{ik} = Random error associated with observation ijk that was assumed to be normally and independently distributed

Data collected during the study on the starter, finisher and overall growth traits in addition to nutrient utilization efficiency, size of visceral organs and apparent nutrient digestibility were subject to analysis of variance for a completely randomized design (CRD) using a general linear model procedure of SAS[30]. The statistical model consisted of the equation:

$$Y_{ij} = \mu + D_i + E_{ij}.$$

Where

Y_{ij} = Observed value of a dependent variable
μ = Overall population mean
D_i = Effect of dietary treatments
E_{ij} = Residual error associated with observation ij assumed to be normally and independently distributed. For all statistical tests, significance was declared at $p<0.05$

The mean separation was done using the LSD range test.

RESULTS

Protein efficiency ratio (PER) and energy efficiency ratio (EER): The PER and EER of birds fed maize-soybean meal-based diets supplemented with different levels of *Aspergillus* xylanase are presented in Table 3. Birds fed XYL20 had the highest PER and EER compared with those fed with the other levels of *Aspergillus* xylanase. Daily protein consumption was lowest ($p<0.05$) in birds fed XYL20 during both the starting and finishing phases. The daily PER was higher ($p<0.05$) in birds fed both XYL15 and XYL20 while daily metabolizable energy intake did not differ ($p>0.05$). Daily EER was highest ($p<0.05$) in birds fed XYL20, which was better when compared with birds that received dietary treatment XYL0, XYL15 and XYL25. Metabolizable energy intake (MEI) was less ($p<0.05$) for birds fed XYL0, XYL20 and XYL25 during the starting phase while birds fed XYL10 and XYL15 had more ($p<0.05$) MEI. Hence, birds fed all of the dietary treatments (XYL0, 10, 15 and 25) used in the present study consumed more ($p<0.05$) MEI at finisher and overall phases except for birds fed dietary treatment XYL20 that consumed less.

Apparent nutrient digestibility: Apparent nutrient digestibility data for birds fed with *Aspergillus* xylanase are presented in Table 4. All the nutrient digestibility parameters were affected ($p<0.05$) by *Aspergillus* xylanase supplementation except for crude fat. The addition of *Aspergillus* xylanase increased ($p<0.05$) the digestibility of crude fiber, crude protein and dry matter. Crude fiber and dry matter digestibility rates were higher ($p<0.05$) for birds fed XYL20 and 25 although statistically similar to birds fed XYL15. Crude protein digestibility was enhanced ($p<0.05$) by

Table 3: The effect of dietary *Aspergillus* xylanase supplementation on protein and energy efficiency ratio of broiler birds

	Diets					
	XYL0	XYL10	XYL15	XYL20	XYL25	SEM
DPC (g)	22.93^{a}	23.38^{a}	23.25^{a}	21.54^{b}	23.12^{a}	0.08
DPER (g)	0.06^{b}	0.06^{b}	0.07^{a}	0.07^{a}	0.06^{b}	0.00
DMEI (kcal bird^{-1})	330.42	336.04	334.90	309.75	333.38	0.85
DEER (kcal/100 kcal)	0.45^{ab}	0.40^{b}	0.44^{ab}	0.52^{a}	0.44^{ab}	0.00
Starter phase						
PC (g)	336.53^{bc}	359.10^{a}	350.67^{ab}	323.87^{c}	337.53^{bc}	0.87
PER (g)	2.70^{b}	2.60^{b}	2.65^{b}	3.22^{a}	2.68^{b}	0.01
MEI (kcal bird^{-1})	4226.39^{b}	4503.56^{a}	4401.13^{a}	4059.25^{b}	4234.30^{b}	8.78
EER(gain/100 kcal)	21.49^{b}	20.75^{b}	21.14^{b}	25.59^{a}	22.18^{b}	0.11
Finisher phase						
PC (g)	626.69^{a}	623.01^{a}	626.12^{a}	580.99^{b}	633.63^{a}	2.10
PER (g)	2.71^{b}	2.73^{b}	2.70^{b}	3.09^{a}	2.60^{b}	0.01
MEI (kcal bird^{-1})	9651.38^{a}	9610.02^{a}	9664.55^{a}	8950.30^{b}	9767.78^{a}	14.89
EER(gain/100 kcal)	17.59^{b}	17.68^{b}	17.49^{b}	20.01^{a}	16.90^{b}	0.07
Overall phase						
PC (g)	963.22^{a}	982.10^{a}	976.79^{a}	904.86^{b}	971.16^{a}	1.19
PER (g)	2.70^{b}	2.68^{b}	2.69^{b}	3.13^{a}	2.67^{b}	0.01
MEI (kcal bird^{-1})	13877.77^{ab}	14113.58^{a}	14065.68^{a}	13009.55^{b}	14002.08^{a}	38.67
EER (gain/100 kcal)	18.78^{b}	18.66^{b}	18.63^{b}	21.75^{a}	18.49^{b}	0.07

^{a,b,c}Row means with different superscripts differ significantly. SEM: Standard error of the mean, DPER: Daily protein efficiency ratio, DMEI: Daily metabolizable energy intake, DEER: Daily energy efficiency ratio, PC: Protein consumption, PER: Protein efficiency ratio, MEI: Metabolizable energy intake, EER: Energy efficiency ratio, XYL0: Basal diet: BD (without *Aspergillus* xylanase; XYL), XYL10: BD+1 g XYL, XYL15: BD+1.5 g XYL, XYL20: BD+2 g XYL, XYL25: BD+2.5 g XYL

Table 4: Effects of xylanase supplemented finisher diets on apparent digestibility (%) of dry matter, crude fiber, crude protein and crude fat in broiler chickens

	Diets					
	XYL0	XYL10	XYL15	XYL20	XYL25	SEM
Cruder fiber	13.06^{b}	12.99^{b}	16.46^{ab}	18.99^{a}	19.55^{a}	0.18
Crude protein	79.42^{b}	81.55^{b}	85.99^{a}	87.09^{a}	88.97^{a}	0.51
Crude fat	78.85	79.79	80.04	80.56	79.29	0.49
Dry matter	76.07^{b}	75.01^{b}	78.03^{ab}	82.96^{a}	83.73^{a}	0.52

^{a,b,c}Row means with different superscripts differ significantly. SEM: Standard error of the mean, XYL0: Basal diet: BD (without *Aspergillus* xylanase; XYL), XYL10: BD+1 g XYL, XYL15: BD+1.5 g XYL, XYL20: BD+2 g XYL, XYL25: BD+2.5 g XYL

Aspergillus xylanase supplementation for birds fed XYL15, XYL20 and XYL25 when compared with their counterparts that received XYL0 and XYL10.

Growth performance: The effect of *Aspergillus* xylanase (ASXY) enzyme on broiler birds' body weight gain (BWG) is presented in Fig. 1-3 for starting, finishing and overall phases, respectively. Fig. 1-3 show that broilers BW during the starting, finishing and overall feeding trials present a similar trend, in which case the highest inclusion level (2.5 g XYL kg^{-1} feed) of ASXY enzyme in different stages (phases) of the feeding trial did not cause the largest response. The optimum inclusion level of ASXY for body weight gain was 2 g XYL kg^{-1} feed in all the phases of production.

Table 5 presents the broiler birds' data for feed intake (FI), feed conversion ratio (FCR) and BWG. No significant ($p>0.05$) interactions between diet and week was observed for FI and FCR. Dietary treatment XYL20 promoted the highest ($p<0.05$) BWG in the final week (week 6) of the feeding trial, while no differences were observed for BWG in other weeks (weeks 1-5).

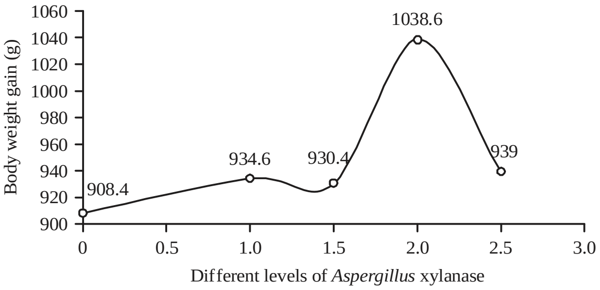

Fig. 1: Effect of *Aspergillus* xylanase enzyme inclusion on body weight gain of broiler chickens during the starter phase

Different growth performance phases of broiler birds fed maize-soybean meal-based diets supplemented with ASXY

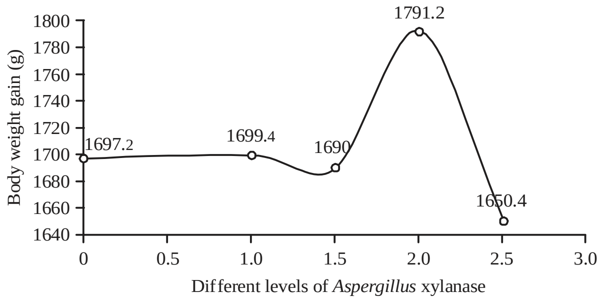

Fig. 2: Effect of *Aspergillus* xylanase enzyme inclusion on body weight gain of broiler chickens during the finisher phase

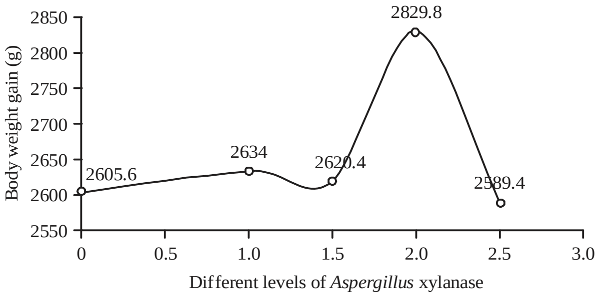

Fig. 3: Effect of *Aspergillus* xylanase enzyme inclusion on body weight gain of broiler chickens during the overall phase

are presented in Table 6. All of the growth traits considered in this study were affected ($p<0.05$) by the inclusion of dietary ASXY, except for BWG during the finishing phase. Birds fed XYL20 consumed less feed during the starting, finishing and overall feeding trial phases when compared with birds fed other levels of ASXY.

The average daily feed intake of birds fed XYL20 was lower but similar to birds fed XYL25 when compared with other treatments used in the study. During the starting phase, birds fed XYL10 and 15 consumed more ($p<0.05$) feed. Daily weight gain and BWG were highest ($p<0.05$) for birds fed XYL20 during the starting and overall phases (entire feeding period) but similar to birds fed XYL25. Birds fed XYL20 showed greater improvement in FCR during the starting and the overall feeding periods.

Carcass characteristics of broiler chickens: The meat yield traits of broiler birds fed maize-soybean meal diet with different supplemental levels of ASXY is presented in Table 7. The weights of carcasses, necks, wings, drumsticks, thighs, breasts and head were affected ($p<0.05$) by the addition of ASXY while shank and vertebrate weights did not differ ($p>0.05$). Birds fed XYL20 had the highest ($p<0.05$) values for thighs, breasts, wings and overall carcass weights compared with birds fed other treatments used in the present study.

Table 5: Average weekly feed intake (g bird^{-1}), average weekly weight gain (g bird^{-1}) and weekly feed conversion ratio (FCR) in broiler birds fed dietary *Aspergillus* xylanase

	Diets					
	XYL0	XYL10	XYL15	XYL20	XYL25	SEM
Feed intake (g bird^{-1})						
Week 1	186.20	211.40	199.00	183.40	189.00	0.54
Week 2	513.80	554.00	541.00	502.60	498.40	0.63
Week 3	714.00	743.40	729.40	674.80	730.80	1.39
Week 4	877.80	884.80	887.60	828.80	909.40	8.38
Week 5	1050.00	1010.80	1036.00	966.60	1038.80	2.67
Week 6	1174.60	1188.60	1176.00	1080.80	1188.60	2.89
Body weight (g bird^{-1})						
Week 1	199.00	210.20	201.60	202.00	208.20	0.67
Week 2	494.60	507.40	511.20	564.80	512.60	1.19
Week 3	953.40	979.60	974.40	1082.60	984.00	2.62
Week 4	1549.20	1585.20	1558.60	1629.00	1561.80	4.69
Week 5	2201.60	2248.80	2201.20	2333.60	2220.80	6.19
Week 6	2650.60^{b}	2679.00^{b}	2664.40^{b}	2873.80^{a}	2734.40^{ab}	8.15
FCR						
Week 1	0.94	1.01	1.01	0.91	0.91	0.13
Week 2	1.04	1.09	1.04	0.89	0.97	0.12
Week 3	0.75	0.76	0.75	0.63	0.74	0.08
Week 4	0.57	0.56	0.57	0.51	0.58	0.05
Week 5	0.48	0.45	0.47	0.41	0.47	0.02
Week 6	0.44	0.45	0.44	0.37	0.45	0.02

^{a,b,c}Row means with different superscripts differ significantly. SEM: Standard error of the mean, FCR: Feed conversion ratio, XYL0: Basal diet: BD (without *Aspergillus* xylanase; XYL), XYL10: BD+1 g XYL, XYL15: BD+1.5 g XYL, XYL20: BD+2 g XYL, XYL25: BD+2.5 g XYL

Table 6: The effect of dietary xylanase supplementation on feed intake, body weight gain and feed conversion ratio of broiler birds

	Diets					
	XYL0	XYL10	XYL15	XYL20	XYL25	SEM
Daily performance						
Day old weight (g)	45.00	45.00	44.00	44.00	45.00	0.140
Daily feed intake (g)	107.53^{a}	109.36^{a}	108.81^{a}	100.88^{b}	103.45^{ab}	0.750
Daily weight gain (g)	62.04^{b}	62.71^{b}	62.39^{b}	67.37^{a}	64.65^{ab}	0.450
Starter phase						
Feed intake (g)	1414.00^{bc}	1508.80^{a}	1473.40^{ab}	1360.80^{c}	1418.20^{bc}	5.750
Body weight gain (g)	908.40^{b}	934.60^{b}	930.40^{b}	1038.60^{a}	979.00^{ab}	4.690
FCR (g g^{-1})	1.56^{ab}	1.62^{a}	1.59^{ab}	1.31^{c}	1.51^{b}	0.010
Finisher phase						
Feed intake (g)	3102.40^{a}	3084.20^{a}	3099.60^{a}	2876.20^{b}	3136.80^{a}	9.750
Body weight gain (g)	1697.20	1699.40	1690.00	1791.20	1650.40	6.980
FCR (g g^{-1})	1.83^{a}	1.82^{a}	1.84^{a}	1.61^{b}	1.87^{a}	0.010
Overall phase						
Feed intake (g)	4516.40^{a}	4593.00^{a}	4573.00^{a}	4237.00^{b}	4555.00^{a}	10.46
Body weight gain (g)	2605.60^{b}	2634.00^{b}	2620.40^{b}	2829.80^{a}	2789.40^{ab}	8.330
FCR (g g^{-1})	1.74^{a}	1.75^{a}	1.75^{a}	1.50^{b}	1.76^{a}	0.010

^{a,b,c}Row means with different superscripts differ significantly. SEM: Standard error of the mean, FCR: Feed conversion ratio, XYL0: Basal diet: BD (without *Aspergillus* xylanase; XYL), XYL10: BD+1 g XYL, XYL15: BD+1.5 g XYL, XYL20: BD+2 g XYL, XYL25: BD+2.5 g XYL

Table 7: The effect of dietary *Aspergillus* xylanase supplementation on carcass characteristics of broiler birds

	Diets					
	XYL0	XYL10	XYL15	XYL20	XYL25	SEM
Carcass weight (g)	2182.18^{c}	2204.20^{ab}	2310.31^{b}	2452.45^{a}	2268.26^{bc}	13.97
Neck weight (g)	74.00^{ab}	67.87^{b}	73.80^{ab}	79.67^{a}	66.93^{b}	1.320
Wing weight (g)	81.13^{b}	82.27^{b}	80.73^{b}	91.67^{a}	80.40^{b}	1.060
Drumstick weight (g)	96.00^{b}	101.80^{ab}	99.73^{ab}	117.67^{a}	100.80^{ab}	1.570
Thigh weight (g)	112.33^{b}	100.94^{b}	100.00^{b}	128.67^{a}	111.08^{b}	1.980
Breast weight (g)	499.27^{b}	489.47^{b}	493.13^{b}	637.53^{a}	511.20^{b}	4.760
Vertebrate weight (g)	180.60	182.33	185.00	188.86	185.87	2.450
Shank weight (g)	35.53	37.87	38.33	39.06	35.13	0.590
Head weight (g)	44.13^{b}	48.93^{ab}	47.87^{ab}	50.13^{a}	44.67^{b}	0.670

^{a,b,c}Row means with different superscripts differ significantly. SEM: Standard error of the mean, XYL0: Basal diet: BD (without *Aspergillus* xylanase; XYL), XYL10: BD+1 g XYL, XYL15: BD+1.5 g XYL, XYL20: BD+2 g XYL, XYL25: BD+2.5 g XYL

Neck weights were highest ($p<0.05$) for XYL20-fed birds although statistically similar to birds fed XYL0 and 15. Drumsticks recorded an improved ($p<0.05$) value for birds fed XYL20 but they are statistically similar to their counterparts that received dietary treatment XYL10, XYL15 and XYL25. Again, head weights were least ($p<0.05$) for birds fed XYL0 and 25, while those that received XYL20 recorded increased head weight values although statistically similar to their counterparts that received XYL10 and 15. Generally, birds fed XYL20 were observed to have improved ($p<0.05$) retail cut yields of necks, wings, drumsticks, thighs, breasts, heads and overall carcasses when compared with birds fed other XYL levels.

Size of visceral organs: As Table 8 indicates, ASXY supplementation to maize-soybean meal significantly ($p<0.05$) caused an increase in spleen weights and decrease in small intestine lengths, while all of the other organs were not affected ($p>0.05$). As ASXY inclusion level increased, spleen weights of birds also increased ($p<0.05$). Small intestine lengths (SIL) were longest (163.00 cm; $p<0.05$) for birds fed XYL0. The SIL of birds that received XYL10 and 15 were 159.5 cm and 157.24 cm respectively, while the lowest SIL value was recorded for birds fed XYL20 and 25 (147.54 and 150.65 cm, respectively).

DISCUSSION

Protein and energy utilization efficiency: The higher efficiency of nutrient utilization for birds fed dietary treatment XYL20 may be the reason for the observed improvement in performance (Table 6) as compared to birds that were fed other dietary treatments used in the present study. This finding is in agreement with the studies of Del Alamo *et al.*[31], Hajati[32] and El-Katcha *et al.*[20], they found that exogenous enzyme inclusion in the corn-soybean meal diets of broilers

Table 8: The effect of dietary *Aspergillus* xylanase supplementation on size of visceral organs of broiler birds

	Diets					
	XYL0	XYL10	XYL15	XYL20	XYL25	SEM
Full gizzard (g)	43.80	43.07	47.40	46.20	45.13	0.08
Empty gizzard (g)	33.73	33.27	35.27	34.67	35.73	0.05
Heart (g)	10.80	10.40	11.27	11.13	11.60	0.02
Liver (g)	35.26	36.40	37.47	38.13	36.66	0.06
LI weight	1.79	1.87	1.83	1.81	1.86	0.01
LI length	6.11	6.06	5.68	5.59	6.02	0.03
Spleen (g)	1.43^{c}	1.60^{c}	2.06^{b}	2.73^{a}	2.56^{a}	0.01
SI length (cm)	163.99^{a}	159.50^{ab}	157.24^{ab}	147.54^{b}	150.65^{b}	0.86
SI weight (g)	56.20	53.07	52.30	53.20	52.23	0.10
Proventriculus (g)	8.73	8.30	8.41	8.50	8.60	0.04

^{a,b,c,d}Row means with different superscripts differ significantly. SEM: Standard error of the mean. Organs; SI length: Small intestine length, SI weight: Small intestine weight, XYL0: Basal diet: BD (without *Aspergillus* xylanase; XYL), XYL10: BD+1 g XYL, XYL15: BD+1.5 g XYL, XYL20: BD+2 g XYL, XYL25: BD+2.5 g XYL

increased both energy and protein efficiency, reduced feed intake and improved both BWG and FCR. El-Katcha *et al.*[20] observed that the improvement in the performance was closely linked with the nutrient improvements utilization from the feed. These effects are probably mediated through changes occurring in the cell wall architecture achieved by the hydrolysis of structurally important arabinoxylans, which may release encapsulated nutrients. The results of this study are in agreement with O'Neill *et al.*[33,34] who observed that an increase in feed use efficiency may have resulted in a significant increase in BWG and an improved FCR. Cowieson *et al.*[35] reported an improvement in FCR and BWG in broilers that were fed XYL-supplemented maize diets that resulted in an improved nutrient utilization. The significant improvement in nutrient digestibility fostered by the supplementation of *Aspergillus* xylanase recorded in this study supports the mechanism of increased feed utilization efficiency, that has also been observed by Kiarie *et al.*[18].

Apparent nutrient digestibility: The higher inclusion levels of dietary *Aspergillus* xylanase in maize-soybean meal diets of broilers enhanced the digestibility of crude fiber, crude protein and dry matter possibly because xylanase promoted an increase in the activities of endogenous digestive enzymes by increasing the availability of substrates. Similarly, Shakouri *et al.*[36] reported the positive effects of exogenous enzyme addition on nutrient digestibility and FCR. Kiarie *et al.*[18] also suggested that xylanase supplementation does not only increase nutrient digestibility but also the production of cecal volatile fatty acids (VFA) as a result of the increased flow of xylo-oligomers into the ceca. Microbial metabolites such as VFAs have been identified as apparent indicators of exogenous enzyme-inducing the *in situ* generation of fermentative oligosaccharides[37]. The results of the current study are in line with the findings of Scott *et al.*[38], who reported that the addition of exogenous enzymes to wheat and maize-based diets increased nutrient digestibility in broilers. However, a contradictory result was recorded by Bhuiyan *et al.*[39], who found no significant changes in the nutrient digestibility in birds that had maize diets supplemented with high levels of xylanase. Water-soluble arabinoxylans are the major anti-nutritive factors of NSPs, which increase digesta viscosity and modulate the gut micro-flora thereby interfering with digestibility and the absorption of nutrients[2,19]. Our results are partially in line with the findings of Esmaeilipour *et al.*[40], who reported that xylanase significantly decreased the viscosity of the digesta and improved the apparent nutrient digestibility of the dry matter, crude protein and energy of the wheat-based diets of broilers. In a previous study, Gao *et al.*[41] reported that the addition of xylanase to wheat-based diets of broilers increased the apparent fat digestibility. However, in this study *Aspergillus* xylanase had no effect on crude fat digestibility, a finding that is in agreement with reports by Zhang *et al.*[19], Esmaeilipour *et al.*[40] and Bedford[42]. Therefore, the present study shows that the *Aspergillus* xylanase can reduce protein and starch entrapment thereby increasing their digestibility, mostly of protein and starch, rather than fat.

Growth performance: The diet × week interaction effect on BWG revealed that the addition of XYL20 to the diet promoted the highest BWG at week 6. This is similar to the findings of Jackson *et al.*[43] and Lee *et al.*[44], Jia *et al.*[45], they suggested that BWG was more effectively increased when broiler diets were supplemented with the *Bacillus* xylanase enzyme in the finisher phase than in the starter phase. These outcomes demonstrated that some of the anti-nutrients (NSPs) including glucans, phytates and mannans may not be adequately digested and absorbed by the villi of young birds. Olukosi *et al.*[46] and Wyatt *et al.*[47] suggested that younger animals were hampered in their growth since they cannot secrete a sufficient number of endogenous enzymes because their body systems cannot efficiently manage exogenous digestive enzymes. This could clearly be the reason why we see enhanced, obvious performance with mature or finisher birds. Some authors have reported the presence of relatively shorter villi in younger birds which resulted in reduced endogenous enzyme secretion at the tip of the villi, thereby resulting in poor digestion and absorption of nutrients in addition to an unhealthy gut[48,49].

The response curves in Fig. 1-3 showed that a supplementary dietary level of 2 g kg^{-1} of *Aspergillus* xylanase in the birds feed was the maximum inclusion level that caused the largest BWG response. This indicates that this specific inclusion level produced the highest enzyme activities on the substrates. According to Kanti and Sudiana[50], any level of enzyme supplementation that produced the maximum enzyme activities on the substrate usually produce the largest response. The dietary supplementation of XYL20 (2 g kg^{-1} feed) improved weight gain and FCR and reduced FI throughout the feeding trial (Table 6). This is in agreement with the results of other researchers who found that an increase in BWG may be related to the supplementation of carbohydrases[32,51]. Previous reports by Zhang *et al.*[19], Esmaeilipour *et al.*[40], Gao *et al.*[41,52] showed that the supplementation of *Bacillus* xylanase in wheat-based diets significantly increased the broilers' BWG and improved their FCR. Meanwhile, the data in the present study were not in agreement with those of Luo *et al.*[17] and Gao *et al.*[52], they found no differences in the FCR of broilers whose diet was supplemented with xylanase. Some studies have shown that the addition of xylanase resulted in improved broiler performance[9,12,15]. However, other authors found no effects on growth performance from the dietary supplementation of exogenous xylanase[3,53]. However, the improved performance of birds fed XYL20 recorded in the present study may be due to the reduction of the anti-nutritive effects of NSPs and the resulting release of nutrients for the birds. Other authors share the same opinion[2,54,55], they recorded that more nutrients were made available to the birds which may have improved the BWG as a result of reduction of NSPs in the diets. Xylanase may increase access to entrapped nutrients by digesting some fractions of the plant cell walls of grains, allowing endogenous amylase access to previously trapped starch fractions, thereby preventing loss of previously inaccessible "resistant starch"[4,56]. The reduction in the viscosity of the digesta is the primary effect of xylanase with the release of sugars being a secondary effect[57]. Cereals contain up to 15 % NSPs in their cell walls, which is made up of soluble and insoluble NSP[58]. The insoluble component of NSP comprises the bulk of the total fiber in the diets of broilers and these insoluble components become a wall or cage that keeps nutrients trapped inside. It has an effect on nutrient digestion and utilization in non-ruminant animals[59]. On the other hand, the soluble component of NSPs, i.e., mainly arabinoxylans, only hinders nutrient digestion and absorption by increasing gut viscosity. This components also alters the digestive tract functions by modifying the secretion of endogenous digestive enzymes, water and electrolyte secretion in addition to elevating fermentation in the small intestine[19]. A study by Barekatain *et al.*[57] showed that diets containing sorghum brewer's dried grains that included xylanase supplementation significantly reduced the concentration of insoluble NSPs and increased the concentration of free sugars (i.e., arabinose and xylose). They also reported that the availability of these free sugars may have provided nutrients to birds, which resulted in improved FCR and BWG[23].

Carcass characteristics of broiler chickens: The increased proportional weights of retail cuts from broiler chickens fed XYL20 may be due to the peak bio-activities of the *Aspergillus* xylanase enzyme that resulted in an increase in broiler muscle (tissue) development. This is in line with the findings of Kanti and Sudiana[50], they reported that any enzyme level that produced the maximum enzyme activities on the substrate usually cause the largest response. The reduced weight of carcasses and other yields of retail cuts for birds fed XYL0 may be due to a reasonable increase in fiber content and a possible increase in the level of anti-nutrients in the diet. The results of this study support an earlier report by Ogunsipe *et al.*[60], who revealed that rich fiber diets reduce the slaughter weights of rabbits and broilers. Again, Moharrery and Mohammad[61] reported that higher fiber diets disrupt intestinal micro-villus and depress nutrient absorption that may hinder muscle (tissue) development. Xylanase enzyme supplementation to fibrous diets improved the growth rate, which increased the carcass and retail cut yields[62]. The results of the present research are in line with the findings of Hajati[32], Wang *et al.*[62] and Alam *et al.*[63], they reported an increased carcass yield with the addition of exogenous enzymes in wheat-based diets which they attributed to higher tissue (muscle) development in the carcass and breast. On the other hand, the reports of the present study contradict the findings of Nadeem *et al.*[64], who revealed that the yields of carcasses and retail cuts were not significantly different when the birds were fed xylanase enzyme-supplemented diets.

Size of visceral organs: This study showed that increasing the supplemental levels of *Aspergillus* xylanase was correlated to a lowered lengths of small intestines in broilers. Yasar and Forbes[65] reported that the decrease in relative length and weight of the small intestine was probably caused by a decrease in the thickness (i.e., viscosity) of the contents of the small intestine and a reduction in the crypt cell proliferation rate. Some authors maintain that the reduction in the length may be associated with a decrease in the viscosity of the gut contents and the concentration of volatile fatty acids in the ceca as well as a rapid passage rate of the digesta and its greater dilution with water[65,66]. They also suggested that the reduction in gut size represented a considerable reduction in the nutrient cost of maintaining the integrity of the gut and a potential to increase the efficiency of utilization of nutrients for growth. The present study showed that higher supplementation levels of *Aspergillus* xylanase in maize-soybean broiler diets significantly increased spleen weights, which may suggest that enzyme supplementation accelerated the development of an immune-related organ. The spleen produces antibodies[67] and forms a reservoir that contains over half of the body’s monocytes[68]. Meanwhile, monocytes are phagocytic in action, i.e., they ingest or engulf germs[23,69]. Gao *et al.*[41] showed that the spleen is an immune defense organ that is part of the peripheral lymphoid tissue. The fact that the spleen increased in weight as the inclusion levels of *Aspergillus* xylanase increased which showed that the supplemented enzyme may have positively influenced the immune capacity of the birds. Another author has reported that the spleen removes old red blood cells and holds a reserve of blood that can be valuable in case of hemorrhagic shock[70]. It should be noted that the use of *Aspergillus* xylanase significantly influenced the performance of broiler chickens. The study have shown that supplementing 2 g XYL kg^{-1} of broiler feed resulted in an improved performance compared to other levels of *Aspergillus* xylanase used in the present study. Again, at this level (2 g XYL kg^{-1} feed), there seem to be a maximum activities of *Aspergillus* xylanase on the substrate which was reflected in the overall performance of broiler chickens fed XYL20. However, the findings of the present study revealed that feeding maize-soybean meal based diets supplemented with 2 g *Aspergillus* xylanase improved broiler performance.

CONCLUSION

The optimum inclusion level of *Aspergillus* xylanase based on weight gain and FCR of the broiler chicken was 2 g XYL kg^{-1} feed (XYL20). Based on the results obtained from this study, dietary treatment XYL20 enhanced PER, EER and apparent nutrient digestibility. Dietary treatment XYL20 improved broiler growth performance and carcass traits compared with those that received other dietary treatments. *Aspergillus* xylanase supplementation also accelerated the development of the immune organ (spleen) of broilers compared with those that received the control diet.

SIGNIFICANCE STATEMENT

This study discovers the potential of *Aspergillus* xylanase in maize-soybean meal diet. *Aspergillus xylanase* is a microbial exogenous enzyme that can be beneficial for feed manufactures and poultry producers with regards to optimum broiler performance. This study will help researchers uncover the critical areas of interest with regards to optimum broiler performance of microbial exogenous enzyme (*Aspergillus* xylanase) that can further unlock the nutritional potentials of other basal diet such as rye-, barley- and wheat-based diets. Hence, a new theory on optimum inclusion levels of *Aspergillus* xylanase and the maize-soybean meal combination has been achieved in the present study and possibly, other combinations with rye-, barley- and wheat-based diets examined in further studies may be useful.

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