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Effect of Dietary Supplementation of *Aspergillus* Xylanase on Broiler Chickens Performance

Research Article

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ABSTRACT

The effect of *Aspergillus* xylanase (ASXYL) supplementation to maize-soybean diets on serum aspartate aminotransferase, serum alanine aminotransferase, microbial examination, growth traits, carcass characteristics and meat quality traits of broiler chickens was investigated. Three hundred one-day-old mixed sex "Cobb 500" broiler chicks were allotted to 5 dietary treatments with 5 replicates of 12 birds each. The treatments include, ASXYL0 (0 g/kg), ASXYL10 (1 g/kg), ASXYL15 (1.5 g/kg), ASXYL20 (2 g/kg) and ASXYL25 (2.5 g/kg). Birds fed ASXYL20 had the highest (P<0.05) body weight with an improved feed conversion ratio (FCR) and a higher values for thigh, breast, wing and carcass yields. Neck weight was high (P<0.05) for fed birds ASXYL0, ASXYL15 and ASXYL20. Drumstick recorded higher (P<0.05) value for birds fed ASXYL20 though, similar to ASXYL10, ASXYL15 and ASXYL25. Drip and cooking loss decreased (P<0.05) with increased supplemental levels of ASXY while shear force increased (P<0.05) as ASXYL supplementation increased. Urea, aspartate aminotransferase and alanine aminotransferase decreased (P<0.05) with increased supplemental levels of ASXYL while glucose level increased (P<0.05) with increased levels of ASXYL supplementation. Supplementary ASXYL influenced (P<0.05) the proliferation of *Lactobacillus* counts in ileum and caecum while no difference (P>0.05) was observed on the population of *Bifidobacteria* and *Escherichia coli* in both ileum and caecum of broilers at the end of the feeding trial. It was concluded that dietary ASXYL20 produced a much improved body weight and retail cut yields. Again, the bio-markers showed that ASXYL improved the health status of broiler chicken and the tested enzyme influenced a positive intestinal environment.

KEY WORDS *Aspergillus* xylanase, carcass weight, glucose, health status, *Lactobacillus*.

INTRODUCTION

Poultry farming is one of the most important sectors in the global agribusiness. This sector increased significantly after inclusion in the production chain in the 1980 s mainly as a result of increased adoption of technology in the sector. The mechanisms of technological advances in nutrition with the inclusion of exogenous enzymes in broiler diets allow greater assimilation of nutrients resulting in increased meat yield as a result of improved body weight. Research studies have reported the beneficial impact of exogenous xylanase enzymes on the performance and nutrient digestibility of broiler birds fed non-conventional feedstuffs (Mathlouthi *et al.* 2002; Oyeagu *et al.* 2016). Broiler ration in South Africa and most part of the world are almost entirely formulated from two basic ingredients; maize, which is an excellent

energy source and soybean meal, which contributes a high-quality protein and with great amino acid availability (Opalinski *et al.* 2006). However, it is known that the nutrients originated from these ingredients are not absorbed properly, mainly due to the presence of anti-nutritional factors such as non-starch polysaccharides (NSPs) which are inherent in the plant cell wall (Oyeagu *et al.* 2015). Due to the chemical structure of the plant cell wall matrix, NSPs degrading enzymes has been recommended to enhance poultry performance. Exogenous xylanase enzyme supplementation has been documented to be effective in breaking polymeric chains of NSPs and hence improve the nutritive value of feedstuffs (Giraldo *et al.* 2008). Therefore adding NSPs – degrading enzymes in poultry diet has increased considerably in recent years. Birds do not produce enzymes such as xylanase for the digestion of NSPs. Supplementation of xylanase enzymes may not only reduce the anti-nutritive effects of NSPs, but also releases some nutrients from these, which could be utilized by birds (Oyeagu *et al.* 2016). Again, these NSPs – degrading enzymes has been reported to improve *Lactobacillus* and *Bifidobacteria* counts in caecum of broilers fed corn-soybean meal based diet (Nian *et al.* 2011).

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 carcass yield of broiler chickens (Hajati *et al.* 2009). Nutritional status is an important factor in the regulation of plasma biomarkers in broiler chickens (Buyse *et al.* 2002; Swennen *et al.* 2005; Gao *et al.* 2008). Evaluation of plasma biochemistry in birds allows for the identification of metabolic alterations as a result of management conditions such as diets (Alonso-Alvarez and Ferrer, 2001). The use of blood examination as a way of assessing the health status of animals has been documented (Muhammed *et al.* 2000; Owoyele *et al.* 2003). Haematobiochemical examination plays a vital role in the physiological, nutritional and pathological status of organisms (Muhammed *et al.* 2000). They range from giving the level of the blood to detecting ailment or disorders through them. It had been reported that biochemical changes as a result of toxins have effects on blood parameters (Karnish, 2003).

The effect of different diets (barley-based, wheat-based, and non-conventional feed) supplemented with different mono-enzymes or multi-enzymes has been evaluated on the blood parameters of broiler (Muhammed *et al.* 2000; Owoyele *et al.* 2003; Oyeagu *et al.* 2016; Oyeagu *et al.* 2019a), but there is little information on serum biomarkers, ileum and caecal micro-flora, carcass characteristics and meat quality traits of broiler chickens fed *Aspergillus* xylanase.

The present study, therefore, sought to examine the effect of *Aspergillus* xylanase on serum biomarkers, ileum and caecal micro-flora, growth traits, carcass characteristics and meat quality traits in broiler chickens fed maize-soybean meal 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.

Study site

This study was conducted at the poultry unit of North-West University experimental farm (Molelwane), in the North West 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 to 35 ˚C and average annual rainfall ranging from 200 to 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.

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 (Aquilina, 2016). This strain is deposited at the German Collection of Microorganisms and Cell Cultures (DSMZ) with the accession number DSM 26372 (Aquilina, 2010b). According to the manufacturer (Aquilina, 2010a), the bulk density is approximately 1.1 g/ml and the average particle size is approximately 600 µm with enzyme activity of 1000 FXU/t (1000 g/t).

Experimental diets

The feeding strategy consisted of starter (0 to 21 d) and finisher (22 to 42 d) basal diets (Tables 1 and 2), which were formulated to meet the birds' dietary nutrient requirements (NRC, 1994). At each feeding phase (starter and finisher), five dietary treatments of iso-nitrogenous and iso-caloric were formulated through the addition of *Aspergillus* xylanase (ASXYL) enzyme at five different levels.

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

Ingredients	Experimental diets				
	ASXYL0	ASXYL10	ASXYL15	ASXYL20	ASXYL25
Yellow maize	62.98	62.98	62.98	62.98	62.98
Soybean meal	27.48	27.48	27.48	27.48	27.48
Sunflower meal	4.00	4.00	4.00	4.00	4.00
Fish meal	2.50	2.50	2.50	2.50	2.50
Canola oil	0.11	0.11	0.11	0.11	0.11
Limestone	1.32	1.32	1.32	1.32	1.32
Mono-calcium phosphate	0.44	0.44	0.44	0.44	0.44
Salt	0.28	0.28	0.28	0.28	0.28
Methionine	0.23	0.23	0.23	0.23	0.23
Threonine	0.05	0.05	0.05	0.05	0.05
Lysine	0.32	0.32	0.32	0.32	0.32
Choline Cl	0.10	0.10	0.10	0.10	0.10
Vitamin and mineral premix[1]	0.20	0.20	0.20	0.20	0.20
Maxiban[2]	0.05	0.05	0.05	0.05	0.05
Surmax[3]	0.04	0.04	0.04	0.04	0.04
Total	100.00	100.00	100.00	100.00	100.00
Xylanase	0.00	0.10	0.15	0.20	0.25
Chemical composition					
Moisture (%)	11.11	10.89	1.00	11.08	11.01
Metabolizable energy (MJ/kg)	12.79	12.71	12.79	12.68	12.76
Crude protein (%)	23.83	23.90	23.79	23.85	23.88
Crude fat (%)	4.01	4.03	3.99	4.11	4.08
Neutral detergent fibre (%)	15.32	15.27	15.39	15.28	15.30
Acid detergent fibre (%)	3.85	3.95	3.97	4.00	4.02
Calcium (%)	0.93	0.94	0.92	0.93	0.94
Phosphorus (%)	0.66	0.68	0.65	0.69	0.67

ASXYL0: basal diet (without *Aspergillus* xylanase (XYL)); ASXYL10: basal diet + 1 g XYL/kg feed; ASXYL15: basal diet + 1.5 g XYL/kg feed; ASXYL20: basal diet + 2 g XYL/kg feed and ASXYL25: basal diet + 2.5 g XYL/kg feed.
[1] Vitamin and mineral premix (2.5 kg of vitamin premix) contained: Retinal: 2700 mg; Calcidiol: 400 mg; Tocopheryl acetate: 18 g; Menadione: 2000 mg; Thiamine: 1800 mg; Riboflavin: 6600 mg; Niacin: 10 g; Calcium pantothenate: 30 g; Pyridoxine: 3 g; folic acid: 1 g; Cobalamin: 15 mg; Choline chloride: 250 g; Biotin: 100 mg; Mn: 100 g; Fe: 50 g; Zn: 100 g; Cu: 10 g; I: 1 g and Se: 200 mg.
[2] 1000 g of Maxiban contained: Narasin: 80 g/kg and Nicarbazin: 80 g/kg.
[3] 1000g of Surmax contained: Avilamycin: 100 g/kg.

The five experimental diets formulated were, ASXYL0 (only basal diet; BD), ASXYL10 (BD+1 g ASXYL/kg feed), ASXYL15 (BD+1.5 g ASXYL/kg feed), ASXYL20 (BD+2 g ASXY/kg feed) and ASXYL25 (BD+2.5 g ASXY/kg feed) for both starter and finisher phases. The ingredient and chemical composition of the five experimental diets for starter and finisher phases are presented in Tables 1 and 2, respectively. The chemical (proximate) composition of the experimental diets was analyzed according to AOAC (2006) methods with average crude protein (CP) and metabolizable energy (ME) of 23.70 CP and 12.60 MJ of ME/kg respectively for starter chicks while an average of 19.70 CP and 13.00 MJ of ME/kg was recorded for finisher birds.

Experimental birds and management

A total of three hundred, one day old, non-sexed broiler birds (Cobb 500®) were used in the present study. Sixty birds (five replication of twelve birds in each replicate per treatment group) were assigned randomly to one of the five experimental diets (ASXYL0, ASXYL10, ASXYL15, ASXYL20 and ASXYL25). Each experimental diet was replicated into 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 as follows; day 1 – intra ocular New castle disease vaccine, week 2 – Gumboro disease vaccine, week 3 – Lasota (New castle disease vaccine), week 4 – Gumboro disease vaccine, and week 5 – fowl pox vaccine. A stress pack was administered to the birds via drinking water at 100 g/50 liters (according to manufacturer's recommendation) to boost appetite and energy supply. Dietary treatments and clean water were provided *ad libitum* in a six-week feeding trial.

Serum biochemical profile

Before blood collection, birds were feed-fasted for 4 h in an attempt to allow for the stabilization of the various plasma constituents.

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

Ingredients	Experimental diets				
	ASXYL0	ASXYL10	ASXYL15	ASXYL20	ASXYL25
Yellow maize	72.36	72.36	72.36	72.36	72.36
Soybean meal	24.55	24.55	24.55	24.55	24.55
Canola oil	0.24	0.24	0.24	0.24	0.24
Limestone	1.25	1.25	1.25	1.25	1.25
Mono-calcium phosphate	0.17	0.17	0.17	0.17	0.17
Salt	0.39	0.39	0.39	0.39	0.39
Methionine	0.21	0.21	0.21	0.21	0.21
Tryptophan	0.05	0.05	0.05	0.05	0.05
Threonine	0.05	0.05	0.05	0.05	0.05
Lysine	0.34	0.34	0.34	0.34	0.34
Choline Cl	0.10	0.10	0.10	0.10	0.10
Vitamin and mineral premix[1]	0.20	0.20	0.20	0.20	0.20
Maxiban[2]	0.05	0.05	0.05	0.05	0.05
Surmax[3]	0.04	0.04	0.04	0.04	0.04
Total	100.00	100.00	100.00	100.00	100.00
Xylanase	0.00	0.10	0.15	0.20	0.25
Chemical composition					
Moisture (%)	11.01	11.03	10.97	11.10	11.13
Metabolizable energy (MJ/kg)	13.08	13.04	13.01	13.03	13.05
Crude protein (%)	19.75	19.91	19.70	19.83	19.85
Crude fat (%)	4.08	4.12	4.12	4.15	4.09
Neutral detergent fibre (%)	15.93	18.32	18.28	18.21	18.02
Acid detergent fibre (%)	4.85	4.97	5.02	4.95	5.00
Calcium (%)	0.74	0.72	0.74	0.73	0.73
Phosphorus (%)	0.41	0.42	0.41	0.41	0.41

ASXYL0: basal diet (without *Aspergillus* xylanase (XYL)); ASXYL10: basal diet + 1 g XYL/kg feed; ASXYL15: basal diet + 1.5 g XYL/kg feed; ASXYL20: basal diet + 2 g XYL/kg feed and ASXYL25: basal diet + 2.5 g XYL/kg feed.
[1] Vitamin and mineral premix (2.5 kg of vitamin premix) contained: Retinal: 2700 mg; Calcidiol: 400 mg; Tocopheryl acetate: 18 g; Menadione: 2000 mg; Thiamine: 1800 mg; Riboflavin: 6600 mg; Niacin: 10 g; Calcium pantothenate: 30 g; Pyridoxine: 3 g; folic acid: 1 g; Cobalamin: 15 mg; Choline chloride: 250 g; Biotin: 100 mg; Mn: 100 g; Fe: 50 g; Zn: 100 g; Cu: 10 g; I: 1 g and Se: 200 mg.
[2] 1000 g of Maxiban contained: Narasin: 80 g/kg and Nicarbazin: 80 g/kg.
[3] 1000g of Surmax contained: Avilamycin: 100 g/kg.

Blood was collected in the morning to further reduce the variability of the measured plasma constituents. At 42 days of age, five birds were chosen randomly from each experimental pen and 2 mL of blood was collected from the wing vein using a sterile syringe and needles. Blood collected was emptied into a labelled treated vacutainer tubes. Red-top tubes without anticoagulant were used for serum biochemical analysis.

The blood was stored for 10 minutes at room temperature. After centrifugation (20 minutes, 1500 rpm), the serum was collected into 0.5 mL centrifuge tube and stored at -20 ˚C pending determination of serum metabolites. All analyses were conducted within 48 h after collection. The serum metabolites considered are alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein, albumin, urea and cholesterol were assayed using automated Idexx Vet Test Chemistry Analyser (IDEXX Laboratories, Inc) in the Animal Health Laboratory at the Centre for Animal Health Studies North-West University, Marfikeng.

Growth performance

Average daily feed intake (ADFI) per bird was measured from day 1 to day 42 of age by subtracting the weight of the feed left over from that of the feed offered, and dividing the difference by the total number of birds in the pen. Average live-weight was measured weekly by weighing all the birds in each pen using a 10100 g capacity precision weighing balance with model, A and D Weighing GF-10 K industrial balance, made in Japan. The feed conversion ratio (FCR) was calculated as follows: FCR= feed intake/weight gain.

Slaughter procedure

At 42 days of age, all chickens were taken to Rooigrond poultry abattoir (North-West province, South Africa) for slaughter. The chickens were starved for 12 hours before slaughter. All the chickens were humanely gas stunned by exposing them to relatively low concentrations of carbon dioxide (<40% by volume in air), and then, once they were unconscious, exposed to a higher concentration (approximately 80% to 90% by volume in air).

At the abattoir, all the chickens were hung onto a movable metal rack that holds them upside down by their feet. Chickens were then slaughtered by cutting the jugular vein with a sharp knife and they were left hanging until bleeding stopped.

Carcass characteristics

Five birds per replicate were randomly selected for determination of carcass characteristics and meat quality. Immediately after slaughter, the feathers were plucked and the gastro intestinal tract (GIT) was removed. The carcasses were then weighed to obtain the carcass weight of the birds. For the measurement of carcass cuts, head and shanks were removed close to the scull and at hock joint, respectively. Wings were removed by cutting at the humeoscapular joint, the cuts were made through the 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.

Meat pH and temperature measurements

Meat pH and temperature were recorded immediately after slaughter and 24 h post slaughter on the breast muscle (central area of the breast) using a Corning Model 4 pH-temperature meter (Corning Glass Works, Medfield, MA) equipped with an Ingold spear-type electrode (Ingold Messtechnik AG, Udorf, Switzerland) according to Stanford *et al.* (2003). After every 20 measurements, the pH meter was calibrated with pH 4, pH 7 and pH 10 standard solutions (Ingold Messtechnik AG, Udorf, Switzerland).

Meat colour

Colour of the meat (L^*=Lightness, a^*=Redness and b^*=Yellowness) was determined 24 hours after slaughter, using a Minolta colour-guide (BYK-Gardener GmbH, Geretsried, Germany), on a 20 mm diameter measurement area and illuminant D65-day light, 10 degree observation angle. The colour meter was calibrated using the green standard before measurements. Colour recording was done on the surface of the breast muscle, which was allowed to bloom for 1 hour on a polystyrene tray at 4 ˚C.

Water holding capacity (WHC)

The pressure method as described by Delezie *et al.* (2007) was used to determine the WHC. About 100 g meat sample from pectoral major (PM) was cut and weighed to obtain the initial weight using a digital scale sensitive up to 0.01 g. The meat sample was then placed in between 2 filter papers, placed on a flat surface and approximately 60 kg weight was applied on the sample for 5 min. Thereafter, the meat sample was re-weighed. The WHC was calculated as the ratio of the amount of water retained over the initial sample weight.

Drip loss

Approximately 30 g meat strips were sampled from the breast muscle parallel to the fibre direction then weighed to get the wet sample using a digital scale sensitive to 0.01 g. The samples were suspended inside a plastic container and sealed under atmospheric pressure. The samples were then held at 2 ˚C for 72 hours after which they were removed from the container. The samples were blotted with paper towels to remove excess surface moisture and were then re-weighed. The drip loss was then calculated by subtracting the blotted sample weight from the initial sample weight. The drip loss was expressed as a percentage of the initial sample weight (Honikel and Hamm, 1994).

Cooking loss measurement

Raw meat cubes were cut from the breast muscle, weighed in natura, then placed in a plastic bag and cooked in a water bath at 75 ˚C for 45 minutes (Rizz *et al.* 2007). The samples were then cooled in running water for 15 min, dried with soft tissue and weighed (Sanka and Mbaga, 2014). Cooking loss was calculated as percentage loss of weight during cooking relative to the weight of raw muscle (Petracci and Baéza, 2009) according to the following formula:

Cooking loss (%)= ((weight before cooking–weight after cooking)/(weight before cooking)) × 100

Tenderness

Breast muscles were wrapped in aluminum foil and baked to reach an internal temperature of 85 ˚C, which was maintained for 30 minutes. Warner Bratzler shear device mounted on an Universal Instron apparatus (cross head speed=200 mm/minute, one shear in the centre of each core). Smaller samples were then cut parallel to the muscle fibres with the aid of a Meullenet - Owens Razor Shear Blade (A/MORS) with a diameter of 1.2 cm. The reported value represented the average peak force measurements of each sample in Newtons. The shear force was recorded using the Texture analyser (TA XT plus).

Cecal and ileum micro flora composition

Five birds per treatment at the age of 42 days were killed by severing the jugular vein. The abdominal cavity was opened, and the entire gastro intestinal tract was removed aseptically. All digesta contents of ileum, caecum and colon were collected immediately under aseptic conditions into sterile glass bags and put on ice before they were transported to the laboratory for enumeration of microbial populations. Ceacal and ileum digesta contents were emptied

aseptically in a new sterile bag and were immediately diluted 10-fold (ie 10% wt/vol) with sterile ice-cold anoxic PBS (0.1 m; pH 7.0) and subsequently homogenized for 3 min in a stomacher (Bagmixer 100 Minimix, Interscience, Arpents, France). Each ceacal and ileum digesta homogenate was serially diluted from 10^{-1} to 10^{-7}. Dilutions were subsequently plated on duplicate selective agar media for enumeration of target bacterial groups. In particular, *E. Coli*, *Lactobacillus* spp. and *Bifidobacterium* spp. were enumerated using VRB agar (MERCK, 1.01406), Rogosa agar (MERCK, 1.10660), and Beerens agar respectively according to Tuohy *et al.* (2002).

Plates were incubated at 39 ˚C for 48 to 120 hours anaerobically (Beerens, Rogosa agars) or 24 to 48 hours anaerobically at 37 ˚C (VRB agar). The bacterial colonies were enumerated, and the average number of live bacteria was calculated based on the weight of original ileum and caecum contents. All quantitative data were converted into logarithmic colony forming units (cfu/g), Koc *et al.* (2010).

Statistical design and analysis

Data collected during the study were subjected to analysis of variance (ANOVA) for completely randomized design (CRD) as described by Steel and Torrie (1980) using general linear model Procedure of SAS (2010). The statistical model used to test the effects of treatment on growth traits, meat quality traits, carcass characteristics, serum biochemical profile, and gut micro-flora was:

$$Y_{ij} = \mu + A_i + E_{ij}$$

Where:
Y_{ij}: observed value of a dependent variable.
μ: overall mean.
A_i: effect of different levels of dietary *Aspergillus* xylanase enzyme.
E_{ij}: residual error.

The differences between means were tested for significance at $P < 0.05$ using least significant difference (LSD) range test.

RESULTS AND DISCUSSION

Serum biochemical profile

Table 3 represents the serum biochemical traits of broiler chickens fed different inclusion levels of *Aspergillus* xylanase (ASXYL). The addition of ASXYL altered (P<0.05) the concentrations of serum glucose, urea and serum enzymes (aspartate aminotransferase (AST) and alanine aminotransferase (ALT) while total protein, cholesterol and alkaline phosphatase (ALP) did not differ (P>0.05).

Urea, AST and ALT values decreased (P<0.05) as the levels of ASXYL enzyme increased. Again, glucose level increased (P<0.05) with increased inclusion levels of ASXY enzyme.

Gut micro-flora composition

The population of ileum and caecum microbes of broilers fed maize-soybean meal diet with different inclusion levels of ASXYL is presented in Table 4. ASXYL supplementation had no influence (P>0.05) on the population of *Bifidobacteria* and *Escherichia coli* in both ileum and caecum of broilers at 42 days of age.

However, supplementing ASXYL enzyme increased (P<0.05) the counts of *Lactobacillus* in the ileum and caecum of broiler birds compared to their counterparts that received XYL0 (control diet) that do not contain *Aspergillus* xylanase.

Growth performance

Different phases in growth performance of broiler birds fed maize-soybean meal based diets supplemented with ASXYL are presented in Table 5. All the growth traits considered in this study were affected (P<0.05) by the inclusion of dietary ASXYL except for body weight gain (BWG) during finisher phase. Birds fed ASXYL20 consumed less feed at starter phase, finisher phase and overall feeding trial when compared with birds fed other levels of ASXYL.

Average daily feed intake of birds fed ASXYL20 was lower but similar to birds fed ASXYL25 when compared with other treatments used in the study. Daily weight gain and BWG were highest (P<0.05) for birds fed ASXYL20 during the starter phase and overall phase (entire feeding period) but similar to birds fed ASXYL25. Birds fed ASXYL20 recorded an improved FCR during the starter and the overall feeding period compared with other treatments used in the study.

Carcass characteristics of broiler chickens

The meat yield traits of broiler birds fed maize-soybean meal diet with different supplemental levels of ASXYL enzyme is presented in Table 6. The weights of carcass, neck, wing, drumstick, thigh, breast and head were affected (P<0.05) by the addition of ASXYL while shank and vertebrate weights did not differ (P>0.05). Birds fed ASXYL20 had the highest (P<0.05) values for thigh, breast, wing as well as carcass yields compared with birds fed other treatments used in the present study. Neck weight was highest (P<0.05) for ASXYL20 fed birds, though statistically similar to birds fed ASXYL0 and ASXYL15.

Drumstick recorded an improved (P<0.05) value for birds fed ASXYL20, though similar to their counterparts that received dietary ASXYL10, ASXYL15 and ASXYL25.

Table 3 The effect of dietary *Aspergillus* xylanase supplementation on serum biochemical (SB) metabolites of broiler birds

SB metabolites	Experimental diets					SEM
	ASXYL0	ASXYL10	ASXYL15	ASXYL20	ASXYL25	
Protein (g/L)	25.85	27.82	31.86	30.79	28.97	0.44
Glucose (g/L)	12.79^{bc}	13.81^{b}	14.95^{a}	15.62^{a}	15.58^{a}	0.13
Urea (mmol/L)	0.95^{a}	0.96^{a}	0.81^{b}	0.70^{c}	0.68^{c}	0.00
Cholesterol (mmol/L)	3.88	3.95	4.01	4.11	3.98	0.01
ALP (IU/L)	145.18	148.30	141.22	147.30	144.85	2.88
AST (IU/L)	328.33^{a}	317.63^{a}	275.57^{b}	266.99^{b}	262.59^{b}	5.87
ALT (IU/L)	16.18^{a}	14.00^{b}	12.54^{c}	12.77^{c}	12.85^{c}	0.12

ASXYL0: basal diet (without *Aspergillus* xylanase (XYL)); ASXYL10: basal diet + 1 g XYL/kg feed; ASXYL15: basal diet + 1.5 g XYL/kg feed; ASXYL20: basal diet + 2 g XYL/kg feed and ASXYL25: basal diet + 2.5 g XYL/kg feed.
AST: aspartate aminotransferase; ALT: alanine aminotransferase and ALP: alkaline phosphatase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 4 The effect of dietary *Aspergillus* xylanase supplementation on the counts of bacteria in the ileum and caecum (log_{10} cfu/g) of broilers fed corn-soybean meal diets

Bacteria	Experimental diets					SEM
	ASXYL0	ASXYL10	ASXYL15	ASXYL20	ASXYL25	
Ileum						
Lactobacillus	6.02^{b}	6.94^{ab}	7.86^{a}	8.72^{a}	8.90^{a}	0.08
Bifidobacteria	4.97	4.07	4.13	4.54	4.47	0.03
E. coli	4.17	4.16	3.98	3.92	3.88	0.03
Caecum						
Lactobacillus	7.17^{c}	8.64^{b}	9.09^{a}	9.15^{a}	9.08^{a}	0.09
Bifidobacteria	5.16	5.34	5.48	5.66	5.71	0.06
E. coli	5.92	5.89	5.60	5.45	5.19	0.05

ASXYL0: basal diet (without *Aspergillus* xylanase (XYL)); ASXYL10: basal diet + 1 g XYL/kg feed; ASXYL15: basal diet + 1.5 g XYL/kg feed; ASXYL20: basal diet + 2 g XYL/kg feed and ASXYL25: basal diet + 2.5 g XYL/kg feed.
AST: aspartate aminotransferase; ALT: alanine aminotransferase and ALP: alkaline phosphatase.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 5 The effect of dietary xylanase supplementation on feed intake (FI), weight gain (WG) and feed conversion ratio (FCR) of broiler birds

Item	Experimental diets					SEM
	ASXYL0	ASXYL10	ASXYL15	ASXYL20	ASXYL25	
Daily performance						
Day-old weight (g)	45.00	45.00	44.00	44.00	45.00	0.14
Daily FI (g)	107.53^{a}	109.36^{a}	108.81^{a}	100.88^{b}	103.45^{ab}	0.75
Daily WG (g)	62.04^{b}	62.71^{b}	62.39^{b}	67.37^{a}	64.65^{ab}	0.45
Starter phase (1-21 d)						
FI (g)	1414.00^{bc}	1508.80^{a}	1473.40^{ab}	1360.80^{c}	1418.20^{bc}	5.75
Body WG (g)	908.40^{b}	934.60^{b}	930.40^{b}	1038.60^{a}	979.00^{ab}	4.69
FCR (g/g)	1.56^{ab}	1.62^{a}	1.59^{ab}	1.31^{c}	1.51^{b}	0.01
Finisher phase (22-42 d)						
FI (g)	3102.40^{a}	3084.20^{a}	3099.60^{a}	2876.20^{b}	3136.80^{a}	9.75
Body WG (g)	1697.20	1699.40	1690.00	1791.20	1650.40	6.98
FCR (g/g)	1.83^{a}	1.82^{a}	1.84^{a}	1.61^{b}	1.87^{a}	0.01
Overall phase (1-42 d)						
FI (g)	4516.40^{a}	4593.00^{a}	4573.00^{a}	4237.00^{b}	4555.00^{a}	10.46
Body WG (g)	2605.60^{b}	2634.00^{b}	2620.40^{b}	2829.80^{a}	2789.40^{ab}	8.33
FCR (g/g)	1.74^{a}	1.75^{a}	1.75^{a}	1.50^{b}	1.76^{a}	0.01

ASXYL0: basal diet (without *Aspergillus* xylanase (XYL)); ASXYL10: basal diet + 1 g XYL/kg feed; ASXYL15: basal diet + 1.5 g XYL/kg feed; ASXYL20: basal diet + 2 g XYL/kg feed and ASXYL25: basal diet + 2.5 g XYL/kg feed.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Again, head weight was lowest (P<0.05) for birds fed ASXYL0 and ASXYL25 while those that received ASXYL20 recorded the highest head weight value, though statistically similar to their counterparts that received ASXYL10 and ASXYL15. Generally, birds fed ASXYL20 were observed to have an improved (P<0.05) retail cut yields of neck, wing, drumstick, thigh, breast, head as well as carcass when compared with birds fed other ASXYL levels.

Meat quality attributes

The meat quality traits of broiler chickens fed different in

clusion levels of ASXY are presented in Table 7. Different levels of ASXYL supplementation to maize-soybean meal diet had an influence (P<0.05) on cooking loss, shear force and drip loss while colour, water holding capacity, pHo, pHu and temperature were not affected (P>0.05). Cooking loss value of chicken breast meat was significantly (P<0.05) higher for birds fed ASXYL0, though similar to their counterparts that received ASXYL10. The cooking loss of chicken breast meat recorded the lowest (P<0.05) value for birds fed higher levels of the tested enzyme (ASXYL15, ASXYL20 and ASXYL25). Shear force values of the breast meat increased (P<0.05) as the levels of the tested enzyme increased.

The lowest (P<0.05) shear force value was recorded for control birds (ASXYL0) while ASXYL15, ASXYL20 and ASXYL25 fed birds had the highest (P<0.05) shear force values, though similar to birds fed ASXYL10. The addition of ASXYL enzyme influenced (P<0.05) the reduction of drip loss value, whereas the breast meat of birds fed ASXYL0 and ASXYL10 had the highest (P<0.05) values of drip loss, though statistically similar to their counterparts that received ASXYL15. The lowest (P<0.05) drip loss value was recorded for birds fed ASXYL20 and AS XYL25.

Serum biochemical profile

Blood biochemical parameters can reflect the physiological state of the body. In the present study, we observed that broilers had a higher concentration of blood glucose with increased supplemental levels of ASXYL. Shouqing *et al.* (2015) opined that lactose can be degraded into glucose and galactose by lactase which is mainly synthesized and secreted by the small intestine epithelia cells of piglets. In mammals, glucose is not the only host energy sources, but it promotes the development of brain and neuron, again, excess of the glucose is deposited in the tissue (flesh), hence, developing the retail cut yields (Bano, 2013). The result of the present study did not conform to those of Balamurgan and Chandrasekaran (2010), who found no effect of supplemental multi-enzyme (xylanase and protease) in blood glucose of broiler birds. Chauhan *et al*. (2002) found that no effect was detected on blood glucose when they fed dietary phytase enzyme to broilers. Blood glucose level did not change in the findings of Mithu *et al.* (2017) who supplemented xylanase enzyme to high fibre diet (wheat). Exogenous enzymes used in the diets of broiler birds are very beneficial in improving nutrient digestibility by supplying external enzymes that the birds cannot produce in sufficient quantity by itself, and even though the birds can produce enough quantity of an enzyme by itself, exogenous enzyme may reduce the requirement for the endogenous enzyme, thus making more nutrients and energy available for growth of the birds (Olukosi *et al*. 2007; Oyeagu *et al.* 2019a), hence, this may be the reason for increased blood glucose concentration recorded in the present study at 42 days of age. As the inclusion levels of ASXYL enzyme increased, the concentration of urea decreased.

The lowest urea value was recorded for birds fed ASXYL20 and ASXYL25. Although, urea concentration may not have much value or importance in detecting renal diseases in many avian species (including broilers), but it could be used as a sensitive indicator for dehydration (Lumeij, 1998).

However, high urea plasma levels has been linked to conditions such as dehydration and ureteral obstructions that caused low urine flow (Oloruntola *et al*. 2016). In the present study, birds fed ASXYL0 and ASXYL10 recorded more concentration of blood urea compared with birds fed other levels of ASXYL enzyme. A decreased urea value was reported to be associated with reduced urea synthesis (build-up), reduced mal-absorption, reduced protein malnutrition and reduced advanced liver disease (Peter and Susan, 1991; Oloruntola *et al.* 2016). The result of the present study is in line with the report of Obikaonu *et al.* (2012) who observed a decrease in urea level in broiler birds when the dietary inclusion of neem leaf meal increased from 0% to 10%.

The progressive reduction in the concentration of ALT and AST of broiler birds with increased supplemental levels of ASXYL enzyme indicates a safety inclusion of the tested enzyme in broiler ration at the rates used in this study. Measurement of ALT and AST activities are indicative of liver damage in broiler chicks and is therefore a valuable tool for determination of a safe inclusion rate for feed additives, since diets may influence serum enzymes (Mohamed *et al*. 2014).

Some authors reported that the higher serum AST and ALT concentrations indicate the release of aminotransferase from cytoplasm to blood stream probably due to damage liver or different other tissues (Ahmad *et al.* 2013; Abida *et al*. 2017).

The reduction of the serum enzymes (ALT and AST) with the inclusion of ASXYL highlights the possibility of reducing cases of myocardial problem in broilers as a result of ASXYL enzyme inclusion in the diets. Oloruntola *et al.* (2016) also share the same view. Aspartate aminotransferase and alanine aminotransferase are enzymes found in cardiac muscle, skeletal muscle, kidney, brain, liver and red blood cell, also they are regarded as a biochemical markers for the diagnosis of acute myocardial infection (Gaze, 2007).

Table 6 The effect of dietary *Aspergillus* xylanase supplementation on carcass characteristics of broiler birds at 42 day of age

Carcass characteristics	Experimental diets					SEM
	ASXYL0	ASXYL10	ASXYL15	ASXYL20	ASXYL25	
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}	1.32
Wing weight (g)	81.13^{b}	82.27^{b}	80.73^{b}	91.67^{a}	80.40^{b}	1.06
Drumstick weight (g)	96.00^{b}	101.80^{ab}	99.73^{ab}	117.67^{a}	100.80^{ab}	1.57
Thigh weight (g)	112.33^{b}	100.94^{b}	100.00^{b}	128.67^{a}	111.08^{b}	1.98
Breast weight (g)	499.27^{b}	489.47^{b}	493.13^{b}	637.53^{a}	511.20^{b}	4.76
Vertebrate weight (g)	180.60	182.33	185.00	188.86	185.87	2.45
Shank weight (g)	35.53	37.87	38.33	39.06	35.13	0.59
Head weight (g)	44.13^{b}	48.93^{ab}	47.87^{ab}	50.13^{a}	44.67^{b}	0.67

ASXYL0: basal diet (without *Aspergillus* xylanase (XYL)); ASXYL10: basal diet + 1 g XYL/kg feed; ASXYL15: basal diet + 1.5 g XYL/kg feed; ASXYL20: basal diet + 2 g XYL/kg feed and ASXYL25: basal diet + 2.5 g XYL/kg feed.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

Table 7 The effect of dietary *Aspergillus* xylanase supplementation on meat quality attributes of broiler birds at 42 day of age

Meat quality attributes	Experimental diets					SEM
	ASXYL0	ASXYL10	ASXYL15	ASXYL20	ASXYL25	
Colour						
a* (redness)	1.62	1.45	1.33	1.36	1.20	0.00
b* (yellowness)	6.45	6.98	6.94	6.72	6.75	0.13
L* (lightness)	59.55	58.99	59.97	58.83	59.90	0.67
Cooking loss (%)	20.53^{a}	18.94^{ab}	15.84^{b}	14.89^{b}	15.94^{b}	0.13
Shear force (N)	5.39^{b}	6.76^{ab}	7.46^{a}	7.73^{a}	7.98^{a}	0.07
Drip loss (%)	7.52^{a}	7.29^{a}	6.14^{ab}	5.03^{b}	4.50^{b}	0.02
Water holding capacity (%)	16.81	17.29	17.92	18.10	18.89	0.25
pH_0	6.36	6.27	6.23	6.26	6.20	0.02
pHu	5.78	5.84	5.74	5.83	5.72	0.02
Temperature 45 mins after slaughter (C°)	37.69	37.32	36.76	36.99	37.26	0.46
Temperature 24 hrs after slaughter (C°)	9.77	9.92	9.87	9.23	9.72	0.11

ASXYL0: basal diet (without *Aspergillus* xylanase (XYL)); ASXYL10: basal diet + 1 g XYL/kg feed; ASXYL15: basal diet + 1.5 g XYL/kg feed; ASXYL20: basal diet + 2 g XYL/kg feed and ASXYL25: basal diet + 2.5 g XYL/kg feed.
The means within the same row with at least one common letter, do not have significant difference (P>0.05).
SEM: standard error of the means.

The myocardial degradation has been identified as a major cause of sudden death and serious losses in broiler industry (Kwada *et al.* 1994; Oloruntola *et al.* 2016) which in most cases is the resultant effect of burden imposed on heart due to procedures adopted for growing chickens to produce a fast body weight gain of about 2.5 kg to 3 kg in 42 days (Oloruntola *et al.* 2016).

Gut micro-flora composition

The gut micro-flora has an important role in animal health and production. They positively influence the hosts' gastrointestinal development, digestion, metabolism, pathogen exclusion, immune stimulation and vitamin synthesis (Torok *et al.* 2008; Albazaz and Buyukunal Bal, 2014). In poultry, the micro-organism that colonizes the gastrointestinal tract during the early post-hatch period forms a synergistic relationship with their host. According to Munyaka *et al.* (2016), the gut micro-biota plays an important role in host nutrition and health by promoting digestion and absorption of nutrients, preventing pathogen's colonization, and shaping and maintaining normal mucosal immunity.

Bacterial richness and evenness are the major parameters for defining microbial structure and diversity and it is generally expected that dietary manipulations would influence the intestinal microbial structure and diversity (Kiarie *et al.* (2014). Masey *et al.* (2014) had earlier reported that feed enzymes can influence microbial populations due to the changes imposed on the lumen contents. In the present study, the supplementation of ASXYL enzyme increased the total number of *Lactobacillus* spp. in both ileum and caecum. *Lactobacilli* have a number of biochemical properties, including, the production of antibacterial compounds (Stern *et al.* 2006) and bile salt hydrolase compounds (Knarreborg *et al.* 2002), in addition to their known probiotic roles that help to maintain gut integrity. On the other hand, even though the *E. coli* counts was not influenced by inclusion levels of ASXYL enzyme, there was a progressive drop/decrease in the numerical values reported for *E. coli* as the inclusion levels of ASXYL increased. The result of the present study is in agreement with the findings of Nian *et al.* (2011), who revealed that inclusion of xylanase enzyme in wheat-based diet reduced the number of *Coliform* and *Salmonella* and increased the number of *Lactoba-*

cillus in the ileum. Some studies have shown that xylanase addition could inhibit the proliferation of potentially pathogenic microbial populations in the intestine and improve animal health (Nian *et al.* 2011; Sugiharto, 2016). It is possible that, enzyme supplementation resulted in multiplication of beneficial bacteria in the present study. In this case, exogenous enzyme acts not only by lowering intestinal viscosity, but also leads to development of more competitive bacterial communities with higher intra-bacterial competition and thus additionally limits bacterial interference with nutrient absorption, and may have the effect of reducing potentially pathogenic population (Jozefiak *et al.* 2010). Dietary factors can also influence microbial populations in birds. Some authors (Rodriguez *et al.* 2012) observed higher number of *Escherichia coli* and *Lactobacilli* in the digesta of broilers fed wheat and barley, relative to those fed corn diets. Changes in the intestinal micro-biota of chickens can alter the mucosal structure and thereby influence nutrient absorptive capacity (Munyaka *et al.* 2016). According to Bedford (2000a), xylanase enzymes can change the microbial populations indirectly within the gastrointestinal tract through their utilization of the substrates that bacteria use as a carbon source. The composition of diet affects the gastro-intestinal micro-flora in broilers. The presence of viscous polysaccharides has been shown to increase the intestinal microbial activities associated with poor broiler performance (Hubner *et al.* 2002). Exogenous enzyme supplementation can significantly influence microbial populations in the intestine. NSP – degrading enzymes such as xylanase are hypothesized to work in two steps which are described as an ileal phase and ceacal phase (Bedford, 2000b). During the ileal phase, enzymes remove fermentable substrates. During the ceacal phase, degradation products of sugars such as xylose and xylo-oligomers are fermented by ceacal bacteria, thus stimulating the production of volatile fatty acid and the growth of specific beneficial bacteria (Bedford, 2000b). The study of Engberg *et al.* (2004) revealed that xylanase supplementation to wheat-based diets stimulated growth of lactic acid bacteria in the ileum which was confirmed by high lactic acid concentrations. However, there was a significant difference in *Lactobacillus* counts in both ileum and caecum while the population of *E. coli* and *Bifidobacteria* were not influenced. The results of the present study is slightly contrary with those of Gao *et al.* (2008) who reported that xylanase addition in wheat-based diet had no significant change on the *Lactobacillus*, *Bifidobacteria* and *E. coli* counts in the caecum and ileum of broilers. Our result also did not agree with those of Dingyuan *et al.* (2009) who found no influence of xylanase addition to wheat-based diets on the counts of *Lactobacillus*, *E. coli* and total aerobes in ileum and caecum. However, it should be noted from the present study that the increased supplemental levels of ASXYL allowed the proliferation of *Lactobacillus* in both caecum and ileum.

Growth performance

The dietary ASXYL20 (2 g/kg feed) improved weight gain, FCR and reduced FI throughout the feeding trial (Table 5). This May be the optimum/maximum bio-activity of the tested enzyme was achieved at ASXYL20 level of inclusion compared with other treatments which may have affected the variations in feeding and body weight pattern of the birds. This is in agreement with other researchers who found that the increase in BWG may be due to the use of carbohydrases (Mathlouthi *et al.* 2002; Francesch and Geraert, 2009; Hajati, 2010). Previous reports (Gao *et al.* 2008; Esmaeilipour *et al.* 2011; Zhang *et al.* 2014) showed that the supplementation of *Bacillus* xylanase in wheat-based diets significantly increased BWG and improved FCR of broilers. Feed conversion ratio is a good indicator of how efficient a feed or a feeding strategy can be. The improved growth performance recorded for birds fed ASXYL20 may be that the peak bio-activities of the ASXYL on the substrate are at 2 g *Aspergillus* xylanase per kg diet (ASXYL20). It was reported that the dietary fiber is responsible for accelerating digesta passage rate (Wilfarta *et al.* 2007; Al-Harthi, 2014; Sayehban *et al.* 2015). However, due to the low fiber content of the dietary ASXYL20 as a result of the maximum enzyme activities, broilers that received ASXYL20 may have experienced lower digesta passage rate than those fed other dietary treatments. Previous studies (Sayehban *et al.* 2016) reported that the longer the low fibre digesta in the intestinal tract, the greater the chances for better feed digestion which improves growth performance. According to Engberg *et al.* (2004) and Selle *et al.* (2010), whole wheat (high fibre) feeding reduces the activities of amylase in the pancreatic tissue while the addition of xylanase increased chymotrypsin and lipase activities. Meanwhile, the data in the present study were not in agreement with those of Wang *et al.* (2005), Gao *et al.* (2008) and Dingyuan *et al.* (2009) who found no difference in FCR of broilers supplemented with dietary xylanase. Some studies have shown that addition of xylanase resulted in improved broiler performance (Gracia *et al.* 2003; Jiang *et al.* 2008; Williams *et al.* 2014). However, other authors found no effects on growth performance after supplementing exogenous xylanase enzyme (Kocher *et al.* 2003; Singh *et al.* 2012). However, the improved performance of birds fed ASXYL20 recorded in the present study may be due to the reduction of anti-nutritive effects of NSPs and the release of some nutrients. Some authors share the same opinion (Giraldo *et al.* 2008; Oyeagu *et al.* 2016). Xylanase may increase access to entrapped nutrient components by digest-

ing some fractions of the plant cell walls of grains, allowing α-amylase access to starch fractions and prevent "resistant starch" (Leslie *et al.* 2007; Stefanello *et al.* 2015). The reduction in the viscosity of the digestion is the primary effect of xylanase with the release of sugars being a secondary effect (Barekatain *et al.* 2013). Cereals contain up to 15% NSPs in their cell walls, which is made up of soluble and insoluble NSP (Diebold *et al.* 2004). The insoluble component of NSPs comprises the bulk of the total fibre in the diets and they act to become a wall or cage that keep nutrients trapped inside and it has an adverse effect on nutrient digestion and utilization in non-ruminant animals (Hetland *et al.* 2004). On the other hand, the soluble component of NSPs, mainly arabinoxylans, can only act as physical obstacles to nutrient digestion and absorption by increasing gut viscosity. These components also alter the digestive tract functions by modifying secretion of endogenous digestive enzymes, water and electrolytes as well as elevating fermentation in small intestine (Zhang *et al.* 2014). A study by Barekatain *et al.* (2013) showed that diets containing sorghum brewer's dried grains with xylanase supplementation significantly reduced the concentration of insoluble NSPs and increased the concentration of free sugars (arabinose and xylose). They reported that the availability of these free sugars may have provided nutrients to birds which resulted in improved FCR and BWG (Oyeagu *et al.* 2019a).

Carcass characteristics of broiler chickens

Carcass and cut yield traits determine the purchase decision of chicken meat consumers. The carcass yield is closely linked to adequate food and nutrition of broilers (Cardoso *et al.* 2011). After all, animals with adequate supply of nutrients will deposit effectively muscle. The increased proportional weight of retail cut yields of broiler chickens (including the breast meat) fed 2 g *Aspergillus* xylanase per kg diet (ASXYL20) may have occurred due to the fact that this level provides a better digestibility of the ingredients and, therefore, increases the amount of nutrients available for improved muscle (tissue) development and breast growth, since the breast cutting represents about 40% of the total carcass yield (Dalólio *et al.* 2015). It was noticed from the results of the present study that every other treatment enzyme level over or lower than ASXYL20 did not have the same response as ASXYL20 which may be due to lack of substrate available after the addition of an amount of enzymes greater than or lower than ASXYL20 without considering the nutritional energy matrix and, or, the diet proteins. The result of this study supports earlier report of Ogunsipe *et al.* (2014) who revealed that rich fibre diets reduces slaughter yield in rabbits and broilers. Again, Moharrery and Mohammad (2005) reported that higher fibre diets disrupts intestinal micro-villus and depresses nutrient absorption which may hinder muscle (tissue) development. The dietary supplementation of exogenous enzymes enhanced diet digestibility possibly because it promoted an increase in the activities of the digestive enzymes by increasing the availability of substrates which will increase nutrient absorption for enhanced muscle (tissue) development (Oyeagu *et al.* 2019b). When adding exogenous amylase, xylanase or protease to broiler diets, researchers reported higher activities of pancreatic and intestinal enzymes measured in 14- and 42-d-old broilers (Horvatovic *et al.* 2015). Diets based on barley, maize, sorghum, and wheat without enzymes negatively affected the activity of the intestinal enzymes, while enzyme supplementation did not (Shakouri *et al.* 2008). According to another study (Zdunczyk *et al.* 2009), maltase and sucrase activities was reduced when turkeys were fed diets with high fiber levels. It was reported that xylanase enzyme supplementation to fibrous diets improved the growth rate which increased the carcass and retail cut yields (Wang *et al.* 2005). The results of the present research are in line with the findings of Alam *et al.* (2003); Wang *et al.* (2005) and Hajati (2010). They reported an increased carcass yield by the addition of exogenous enzymes in wheat-based diets and they attributed it to higher tissue (muscle) development in carcass and also breast muscle. On the other hand, the reports of the present study contradicts the findings of Nadeem *et al.* (2005) and Pattaniak *et al.* (2011) who revealed that carcass yield and retail cuts had no significant difference among xylanase enzyme supplemental diets.

Meat quality attributes

According to Petracci and Baéza (2011), broiler meat has some important intrinsic attribute such as, appearance, texture, succulence and flavor. Again, the functionality of broiler meat is the coloring which is the most important factor that affects the choice of consumers (Werner *et al.* 2009). The results of the present study showed that there was no change or distortion in the chicken meat colour. It was reported that meat quality parameters depends directly from the management conditions, especially the type of feed provided to the birds (Neves *et al.* 2014). The addition of exogenous enzymes in the feed of broilers, probably, improves nutrient digestibility efficiency and promotes greater carcass yield and meat deposition (Allouche *et al.* 2015). The increase in protein deposition may change the type and shape of fiber to be deposited on the substrate, and may therefore change the meat quality parameters (Fatufe *et al.* 2004; Dalólio *et al.* 2016). The results of the present study did not agree with the studies of Tabook *et al.* (2006), Werner *et al.* (2009), Hana *et al.* (2010) and Dalolio *et al.* (2016), who found no effect on meat quality traits such as,

pH, water holding capacity, shear force among others when they expose chickens to different dietary multi-enzyme (xylanase and protease) supplementation. The results of the present study showed that, there was a progressive increase in the numerical values of water holding capacity as the inclusion levels of the tested enzyme increased. The higher values of cooking loss and drip loss of chicken breast meat recorded for birds fed ASXYL0 showed that reasonable amount of nutrient in the meat are lost due to increased water loss (exudate). Omojola *et al.* (2014) also shared the same view, they opined that meat with higher nutrient loss as a result of increased exudate (water loss) should be referred to as poor quality meat. This suggests that higher levels of ASXYL enzyme supplementation in the present study reduced nutrient loss as well as increased the cooking yield. This may be due to the specific hydrolyzing activity of ASXYL (from *Aspergillus strain E A.1*) used in this study because microbial xylanase (wild or modified) from nonpathogenic and safe microorganisms improves meat quality (Alaa *et al.* 2014), unlike the traditional plant xylanases that tend to have broad specificities and indiscriminately break down connective tissue and myofibrillar proteins (Foegeding and Larick, 1986; Miller *et al.* 1989; Ashie *et al.* 2002). Meanwhile, Gorsuch and Alvarado (2010) stated that cooking loss and / or drip loss is a measurement of water holding capacity of the muscle. They recorded a shear force of 1.7 to 3.7 in their study and they considered the meat as a tender meat. However, shear force is an indication of the degree of toughness or tenderness. In the present study, the shear force values ranges between 5.39 and 7.98 and the highest value was recorded when the supplemental levels of ASXYL increased. This is an indication that increased force was required to shear through the breast meat of birds fed higher supplemental levels of ASXYL. Gajana *et al.* (2013) recorded a similar result and they attributed it to the pre-slaughter glycogen depletion which may have resulted in meat with a higher ultimate pH (pHu) (Kannan *et al.* 2002) and therefore tougher meat that will require more force to shear it. Meat tenderness is related to ultimate pH (pHu) value and meat colour (Byrne *et al.* 2000; Strydom *et al.* 2000; Vestergaard *et al.* 2000), although there are some cases where such relationship may not be significant (Muchenje *et al.* 2008). In the present study, the ultimate pH (pHu) did not differ even when there was a change in the shear force. According to Muchenje *et al.* (2008), an increase in pHu does not necessarily result in tougher meat as other parameters with regards to meat tenderness. Meat tenderness varies, mainly as a result of changes to the myofibrillar protein structure of muscle in the period between animal slaughter and meat consumption (Muir *et al.* 2000; Muchenje *et al.* 2009). Gajana *et al.* (2013) revealed that the positive correlation between cooking loss or evaporation loss and shear force values may imply that increased evaporation loss or cooking loss has the influence of decreasing tenderness and juiciness in meat as it results in tough muscles (tissue) that may be difficult to chew. Some researchers found that increase in cooking loss may lead to more nutrient loss (Omojola *et al.* 2014) from the meat and more protein toughening due to denaturation (Barbut *et al.* 2005).

CONCLUSION

From the results of the present study, it can be concluded that dietary ASXYL20 improved the growth traits, retail-cut yields as well as carcass yield of broiler chickens compared with the control (ASXYL0) group. Moreover, ASXYL supplementation in maize-soybean meal diet did not show any detrimental effect on gut health and liver or kidney functions, while it also showed some positive potential on meat quality traits of chicken meat.

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REFERENCES

Abida N., Amber K., Zunaira A., Anam A., Bushra I., Shaneel K., Laiba R., Ramsha A. and Faiza N. (2017). Effect of supplementation of xylanase on feed efficiency and serum biochemistry in broilers. *Res. J. Pharmacol. Pharmacol.* **1,** 1-9.

Ahmad Z., Butt M.S., Hussain R., Ahmed A. and Riaz M. (2013). Effect of oral application of xylanase on some hematological and serum biochemical parameters in broilers. *Pakistan Vet. J.* **33,** 388-390.

Alaa A.B., David L.H., Geert G., Adnan A.B. and Philip F. (2014). Exogenous proteases for meat tenderization. *Crit. Rev. Food Sci. Nutr*. **54,** 1012-1031.

Alam M.J., Howlider M.A.R., Pramanik M.A.H. and Haque M.A. (2003). Effect of exogenous enzyme in diet on broiler performance. *Int. J. Poult. Sci*. **2,** 168-173.

Albazaz I.R. and Büyükünal Bal E.B. (2014). Micro-flora of digestive tract in poultry. *KSU J. Nat. Sci*. **17,** 39-42.

Al-Harthi M.A. (2014). The chemical composition and nutrient profiles and energy values of olive cake for poultry diets. *Life Sci. J.* **11(9),** 159-165.

Allouche L., Madani T., Hamouda A., Boucherit M.R., Taleb H., Samah O., Rahamani K. and Touabati A. (2015). Effect of addition of exogenous enzymes in hypocaloric diet in broiler chicken on performance, biochemical parameters and meat

characteristics. *Biotechnol. Anim. Husband*. **31,** 551-565.

Alonso-Alvarez C. and Ferrer M.A. (2001). Biochemical study of fasting, sub-feeding and recovery process in yellow-legged gulls. *Physiol. Biochem. Zool*. **74,** 703-713.

AOAC. (2006). Official Methods of Analysis. 18th Ed. Association of Official Analytical Chemists, Arlington, Washington, DC., USA.

Aquilina G., Azimonti G., Bampidis V., de Lourdes Bastos M., Bories G., Chesson A., Sandro Cocconcelli P., Flachowsky G., Gropp J., Kolar B., Kouba M., López Puente S., López‐Alonso M., Mantovani A., Mayo B., Ramos F., Rychen G., Saarela M., Edoardo Villa R., John Wallace R. and Wester P. (2016). Safety and efficacy of RONOZYME® WX (endo-1,4-betaxylanase) as a feed additive for chickens and turkeys for fattening, minor poultry species for fattening, weaned piglets and pigs for fattening. *European Food Safety Author. J*. **14,** 1-14.

Aquilina G., Bories G., Brantom P., Chesson A., Sandro Cocconcelli P., de Knecht J., Albert Dierick N., Antoni Gralak M., Gropp J., Halle I., Kroker R., Leng L., Lindgren S., Lundebye Haldorsen A.K., Mantovani A., Mézes M., Renshaw D. and Saarela M. (2010a). Scientific Opinion on Ronozyme® P (6-phytase) as feed additive for salmonids. *European Food Safety Author. J*. **8,** 13-27.

Aquilina G., Bories G., Brantom P., Chesson A., Sandro Cocconcelli P., de Knecht J., Albert Dierick N., Antoni Gralak M., Gropp J., Halle I., Kroker R., Leng L., Lindgren S., Lundebye Haldorsen A.K., Mantovani A., Mézes M., Renshaw D. and Saarela M. (2010b). Scientific Opinion on Ronozyme® P (6-phytase) as feed additive for chickens and turkeys for fattening, laying hens, piglets (weaned), pigs for fattening and sows (poultry and pigs). *European Food Safety Author. J*. **8,** 1-12.

Ashie I.N.A., Sorensen T.L. and Nielsen P.M. (2002). Effects of papain and a microbial enzyme on meat proteins and beef tenderness. *J. Food Sci.* **67,** 2138-2142.

Balamurgan R. and Chandrasekaran D. (2010). Effect of multi-enzyme supplementation on weight gain, feed intake, feed efficiency and blood glucose in broiler chicken. *Indian J. Sci. Technol.* **3,** 193-195.

Bano G. (2013). Glucose homeostasis, obesity and diabetes. *Best Pract. Res. Clin. Obstet. Gynaecol*. **27,** 715-726.

Barbut S., Zhang L. and Marcone M. (2005). Effects of pale, normal, and dark chicken breast meat on microstructure, extractable proteins, and cooking of marinated fillets. *Poult. Sci.* **84(5),** 797-802.

Barekatain M.R., Antipatis C., Choct M. and Iji P.A. (2013). Interaction between protease and xylanase in broiler chicken diets containing sorghum distillers' dried grains with soluble. *Anim. Feed Sci. Technol*. **182,** 71-81.

Bedford M.R. (2000a). Exogenous enzymes in monogastric nutrition-their current value and future benefits. *Anim. Feed Sci. Technol.* **86,** 1-13.

Bedford M.R. (2000b). Removal of antibiotic growth promoters from poultry diets: Implications and strategies to minimize subsequent problems. *World's Poult. Sci. J*. **56,** 347-365.

Buyse J., Janssens K., Van der Geyten S., Van As P., Decuypere E. and Darras V.M. (2002). Pre- and postprandial changes in plasma hormone and metabolite levels and hepatic deiodinase activities in meal-fed broiler chickens. *Br. J. Nutr*. **88,** 641-653.

Byrne C.E., Troy D.J. and Buckley D.J. (2000). Postmortem changes in muscle electrical properties of bovine m. longissimus dorsi and their relationship to meat quality attributes and pH fall. *Meat Sci.* **54,** 23-34.

Cardoso D.M., Maciel M.P., Passos D.P., Silva F.V., Reis S.T. and Aiura F.S. (2011). Efeito do uso de complexo enzimático em rações para frangos de corte. *Arch. Zootec*. **60(232),** 1053-1064.

Chauhan S.S., Hassen H. and Singh M. (2002). Studies on the effect of phytase enzyme supplementation on blood parameters in broilers. Pp. 294 in 4th Biennial Conf. Anim. Nutr. Assoc., Hisar, India.

Dalólio F.S., Moreira J., Vaz Diego P., Albino L.F.T., Valadares L.R., Pires A.V.P. and Sandra R.F. (2016). Exogenous enzymes in diets for broilers. *Rev. Brasileira Saúde Prod. Anim. Salvador.* **17,** 149-161.

Dalólio F.S., Vaz D.P., Moreira J., Albino L.F.T. and Valadares L.R. (2015). Carcass characteristics of broilers fed enzyme complex. *Biotechnol. Anim. Husband*. **31,** 153-162.

Delezie E., Swennen Q., Buyse J. and Decuypere E. (2007). The effect of feed withdrawal and crating density in transit on metabolism and meat quality of broilers at slaughter weight. *Poult. Sci.* **86,** 1414-1423.

Diebold G., Mosenthin R., Piepho H.P. and Sauer W.C. (2004). Effect of supplementation of xylanase and phospholipase to a wheat-based diet for weanling pigs on nutrient digestibility and concentrations of microbial metabolites in ileal digesta and feces. *J. Anim. Sci.* **82,** 2647-2656.

Dingyuan L., Fengxia Y., Xiaojun Y., Junhu Y., Baojun S. and Zhenfeng Z. (2009). Effects of xylanase on performance, blood parameters, intestinal morphology, microflora and digestive enzyme activities of broilers fed wheat-based diets. *Asian-Australasian J. Anim. Sci*. **22,** 1288-1295.

Engberg R.M., Hedemann M.S., Steenfeldt S. and Jensen B.B. (2004). Influence of whole wheat and xylanase on broiler performance and microbial composition and activity in the digestive tract. *Poult. Sci*. **83,** 925-938.

Esmaeilipour O., Shivazad M., Moravej H., Aminzadeh S., Rezaian M. and Van Krimpen M.M. (2011). Effects of xylanase and citric acid on the performance, nutrient retention, and characteristics of gastrointestinal tract of broilers fed low phosphorus wheat-based diets. *Poult. Sci.* **90,** 1975-1982.

Fatufe A.A., Timmler R. and Rodehutscord M. (2004). Response to lysine intake in composition of body weight gain and efficiency of lysine utilization of growing male chickens from two genotypes. *Poult. Sci*. **83,** 1314-1324.

Foegeding E.A. and Larick D.K. (1986). Tenderization of beef with bacterial collagenase. *Meat Sci*. **19,** 201-214.

Francesch M. and Geraert P.A. (2009). Enzyme complex containing carbohydrases and phytase improves growth performance and bone mineralization of broilers fed reduced nutrient corn-soybean-based diets. *Poult. Sci.* **88,** 1915-1924.

Gajana C.S., Nkukwana T.T., Marume U. and Muchenje V. (2013). Effects of transportation time, distance, stocking density, temperature and lairage time on incidences of pale

soft exudative (PSE) and the physico-chemical characteristics of pork. *Meat Sci.* **95,** 520-525.

Gao F., Jiang Y., Zhou G.H. and Han Z.K. (2008). The effects of xylanase supplementation on performance, characteristics of the gastrointestinal tract, blood parameters and gut micro-flora in broilers fed on wheat-based diets. *Anim. Feed Sci. Technol*. **142,** 173-184.

Gaze D.C. (2007). The role of existing and novel cardiac biomarkers for cardio-protection. *Curr. Opin. Investig. Drugs*. **8,** 711-717.

Giraldo L.A., Tejido M.L., Ranilla M.J., Ramos S. and Carro M.D. (2008). Influence of direct-fed fibrolytic enzymes on diet digestibility and ruminal activity in sheep fed grass hay-based diet. *J. Anim. Sci*. **86,** 1617-1623.

Gorsuch V. and Alvarado C.Z. (2010). Postrigor tumble marination strategies for improving color and water holding capacity in normal and pale broiler breast fillets. *Poult. Sci.* **89,** 1002-1008.

Gracia M.I., Aran´ıbar M.J., L´azaro L., Medel P. and Mateos G.G. (2003). Alpha-amylase supplementation of broiler diets based on corn. *Poult. Sci*. **82,** 436-442.

Hajati H. (2010). Effects of enzyme supplementation on performance, carcass characteristics, carcass composition and some blood parameters of broiler chicken. *American J. Anim. Vet. Sci.* **5,** 221-227.

Hajati H., Rezaei M. and Sayyahzadeh H. (2009). The effects of enzyme supplementation on performance, carcass characteristics and some blood parameters of broilers fed on corn-soybean meal-wheat diets. *Int. J. Poult. Sci*. **8,** 1199-1205.

Hana A.H.Z., Mohammad A.R.J. and Majdi A.A.I. (2010). The influence of supplemental multi-enzyme feed additive on the performance, carcass characteristics and meat quality traits of broiler chickens. *Int. J. Poult. Sci*. **9,** 126-133.

Hetland H., Choct M. and Svihus B. (2004). Role of insoluble non-starch polysaccharides in poultry nutrition. *World's Poult. Sci. J*. **60,** 415-422.

Honikel K.O. and Hamm R. (1994). Measurement of water-holding capacity and juiceness. Pp. 125-161 in Quality Attributes and Their Measurement in Meat, Poultry and Fish Products. A.M. Pearson and T.R. Dutson, Eds. Blackie Academic and Professional, New York, USA.

Horvatovic M.P., Glamocic D., Zikic D. and Hadnadjev T.D. (2015). Performance and some intestinal functions of broilers fed diets with different inclusion levels of sunflower meal and supplemented or not with enzymes. *Brazilian J. Poult. Sci*. **17(1),** 25-30.

Hubner K., Vahjen W. and Simon O. (2002). Bacterial responses to different dietary cereal type and xylanase supplementation in the intestine of broiler chicken. *Arch. Anim. Nutr.* **56,** 167-187.

Jiang Z., Zhou Y., Lu F., Han Z. and Wang T. (2008). Effects of different levels of supplementary alpha-amylase on digestive enzyme activities and pancreatic amylase mRNA expression of young broilers. *Asian-australasian J. Anim. Sci.* **21,** 97-102.

Jozefiak D., Rutkowski A., Kaczmarek S., Jensen B.B., Engberg R.M. and Hojbjerg O. (2010). Effect of β-glucanase and xylanase supplementation of barley- and rye-based diets on caecal microbiota of broiler chickens. *British Poult. Sci.* **51,** 546-557.

Kannan G., Chawan C.B., Kouakou B. and Gelaye B. (2002). Influence of packaging method and storage time on shear value and mechanical strength of intramuscular connective tissue of chevron. *J. Anim. Sci.* **80,** 2383-2389.

Karnish A.R. (2003). Immune Regulation in Health and Disease. Academic Press, San Diego, USA.

Kiarie E., Romero L.F. and Ravindran V. (2014). Growth performance, nutrient utilization, and digesta characteristics in broiler chickens fed corn or wheat diets without or with supplemental xylanase. *Poult. Sci.* **93,** 1186-1196.

Knarreborg A., Engberg R.M., Jensen S.K. and Jensen B.B. (2002). Quantitative determination of bile salt hydrolase activity in bacteria isolated from the small intestine of chickens. *Environ. Appl. Microbiol*. **68,** 6425-6428.

Koc H., Samli A., Okur M., Ozduven H.A. and Senkoylu N. (2010). Effects of *Saccharomyses cerevisiae* and/or mannan oligosaccharides on performance, blood parameters and intestinal micro-biota of broiler chicks. *Bulgarian J. Agric. Sci*. **16,** 643-650.

Kocher A., Choct M., Ross G., Broz J. and Chung T.K. (2003). Effects of enzyme combinations on apparent metabolizable energy of corn-soybean meal based diets in broilers. *J. Appl. Poult. Res.* **12,** 275-283.

Kwada M., Hirosawa R., Yanai T.M. and Ueda K. (1994). Cardiac lesions in broilers which died without clinical signs. *Avian Pathol*. **23,** 503-511.

Leslie M.A., Moran Jr, E.T. and Bedford M.R. (2007). The effect of phytase and glucanase on the ileal digestible energy of corn and soybean meal fed to broilers. *Poult. Sci*. **86,** 2350-2357.

Lumeij J.T. (1998). A contribution to clinical investigative methods for birds, with special reference to the racing pigeon (*Columbial livia domestica*). Ph D. Thesis. University of Utrecht, Utrecht, The Netherlands.

Masey O'Neill H.V., Smith J.A. and Bedford M.R. (2014). Multicarbohydrase for non-ruminants. *Asian Australasian J. Anim. Sci*. **27,** 290-301.

Mathlouthi N., Serge M., Luc S., Bernard Q. and Michel L. (2002). Effects of xylanase and -glucanase addition on performance, nutrient digestibility, and physico-chemical conditions in the small intestine contents and caecal micro-flora of broiler chickens fed a wheat and barley-based diet. *Anim. Res.* **51,** 395-406.

Miller A.J., Strange E.D. and Whiting R.C. (1989). Improved tenderness of restructured beef steaks by microbial collagenase derived from Vibrio B-30. *J. Food Sci*. **54,** 855-857.

Mithu D., Robin B., Sarma D.N., Arundhati B. and Amonge T.K. (2017). Growth performance, serum biochemistry and carcass characteristics of broiler birds fed high fibre diet supplemented with exogenous enzyme. *Int. J. Chem. Stud*. **5,** 310-313.

Mohamed I.E., Mosaad A.S., Hany F.E. and El-Sayed R.K. (2014). Growth performance, blood parameters, immune response and carcass traits of broiler chicks fed on graded levels of wheat instead of corn without or with enzyme supplementation. *Alexandria J. Vet. Sci*. **40,** 95-111.

Moharrery A. and Mohammad A.A. (2005). Effect of diet

containing different qualities of barley on growth performance serum amylase and intestinal villus morphology. *Int. J. Poult. Sci.* **4,** 549-556.

Muchenje V., Dzama K., Chimonyo M., Raats J.G. and Strydom P.E. (2008). Meat quality of Nguni, Bonsmara and Aberdeen Angus steers raised on natural pasture in the Eastern Cape, South Africa. *Meat Sci.* **79,** 20-28.

Muchenje V., Dzama K., Chimonyo M., Strydom P.E., Hugo A. and Raats J.G. (2009). Some biochemical aspects pertaining to beef eating quality and consumer health: A review. *Food Chem.* **112,** 279-289.

Muhammed N.O., Adeyina A.O. and Peters O.M. (2000). Nutritional evaluation of fungi treated cocoa bean shell. *Nigerian J. Pure Appl. Sci.* **5,** 1059-1064.

Muir P.D., Wallace G.J., Dobbie P.M. and Bown M.D. (2000). A comparison of animal performance and carcass and meat quality characteristics in Hereford, Hereford x Friesian, and Friesian steers grazed together at pasture. *New Zealand J. Agric. Res*. **43,** 193-205.

Munyaka P.M., Nandha N.K., Kiarie E., Nyachoti C.M. and Khafipour E. (2016). Impact of combined β-glucanase and xylanase enzymes on growth performance, nutrients utilization and gut microbiota in broiler chickens fed corn or wheat-based diets. *Poult. Sci*. **95,** 528-540.

Nadeem M.A., Anjum M.I., Khan A.G. and Azim A. (2005). Effect of dietary supplementation of non-starch polysaccharide degrading enzyme on growth performance of broiler chicks. *Pakistan Vet. J.* **25,** 183-188.

Neves D.P., Banhazi T.M. and Nääs I.A. (2014). Feeding behaviour of broiler chickens: a review on the biochemical characteristics. *Rev. Brasileira de Ciên. Avíc.* **16,** 1-16.

Nian F., Guo Y.M., Ru Y.J., Peron A. and Li F.D. (2011). Effect of xylanase supplementation on the net energy for production, performance and gut micro-flora of broilers fed corn/soy-based diet. *Asian-Australasian J. Anim. Sci*. **24,** 1282-1287.

NRC. (1994). Nutrient Requirements of Poultry, 9th Rev. Ed. National Academy Press, Washington, DC., USA.

Obikaonu H.O., Okoli I.C., Opara M.N., Okoro V.M.O., Ogbuewu I.P., Etuk E.B. and Udedibie A.B.I. (2012). Haematological and serum biochemical indices of starter broilers fed leaf meal of neem (*Azadirachta indica*). *J. Agric. Technol*. **8,** 71-79.

Ogunsipe M.H., Agbede J.O. and Adedeji O.A. (2014). Performance response, carcass evaluation and economic benefit of rabbits fed sorghum offal-based diets. *African J. Food Agric. Nutr. Dev.* **14,** 8585-8601.

Oloruntola O.D., Ayodele S.O., Agbede J.O. and Oloruntola D.A. (2016). Effect of feeding broiler chickens with diets containing *Alchornea cordifolia* leaf meal and enzyme supplementation. *Archiv. Zootec*. **65,** 489-498.

Olukosi O.A., Cowieson A.J. and Adeola O. (2007). Age-related influence of a cocktail of xylanase, amylase and protease or phytase individually or in combination in broilers. *Poult. Sci*. **86,** 77-86.

Omojola A.B., Otunla T.A., Olusola O.O., Adebiyi O.A. and Ologhobo A.D. (2014). Performance and carcass characteristics of broiler chicken fed soybean and sesame/soybean based diets supplemented with or without microbial phytase. *American J. Exp. Agric.* **4,** 1637-1648.

Opalinski M., Maiorka A., Cunha F., Silva E.C.M. and Borges S.A. (2006). Adição de níveis crescentes de complexo enzimático em rações com soja integral desativada para frangos de corte. *Arch. Vet. Sci*. **11,** 31-35.

Owoyele B.V., Adebayo J.O., Muhammad N.O. and Ebunlomo A.O. (2003). Effect of different processing techniques of turban meal on some hematological indices in rats. *Nigerian J. Pure App. Sci.* **18,** 1340-1345.

Oyeagu C.E., Ugwuanyi C.L., Onwujiariri E., Osita C.O., Akuru E.A., Ani A.O., Idamokoro M.E and Falowo A.B. (2019a). Blood bio-markers, growth traits, carcass characteristics and income over feed cost of broiler birds fed enzyme fortified brewer's dried grain. *Pakistan J. Nutr.* **18(9),** 834-844.

Oyeagu C.E., Mlambo V. and Muchenje V. (2019b). Effect of dietary *Aspergillus* xylanase on nutrient digestibility and utilization performance and size of internal organs in broiler chickens offered maize-soybean meal based-diets. *Pakistan J. Nutr.* **18(9),** 852-865.

Oyeagu C.E., Ani A.O., Egbu C.F., Akpolu E.S., Iwuchukwu J.C. and Omumuabuike J.N. (2015). Performance of broiler finisher birds fed toasted bambara nut (*Vigna subterranean verdc*) offal with supplementary enzyme. *Asian J. Sci. Technol.* **6,** 934-939.

Oyeagu C.E., Ani A.O., Egbu C.F., Udeh F.U., Omumuabuike J.N. and Iwuchukwu, J.C. (2016). The effect of feeding toasted bambara nut (*Vigna subterranean verdc*) offal and supplementary enzyme on performance of broiler chicks. *J. Trop. Agric.* **93,** 271-283.

Pattaniak J.R., Das S.K., Mishra S.K., Panda N., Pati P.K. and Dehuri P.K. (2011). Effect of fiber degrading multi-enzyme on the performance of commercial broiler. *Indian. J Anim. Sci.* **81,** 1052-1059.

Peter M.L. and Susan C.E.F. (1991). Interpretation of laboratory results. *Australian Vet. Practit.* **21,** 100-108.

Petracci M. and Baéza E. (2009). Harmonization of methodology of assessment of poultry meat quality features. Working paper of WPSA Working Group 5 Poultry Meat July.

Petracci M. and Baéza E. (2011). Harmonization of methodologies for the assessment of poultry meat quality features. *World`s Poult. Sci. J*. **67,** 137-151.

Rizz C., Marangon A. and Chiericat G.M. (2007). Effect of genotype on slaughtering performance and meat physical and sensory characteristics of organic laying chicken. *Poult. Sci*. **86,** 128-135.

Rodriguez M.L., Rebol´e A., Velasco S., Ortiz L.T., Trevino J. and Alzueta C. (2012). Wheat- and barley-based diets with or without additives influence broiler chicken performance, nutrient digestibility and intestinal microflora. *J. Sci. Food Agric*. **92,** 184-190.

Sanka Y.D. and Mbaga S.H. (2014). Evaluation of Tanzanian local chicken reared under intensive and semi-intensive systems: II. Meat quality attributes. *Livest. Res. Rural Dev.* Available at: http://www.lrrd.org/lrrd26/9/sank26156. html.

SAS Institute. (2010). SAS®/STAT Software, Release 9.4. SAS Institute, Inc., Cary, NC. USA.

Sayehban P., Seidavi A., Dadashbeiki M., Ghorbani A., Araújo W.A.G. and Albino L.F.T. (2015). Effects of different dietary

levels of two types of olive pulp and exogenous enzyme supplementation on the gastro-intestinal tract size, immunology and hematology of broilers. *Brazilian J. Poult. Sci.* **17,** 73-85.

Sayehban P., Seidavi A., Dadashbeiki M., Ghorbani A., Araújo W.A.G. and Albino L.F.T. (2016). Effects of different levels of two types of olive pulp with or without exogenous enzyme supplementation on broiler performance and economic parameters. *Brazilian J. Poult. Sci*. **18(3),** 489-499.

Selle P.H., Cadogan D.J., Ru Y.J. and Partridge G.G. (2010). Impact of exogenous enzymes in Sorghum or wheat based broiler diets on nutrient utilization and growth performance. *Int. J. Poult. Sci.* **9(1),** 53-58.

Shakouri M.D., Iji P.A., Mikkelsen L.L. and Cowieson A.J. (2008). Intestinal function and gut microflora of broiler chickens as influenced by cereal grains and microbial enzyme supplementation. *J. Anim. Physiol. Anim. Nutr*. **93,** 647-658.

Shouqing M., Meilei M., Chunlong M., Kaifan Y. and Weiyun Z. (2015). Comparisons of blood biochemical parameters, digestive enzyme activities and volatile fatty acid profile between Meishan and Yorkshire piglets. *J. Anim. Nutr*. **1,** 289-292.

Singh A., Masey O'Neill H.V., Ghosh T.K., Bedford M.R. and Haldar S. (2012). Effects of xylanase supplementation on performance, total volatile fatty acids and selected bacterial population in caeca, metabolic indices and peptide YY concentrations in serum of broiler chickens fed energy restricted maize-soybean based diets. *Anim. Feed Sci. Technol.* **177,** 194-203.

Stanford K., Aalhus J.L., Dugan M.E.R., Wallins G.L., Sharma R. and McAllister T.A. (2003). Effects of feeding transgenic canola on apparent digestibility, growth performance and carcass characteristics of lambs. *Canadian J. Anim. Sci*. **83,** 299-305.

Steel R.G.D. and Torrie J.H. (1980). Principles and Procedures of Atatistics. A Biometric Approach. McGraw-Hill Publishers, New York.

Stefanello C., Vieira S.L., Santiago G.O., Kindlein L., Sorbara J.O.B. and Cowieson A.J. (2015). Starch digestibility, energy utilization, and growth performance of broilers fed corn-soybean basal diets supplemented with enzymes. *Poult. Sci.* **94,** 2472-2479.

Stern N.J., Svetoch E.A., Eruslanov B.V., Perelygin V.V., Mitsevich E.V., Mitsevich I.P., Pokhilenko V., Levchuk V.P., Svetoch O.E. and Seal B.S. (2006). Isolation of a *Lactobacillus salivarius* strain and purification of its bacteriocin, which is inhibitory to Campylobacter jejuni in the chicken gastrointestinal system. *Antimicrob. Agents Chemother*. **50,** 3111-3116.

Strydom P.E., Naude R.T., Smith M.F., Scholtz M.M. and van Wyk J.B. (2000). Characterization of indigenous African cattle breeds in relation to meat quality traits. *Meat Sci.* **55,** 79-88.

Sugiharto S. (2016). Role of nutraceuticals in gut health and growth performance of poultry. *J. Saudi Soc. Agric. Sci.* **15,** 99-111.

Swennen Q., Janssens G.P.J., Millet S., Vansant G., Decuypere E. and Buyse J. (2005). Effects of substitution between fat and protein on food intake and its regulatory mechanisms in broiler chickens: Endocrine functioning and intermediary metabolism. *Poult. Sci*. **84,** 1051-1057.

Tabook N.M., Kadim I.T., Mahgoub O. and Al-Marzooqi W. (2006). The effect of date fibre supplemented with an exogenous enzyme on the performance and meat quality of broiler chickens. *British Poult. Sci.* **47,** 73-82.

Torok V.A., Kathy O., Maylene L. and Robert J.H. (2008). Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism. *Appl. Environ. Microbio.* **74,** 783-791.

Tuohy K.M., Ziemer C.J., Klinder A., Knobel Y., Pool-Zobel B.L. and Gibson G.R. (2002). A human volunteer study to determine the prebiotic effects of lactulose powder on human colonic micro-biota. *Microbio. Ecol. Health Dis*. **14,** 165-173.

Vestergaard M., Therkildsen M., Henckel P., Jensen L.R., Andersen H.R. and Sejrsen K. (2000). Influence of feeding intensity, grazing and finishing feeding on meat and eating quality of young bulls and the relationship between muscle fibre characteristics, fibre fragmentation and meat tenderness. *Meat Sci.* **54,** 187-195.

Wang Z.R., Qiao S.Y., Lu W.Q. and Li D.F. (2005). Effects of enzyme supplementation on performance, nutrient digestibility, gastrointestinal morphology, and volatile fatty acid profiles in the hindgut of broilers fed wheat-based diets. *Poult. Sci*. **84,** 875-881.

Werner C., Janisch S., Kuembet U. and Wicke M. (2009). Comparative study of the quality of broiler and turkey meat. *British Poult. Sci*. **50,** 318-324.

Wilfarta A., Montagne L., Simmins H., Noblet J. and van Milgen H. (2007). Digesta transit in different segments of the gastrointestinal tract of pigs as affected by insoluble fibre supplied by wheat bran. *British J. Nutr*. **98(1),** 54-62.

Williams M.P., Klein J.T., Wyatt C.L., York T.W. and Lee J.T. (2014). Evaluation of xylanase in low-energy broiler diets. *J. Appl. Poult. Res*. **23,** 188-195.

Zdunczyk Z., Jankowski J., Juskiewicz J., Lecewicz J. and Slominski B. (2009). Application of soybean meal, soy protein concentrate and isolate differing in α-galactosides content to low- and high-fibre diets in growing turkeys. *J. Anim. Physiol. Anim. Nutr*. **85,** 1-10.

Zhang L., Xu J., Lei L., Jiang Y., Gao F. and Zhou G.H. (2014). Effects of xylanase supplementation on growth performance, nutrient digestibility and non-starch polysaccharide degradation in different sections of the gastrointestinal tract of broilers Fed wheat-based diets. *Asian-Australasian J. Anim. Sci.* **27,** 855-861.