Influence of Exogenous Enzymes and Probiotics on Haemato-Biochemical Indices and Antioxidant Capacity of Broiler Chickens fed Sorghum Based Diet

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Abstract

Sorghum is one of the most important cereal crops worldwide, it can be used for both human consumption and livestock feed. Sorghum contains some anti-nutritional factors such as tannin and phytic acid which inhibit the use of some nutrients like protein and energy. The inclusion of probiotics and exogenous enzymes can help to alleviate the presence of anti-nutritional factors in feedstuffs and improve growth performances. Therefore, this study was aimed at investigating the effects of probiotics and exogenous enzymes on selected indicators of blood biochemical parameters and antioxidant status of broiler birds fed sorghum-based diet. A total of 396 1-day-old unsexed broiler chicks were randomly assigned in a completely randomized design and allotted to six (6) experimental treatments (T) with three (3) replicates and each replicate having twenty-two (22) birds respectively. The treatment groups included: T1: maize based, T2: Sorghum based, T3: Sorg+ProAct+ENV, T4: Sorg+G2G+ENV, T5: Sorg+XAP+ENV, T6: Sorg+ENV. The results for the haematological indices showed significant (P<0.05) difference in values for packed cell volume and T1 and T2 had the lowest values. Serum total protein and globulins were also significantly (P<0.05) influenced by the dietary treatments. Total antioxidant capacity showed that GSH, SOD and MDA were not significantly (P>0.05) affected by the experimental diets in the chickens. In conclusion, the use of exogenous enzymes and probiotics in sorghum-based diet improved the parked cell volume and serum total protein of experimental broiler chickens while the dietary treatment had no effect on the total antioxidant capacity.

Keywords: Antioxidant Status, Exogenous enzymes, Immunity, Probiotics

Running title: Influence of exogenous enzymes and probiotics in broiler chickens fed sorghum-based diet

Influence Des Enzymes Exogènes Et Des Probiotiques Sur Les Indices Biochimiques Sanguins Et La Capacité Antioxydante Des Poulets De Chair Nourris Avec Un Régime A Base De Sorgho

Résumé

Le sorgho est l'une des céréales les plus importantes au monde. Il peut être utilisé pour la consommation humaine et l'alimentation du bétail. Cependant, le sorgho contient des facteurs antinutritionnels tels que les tanins et l'acide phytique qui limitent l'utilisation de certains nutriments comme les protéines et l'énergie. L'ajout de probiotiques et d'enzymes exogènes peut aider à atténuer la présence de ces facteurs antinutritionnels dans les aliments pour animaux et améliorer leurs performances de croissance. Par conséquent, cette étude visait à étudier les effets des probiotiques et

des enzymes exogènes sur certains indicateurs des paramètres biochimiques sanguins et sur le statut antioxydant des poulets de chair nourris avec un régime à base de sorgho.
Un total de 396 poussins d'un jour non sexés ont été répartis aléatoirement selon un dispositif entièrement randomisé et attribués à six (6) traitements expérimentaux (T) avec trois (3) répétitions par traitement et vingt-deux (22) oiseaux par répétition. Les groupes de traitement comprenaient : T1 : à base de maïs, T2 : à base de sorgho, T3 : Sorgho+ProAct+ENV, T4 : Sorgho+G2G+ENV, T5 : Sorgho+XAP+ENV, T6 : Sorgho+ENV. Les résultats des indices hématologiques ont montré une différence significative (P<0,05) pour l'hématocrite, les valeurs les plus faibles étant observées dans les groupes T1 et T2. La protéine sérique totale et les globulines ont également été significativement influencées (P<0,05) par les régimes alimentaires. La capacité antioxydante totale a montré que le GSH, la SOD et le MDA n'étaient pas significativement affectés (P>0,05) par les régimes expérimentaux chez les poulets. En conclusion, l'utilisation d'enzymes exogènes et de probiotiques dans un régime à base de sorgho a amélioré l'hématocrite et la protéine sérique totale des poulets de chair expérimentaux, tandis que le régime alimentaire n'a eu aucun effet sur la capacité antioxydante totale.

Mots-clés : Statut antioxydant, Enzymes exogènes, Immunité, Probiotiques

Introduction

Sorghum is a staple food widely grown and utilized around the world. It is cultivated for its versatile use by humans and animals. Sorghum is a rich source of vitamins, macro- and microelements, including phenolic acids, flavonoids and sterols (Frankowski *et al*., 2022). As livestock feed, sorghum plant produces grains that can be a nutritionally equivalent substitute for corn (Etuk *et al*., 2012) however, the use of sorghum-based diet as shown to be of detrimental effects in animal performance and health because of the presence of anti-nutritional factors such as phytate, kafirin and tannins, which limits the utilization of the grain as energy source, reduce palatability, and also substantially reduce digestibility of proteins once ingested which thus, reduces animal performance and poor digestibility of nutrients (Hariprasanna *et al*., 2015; Amy *et al*., 2020; Habibi *et al*., 2024). To address these problems, attention has been shifted to the use of natural growth promoters such as organic acids, prebiotics, feed enzymes and probiotics, which help to improve the digestion and absorption of nutrients, improve feed efficiency, increase growth performance and rapid development of a healthy gut microflora.

Feed additives are non-nutritive natural products added to animal diets to improve feed quality and improve animal performance and health (Ayalew *et al*., 2022). Feed additives that are frequently used are prebiotics, organic acids, phytobiotics, mannanoligosaccharides, probiotics and enzymes (Lagua *et al*., 2021).

Enzymes are proteins that facilitate specific chemical reactions and work on specific substrates. Exogenous enzymes that are widely used in broiler feed production include pectinases, amylases, cellulase, xylanases, associated enzyme phytases, proteases, and lipases as they help to improve health, nutrient utilization, feed efficiency, and decreased nutrients excreted (Alqhtani *et al*., 2022). The use of enzymes for broilers has proven to be beneficial nutritionally, economically, and environmentally (Doskovic *et al*., 2013). The use of enzymes singly or using a multi-enzyme composed of xylanase, protease and amylase in chicken diets, resulted in high feed utilization efficiency, reduction of digesta viscosity, enhanced digestion and absorption of nutrients, and increased feed intake and

weight gain (O'Neil *et* al., 2017; Oladipo *et al*., 2023).

Probiotics are live micro-organisms, which when supplemented in sufficient quantities, grant health benefits to their host (FAO/WHO, 2001). Probiotics feed supplementation have shown to improve growth, feed efficiency, and intestinal health (Abdel-Moneim *et al*., 2020). Probiotics has shown to improve the gastrointestinal microecological environment; and enhance the internal immunity as well as antioxidant capacity, thereby inhibiting the adhesion of pathogenic bacteria in broiler chickens (Inatomi and Otomaru, 2018).

Previous studies showed that dietary combination of probiotics and exogenous enzymes hold several promises in improving growth performance, intestinal morphology, energy and protein utilisation, and gut microflora in broiler chickens fed sorghum-based diet (Oladipo *et al*., 2019; Daramola *et al*., 2020). The inclusion of sorghum up to 100% replacement level for maize with exogenous enzymes supplementation improved the performance of chickens without adverse effect (Gidado *et al*., 2020). Similarly, Morgan *et al*. (2022) stated that feed efficiency in birds fed sorghum-based diets improved as a consequence of supplementing with fermentable fibre , xylanase and xylo-oligosaccharides.

The possible benefits of supplementation of exogenous enzymes to feed are improving nutrient utilization and animal performance but exogenous enzymes have a limit to which it can enhance digestive performance base on the pH and retention time within the gastrointestinal tract meanwhile probiotics supplementation can cause high feed digestibility through secretion of digestive enzymes in the gastrointestinal tract. Herein, we hypothesized that the mixture of exogenous enzymes and probiotics may improve feed utilization and cause an improvement in blood parameters and antioxidant system. This experiment was conducted to compare the effect of probiotic and exogenous enzymes on antioxidant system and some blood parameters of broiler chickens fed sorghum-based diet.

Materials and methods

Experimental site

The experiment was conducted at the Poultry Research Unit, National Veterinary Research Institute, Vom, Plateau State, Nigeria. The study area is located on Latitude 09° 44' N and Longitude 08°45' E with a physical feature of rocky granite of old volcanoes at altitude of 4200ft (1285m) above sea level with a mean annual rainfall ranging between 1300 to 1500mm and average daily temperature ranges between 17ºC to 28.6ºC. The wet season extends from late April to middle October and relative humidity which ranges from 22% in January to 78% in July/August. Mean monthly sunshine hours ranges from 177 - 288.3.

Test Materials, Sources and Constituents

The test materials used in the current study were Ronozyme ProAct® and Roxazyme G2G®, which contains serine-protease and endo-1,4 β-glucanase, respectively (DSM Nutritional Products, Stanley, USA). The multienzyme used was AxtraXAP 101 GT® containing a blend of xylanase, amylase and Protease (Danisco Animal Nutrition, DuPont, Malborough, UK). EnvivaPro 202 TPT® served as the probiotic which contains Bacillus amyloliquifaciens® (Danisco Animal Nutrition, DuPont, Malborough, UK).

Experimental Design and Management of Birds

Three hundred and ninety-six (396) 1-day-old unsexed Ross broiler chicks were weighed, and randomly allotted to six dietary treatments with three replicates and each having twenty-two birds in a completely randomized design (CRD). Treatment groups were fed with

maize-based diet, sorghum based diet, Sorghum+ProAct+ENV Sorghum+G2G+ENV, Sorghum+XAP+ENV and Sorghum+ENV.

Table 1: Composition of finisher broiler chicken diets containing exogenous enzymes and probiotics in combination

Feed Ingredients (Kg)	T1 Maize	T2 Sorg	T3 Sorg + ProAct + ENV	T4 Sorg + G2G + ENV	T5 Sorg + XAP+ ENV	T6 Sorg+ ENV
Maize	59.00	0.00	0.00	0.00	0.00	0.00
Sorghum	0.00	59.00	59.00	59.00	59.00	59.00
Soya bean meal	17.00	17.00	17.00	17.00	17.00	17.00
Groundnut cake	13.00	11.00	11.00	11.00	11.00	11.00
Maize offal	7.00	9.00	9.00	9.00	9.00	9.00
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Limestone	0.70	0.70	0.70	0.70	0.70	0.70
Common salt	0.30	0.30	0.30	0.30	0.30	0.30
Vit/Mineral Premix*	0.25	0.25	0.25	0.25	0.25	0.25
DL-Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.05	0.05	0.05	0.05	0.05	0.05
Ronozyme® ProAct^{+}	0.00	0.00	0.02	0.00	0.00	0.00
Roxazyme® G2G^{+}	0.00	0.00	0.00	0.02	0.00	0.00
Axtra® XAP 101 TPT^{+}	0.00	0.00	0.00	0.00	0.01	0.00
Enviva® PRO 202 GT^{+}	0.00	0.00	0.006	0.006	0.006	0.006
TOTAL	100.00	100.00	100.00	100.00	100.00	100.00
Calculated analysis						
ME (Kcal/Kg DM)	2950	2900	2900	2900	2900	2900
Crude protein (%)	20.00	20.17	20.17	20.17	20.17	20.17
Crude fibre (%)	4.27	4.19	4.19	4.19	4.19	4.19
Ether extract (%)	3.36	2.15	2.15	2.15	2.15	2.15
Calcium (%)	1.21	1.23	1.23	1.23	1.23	1.23
Phosphorus (%)	0.77	0.81	0.81	0.81	0.81	0.81
Lysine (%)	1.12	1.16	1.16	1.16	1.16	1.16
Methionine (%)	0.48	0.49	0.49	0.49	0.49	0.49
Cost/kg diet (₦/kg)	107.17	106.47	113.41	112.58	112.58	110.91

*Nutripoult broiler premix each 2.5kg cotains: Vit A, 10,000,000 IU; Vit D3, 2,000,000 IU; Vit E, 40,000mg; Vit K3, 2000mg; Vit B1, 1500 mg; Vit B2, 5000mg; Vitamin B6 4000 mg; Vit B12, 20mg; Niacin, 40,000mg; Calpan, 10,000 mg; Folic acid, 10,000mg; Biotin, 100mg; Chlorine chloride, 30,000mg; Iodine, 800mg; iron, 40,000mg; Manganese, 80,000mg; Cobalt, 300; Copper, 80,000; Selenium, 200mg; Zinc, 60,000mg; Antioxidant, 100,000mg**;** Sorg: sorghum**,** ProAct: Proact enzyme**,** G2G: G2G enzyme**,** XAP: Axtra® XAP 101 TPT enzyme**,** ENV: Enviva® PRO 202 GT. $^{+}$Feed additives exclusive of 100kg diet.

The experimental diets were formulated to meet the nutritional requirements of broilers according to the NRC, 1994. All birds were housed in deep litter pens and managed with all necessary routine management practices with feed and water provided ad libitum.

Data Collection

Haematological and Serum Biochemical Indices

At the end of the 42nd day, 4 mL of blood was collected from six birds per treatment via the jugular vein section and placed in ethylene-diamine tetra-acetic acid (EDTA) test tubes for haematological analyses (0.5 mL) and in plain test tubes without an anticoagulant (2.5 mL). The plain tubes were centrifuged at 1500 x g at 4°C for 10 min to obtain sera for the analyses of proteins, lipid profiles and oxidative stress parameters.

Full Blood Count

The packed cell volume (PCV), haemoglobin concentration (Hb), total leucocyte counts (WBC), neutrophils and lymphocytes were evaluated using a Vet haemoanalyzer (Mindray BC 300, Mindray Corp., China), according to the manufacturer's instructions. The neutrophil/lymphocyte ratio was calculated as described by Minka and Ayo (2011).

Determination of Serum Proteins

The serum total protein and albumin were determined using a commercial assay kit (Agape Diagnostics, Switzerland GmbH) according to the manufacturer's specification.

Assessment of Serum Lipid Profiles

Total cholesterol [TC], triglycerides [TG] and high-density lipoprotein cholesterol [HDLC] were determined using commercial assay kits (Agape Diagnostics, Switzerland GmbH) according to the manufacturer's specification. The low-density lipoprotein cholesterol (LDLC) and very low-density lipoprotein cholesterol (VLDLc) were calculated using the Friedwald formula (Friedwald *et al*., 1972): "LDLc =TC-HDLc – (0.20 x TG)," "VLDLc = TG/5". The atherogenic index (AI) was calculated according to the equation described by Lee and Nieman (1996): "AI =TC-HDLc/HDLc".

Assessment of Oxidative Stress Parameters

Oxidative stress parameters such as malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH) were analysed according to method modified by Gotep *et al*. (2016).

Statistical analysis

Data obtained in the experiment were statistically analysed using the General Linear Model Procedure of Statistical Analysis software package. Significant difference between treatments means were separated using Tukey Test (SAS, 2002).

Results and Discussion

Haematological parameters of broiler chickens fed sorghum-based diets with enzymes and probiotics in combination

The effect of enzymes and probiotics in combination on haematological parameters of broiler chickens is shown on Table 2. The result showed significant ($P<0.05$) difference in the packed cell volume with T1 (maize based diet) having the least value and also below the reference range (Jain, 1993) followed by the sorghum-based diet without enzyme and probiotics in combination but within the reference range. The observed result for Hb showed birds fed sorghum-based diet alone (T2) had the least value and was also below the reference range reported by Jain (1993). Rastogi (2007) stated that PCV and Hb concentration are generally affected by inadequate intake of energy and protein, with lower values indicating anaemia. Therefore, the addition of enzyme and probiotics in combination improved nutrient availability for the birds. The values obtained for red blood cell count for chickens in T1 and T2 groups

were within the range of (2 -3 $\times 10^6$/L) for healthy chickens, while T3, T4, T5 and T6 where a little below the normal range. Rastogi (2007) reported that low erythrocyte count implies low oxygen transport while high erythrocyte count (polycythemia) indicates that the blood is thick and this can impair the ability of the blood to carry oxygen.

The results for heterophils, lymphocytes and monocytes showed no significant (P>0.05) difference across treatment diets. The results for heterophils and monocytes were below the reference range recorded by Jain (1993) which probably was as a result of environmental factors. Lymphocytes, mean corpuscular haemoglobin, mean corpuscular hemoglobin concentration, and mean corpuscular volume were all above the normal range and this is in line with the findings of Jin *et al*. (1997) who reported that probiotics increased the haematological profile of poultry either due to its direct effects on haemopoetic organs or the indirect effects on the intestinal microflora.

The interaction effect of exogenous enzymes and probiotics showed significant (P< 0.05) influence on PCV and WBC which implies that both feed additives can help build up white blood cells

Table 2: Haematological parameters of broiler chickens fed sorghum-based diets with enzymes and probiotics in combination

Parameters	T1 Maize	T2 Sorg	T3 Sorg + ProAct + ENV	T4 Sorg + G2G + ENV	T5 Sorg+ XAP + ENV	T6 Sorg + ENV	SEM	Ref. Range
PCV (%)	21.00^{c}	23.00^{b}	25.66^{a}	26.33^{a}	23.66^{b}	25.33^{a}	1.38	22.00 -35.00†
Hb (g/dL)	9.00	6.66	7.96	7.83	8.53	7.13	1.57	7.00 -13.00†
RBC ($\times 10^6$/L)	2.33	2.03	1.58	1.90	1.73	1.70	0.21	2.00-3.00††
WBC ($\times 10^9$/L)	9.53^{b}	7.83^{c}	10.20^{a}	10.13^{a}	10.37^{a}	8.17^{c}	0.58	1.90 – 9.50†
Heterophils (%)	12.00	10.33	14.00	14.66	11.00	15.66	2.43	15.00-40.00†
Lymphocytes (%)	87.66	89.33	85.66	84.66	86.66	82.00	2.76	45.00-70.00†
Monocytes (%)	0.33	0.00	0.33	0.33	0.33	0.00	0.27	1.00-7.00††
MCH (*ρg*)	67.67^{c}	64.66^{c}	142.14^{a}	75.89^{b}	116.84^{b}	101.86^{b}	1.62	27.00-41.00†
MCV (*fl*)	157.89^{c}	223.30^{c}	458.21^{a}	292.56^{b}	324.11^{b}	361.86^{b}	61.59	81.00-122.00†
MCHC (%)	42.86^{a}	28.96^{d}	31.02^{c}	25.94^{e}	36.05^{b}	28.15^{d}	0.60	27.20-33.80†

a,b,c,d,e: Means with different superscripts on the same row are significantly different (P<0.05), SEM: Standard error of means, ProAct: Ronozyme® ProAct enzyme, G2G: Roxazyme® G2G enzyme, XAP: Axtra® XAP 101 TPT enzyme, ENV: Enviva® PRO 202 GT, PCV: Packed cell volume, Hb: Haemoglobin, RBC: Red blood cell, WBC: White blood cell, MCHC: Mean Corpuscular Haemoglobin Concentration, MCH: Mean Corpuscular, Haemoglobin, MCV: Mean Corpuscular Volume, Reference range: Simraks *et al* (2004)†, Jain (1993)††

Serum proteins of broiler chickens fed sorghum-based diets with enzymes and probiotics in combination

The result of serum proteins presented in Table 3, showed that serum total protein and globulin were significantly (P<0.05) influenced by the dietary treatments, while, albumin and albumin/globulin ratio did not differ significantly (P>0.05).

The result for serum total protein followed similar trend with serum globulin in broiler chickens in Treatment 5 (sorghum-based diet in combination with multi enzyme and probiotics) having significantly higher (P< 0.05) value of total protein (45.80 g/dL) and globulin (33.44 g/dL). The improved total protein and globulin as observed in this study suggested that the diet is capable of supplying the proteins needed by the birds. The enhanced values for total protein and globulin as shown in this result could be attributed to efficacy of the multi enzyme to enhance feed utilization by causing degradation of fibre and non-starch polysaccharide (NSP) components of

sorghum- based diets into soluble metabolizable energy (Choct, 2006).

The above result is in line with the findings of Abudabo (2012) who stated that enzyme supplementation improved serum total protein significantly suggesting that the efficiency of dietary protein increased in broiler chickens fed diets with protease enzyme. In the same vein, Haghighi *et al*. (2005) demonstrated that administration of probiotics enhances serum and intestinal natural antibodies to several foreign antigens in chickens. This could be because the microbes present in probiotics would secrete amylase, protease, and lipase, which would enhance the catalytic activities of the endogenous enzymes to liberate more energy from hydrolysing the energy sources in the feed ingredients.

Table 3: Serum proteins of broiler chickens fed sorghum-based diets with enzymes and probiotics in combination

Parameters	T1 Maize	T2 Sorg	T3 Sorg+ ProAct+ ENV	T4 Sorg+ G2G+ ENV	T5 Sorg+ XAP+ ENV	T6 Sorg+ ENV	SEM	Ref. range
Total protein (g/dL)	30.41^{b}	33.39^{b}	38.89^{b}	35.25^{b}	45.80^{a}	36.76^{b}	4.08	30.00- 49.00
Albumin (g/dL)	8.58	9.56	8.27	10.07	12.36	8.43	2.50	10.00-16.00
Globulin (g/dL)	21.83^{b}	23.82^{b}	30.62^{a}	25.17^{b}	33.44^{a}	28.33^{b}	4.57	19.00-29.00
Albumin/Globulin ratio	0.40	0.41	0.27	0.40	0.38	0.31	0.11	0.35-0.75

a,b,c : Means with different superscripts on the same row are significantly different (P<0.05), SEM: Standard error of means, Sorg: Sorghum, ProAct: ProAct enzyme, G2G: G2G enzyme, XAP: Axtra XAP 101 TPT enzyme, ENV: Enviva PRO 202 GT, Ref: Reference

However, Yuan *et al*. (2008) found that comparing with the control group, the addition of enzyme complex to broiler diet had no significant effects on serum protein parameters while the contributory factors causing these differences reported in other studies could be attributed to the differences in the inclusion levels of enzymes and probiotics, sources of feed additives, or the microbial environment in which the birds were reared.

The results for albumin and Albumin/Globulin ratio showed no significant (P>0.05) improvement which is in accordance with the findings of Chen *et al*. (2005) who reported that probiotic supplementation did not have any effect on albumin and haematological parameters.

Lipid profile of broiler chickens fed sorghum- based diets with enzymes and probiotics in combination

The effect of probiotics and enzyme combination in sorghum- based diets on lipid profile of broiler chickens is presented in Table 4. The result for Cholesterol showed no significant (P>0.05) effect of diet. The birds fed sorghum- based diets with and without enzyme and probiotics in combination (T2, T3, T4, T5 and T6) showed a decrease in the value of cholesterol compared to the control (T1). This result is in line with report of Iqramu *et al*. (2017) who stated that serum total cholesterol and triglycerides were reduced significantly by dietary supplementation of probiotic containing *L. sprongene* at 100 mg per kg diet. This significant reduction can be attributed to reduced absorption or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation (Mohan *et al*., 1995) and high bile salt in *Lactobacillus* with hydrolytic activity, which is responsible for deconjugation of bile salts (Surono,2003).

Table 4: Lipid profile of broiler chickens fed sorghum-based diets with enzymes and probiotics in combination

Parameters	T1 Maize	T2 Sorg	T3 Sorg+ ProAct+ ENV	T4 Sorg+ G2G+ ENV	T5 Sorg+ XAP+ ENV	T6 Sorg+ ENV	SEM
Cholesterol (mg/dL)	158.58	142.96	154.63	146.46	135.43	145.00	20.42
Triglycerides (mg/dL)	29.16[b]	28.94[b]	25.44[b]	58.17[a]	23.85[b]	15.92[b]	18.91
HDL (mg/dL)	82.20	89.07	84.72	88.32	85.96	85.57	14.25
LDL (mg/dL)	70.55	48.10	64.81	46.51	44.69	56.24	21.41
VLDL (mg/dL)	5.83[b]	5.79[b]	5.09[b]	11.63[a]	4.77[b]	3.18[b]	3.78
Atherogenic index	157.58	141.96	153.63	145.46	134.43	144.00	20.42
Tg/HDL ratio	0.33[b]	0.33[b]	0.30[b]	0.64[a]	0.29[b]	0.17[b]	0.18

a,b,c : Means with different superscripts on the same row are significantly different (P<0.05) SEM: Standard error of means, Sorg: Sorghum, ProAct: Ronozyme® ProAct enzyme, G2G: Roxazyme® G2G enzyme, XAP: Axtra® XAP 101 TPT enzyme, ENV: Enviva® PRO 202 GT, HDL: High density lipoprotein, LDL: Low Density Lipoprotein, VLDL: Very low density lipoprotein, Tg/HDL: Triglycerides/ High density lipoprotein ratio

The mean triglycerides levels were significantly (p<0.05) highest in broilers in the T4 group when compared with all other treatments. This result is in line with the observation of Abeer and Mosaad (2015) who reported significant decrease in serum triglycerides level in broiler chickens on probiotic and or prebiotic supplementation. The increase in value of triglycerides in birds in T4 dietary group may be as a result of poor absorption of fat in the gastrointestinal tract (GIT). probiotics in combination (T2, T3, T4, T5 and T6) showed a decrease in the value of cholesterol compared to the control (T1). This result is in line with report of Iqramu *et al*. (2017) who stated that serum total cholesterol and triglycerides were reduced significantly by dietary supplementation of probiotic containing *L. sprongene* at 100 mg per kg diet. This significant reduction can be attributed to reduced absorption or synthesis of cholesterol in the gastro-intestinal tract by probiotic supplementation (Mohan *et al*., 1995).

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There was no significant (p>0.05) difference in the HDL and LDL levels of birds fed sorghum based diets with and without probiotics and enzyme (T2,T3,T4,T5 and T6) among the treatment groups compared with the control (T1). However, chickens fed sorghum-based diets had increased level of HDL and a reduced level of LDL. Supplementation of probiotics in broiler chicken diet changed the lipoprotein metabolism of birds favourably with more marked decrease in LDL cholesterol and increased HDL cholesterol concentration. This agrees with the reports of Kalavathy *et al*. (2010) who stated that probiotic supplementation decreased serum LDL level but had no significant effect on serum HDL level of broiler chickens. The use of sorghum

also increased the HDL level in broiler chickens and reduced the LDL level which means that sorghum do not have any adverse on the lipid profile of birds

Total antioxidant capacity of broiler chickens fed sorghum- based diets with and without enzymes and probiotics in combination

The effect of probiotics and enzymes in combination in sorghum-based diet on antioxidative status of broiler chickens is shown in Table 5. The result of all parameters measured (GSH, SOD and MDA) showed no significant (P> 0.05) effect of diets. The serum GSH concentration was numerically higher in broilers fed diet supplemented with multi enzymes and probiotics (T5). The result indicated the synergetic effect of multi-enzyme and probiotics on the health status of birds. This shows that the combination of the feed additive could improve the antioxidant activities and reduce the damage caused by free radicals in the birds. Similarly, the activity of SOD also showed the synergetic effect of multi enzyme and probiotics (T5). Previous studies have shown that inclusion of probiotics in the diets of chickens tremendously increased the activity of SOD by reducing oxidative stress (Anwar *et al*., 2012; Ogbuagu *et al*., 2018). The slight increase in serum SOD activity is confirmed by improvements in the growth performance of birds in the current study. Experimental birds fed with sorghum-based diet with multi enzyme and probiotics (T5) performed better in final weight and weight gain compared to birds fed sorghum without feed additives (T2) and birds fed sorghum and probiotics alone (T6). These results, showed that the combination of probiotics and enzyme can inhibit excess of oxidative free radicals responsible for cell damage and thereby improve the growth performance of broilers. Numerically, the result for MDA values showed that chickens on treatment diet 4 (sorghum+ G2G+ ENV) had adecrease in MDA concentration. Although, the levels of GSH and SOD were low in T4, that of MDA was lower. Furthermore, the use of both Bacillus amyloliquefaciens, as probiotics,
and as external mixture of digestive enzymes serine-protease, endo-1,4 β-glucanase and a blend of xylanase, amylase, and protease is likely show pronounced cumulative beneficial effect on broiler's performance by reducing the negative impact of heat stress on chickens and reducing lipid peroxidation

Table 5: Total antioxidant capacity of broiler chickens fed sorghum-based diets with enzymes and probiotics in combination

Parameters	T1 Maize	T2 Sorg	T3 Sorg + ProAct + ENV	T4 Sorg + G2G + ENV	T5 Sorg + XAP + ENV	T6 Sorg +ENV	SEM
GSH (µg/mL)	7.40	7.60	6.33	0.73	8.33	3.60	3.27
SOD (min/mg)	2.19	2.35	0.89	1.34	2.96	2.59	1.38
MDA (µmol/L/mg)	269.98	294.51	331.93	116.56	177.67	321.79	190.20

SEM: Standard error of means ProAct: Ronozyme® ProAct enzyme, G2G: Roxazyme® G2G enzyme, XAP: Axtra® XAP 101 TPT enzyme, ENV: Enviva® PRO 202 GT, PER: Protein efficiency ratio, GSH: Glutathione SOD: Superoxide dismutase, MAD: Malondialdehyde

Conclusion

Based on the results of present study, dietary addition of probiotics and exogenous enzymes in sorghum-based diet had no detrimental effect on the haematology and serum biochemical profile of the experimental broiler chickens and also did not influence antioxidant status of the birds.

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