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EFFECT OF ENZYME INCLUSION ON GROWTH PERFORMANCE HAEMATOLOGICAL AND SERUM BIOCHEMICAL RESPONSES OF FINISHER BROILER CHICKENS FED SORGHUM BASED DIETS.

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ABSTRACT

One hundred and five day – old broiler chickens of mixed sexes were used to investigate the effect of different levels of sorghum as a replacement for maize on growth performance, haematological and serum biochemical indices. Five diets were formulated to contain 0% (Control), 25%, 50%, 75% and 100% replacement levels of Sorghum in a completely randomized design. The experiment lasted for 56 days. Results showed that birds on 25% and 50% replacement levels of Sorghum had average daily weight gain, feed intake and feed conversion ratio that were comparable (P>0.05) to those on the control diet. Packed cell volume, red blood cell, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin were significantly ($p < 0.05$) influenced by varying inclusion level of sorghum as a replacement for maize. Birds fed diet containing 50% level of sorghum replacement (D3) with enzyme inclusion recorded higher ($p < 0.05$) statistical values of 44.30g/l, 15.25g/l, 29.05g/l and 4.60mmol/l for total protein, albumin, globulin and cholesterol respectively. Birds fed diet containing 100% sorghum (D5) with enzyme recorded least ($p < 0.05$) value of 31.85g/l for total protein compared to 44.30 g/l obtained in the birds fed D3. It was concluded that sorghum with enzyme inclusion as a replacement for maize could be efficiently utilized and tolerated by broiler chickens at 25% and 50% inclusion levels for improved performance without any deleterious effect. However, further investigation into other methods that can efficiently aid bio-availability of nutrients from higher inclusion levels of sorghum as a replacement for maize needs to be carried out.

Key words: Sorghum, phytase, haematology, serum biochemistry, broiler.

INTRODUCTION

Broiler production is the quickest means of achieving high-quality animal protein in the shortest possible time. In raising poultry, feed expenditure accounts for about 60-70% of the total cost (Nworgu *et al*., 1999). High feed cost is the major constraint for further progress of the poultry industry (Esonu *et al*., 2006; Sharif *et al*., 2012). Cereal grains account for about 50-60% of a typical broiler diet where this feed ingredients serve as a principal energy source for poultry. Maize has remained the chief energy source in compounded diets and constitutes about 50% of poultry diet (Ajaja *et al*., 2002). In poultry nutrition, energy is used for the provision of body heat, maintenance, growth and production (Inaku *et al*., 2011). However, dependence on corn grain becomes a problem in feed formulations due to its unavailability, expensive or poor quality and competition between human beings and poultry. This has

compelled the nutritionists to explore new and non-conventional feedstuffs.

In regard to the nutritive value, cost and availability, sorghum can compete favourably as the next alternative to maize in poultry feed (Hancock, 2000). However, presence of high level of tannins, variable amounts of phytate and possibly polyphenols in the sorghum grain may act as anti-nutritional factors (Doka *et al*., 2004; Selle *et al*., 2010). These factors can negatively influence the nutritive properties of sorghum and therefore cause negative effects on feed intake, palatability, digestibility of nutrients and growth performance (Hassan *et al*., 2003; Kim and Miller, 2005). According to literature, researchers used sorghum in chicken diets (Robertson and Perez-Maldonado, 2006; Campos *et al*., 2007; Medugu *et al*., 2010) but revealed contradictory results. One of the methods of tackling problem of anti-nutritional factors is the use of enzymes as feed additives to improve the growth, nutrient digestibility, improvement of feed efficiency and as a result to increase economic indicators (Selle *et al*., 2010; Ibrahim *et al*., 2012).

Phytase enzymes improve the digestibility and availability of proteins, optimizing diet costs by allowing lower quality protein feed ingredients to be used in the diet. This helps reduction in feed costs, as grain prices continue to fluctuate. Researchers have also been digging into the additional benefits of the protease enzyme and its link to gut health and bacteria balance in poultry. Phytase enzymes are important factors in protein digestion as they hydrolyse the less digestible proteins in animal feeds and break them down into more usable peptides (Cadogan *et al.,* 2005). Therefore this study was conducted to evaluate the effect of protease enzyme on growth performance haematological and serum biochemical responses of finisher broiler chickens fed sorghum based diets.

MATERIALS AND METHODS

Design and Management of the birds

A total of one hundred and five unsexed day old broiler chicks after one week of acclimatization were randomly allotted to five treatment groups of twenty one birds each. The birds were replicated three times with 7 birds per replicate in a complete randomized designed (CRD). Birds were raised on deep litter system for the period of the experiment. Feed and water were provided and routine medication and management were duly followed.

Experimental Diets

Five isonitrogenous diet were formulated for broiler chickens during the period of study. Diet 1 (control diet) contained 100% of maize (i.e 0% sorghum). In the other remaining four diets, 25%, 50%, 75% and 100% of maize in the control diet was quantitatively replaced with sorghum as diet 2, 3, 4 and 5 respectively. Diet 2, 3, 4 and 5 were supplemented with 0.05kg phytase enzyme as presented in Table 1 and 2.

Table 1: Percentage composition of experimental diets for broiler chicks (0 – 4 weeks)

Ingredients	**0%**	**25%**	**50%**	**75%**	**100%**
Maize	52.00	39.00	26.00	13.00	0.00
Sorghum	0.00	13.00	26.00	39.00	53.00
Soya bean meal	19.00	19.00	21.30	19.50	23.40
Groundnut cake	16.70	17.70	14.00	15.40	11.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Rice offal	4.95	4.95	5.35	5.75	6.25
Bone meal	2.00	2.00	2.00	2.00	2.00
Limestone	1.50	1.50	1.50	1.50	1.50
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20
Salt	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25
Phytase enzyme	0.00	0.05	0.05	0.05	0.05
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Metabolizable Energy (kcal/kg)	2862.70	2835.40	2801.63	2769.90	2734.00
Crude protein (%)	23.54	23.78	23.85	23.96	23.97
Fibre (%)	3.03	3.02	3.01	3.01	2.99
Fat (%)	4.11	4.10	3.90	3.82	3.57
Calcium (%)	1.19	1.19	1.20	1.30	1.21
Phosphorus (%)	0.47	0.49	0.53	0.56	0.59
Lysine (%)	1.30	1.26	1.30	1.28	1.33
Methionine (%)	0.55	0.54	0.53	0.51	0.50

Table 2: Percentage composition of experimental diets for finisher broiler chickens

Ingredients	**0%**	**25%**	**50%**	**75%**	**100%**
Maize	58.00	43.50	29.00	14.50	0.00
Sorghum	0.00	14.50	29.00	43.50	58.00
Palm oil	2.00	2.50	3.00	3.00	3.00
Soya bean meal	12.00	12.00	12.00	12.00	13.90
Groundnut cake	15.70	15.20	14.70	14.20	11.80
Fish meal	2.00	2.00	2.00	2.00	2.00
Rice offal	5.95	5.95	5.95	6.45	6.45
Bone meal	2.00	2.00	2.00	2.00	2.00
Limestone	1.50	150	1.50	1.00	1.50
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.20	0.20	0.20	0.20	0.20
Salt	0.25	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25	0.25
Phytase enzyme	0.00	0.05	0.05	0.05	0.05
Total	**100.00**	**100.00**	**100.00**	**100.00**	**100.00**
Calculated analysis					
Metabolizable Energy (kcal/kg)	2997.19	2991.38	29.86.89	2949.79	2911.73
Crude protein (%)	20.00	20.00	20.05	20.13	20.19
Fibre (%)	2.76	2.74	2.71	2.70	2.70
Fat (%)	4.16	3.98	3.81	3.66	3.46
Calcium (%)	1.16	1.16	1.16	1.17	1.17
Phosphorus (%)	0.44	0.47	0.50	0.54	0.57
Lysine (%)	0.98	0.99	0.99	1.00	1.17
Methionine (%)	0.49	0.48	0.46	0.45	0.44

DATA COLLECTION

Growth Performance

The chicks were weighed at the commencement of the study and subsequently on weekly basis for eight weeks. The feed intake was calculated on daily basis as feed offered minus feed leftover in the feeding trough.

$$Feed\ intake\ /bird\ (g) = \frac{amount\ of\ feed\ offered\ (g) - amount\ of\ feed\ leftover\ (g)}{number\ of\ birds}$$

Collection of blood sample

At the end of the feeding trial, 4ml of blood was collected from one bird per replicate that is, three from each treatment. The blood was collected through the wing vein with a syringe. 2ml of blood sample was transferred into a bottle containing Ethylene Diamine Tetra Acetic Acid (EDTA) for haematological analyses. The parameters determined include Packed Cell Volume (PCV), White Blood Cell (WBC), Haemoglobin (Hb), Red Blood Cell (RBC), Mean Cell Haemoglobin Concentration (MCHC), Mean Cell Haemoglobin (MCH) and Mean Cell Volume (MCV)

Another two meals of blood sample collected was transferred into a plain bottle for serum analysis. The serum metabolite such as Serum Total Protein, Albumin, Globulin, Cholesterol, Creatinine, and Uric Acid were determined.

Statistical Analysis

Data collected were subjected to analysis of variance using the SPSS software (2012). Where analysis of variance indicated significant treatment effects, means were compared using Duncan's New Multiple Range Test (DNMRT) (SPSS, 2012).

RESULTS AND DISCUSSION

The parameters measured to determine the growth response of broiler chickens is presented in Table 3. With the exception of initial weight, all parameters measured were significantly ($P<0.05$) influenced by dietary treatments. The results did not show definite pattern with birds fed diet containing 25% and 50% replacement levels of maize by sorghum recorded the highest and birds on diet containing 100% replacement level of maize by sorghum being the least across parameters measured. The variation observed with 100% sorghum replacement level recording the least values despite enzyme inclusion could be attributed to high fibre content coupled with anti-nutritional factors.

Statistically, similar values were recorded for birds fed 25% and 50% sorghum with enzyme inclusion. Numerically, values obtained for birds on 25% sorghum with enzyme inclusion were higher across the parameters measured. This observation is in contrary to the result obtained by Torneker *et al.* (2009) who reported highest statistical values for final weight, weight gained and daily feed intake in the birds fed diet containing 50% replacement level of maize by sorghum compared to 0% and 25% replacement levels. Improved performance with exogenous enzyme inclusion at was reported by Oladipo *et al.* (2015) when broiler chickens were fed diets containing 55.80 % sorghum as a replacement for maize with protease and phytase inclusion at 0.2 kg/tonne.

Improved final weight with enzyme inclusion as observed in this study could be attributed to ability of exogenous enzyme to hydrolyse non – starch polysaccharides and consequently reduced

the negative effect of polysaccharide by making the nutrients available for birds.

The improved value for weight gain as observed in this study could be associated with efficient feed utilization. Enzyme improved the overall feed utilization by causing degradation of fibre and non – starch polysaccharide (NSP) component of sorghum based diet into soluble metabolizable energy (Choct, 2006). Sekoni *et al.* (2008) reported an increase in crude fibre digestibility and stated that enzyme acted on cellulose, glucoxylans and arabinoxylans thereby increased energy content. Observed daily feed intake and feed conversion ratio obtained in this study significantly (P<0.05) improved. This improvement in feed intake could be attributed to the ability of enzyme to reduce the viscosity of gut contents thereby reduced transit period and consequently improve feed intake (Ani and Nnamani, 2011).

Feed conversion ratio (FCR) improved with birds across dietary treatments showed range values of 2.20 – 2.69. These values are close to the normal range of 2.98 – 5.53 for broiler chickens as reported by Isikwenu *et al.* (2010). Improved FCR could be attributed to fat digestibility and consequently bioavailability of fat soluble vitamins which invariably enhanced energy metabolism (Sekoni *et al.* 2008).

Table 3: Growth response of broiler chickens fed maize-sorghum based diets with enzyme inclusion

Parameter	0% Sorghum (control) D1	25% Sorghum + 0.05 Phytase D2	50% Sorghum + 0.05 Phytase D3	75% Sorghum + 0.05 Phytase D4	100% Sorghum + 0.05 Phytase D5	SEM
Initial weight (g)	145.18	144.54	144.81	145.55	144.95	1.14
Final weight (g)	1900.20^{ab}	2022.00^{a}	1966.70^{a}	1910.90^{ab}	1627.85^{b}	50.77
Weight gain (g/bird)	1755.00^{ab}	1877.50^{a}	1821.80^{a}	1765.30^{ab}	1482.90^{b}	50.79
Daily weight gain (g/bird)	31.34^{ab}	33.53^{a}	32.53^{a}	31.52^{ab}	26.48^{b}	0.91
Daily feed intake (g/bird)	72.01^{a}	73.65^{a}	72.88^{a}	72.69^{a}	70.98^{b}	0.31
FCR	2.34^{ab}	2.20^{b}	2.25^{b}	2.33^{ab}	2.69^{a}	0.06

^{ab}Mean values in the same row having different superscripts are significantly different (P<0.05)

The results of haematological parameters of broiler chickens fed maize-sorghum based diets with enzyme inclusion are presented in Table 4. Packed cell volume, red blood cell, mean corpuscular haemoglobin concentration and mean corpuscular haemoglobin were significantly ($p < 0.05$) influenced by varying inclusion level of sorghum as a replacement for maize. Birds fed 100% sorghum (D4) with enzyme inclusion recorded highest ($p < 0.05$) value of 23.00% for PCV compared to 21.00% obtained for birds fed 0% sorghum (control). Statistical similar values were recorded for birds fed diet containing 25%, 50% and 75% levels of sorghum replacement. PCV values ranging from 21.00% -23.00% in the current study were lower

than the 27.67% - 30.00% reported by Oladipo *et al*. (2015) when broiler chickens were fed sorghum based diet supplemented with phytase and protease enzyme. Similar pattern was observed for red blood cells with 100% sorghum showed higher ($p < 0.05$) statistical value of 2.60 $\times 10^{14}$/L and control diet being the least with 2.30 $\times 10^{14}$/L. Birds fed diets containing 25%, 50% and 75% sorghum with enzyme revealed similar statistical value of 2.45 $\times 10^{14}$/L, 2.40 $\times 10^{14}$/L and 2.40 $\times 10^{14}$/L respectively for red blood cells. Values ranging from 2.30 $\times 10^{14}$/L – 2.60 $\times 10^{14}$/L obtained for RBC were lower compared to 4.23 $\times 10^{14}$/L – 5.67 $\times 10^{14}$/L reported by Oladipo *et al.* (2015). Results obtained for MCHC showed higher ($p < 0.05$) value of 37.20 g/dl for birds on control diet compared to other dietary groups. Birds fed diet contain 75% and 100% replacement level of sorghum recorded close value of 35.35g/dl Vs 35.30g/dl for MCHC. Ranged value of 34.75g/dl – 37.20g/dl recorded is close 33.30g/dl – 33.60g/dl recorded for birds (Ugwuene, 2011).Significant range value of 31.15pg – 33.90pg were recorded for MCH across dietary levels. Birds fed control diet obtained higher ($p < 0.05$) value while birds fed diet containing 100% sorghum showed least value at comparative level. Varying levels of replacement of maize with sorghum had no significant ($p > 0.05$) influence on haemoglobin and MCV. Values ranging from 7.60g/dl – 8.10g/dl and 88.50fl – 92.20fl obtained for Hb and MCV respectively in this study suggested that birds were not affected with allergic effect and had enough oxygen carrying capacity for metabolic activities. The haemoglobin value obtained in this study were close to the range value of 9.17g/dl – 9.97g/dl reported by Oladipo *et al*. (2015) when broiler chickens were fed sorghum based diet supplemented with enzyme.

Table 4: Haematological parameters of finisher broiler chickens fed maize-sorghum based diets with enzyme inclusion

Parameter	0% Sorghum (control) D1	25% Sorghum + 0.05 Phytase D2	50% Sorghum + 0.05 Phytase D3	75% Sorghum + 0.05 Phytase D4	100% Sorghum + 0.05 Phytase D5	SEM
PCV (%)	21.00^{b}	22.50^{ab}	22.00^{ab}	21.50^{ab}	23.00^{a}	0.26
HB (g/dl)	7.80	8.10	7.65	7.60	8.10	0.08
RBC ($\times 10^{14}$/L)	2.30^{b}	2.45^{ab}	2.40^{ab}	2.40^{ab}	2.60^{a}	0.04
WBC ($\times 10^{9}$/L)	3.60	3.40	2.80	4.20	3.65	0.22
MCHC (g/dl)	37.20^{a}	36.00^{b}	34.75^{c}	35.35^{bc}	35.30^{bc}	0.24
MCH (pg)	33.90^{a}	33.20^{ab}	31.90^{bc}	31.70^{bc}	31.15^{c}	0.33
MCV (fl)	91.30	92.20	91.85	89.65	88.50	0.64

^{abc}Mean values in the same row having different superscripts are significantly different (P<0.05)
Hb = Haemoglobin, PCV = Packed Cell Volume, RBC = Red Blood Cell, WBC = White Blood Cell, MCV = Mean Cell Volume, MCHC = Mean Corpuscular Haemoglobin Concentration, MCH = Mean Corpuscular Haemoglobin

The results of serum biochemical indices of broiler chicken fed varying levels of sorghum with enzyme as a replacement for maize are presented in Table 5. Varying levels of replacement of maize by sorghum with enzyme had significant ($p < 0.05$) effect on total protein, albumin, globulin, cholesterol and uric acid. Birds fed diet containing 50% level of sorghum (D3) replacement with enzyme inclusion recorded higher ($p < 0.05$) statistical values of

44.30g/l, 15.25g/l, 29.05g/l and 4.60mmol/l for total protein, albumin, globulin and cholesterol respectively. Birds fed diet containing 100% sorghum (D5) with enzyme recorded least ($p < 0.05$) value of 31.85 for total protein. With the exception of birds on 50% sorghum level of replacement, statistical similar values were obtained across dietary groups for albumin. Birds fed 100% sorghum recorded least value of 20.45g/l for globulin. Significant improved total protein and globulin as observed in this study suggested adequacy of the diet in supplying of protein needed by the birds. Observed improved values for total protein and globulin as shown in this result could be attributed to efficacy of enzyme to enhance overall feed utilization by causing degradation of fibre and non-starch polysaccharide (NSP) components of the sorghum based diet into soluble metabolizable energy (Choct, 2006). Exogenous enzymes are commonly associated with hydrolysis of non-starch polysaccharide by making the nutrient available for the rabbits for tissue build up.

Birds fed 75% sorghum with enzyme and Control diet revealed least ($p < 0.05$) values of 3.85mmol/l and 3.30mmol/l respectively for cholesterol. Birds fed Control diet recorded higher ($p < 0.05$) value for uric acid compared to other dietary treatment groups. Elevated uric acid is an indication of imbalance of amino acid in the diet and consequently energy wastage (Nworgu *et al.,* 2007). Amino acids are required for biosynthesis of protein. The specific number of amino acids required must be present for synthesis of protein. However, observation in this study could be attributed to inefficient digestion and absorption of nutrient in the birds fed control diet. Imbalance of number of amino acids results to non-formation of protein and degradation of nitrogenous compound thereby consequently elevated nitrogenous waste.

Table 5: Serum biochemistry parameters of finisher broiler chickens fed maize-sorghum based diets with enzyme inclusion

Parameter	0% Sorghum (control) D1	25% Sorghum + 0.05 Phytase D2	50% Sorghum + 0.05 Phytase D3	75% Sorghum + 0.05 Phytase D4	100% Sorghum + 0.05 Phytase D5	SEM
Glucose (mmol/l)	11.00	12.60	12.10	12.70	11.25	0.30
Total protein (g/l)	33.60^{bc}	38.55^{b}	44.30^{a}	36.90^{b}	31.85^{c}	1.31
Albumin (g/l)	10.95^{b}	11.25^{b}	15.25^{a}	12.20^{b}	11.40^{b}	0.47
Globulin (g/l)	22.65^{bc}	27.30^{ab}	29.05^{a}	24.70^{abc}	20.45^{c}	1.01
Cholesterol (mmol/l)	3.30^{c}	3.70^{bc}	4.60^{a}	3.85^{b}	4.70^{a}	0.15
Creatinine (mmol/l)	24.30	33.05	27.15	28.55	32.00	1.44
Uric acid (mmol/l)	268.85^{a}	154.30^{b}	152.75^{b}	109.75^{b}	159.10^{b}	15.96

^{abc}Mean values in the same row having different superscripts are significantly different ($P<0.05$)

CONCLUSION AND RECOMMENDATIONS

From the results obtained in this study, it revealed that replacement of maize by sorghum with enzyme supplementation improved growth performance haematological and serum biochemical indices of broiler chickens. Sorghum can be used to replace maize at 25% and 50% replacement levels in broiler ration without any negative effect on performance. Researcher efforts should be geared toward complete replacement of maize by sorghum using every available method of making nutrient in sorghum available for birds.

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