PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2019.834.844

Research Article
Blood Bio-Markers, Growth Traits, Carcass Characteristics and Income Over Feed Cost of Broiler Birds Fed Enzyme Fortified Dried Brewer's Grain

[1]C.E. Oyeagu, [2]C.L. Ugwuanyi, [2]E. Onwujiariri, [3]C.O. Osita, [1,3]E.A. Akuru, [3]A.O. Ani, [1]E. M. Idamokoro and [1]A.B. Falowo

[1]Department of Livestock and Pasture Science, University of Fort Hare, Private Bag X1314, Alice 5700, South Africa
[2]Department of Animal Science, Federal Collage of Agriculture, Ishiagu, Ebonyi State, Nigeria
[3]Department of Animal Science, University of Nigeria, Nsukka, Nigeria

Abstract

Background and Objective: In order to reduce high cost of feed which will step-down the cost of poultry products and encourage small scale and medium scale poultry production, efforts must be made by animal nutritionists to exploit and use some unconventional feed ingredients. Most of these unconventional feed ingredients such as brewer's dried grain pose some threats (high fiber) with regards to their utilization by birds. Hence, the use of feed enzymes to reduce these threats for optimum productivity becomes necessary. The aim of the study was to determine the effect of different levels of enzyme fortified dried brewer's grain (EDBG) on blood bio-markers, growth performance, carcass traits; and income over feed cost in a five week trial. **Materials and Methods:** A total of 300 day old non-sexed "Anak strain" chicks were randomly assigned to four dietary treatments with five replicates of 15 birds each. The treatments include, EDBG0, EDBG3, EDBG6 and EDBG9 for 0, 3, 6 and 9% levels of EDBG for both starter and finisher phases. **Results:** Growth traits for starter phase was better ($p<0.05$) for birds fed control (EDBG0) diet while birds fed EDBG3 recorded an improved ($p<0.05$) growth traits during the finisher phase of the feeding trial. Carcass yield, thigh, breast and drumstick weights of birds had the highest ($p<0.05$) values for birds fed EDBG3, while birds fed EDBG9 recorded the lowest ($p<0.05$) carcass, thigh, breast and drumstick weights. The blood bio-marker examination differ ($p<0.05$) among treatments for Eosinophil, Monocyte, Lymphocyte, White blood cell, Hemoglobin and Red blood cell. Hemoglobin concentration and RBC was highest ($p<0.05$) for birds fed EDBG0 and EDBG3. Birds fed EDBG3 recorded the highest ($p<0.05$) revenue from a bird produced and a better income over feed cost. Although, cost of total feed consumed was the highest for birds fed EDBG0 and EDBG3 but similar to those fed EDBG6. **Conclusion:** With regards to improved growth traits, improved cut yields and a better production cost as well as stable health status of broilers, 3% level of Enzyme fortified dried brewer's grain can be used safely.

Key words: Broiler chicks, feed cost, hematology, poultry production, weight gain

Received: August 13, 2018 Accepted: December 11, 2018 Published: August 15, 2019

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

Corresponding Author: C.E. Oyeagu, Department of Livestock and Pasture Science, University of Fort Hare, Private Bag X1314 Alice 5700, South Africa Tel: +27738852288

Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

A major problem for poultry production in under-developed countries is the high cost of poultry feeds due to high cost of feed ingredients[1], which is a result of the major conventional feed ingredients being used by both humans and animals[2]. This situation does not encourage the proliferation of the poultry industry, which may contribute to increase in unemployment, food insecurity and poverty. Reducing the high cost of poultry diets will help reduce the cost of poultry products. One of the methods to achieve this is the utilization of cheap and readily available alternative feedstuffs, such as dried brewer's grain (DBG), which is the extracted residue of cereal grain resulting from the manufacture of beer[3], for which there is currently no human or industrial use.

The production of 1000 L of beer can generate 35-45 kg of brewer's waste as a residue[4]. There are an increasing number of breweries and a large volume of DBG is generated yearly. DBG has good properties to support poultry production, such as more than 20% crude protein content and fairly rich levels of essential amino acids (0.9% lysine, 0.4% methionine, 0.4% tryptophane, 1.2% phenylalanine, 1.1% threonine and 1.6% valine). It is also a concentrated source of digestible fiber, B vitamins (water-soluble vitamins) and phosphorus. Nevertheless, it still has a considerable amount of indigestible fiber[5,6].

Poultry cannot fully digest high-fiber diets because they lack the necessary digestive mechanisms. However, this problem can be reduced by adding exogenous enzymes to monogastric diets[7,8]. Enzymes improve the digestion of fibrous diets and also prevent excreta output of some pollutants, such as phosphate and nitrogen compounds, including ammonia[9,10]. Therefore, this study was designed to investigate the feeding value of enzyme-fortified DBG as a replacement for maize in broiler feed.

MATERIALS AND METHODS

Ethical considerations: Ethical principles were taken into consideration during the study to adhere to the national and international standards governing research of this nature with regard to the use of research animals. Permission to use animals was obtained from the Ethical Clearance Committee of the Federal Collage of Agriculture, Ishiagu, Ebonyi State, Nigeria.

Study site: The experiment was conducted at the poultry unit of Federal College of Agriculture, Ishiagu, Ivo Local Government Area of Ebonyi State, Nigeria. Ishiagu lies at a latitude of 06°22[1] North and longitude of 07°24[1] East. It has an annual rainfall of 1567.05-1846.98 mm. The natural day lengths are 12-13 h and the mean minimum and maximum daily temperatures are 20.99 and 30.33°C, respectively. The relative humidity range is 46.68-76.20%. Ishiagu is part of the humid tropical rainforest zone of South-Eastern Nigeria. The entire study lasted for six weeks.

Experimental diet: Undried brewer's grain was bought in bulk from Nigeria Brewery PLC, Enugu State, Nigeria. The collected product had a moisture content of about 80%, which increased its bulkiness. Hence, it needs to be dried before incorporation in poultry feed. Sun-drying is the most common method used, which requires large space and large polythene sheets. During the drying process, the wet grains have to be spread in a thin layer and frequently turned to avoid fermentation, which could decrease the nutritive value of the product. The DBG was sun dried to 85% percent dry matter (15% moisture content). The dried particles were then broken down to obtain a homogeneous texture.

The DBG was substituted for maize at four different levels for both starter and finisher diets (Table 1 and 2). The exogenous enzyme was included at a constant level of 2 g kg^{-1} feed in all the experimental diets except for the control diets for both starter and broiler finisher diets. The enzymes were provided by Roxazyme G (DSM Nutritional Products, Johannesburg, South Africa), which is an enzyme complex derived from *Trichoderma viride* and contains beta-glucanases, cellulases and xylanase. The enzyme complex was selected because of its intrinsic bioactive characteristics. According to the manufacturer, the enzyme retains more than 90% residual activity after 2 h at 40°C (DSM) in peptic and acidic conditions (pH).

The four experimental diets are referred to as EDBG0, EDBG3, EDBG6 and EDBG9, which contain 0, 3, 6 and 9% enzyme-fortified DBG (EDBG) for both the starter and finisher phases. The EDBG0 diet was the control diet. Table 1 and 2 show the ingredients (%) and approximate chemical composition (g kg^{-1} DM) of the diets.

Experimental birds and management: A total of 300 day-old non-sexed Anak-strain chicks were used for the study. Seventy-five birds were assigned randomly to one of the four experimental diets for both starter and finisher phases. Each experimental diet was replicated in five experimental pens with 15 birds per pen. The birds were housed in cages measuring 3x3x3m with wood shavings as litter. The birds were provided with feed and water *ad libitum* in a six-week

Table 1: Ingredients, calculated and chemical composition of experimental diets for broiler chicks (0-3 weeks)

Treatments	EDBG0	EDBG3	EDBG6	EDBG9
Maize	53.00	50.00	47.00	44.00
DBG	0.00	3.00	6.00	9.00
Soy bean meal	11.00	11.00	11.00	11.00
Groundnut cake	21.00	20.90	20.90	20.90
Fish meal	2.00	2.00	2.00	2.00
Wheat offal	8.00	8.00	8.00	8.00
Oyster shell	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Enzyme	0.00	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein (%)	22.80	23.02	23.41	23.93
Crude fibre (%)	4.02	5.98	6.97	7.96
ME (kcal kg^{-1})	3100.00	2990.00	2884.00	2801.00
Chemical composition				
Crude protein (%)	22.09	22.45	22.88	23.09
Crude fibre (%)	4.80	5.93	6.78	7.09
Ether extract (%)	4.60	6.20	8.40	9.50
Ash (%)	10.78	10.86	11.22	11.64
Dry matter (%)	91.95	91.90	91.91	91.93
Moisture	8.05	8.10	8.09	8.07
Nitrogen free extract (%)	53.68	52.41	50.76	47.89
Ether extract (%)	4.60	6.20	8.40	9.50

ME: Metabolizable energy, DBG: Dried brewer's grain, EDBG: Enzyme fortified dried brewer's grain, EDBG0: Basal diet: BD (without enzyme fortified DBG). EDBG3: BD+3% enzyme fortified DBG, EDBG6: BD+6% enzyme fortified DBG, EDBG9: BD+9 % enzyme fortified DBG

Table 2: Ingredients, calculated and chemical composition of experimental diets for finisher birds (4-6 weeks)

Treatments	EDBG0	EDBG3	EDBG6	EDBG9
Maize	64.00	61.00	58.00	55.00
DBG	0.00	3.00	6.00	9.00
Soy bean meal	8.00	8.00	8.00	8.00
Groundnut cake	11.00	10.90	10.90	10.90
Fish meal	2.00	2.00	2.00	2.00
Wheat offal	10.00	10.00	10.00	10.00
Oyster shell	2.00	2.00	2.00	2.00
Bone meal	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Enzyme	0.00	0.02	0.02	0.02
Total	100.00	100.00	100.00	100.00
Calculated analysis				
Crude protein (%)	20.40	20.39	20.41	20.43
Crude fibre (%)	4.28	6.72	8.66	10.45
ME (kcal kg^{-1})	3088.96	2931.86	2812.06	2701.66
Chemical composition				
Crude protein (%)	19.59	20.00	20.09	20.16
Crude fibre (%)	4.85	6.77	8.66	10.09
Ether extract (%)	4.61	6.30	8.44	10.05
Ash (%)	10.89	10.99	11.45	11.92
Dry matter (%)	91.95	91.90	91.92	91.91
Moisture	8.05	8.10	8.08	8.09
Nitrogen free extract (%)	51.16	50.92	49.01	48.51
Ether extract (%)	4.61	6.30	8.44	10.05

ME: Metabolizable energy, DBG: Dried brewer's grain, EDBG: Enzyme fortified dried brewer's grain, EDBG0: Basal diet: BD (without enzyme fortified DBG), EDBG3: BD+3% enzyme fortified DBG, EDBG6: BD+6% enzyme fortified DBG, EDBG9: BD+9 % enzyme fortified DBG feeding trial. General flock prophylactic management and routine vaccinations were administered as follows: Day 1: Intra-ocular Newcastle disease vaccine, Week 2: Gumboro disease vaccine, Week 3: Lasota Newcastle 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 per 50 L according to the manufacturer's instructions to boost appetite and energy supply.

Measurement of growth and economics parameters: At the beginning of the experiment, birds in each replicate were weighed individually and subsequently on a weekly basis using a 10.1 kg-capacity precision weighing balance (models A and D Weighing GF-10K industrial balance, Japan). Feed intake was determined daily by the weigh-back technique. The feed conversion ratio was determined as the quantity (g) of feed consumed per unit weight (g) gained over the same period. The cost implication (cost of total feed consumed, revenue from a bird produced and income with respect to feed cost) was also considered at the end of the study as follows:

$$\text{Revenue from a bird produced (RBP) (\$)} = \text{Broiler cost per kg} \times \text{total body weight}$$

$$\text{Cost of total feed consumed (CTFC) (\$)} = \text{Feed cost per kg} \times \text{total feed consumed}$$

$$\text{Income over feed cost (\$)} = \text{RBP} - \text{CTFC}$$

Slaughter procedure: At 42 days of age, all chickens were slaughtered. The chickens were stunned by exposing them to relatively low concentrations of carbon dioxide (<40% by volume in air). Once they were unconscious, they were exposed to a higher concentration (approximately 80-90% by volume in air). At the abattoir, all the chickens were hung on a movable metal rack that holds them upside down by their feet. The chickens were then slaughtered by cutting the jugular vein with a sharp knife and left hanging until bleeding stopped.

Carcass characteristics: Immediately after slaughter, the feathers were plucked and the gastro-intestinal tract was removed. The carcasses were then weighed. Five birds per replicate pen were randomly selected for the determination of carcass characteristics. For the measurement of carcass cuts, the head and shanks were removed close to the skull and at the hock joint, respectively. Wings were removed by cutting at the humeroscapular joint. The cuts were made through the rib head to the shoulder girdle and the vertebrae were then removed intact by pulling outwardly[11]. The breast muscle, neck, wings, shanks, thighs, drumsticks and vertebrae were each weighed separately.

Blood collection and evaluation: At the end of the feeding trial (on the 42nd day), 20 birds were selected randomly from each of the treatment groups (four birds per replicate). Blood samples (3 mL) were collected from the wing veins using sterile needles. The blood samples were collected into a labeled sterilized bottle containing the anticoagulant EDTA (ethylene diamine tetra-acetic acid) for a hematological study. The packed cell volume (PCV) and hemoglobin concentration (Hb) were determined using the methods described by Mitruka and Rawnsley[12]. The red blood cell (RBC) and total white blood cell (WBC) counts were also assayed using an automated Idexx Vet Test Chemistry Analyzer (IDEXX Laboratories, Inc.). The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were estimated through calculations according to Mitruka and Rawnsley[12].

Proximate analysis: The formulated diets, maize meal and DBG (Table 3) were milled (Polymix PX-MFC 90 D) to pass through a 1 mm sieve for chemical analyses. The total nitrogen content was determined by the standard macro-Kjeldahl method (AOAC[13]: method no. 984.13) and the result was converted to crude protein by multiplying the N content percentage by a factor of 6.25. Amino acids were determined by hydrolyzing the samples with 6 M HCl (containing phenol)for 24 h at 110±2°C in glass tubes sealed under vacuum. Crude fiber was determined using an ANKOM[2000] Fiber analyzer (ANKOM Technology, New York) with 0.255 N crude fiber acid solution and then with 0.313 N crude fiber base solution. The crude fat and metabolizable energy (ME) contents were predicted using near-infrared reflectance spectroscopy (NIR; SpectraStar XL, Unity Scientific, Australia).

Table 3: Proximate evaluation of maize meal and dried brewer's grain

	Maize meal	Dried brewer's grain
Nutrients		
Crude protein (%)	9.89	20.34
Crude fibre (%)	2.90	11.70
Ether extract (%)	3.11	6.19
Ash (%)	1.96	5.98
Dry matter (%)	90.25	84.46
Moisture	9.75	15.54
Nitrogen free extract (%)	63.43	61.57

Statistical design and analysis: Data were analyzed using one-way analysis of variance (ANOVA) for a Completely Randomized Design (CRD), as described by Steel and Torrie[14]. The analysis was done using the Statistical Package for the Social Sciences[15] for Windows version 17.0. Significantly different means were separated using Duncan's New Multiple Range Test[16] as outlined by Obi[17]. For all statistical tests, significance was determined at p<0.05.

RESULTS

Performance of broiler chicks: The performance of broiler starter (0-3) and finisher (4-6) birds fed EDBG is shown in Table 4. All the growth parameters of broiler starter birds were significantly affected by the treatments (p<0.05) except for total feed intake and daily feed intake (p>0.05). The final weight, daily weight and total weight gain at the end of the third week (starter phase) of the feeding trial were significantly higher for the birds fed the control (EDBG0) (p<0.05), while birds fed EDBG3 had a better final weight, daily weight and total weight gain compared to those fed EDBG6 and EDBG9. The efficiency of converting feed to meat (FCR) was better for birds fed EDBG0 and EDBG3 (p<0.05), with mean values of 1.05 and 1.12, whereas 1.49 and 1.81 were recorded for birds fed EDBG6 and EDBG9, respectively.

Performance of broiler finisher birds: The growth performance of broiler finisher birds (4-6-week feeding trial) fed different levels of EDBG is presented in Table 4. The growth performance parameters were affected by the EDBG. Birds fed EDBG3 showed the highest final body weight, total weight gain and daily weight gain compared with birds fed the control (p<0.05) and those that received higher levels of EDBG (EDBG6 and EDBG9). Birds fed EDBG9 had the lowest daily weight gain of 54.81 g (p<0.05) compared with 83.68 g, 87.71 and 71.37 g recorded for birds fed EDBG0, EDBG3 and EDBG6, respectively. An increase in total and daily feed consumed was observed for birds fed EDBG0 and EDBG6, although the results were statistically similar to those of EDBG3-fed birds. The lowest total feed consumed was recorded for birds fed EDBG9. Birds fed EDBG3 showed a better FCR of 1.79 (p<0.05) compared with birds fed EDBG0, EDBG6 and EDBG9, which had FCR values of 1.92, 2.27 and 2.66, respectively.

Carcass characteristics: Table 5 shows the carcass characteristics of broiler birds fed EDBG. The carcass, drumstick, thigh and breast-meat weights were all affected by the EDBG (p<0.05). However, the neck, wing, vertebrae and shank weights were not influenced (p>0.05). The carcass weight was higher for birds fed EDBG3 compared to those fed other treatments (p<0.05). The thigh weight showed the highest value for birds fed EDBG0 and EDBG3 (p<0.05), while birds fed EDBG9 showed the lowest values. Breast-meat weight was lowest for birds fed EDBG6 and EDBG9. Birds fed EDBG3 had the highest breast-meat weight, although it was statistically similar to those fed EDBG0. The highest drumstick weight was recorded for birds fed EDBG3 (p<0.05), although the results were statistically similar to birds fed EDBG0 and EDBG6.

Blood bio-markers: The hematological traits of broiler chickens fed EDBG are presented in Table 6.

Although, the hematological values obtained fell within the normal range, there were significant differences among treatments for eosinophil count, monocyte count, lymphocyte

Table 4: The growth performance of broiler chicks (0-3 weeks) and finisher bird (0-6 weeks) affected by enzyme fortified dried brewer's grain

Treatments	EDBG0	EDBG3	EDBG6	EDBG9	SEM	p-value
Final body weight (g)	1070.00^{a}	980.00^{b}	760.00^{c}	610.00^{d}	3.95	0.03
Total weight gain (g)	1028.96^{a}	939.32^{b}	717.99^{c}	568.45^{d}	3.35	0.04
Daily weight gain (g)	29.40^{a}	26.84^{b}	20.51^{c}	16.24^{d}	0.25	0.01
Total feed intake (g)	1080.00	1050.00	1070.00	1030.00	5.01	0.10
Daily feed intake (g)	30.85	30.00	30.57	29.42	0.38	0.09
FCR	1.05^{cd}	1.12^{c}	1.49^{b}	1.81^{a}	0.02	0.02
4-6 weeks						
Final body weight (g)	2970.00^{b}	3070.00^{a}	2540.00^{c}	1960.00^{d}	3.32	0.04
Total weight gain (g)	2928.96^{b}	3027.98^{a}	2497.99^{c}	1918.45^{d}	3.40	0.02
Daily weight gain (g)	83.68^{b}	87.71^{a}	71.37^{c}	54.81^{d}	0.16	0.01
Total feed intake (g)	5626.84^{a}	5510.00^{ab}	5665.86^{a}	5094.00^{b}	6.07	0.04
Daily feed intake (g)	160.77^{a}	157.43^{ab}	161.88^{a}	145.54^{b}	1.22	0.03
FCR	1.92^{c}	1.79^{d}	2.27^{b}	2.66^{a}	0.05	0.01

[a,b,c]Row means with different superscripts differ significantly at p<0.05. SME: Standard error of the mean, FCR: Feed conversion ratio. DBG: Dried brewer's grain, EDBG: Enzyme fortified dried brewer's grain, EDBG0: Basal diet: BD (without enzyme fortified DBG), EDBG3: BD+3% enzyme fortified DBG, EDBG6: BD+6% enzyme fortified DBG. EDBG9: BD+9 % enzyme fortified DBG

Table 5: The effect of enzyme fortified dried brewer's grain on carcass characteristics of broiler birds

Treatments	EDBG0	EDBG3	EDBG6	EDBG9	SEM	p-value
Carcass weight (g)	2016.90^{b}	2236.80^{a}	1814.05^{c}	1650.09^{d}	18.97	0.02
Neck weight (g)	86.47	89.20	84.40	88.07	2.32	0.11
Wing weight (g)	85.47	77.07	83.20	76.33	2.06	0.09
Drumstick weight (g)	92.00^{ab}	94.67^{a}	92.27^{ab}	91.60^{b}	2.57	0.01
Thigh weight (g)	109.33^{a}	105.93^{a}	90.33^{b}	86.67^{bc}	2.98	0.04
Breast weight (g)	491.33^{ab}	517.66^{a}	430.20^{b}	428.00^{b}	5.76	0.02
Vertebrate weight (g)	197.93	187.67	184.93	183.27	3.45	0.21
Shank weight (g)	36.20	38.13	37.00	36.27	0.98	0.17

^{a,b,c}Row means with different superscripts differ significantly. SEM: Standard error of the mean, DBG: Dried brewer's grain, EDBG: Enzyme fortified dried brewer's grain, EDBG0: Basal diet: BD (without enzyme fortified DBG), EDBG3: BD+3% enzyme fortified DBG, EDBG6: BD+6% enzyme fortified DBG, EDBG9: BD+9% enzyme fortified DBG

Table 6: Blood bio-marker study of broiler finisher birds fed enzyme fortified dried brewer's grain

Treatments	NBR	EDBG0	EDBG3	EDBG6	EDBG9	SEM	p-value
Eosinophil count (%)	0-2	0.42^{b}	0.41^{b}	0.56^{ab}	0.61^{a}	0.09	0.03
Monocyte count (%)	0-3	0.43^{b}	0.44^{b}	0.55^{a}	0.59^{a}	0.08	0.01
Lymphocyte count (%)	6-17	8.75^{b}	8.96^{b}	9.97^{a}	10.25^{a}	0.37	0.04
Basophil count (%)	1-5	0.17	0.16	0.16	0.15	0.08	0.09
WBC (10^{3} μL^{-1})	10-40	11.88^{b}	14.96^{b}	17.91^{ab}	18.91^{a}	0.54	0.02
PCV (%)	28-48	31.67	30.00	29.86	30.33	0.66	1.00
HB (g dL^{-1})	7-13	9.01^{a}	8.81^{a}	7.54^{b}	7.22^{b}	0.23	0.03
RBC (10^{12} L^{-1})	2.5-5	3.45^{a}	3.20^{a}	2.75^{ab}	2.70^{ab}	0.15	0.01
MCH (pg)	27-40	36.89	35.63	36.93	37.66	3.31	0.08
MCHC (g dL^{-1})	32-36	33.33	33.35	33.47	33.65	0.15	1.11
MCV (fL)	80-120	110.63	108.73	109.49	110.09	2.43	0.09

NBR: Normal blood range (Jain, 1993; Bounous and Stedman 2000, Ghergariu *et al*. 2000; Trîncă *et al*. 2012; BS, 2013). SEM: Standard error of mean, PCV: Packed cell volume, HB: Hemoglobin, RBC: Red blood cell count, MCHC: Mean cell hemoglobin concentration, MCH: Mean cell hemoglobin, MCV: Mean cell volume, WBC: White blood cell, DBG: Dried brewer's grain, EDBG: Enzyme fortified dried brewer's grain, EDBG0: Basal diet: BD (without enzyme fortified DBG), EDBG3: BD+3% enzyme fortified DBG, EDBG6: BD+6% enzyme fortified DBG, EDBG9: BD+9% enzyme fortified DBG

Table 7: Income over feed cost of broiler finisher birds fed enzyme fortified dried brewer's grain

Treatments	EDBG0	EDBG3	EDBG6	EDBG9	SEM	p-value
Feed cost per kg ($)	0.46	0.43	0.35	0.30	_	_
Total body weight (kg)	2.93^{b}	3.03^{a}	2.50^{c}	1.92^{d}	0.32	0.04
Total feed consumed (kg)	5.63^{a}	5.51^{ab}	5.67^{a}	5.09^{b}	0.40	0.01
Broiler cost per kg ($)	3.75	3.75	3.75	3.75	_	_
Cost of total feed consumed ($)	2.59^{a}	2.37^{ab}	1.98^{ab}	1.53^{b}	0.46	0.03
Revenue from a bird produced ($)	10.99^{b}	11.36^{a}	9.38^{b}	7.20^{c}	1.08	0.02
Income over feed cost ($)	8.49^{b}	9.00^{a}	7.40^{c}	5.67^{d}	0.98	0.04

$^{a-d}$Row means with different superscripts differ significantly at p<0.05. NS: Not significant, SME: Standard error of the mean, DBG: Dried brewer's grain, EDBG: Enzyme fortified dried brewer's grain, EDBG0: Basal diet: BD (without enzyme fortified DBG), EDBG3: BD+3% enzyme fortified DBG, EDBG6: BD+6% enzyme fortified DBG, EDBG9: BD+9% enzyme fortified DBG, $: US dollar sign

count, WBC count, hemoglobin and RBC count. There was no difference (p>0.05) among treatments for Basophil count, PCV, MCH, MCHC and MCV. Birds fed EDBG6 and EDBG9 showed the highest monocyte count and lymphocyte count (p<0.05) compared to those fed EDBG0 and EDBG3.

The eosinophil count and WBC count were significantly higher for birds fed EDBG9 (p<0.05; eosinophil count: 0.61; WBC: 18.91), although they were statistically similar to those fed EDBG6 (eosinophil count: 0.56; WBC: 17.91). The hemoglobin concentration was highest for birds fed EDBG0 and EDBG3 (p<0.05). However, birds fed EDBG6 and EDBG9 had similar hemoglobin concentrations (p>0.05). The hemoglobin concentration tended to decrease with the Increased levels of EDBG content. RBC count was the highest for birds fed EDBG0 and EDBG3 (p<0.05), although the results were statistically similar to those obtained with EDBG6 and EDBG9.

Economic implications: The economic implications of feeding EDBG to broiler chickens are presented in Table 7. The cost of total feed consumed was higher (p<0.05) for birds fed EDBG0 but was statistically similar to those that received EDBG3 and EDBG6. A reduced cost of total feed consumed was observed for birds fed EDBG9. Revenue from a bird produced was significantly higher for birds fed EDBG3 (p<0.05) and tended to decrease with increasing amounts of EDBG. Birds fed EDBG3 showed the highest ($P<0.05$) income over feed cost ($9.00) compared with those that received EDBG0, EDBG6 and EDBG9 ($8.49, $7.40 and $5.67, respectively).

DISCUSSION

Performance of broiler chicks: Increasing the EDBG content resulted in poor performance of the birds across the diets. This may have been due to the reduced digestibility of starch, nitrogen and fat as a result of the higher intestinal viscosity in young chicks[18]. The poor performance may also be attributed to the higher concentration of anti-nutrient effects of non-starch polysaccharides (NSPs), which increase the bulk and viscosity of the intestinal content and ultimately counter the digestion and absorption of nutrients in the intestine[19,20]. The better performance shown for birds fed the control diet (EDBG0) compared with other treatments may have been due to the lack of BDG. However, birds fed EDBG3 had better growth traits than those with higher contents of EDBG (EDBG6 and EDBG9). This may be a result of efficient handling of the fiber content by the exogenous enzymes, which could have increased the availability of minerals such as phosphorus, calcium, zinc and copper, as well as the nutrient digestibility for broiler chicks[21,22]. Research has shown that enzymes have the potential to break down the fibrous feed ingredients by disrupting the plant cell walls reducing the viscosity of the gut contents, thereby enhancing the absorption of nutrients[23,24]. Choct also reported improvements in digestibility both *in vitro* and *in vivo*[25].

Performance of broiler finisher birds: The significantly higher final body weight, total weight gain and daily weight gain recorded for birds fed EDBG3 may indicate that the 3% content of EDBG was capable of supplying adequate nutrients for a better growth rate than the control diet (EDBG0). This may have resulted from the efficient bio-activities of cellulases and glycanases contained in the enzyme complex, which might have resulted in cleavage of the NSPs in the DBG into smaller polymers, thereby preventing the formation of viscous digesta and improving nutrient digestibility[26,27,8]. Agbede *et al.*[28] and Shakouri and Kermanshashi[29] showed a similar improvement for broiler birds. Poor performance was recorded for final body weight, daily weight gain, total body weight gain and FCR of birds as the EDBG content increased. The poor performance of birds fed EDBG6 and EDBG9 may be linked to the increased concentration of anti-nutritive factors present in the DBG[30-32].

Enzyme fortification has the potential to improve the performance of broiler birds by at lowest two mechanisms: improving feed consumption and improving the digestibility of nutrients. Both mechanisms can be induced at least partially by a reduction of the viscosity to reduce the retention time of digesta in the gut, resulting in the release of nutrients to the birds for improved growth and efficient conversion of feed to meat[24,33-37]. The conversion efficiency of feed to meat was better for birds fed EDBG3 compared with the birds fed the control diet (EDBG0). This finding is supported by Alam *et al.*[38] and Ani and Oyeagu[39], who opined that the conversion of feed to meat was increased due to better feed utilization.

Exogenous enzymes complement the endogenous enzymes of poultry by causing the NSPs found in cereals and vegetable proteins to undergo hydrolysis, thereby decreasing gut viscosity and improving nutrient absorption[40-42,8]. Feed enzymes also have the potential to change the bacteria population by breaking down the long-chain carbohydrate molecules utilized by some bacteria to colonize the tract. This increases the quality of amino acids from digested protein in the pre-caecal section of the tract, thereby encouraging more nutrient availability to the host[43,28]. The better performance of birds fed EDBG3 may indicate that the amount of enzyme included ($2 g kg^{-1}$) had peak bio-activity in terms of digestion and absorption with that amount of EDBG.

Carcass characteristics: There was a significant effect of EDBG on the carcass, drumstick, thigh and breast-meat weights of the broiler birds. Birds fed EDBG3 performed better compared to those that received the control. This improvement conforms to the earlier assertions of Adeola and Olukosi[44] that enzyme fortification improves cut yields of birds. The breakdown of fibrous material in the DBG by the enzymes used in fortification enables the birds to acquire more nutrients from the feed, which are deposited as tissues in the body. These observations are consistent with the report by Iyayi and Okhankuele[45], who observed a significant variation in weight percentage of the drumstick and breast when they supplemented the diets of broiler finisher chickens with exogenous enzymes.

The ingredients of plant origin offered to the birds have some variations in their chemical structure and the presence of anti-nutrients, such as phytin, hydrocyanic acid and tannins, which often result in poor performance of the birds. This may be the reason for the poor carcass and cut yields of birds fed EDBG9. The lower carcass and cut yields of birds fed EDBG6 and EDBG9 may be due to the overwhelming presence of NSPs and anti-nutrients that could not be degraded by the amount of fortified enzymes used in this study. Adequate supplementation of diets with exogenous enzymes can reduce the adverse effects of some of these compounds[46,47]. Similar results were found by Dalolio *et al.*[48], who observed an effect of enzyme complex supplementation in diets based on wheat meal.

The improvements in cut yield could be very important to the poultry industry because there is more of a tendency to sell cuts than the whole carcass due to the increase in aggregate value. Silveira *et al.*[49] examined the use of enzyme complex in wheat pelleted diets and found an effect on the yield of the leg quarter, which increased by 25% in comparison to birds fed the control diet. However, they found no significant difference in the yield of breast meat. Cardoso *et al.*[50] also did not find any differences in carcass yield for broilers fed multiple enzyme supplements at 42 days of age. The results of the current study are consistent with the findings of Alam *et al.*[39], Wang *et al.*[51] and Hajati[52], who reported increased carcass yields for birds fed adequate levels of enzyme-supplemented diets. They attributed this increase to the higher fat deposition in the carcass and increased breast-meat yield.

Blood bio-markers: Dietary inclusion of EDBG was expected to minimize the cost of feed without impairing the physiological conditions and health status of the chickens. Hematological constituents usually reflect the physiological responsiveness of the animal to its external and internal environment and thus serve as a viable tool for monitoring animal health. In the present study, monocyte count, lymphocyte count, eosinophil count and WBC count seemed to increase with increased EDBG content. Monocytes ingest or engulf germs and are actively motile and phagocytic in action. They leave the blood stream to ingest micro-organisms and other foreign materials that may be introduced into the tissue. Lymphocytes' main function is the production of antibodies, while eosinophils produce anti-toxins against toxins produced by pathogens. They are known to phagocytize particles that form when an antigen and antibodies react[53]. WBCs are immune cells that protect the body against infections.

The increased monocyte count, lymphocyte count, eosinophil count and WBC count with the increased inclusion of EDBG may be an indication that the animals were fighting a disease or stress. This corroborates the findings of Davis *et al.*[54] and Sugiharto *et al.*[55], who shared the same thoughts. Akinwuntimi *et al.*[56] and Obidinma[57] suggested that higher values of these blood traits may also be due to the high fiber content of the diets coupled with the anti-nutritional inhibitors, particularly phytate, which chelates divalent metal utilization in monogastric animal metabolism. These challenges must have caused the reduced body weight and inefficient FCR (Table 4) observed for birds fed high levels of EDBG.

Sugiharto *et al.*[58] argued that the higher monocyte, lymphocyte, eosinophil and WBC counts may imply a greater ability of the chickens to respond to infection. Hemoglobin and RBCs tended to decrease with increased contents of EDBG. The pattern of effects seems to be an inverse of WBC in the present study. RBCs contain molecules of hemoglobin (iron, hemin, protein and globulin). Hemoglobin combines with oxygen in the blood to form oxy-hemoglobin and carries the oxygen to needy tissues. The decreased values of RBC may be the reason for the poor growth performance of birds fed higher contents of EDBG.

Adejinmi *et al.*[59] reported a progressive degradation of erythrocytes (RBCs) due to the presence of anti-nutrients. However, the higher WBC values in broiler finisher birds that consumed more EDBG may be attributed to the stress of anti-nutrients and high-fiber diets, which often cause a reduction in oxygen-carrying capacity (anemia) in the animal's blood, resulting in impairment of the growth performance of chickens[60,61]. This suggests that the constant enzyme content used in the present study (2 g kg^{-1} feed) could not handle the higher contents of DBG efficiently beyond 3%. It is evident that there were induced physiological or health difficulties that resulted in poor growth of birds fed higher contents of EDBG (EDBG6 and EDBG9).

Economic implications: The results showed that the use of EDBG3 could reduce the cost of production and ensure more money for farmers or producers. Bawa *et al.*[62], Ogundipe *et al.*[63], Dodusola[64] and Ani and Oyeagu[39] opined that it is necessary to reduce the cost of production in order to produce affordable poultry meat and eggs for the populace in the face of poverty or dwindling standards of living. Therefore, a recent trend among animal nutritionists has been to use unconventional feedstuffs in order to reduce the production cost, maximize income and still maintain the standards of meat and egg production[65].

DBG is relatively cheap and readily available and there is little or no competition between humans, farm animals and industry. Abeke[66], Toleun and Igba[67] and Dodusola[64] pointed out that the solution to inadequate protein intake of the populace could easily be achieved if the cost of producing poultry meat and eggs (especially feed cost) can be drastically reduced. The present results showed that 3% EDBG should be added for improved growth, cost effectiveness and stable health status of broilers. This study used different levels of DBG with fixed exogenous enzyme supplementation. Hence, future studies should focus on different supplemental contents of exogenous enzyme that can efficiently support higher contents of DBG for increased broiler production.

CONCLUSION

In conclusion, up to 3% EDBG can be substituted in broiler diets for improved performance, stable health status of the birds, better cut yields and improved income over feed cost. However, further research is required to investigate the level of exogenous enzyme that can tolerate higher contents of DBG.

SIGNIFICANCE STATEMENT

This study discover the potentials of dried brewer's grain, which is a non-conventional feed ingredient (that has no human or industrial use for now) that can be beneficial for feed manufactures and poultry producers with regards to minimizing the cost of producing a healthy meat. This study will help the researchers to uncover the critical areas of interest with regards to different exogenous enzyme levels that can further unlock the nutritional potentials of higher inclusion levels of dried brewer's grain. Hence, a new theory on brewer's dried grain and exogenous enzyme combination has been achieved in the present study and possibly other combinations through further study may be arrived at.

ACKNOWLEDGMENTS

Many thanks to the Department of Animal Science, Federal Collage of Agriculture, Ishiagu, Ebonyi State, Nigeria. Again, the authors wish to acknowledge Govan Mbeki Research and Development Centre (GMRDC) University of Fort Hare, Alice, South Africa for their support.

REFERENCES

1. Adeniji, A.A. and O.O. Balogun, 2002. Utilisation of flavour treated blood-rumen content mixture in the diets of laying hens. Niger. J. Anim. Prod., 29: 34-39.
2. Tona, G.O., J.A. Akinlade, R.O. Olabanji and A.B. Adekitan, 2010. Performances and nutrient digestibility of weaned rabbits fed graded levels of piliostigma thonningii leaf meal-based diets. Proceedings of the 35th Annual Conference of Nigerian Society for Animal Production, March 14-17, 2010, University of Ibadan, Ibadan, Nigeria, pp: 278-281.
3. Ironkwe, M.O. and A.M. Bamgbose, 2012. Effect of replacing maize with brewer's dried grain in broiler finisher diet. Bull. Environ. Pharmacol. Life Sci., 1: 17-20.
4. Para, R. and A. Escobar, 1985. Use of Fibrous Agricultural Residues (FAR) in Ruminant Feeding in Latin America. In: Better Utilization of Crop Residues and by-Products in Animal Feeding: Research Guidelines 1. State of Knowledge, (FAO Animal Production and Health Paper No. 50), Preston, T.R., V.L. Kossila, J. Goodwin and S.B. Reed (Eds.)., Food and Agriculture Organization of the United, Rome, Italy, pp: 81-98.
5. Oreopoulou, V. and W. Russ, 2007. Utilization of by-Products and Treatment of Waste in the Food Industry. Springer, New York, ISBN: 9780387335117, Pages: 316.
6. Hussaini, S.J., H.N. Moghaddam and H. Kermanshahi, 2010. The influence of different levels of brewers spent grain and enzyme on performance and digesta viscosity of broiler chicks. J. Anim. Vet. Adv., 9: 2608-2612.
7. Lemme, A., V. Ravindran and W.L. Bryden, 2004. Ileal digestibility of amino acids in feed ingredients for broilers. World's Poult. Sci. J., 60: 423-438.
8. Oyeagu, C.E., A.O. Ani, C.F. Egbu, E.S. Akpolu, J.C. Iwuchukwu and J.N. Omumuabuike, 2015. Performance of broiler finisher birds fed toasted bambara nut (*Vigna subterranean* (L.) Verdc) offal with supplementary Enzyme. Asian J. Sci. Technol., 6: 934-939.
9. Nelson, M.M., 2005. Enzymes in poultry nutrition. Export Executive, Vet-Care, Bangalore, pp: 51.
10. Costa, F.G.P., C.C. Goulart, D.F. Figueiredo, C.F.S. Oliveira and J.H.V. Silva, 2008. Economic and environmental impact of using exogenous enzymes on poultry feeding. Int. J. Poult. Sci., 7: 311-314.
11. Alikwe, P.C.N., A.Y. Faremi and P.A. Egwaikhide, 2011. Biochemical evaluation of serum metabolites, enzymes and haematological indices of broiler chicks fed with varying levels of rumen epithelial scraps in place of fish meal proein. Pak. J. Sci. Ind. Res., Vol. 54, No. 2.
12. Mitruka, B.M. and H.M. Rawnsley, 1977. Clinical Biochemical and Hematological Reference Values in Normal Experimental Animals. Masson Publ., New York, USA., ISBN-13: 9780893520069, pp: 42-45.
13. AOAC., 2006. Official Methods of Analysis. 18th Edn., Association of Official Analytical Chemists Inc., Arlington, TX., USA.
14. Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd Edn., McGraw Hill Book Co., New York, USA., ISBN-13: 9780070609266, Pages: 633.
15. SPSS., 2003. Statistical Package for Social Sciences, Windows Version 8. SPSS Inc., USA.
16. Duncan, D.B., 1955. Multiple range and multiple F tests. Biometrics, 11: 1-42.

17. Obi, I.U., 2002. Statistical Methods of Detecting Differences Between Treatment Means and Research Methodology Issues in Laboratory and Field Experiments. 2nd Edn., Express Publisher Ltd., Enugu, pp: 13-21.
18. Nian, F., Y.M. Guo, Y.J. Ru, F.D. Li and A. Peron, 2011. Effect of exogenous xylanase supplementation on the performance, net energy and gut microflora of broiler chickens fed wheat-based diets. Asian-Aust. J. Anim. Sci., 24: 400-406.
19. Ao, T., A.H. Cantor, A.J. Pescatore, M.J. Ford, J.L. Pierce and K.A. Dawson, 2009. Effect of enzyme supplementation and acidification of diets on nutrient digestibility and growth performance of broiler chicks. Poult. Sci., 88: 111-117.
20. Hedemann, M.S., P.K. Theil and K.E. Bach Knudsen, 2009. The thickness of the intestinal mucous layer in the colon of rats fed various sources of non-digestible carbohydrates is positively correlated with the pool of SCFA but negatively correlated with the proportion of butyric acid in digesta. Br. J. Nutr., 102: 117-125.
21. Saylor, W.W., 2000. Phosphorus reduction techniques used in broiler nutrition. Feedstuffs, 72: 11-15.
22. Jones, D.R., M.T. Musgrove, K.E. Anderson and H.S. Thesmar, 2010. Physical quality and composition of retail shell eggs. Poult. Sci., 89: 582-587.
23. Acamovic, T., 2001. Commercial application of enzyme technology for poultry production. Word's Poult. Sci. J., 57: 225-242.
24. Duranthon, B., 2012. Formulating feeds with enzymes. All About Feed. International Magazine on Animal Nutrition, Processing and Feed Management. Enzyme Special, September, 2012, pp: 14. https://www.allaboutfeed.net/.
25. Choct, M., 2006. Enzymes for the feed industry: Past, present and future. World's Poult. Sci. J., 62: 5-16.
26. Isikwenu, J.O., 2012. Haematological, organs and performance response of cockerel chicks fed urea-treated and fermented brewer's dried grains diets as replacement for groundnut cake. Am. J. Food Nutr., 2: 1-6.
27. Carsten, P., 2013. Reduction of anti-nutritional factors in (pre) starter feed. All About Feed. International Magazine on Animal Nutrition, Processing and Feed Management, Volume 21, No. 2, pp: 25-27.
28. Agbede, J.O., K. Ajaja and V.A Aletor, 2002. Influence of *Roxazyme* G. supplementation on the utilization of sorghum dust-based diets for broiler-chicks. Proceedings of the 27th Annual Conference Nigerian Society for Animal Production, March 17-21, 2002, Federal University of Technology, Akure, Nigeria, pp: 105-108.
29. Shakouri, M.D. and H. Kermanshahi, 2004. Effect of enzyme supplementation in wheat and trickle based diets on broiler performance. Proceedings of the Annual Conference of the British Society of Animal Science, University of York, April 5-7, 2004, York, UK., pp: 273.
30. Ani, A.O., D.O. Omeje and L.C. Ugwuowo, 2012. Effects of raw bambara nut (*Voandzeia subterranean* L.) waste and enzyme complex on growth performance and apparent nutrient retention in broiler chickens. Afr. J. Biotechnol., 11: 11991-11997.
31. Tiago, S., 2012. Phytate as an anti-nutrient for poultry and swine. All About Feed. International Magazine on Animal Nutrition, Processing and Feed Management, Enzyme Special, September, 2012, pp: 4-5. https://www.allaboutfeed.net/.
32. Oyeagu, C.E., A.O. Ani, C.F. Egbu, F.U. Udeh, J.N. Omumuabuike and J.C. Iwuchukwu, 2016. The effect of feeding toasted Bambara nut (*Vigna subterranean* (L.) *verdc*) offal and supplementary enzyme on performance of broiler chicks. Trop. Agric., 93: 271-283.
33. Lazaro, R., M. Garcia, P. Medel and G.G. Mateos, 2003. Influence of enzymes on performance and digestive parameters of broilers fed rye-based diets. Poult. Sci., 82: 132-140.
34. Liu, N., Y.J. Ru, F.D. Li, J.P. Wang and X.Q. Lei, 2009. Effect of dietary phytate and phytase on proteolytic digestion and growth regulation of broilers. Arch. Anim. Nutr., 63: 292-303.
35. Selle, P.H., A.J. Cowieson and V. Ravindran, 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest. Sci., 124: 126-141.
36. Hajati, H., M. Rezaei and H. Sayyahzadeh, 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.
37. Caroline, J., 2012. Versatility of enzyme contributes in reducing pig production costs. All About Feed. International Magazine on Animal Nutrition, Processing and Feed Management. Enzyme Special, September, 2012, pp: 16-17. https://www.allaboutfeed.net/.
38. Alam, M.J., M.A.R. Howlider, M.A.H. Pramanik and M.A. Haque, 2003. Effect of exogenous enzyme in diet on broiler performance. Int. J. Poult. Sci., 2: 168-173.
39. Ani, A.O. and C.E. Oyeagu, 2015. Effect of feed type on the performance of nera black hens in the humid tropical environment. Br. J. Applied Sci. Technol., 10: 1-12.
40. Choct, M. and G. Annison, 1992. The inhibition of nutrient digestion by wheat pentosans. Br. J. Nutr., 67: 123-132.
41. Giraldo, L.A., M.L. Tejido, M.J. Ranilla, S. Ramos, A.R. Mantecon and M.C. Travieso, 2009. Influence of direct-fed exogenous fibrolytic enzyme on ruminal fibrolityc activity in sheep. Options Mediterr. A, 85: 297-302.
42. El-Katcha, M.I., M.A. Soltan, H.F. El-Kanwy and E.S.R. Kawarie, 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.

43. Gunal, M., S. Yasar and J.M. Forbes, 2004. Performance and some digesta parameters of broiler chickens given low or high viscosity wheat-based diets with or without enzyme supplementation. Turk. J. Vet. Anim. Sci., 28: 323-327.
44. Adeola, O. and O.A. Olukosi, 2008. Opportunities and challenge of alternative feed stuffs in poultry production. Niger. Poult. Sci. J., 5: 147-155.
45. Iyayi, E.A. and D.O. Okhankhuele, 2002. Cassava leaf meal and exogenous enzyme as supplements in broiler finisher diets. Trop. Vet., 20: 172-180.
46. Iyayi, E.A. and D.M. Losel, 2004. Cyanide detoxifition in cassava by-products by fungal solid state fermentation. J. Food Technol. Afr., 5: 48-51.
47. Aguihe, P.C., A.S. Kehinde, I.I. Ilaboya and P. Ogialekhe, 2016. Effect of dietary enzyme (Maxigrain®) supplementation on carcass and organ characteristics of broiler finisher chickens fed cassava peel meal based diet. Int. J. Res. Agric. For., 3: 1-6.
48. Dalolio, F.S., J. Moreira, D.P. Vaz, L.F.T. Albino, L.R. Valadares, A.V. Pires and S.R.F. Pinheiro, 2016. Exogenous enzymes in diets for broilers. Rev. Bras. Saude Prod. Anim., 17: 149-161.
49. Silveira, M., J. Zanusso, P. Rossi, F. Rutz and M. Anciuti *et al*., 2010. Efeito da peletização em dietas contendo complexo enzimático para frangos de corte. Ciênc. Anim. Bras., 11: 326-333.
50. Cardoso, D.M., M.P. Maciel, D.P. Passos, F.V. Silva, S.T. Reis and F.S. Aiura, 2011. Efeito do uso de complexo enzimático em rações para frangos de corte. Arch. Zootec., 60: 1053-1064.
51. Wang, Z.R., S.Y. Oiao, W.O. Lu and D.F. Li, 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.
52. Hajati, H., 2010. Effects of enzyme supplementation on performance, carcass characteristics, carcass composition and some blood parameters of broiler chicken. Am. J. Anim. Vet. Sci., 5: 221-227.
53. Adeyemo, G.O. and O.G. Longe, 2007. Effects of graded levels of cottonseed cake on performance, haematological and carcass characteristics of broilers fed from day old to 8 weeks of age. Afr. J. Biotechnol., 6: 1064-1071.
54. Davis, A.K., D.L. Maney and J.C. Maerz, 2008. The use of leukocyte profiles to measure stress in vertebrates: A review for ecologists. Funct. Ecol., 22: 760-772.
55. Sugiharto, S., M.S. Hedemann and C. Lauridsen, 2014. Plasma metabolomic profiles and immune responses of piglets after weaning and challenge with *E. coli*. J. Anim. Sci. Biotechnol., Vol. 5. 10.1186/2049-1891-5-17
56. Akinwutimi, A.H., U.K. Oke and S.F.A. Basiekong, 2004. Observation on blood constituents of broiler finisher birds fed toasted lima bean (*Phaseolus lunatus*). Proceedings of the 9th Annual Conference ASAN, September 13-16, 2004, Eboyi State University, Abakaliki, pp: 60-67.
57. Obidinma, V.N., 2009. Brewers spent grain as energy source in finisher broiler birds and laying hen production. PhD Thesis, Imo State University Owerri, Nigeria.
58. Sugiharto, S., T. Yudiarti and I. Isroli, 2016. Haematological and biochemical parameters of broilers fed cassava pulp fermented with filamentous fungi isolated from the Indonesian fermented dried cassava. Livest. Res. Rural Dev., Vol. 28, No. 4.
59. Adejinmi, O.O., J.O. Adejinmi and I.O.A. Adeleye, 2000. Studies on the heamatology and serum biochemistry of broiler fed varying levels of soldiers fly Larvae meal diets. Trop. Anim. Prod. Invest., 3: 169-179.
60. Aduloju, Y.O., 2000. Blood composition of broiler chicks fed raw or processed full-fat soybeans supplemented with *Saccharomyces cerevisae*. B.Sc. Thesis, Department of Animal Production, Unilorin, Nigeria.
61. Mohammed, N.O. and O.B. Oloyede, 2009. Haematological parameters of broiler chicks fed *Aspergillus niger*-Fermented *Terminalia catappa* seed meal-based diet. Global J. Biotechnol. Biochem., 4: 179-183.
62. Bawa, G.S., T.S.B. Tegbe, S.O. Ogundipe, I.I. Dafwang and E.A. Abu, 2003. The effect of duration of cooking of lablab seeds on the level of some antinutritional factors. Proceedings of the 28th Annual Conference NSAP, (NSAP'03), Ibadan, Nigeria, pp: 213-215.
63. Ogundipe, S.O., F.O. Abeke, A.A. Sekoni, I.I. Dafwang and I.A. Adeyinka, 2003. Effects of duration of cooking on the utilization of *Lablab purpureus* beans by pullet chicks. Proceedings of the 28th Annual Conference of the Nigerian Society for Animal Production, March 16-20, 2003, Ibadan, Nigeria, pp: 233-235.
64. Dudusola, I.O., 2010. Comparative evaluation of internal and external qualities of eggs from quail and guinea fowl. Int. Res. J. Plant Sci., 1: 112-115.
65. Ojeniyi, F.G., M.O. Oke and D.B. Oke, 2010. Effect of methionine and lysine supplementation on the carcass quality of finisher broiler fed dried layers dropping meals as replacement for fish meal. Proceedings of the 35th Annual Conference of Nigerian Society for Animal Production, March 14-17, 2010, University of Ibadan, Ibadan, Nigeria.
66. Abeke, F.O., 2005. Evaluation of the nutritive value of lablab purpureus beans in replacement for groundnut cake in poultry diets. Ph.D thesis, Ahmadu Bello University, Zaria, Nigeria
67. Toleun, C.D. and F. Igba, 2007. Growth and carcass characteristics of broiler chickens fed water soaked and cooked velvet beans (*Mucuna pruriens*) meal. Proceedings of the 32nd Annual NSAP Conference, March 18-21, 2007, Calabar, Nigeria, pp: 240-243.