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Hematological and Serum Biochemical Characteristics of Broiler Chickens Fed Graded Dietary Levels of Cassava Peel Meal And Palm Kernel Cake Based Diets Supplemented With Exogenous Enzyme

Ukorebi, B. A. and John, U. E.
Corresponding author: basseyukorebi@gmail.com; 09051684811
Department Of Animal Science, Cross River University of Technology, Calabar, Nigeria Target Audience: Animal Scientists, Feed millers, Farmers
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Abstract
An eight week study was carried out to evaluate the hematological and serum biochemical characteristics of broiler chickens fed graded dietary levels of cassava peel meal (CPM) and palm kernel cake (PKC) based diets supplemented with exogenous enzyme (Nutrizyme®). A total of126 Anak 2000 broiler chicks (day-old), used in the investigation were randomly assigned six dietary treatments of 21 birds each. The treatments were sub-divided into three replicates containing 7 birds each in a 3x2 factorial design. All hematological parameters but one (Hemoglobin, Red blood cell, Packed cell volume, Mean cell volume, Mean cell hemoglobin, and Mean cell hemoglobin concentration) showed significant (P<0.05) treatment effect, whilst White blood cell was not significantly (P>0.05) affected by treatments. Similarly, Serum parameters: Total serum protein, Albumin, globulin, triglycerides and total cholesterol were significantly (P<0.05) different among the treatments, whilst the serum enzymes: ALP, ALT, and AST, were not significantly (P>0.05) different among the treatment means. Other serum parameters that indicated no significant (P>0.05) treatment effect were: Total bilirubin, direct bilirubin, and uric acid.The result obtained suggests that up to 100% replacement of maize with CPM + PKC combo (in a ratio of 1:1) in broiler chicken diets, with enzyme supplementation could be achieved in rearing broiler chickens without deleterious effects.
Key words: *cassava peel meal, palm kernel cake, enzyme supplementation hematology, serum biochemistry, broiler chickens*

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I. Introduction

Livestock industry is of socio-economic and nutritional importance for nations worldwide. Apart from providing livelihood for a significant population of the farming families of the rural communities, it contributes significantly to the Gross Domestic Product (GDP) of nations [1]. Notably, the success of the industry hinges basically on nutritional and health management of the animals. In recent times in Nigeria, the industry has witnessed a serious setback due largely to inadequate feed supply which stems from unavailability of protein concentrates and energy feed stuffs. Indeed, this problem has been aggravated by the competitive demand for some of the existing feed ingredients like maize, millet and soybean for livestock feeding, human consumption, as well as industrial uses. This dismal trend has been responsible for the prohibitive cost of finished and livestock products generated from the same. Maize as a conventional feedstuff contributes 45 – 55% of most poultry diets as energy source [2], this has adversely affected the cost of pigs and poultry which depend almost entirely on concentrate feeds.

Nigeria, like most countries of the world is witnessing a steady increase in human population, this has no doubt influenced the demand for protein of animal origin [3], which could be met through harnessing the great nutritional potentials of broiler meat. Broilers are fast growing meat type birds. When compared to the beef industry, poultry enjoys a relative advantage of easy management, high turnover, and quick return on investment [4]. The consumption of broiler meat in Nigeria outstrips that of most other livestock products except beef [5]. This is because of the absence of religious and traditional taboos against poultry meat production and consumption, the fast growth of poultry and its relative affordability. As already pointed out however, the biggest constraint to broiler production in Nigeria is cost of feed, which accounts for about 70 – 80% of the recurrent expenditure in intensive broiler production [6]. For broilers to convert feed into profitable products, there must be adequate calorie intake, sufficient supply of amino acids in suitable proportion and the provision of necessary vitamins and minerals. The increasing demand for maize and soybean as staple food for humans and as livestock feed ingredients has pushed their prices to a prohibitive level, as a result, the use of non-conventional feed stuff is gaining more recognition.

[7] had observed that a major solution to the problem of rising cost and scarcity of protein and energy feed stuffs for non-ruminant feeding programs is seeking new and non-conventional feed resources which are able to replace certain portions of maize in broiler rations without any deleterious effect on performance and health.

A cheaper and readily available potential alternative feedstuff is cassava peel. Cassava peel is the outer covering of the tuber which is usually removed and discarded when the tuber is being processed into various human foods such as garri, fufu, and tapioca among others. According to [8], cassava peel contain the following on proximate basis: 82.55% Dry matter, 5.46 crude protein, 18.81%, crude fibre, 1.75% Ether extract, 70.67% Nitrogen Free Extractive (NFE) and 5.68% Ash. In Nigeria, cassava peel is a waste disposal problem, it is allowed to rot away in heaps, thus contributing to environmental pollution and health hazard concern to humans (Oboh, 2006[9]. Cassava peel contain hydrocyanic acid (NCN) which is toxic to monogastrics, this constitutes a limitation to its incorporation in diets meant for monogastric animals However, if properly harnessed by a biotechnological processing, cassava peel can be a useful feed resource to animals. To this end, several processing methods have been experimented [10, 11, 12, 13, 14, 15, 16, 17, 18]. The processing method adopted in this research was sun drying, employed by [10, 14, 15]. The use of cassava peel in the formulation of broiler diets at inclusion rate of 50% has been recommended because of its positive outcome in feeding trial [17].

Another potential non-conventional feedstuff is palm kernel cake (PKC). It is generated as a residue after the extraction of palm kernel oil from palm nuts. Depending on the efficiency of the process of palm kernel oil extraction, PKC can contain 12 – 32% crude protein [18]. Incorporation of PKC in livestock and poultry diets is limited by its high fibre content, its gritty nature, un-palatability, relatively low availability of its amino acids, and high cupper content [19]. According to [20], PKC is suitable for use in feed formulation for swine, poultry and horses. It is cheaper than conventional feed stuffs used as protein sources and is readily available. Reports by [21] posited that up to 121,520 metric tons of PKC is produced in Nigeria annually. According to [19], PKC can be fed to starter and finisher broiler chickens at 28% and 35% inclusion levels, respectively without deleterious effects on performance. However, earlier reports by [20] indicated that broilers can tolerate up to 20% PKC in their diets without recording negative effects in their growth performance and feed efficiency. Research reports [22, 23], indicate that as the level of fibre in monogastric diets increases, digestibility of nitrogen and energy decreases. According to [24], fibre decreases availability of nutrients by reducing the period of exposure of the food to digestive enzymes and absorptive surfaces due to increased rate of passage of the feed induced by fibre its content. A large proportion of the fibre content of PKC is present as non-starch polysaccharide (NPS), these usually act as physical impediment to nutrient hydrolysis by endogenous digestive enzymes, as well as absorption and utilization of nutrients by monogastric animals. Reports by [25]indicated that endogenous enzymes in broilers cannot adequately digest NPS, this results in increased digesta viscosity, low nutrient digestibility and absorption. Research work has suggested that the negative effects of NSPs can be overcome by supplementation of diets with suitable exogenous enzyme preparations [26, 27]. This research was carried out to harness the potentials of exogenous enzyme to improve the digestion, absorption and utilization of cassava peel meal and Palm kernel cake in the diets of broiler chickens.

Exogenous enzymes are commercial enzyme preparations designed to increase the digestibility of pigs and poultry diets rich in fibre. Among the non-nutrient additives, the use of enzymes is considered easy, economical and non-hazardous to human health [28, 29, 30]. The use of exogenous enzymes in poultry diets, has found widespread commercial acceptance as a strategy to improve nutrient utilization and performance, and to reduce feed cost and nutrient excretion. In addition, the inclusion of exogenous enzymes in the diets of animals also results in the reduction of pathogenic micro-flora, and the improvement of health and welfare of birds [31, 32].

Nutrizyme®is a multi-enzyme complex containing hemicellulose, phytase, arabinases, pectinase, amylase, β-glucanases, cellobiase, xylanase and lipase.Commercial feed enzymes with proven efficiency for animal husbandry include, Xylanase, Arabino-xylanase, β-glucanase, Cellulase [33], Proteases [34], and Phospholipase [35]. There is paucity of information on enzyme supplementation of poultry diets containing CPM and PKC to facilitate the liberation of nutrients in the diets such that they will be efficiently utilized by the birds and enhance performance, this is one of the reasons this research was embarked upon.

Studies on the nutrition of animals need to go beyond growth response, nitrogen balance and efficiency of feed utilization. It is also important to monitor the influence of nutrition on the metabolism of cells in the various tissues [36]. This is why hematological and serum biochemical procedures are important in animal nutrition studies. The blood contains several metabolites which provide useful information on nutritional status and clinical investigation of an individual, hence the World health Organization (WHO)recommended the use of blood parameters for medical and nutritional assessments [37, 38]. According to [39], Hematological and serum biochemical components of the blood of an animal have been found to be influenced by the quantity and quality of its feed. Reports by [40] stated that hematological constituents reflect the physiological responsiveness of the animal to its internal and external environments which include feed and feeding. In the same vein, the serum biochemical components of the blood provide information on the hepatocellular and renal integrity of animal subjects, their electrolyte balance, dietary protein quality, and nutritional or metabolic parameters. In general therefore, the blood picture of an animal is a reflection of its general health status and welfare.The foregoing suggest that blood analysis should be an integral part of animal nutrition studies.

II. Materials and Methods

Experimental Site

This study was conducted in the Animal Science unit of the Teaching and Research farm of the faculty of Agriculture and forestry, Cross River University of Technology (CRUTECH) Obubra Campus, Cross River State. The location lies along longitude 8^o – 9^oE and latitude 6^o–7^oN of the equator, with a warm weather and ambient temperature of about 21 - 30^oC and has an annual rainfall of 500 – 1070mm [41].

Cassava Peels/PKC procurement.

Cassava peels were collected from garri producing families in Ovonum Village in Obubra Local Government Area of Cross River State, washed and sun dried for 3 – 5 days to reduce the toxic contents (HCN). The dried cassava peels were milled using hammer mill to produce cassava peel meal.

Palm kernel cake and Nutrizyme were purchased from feed ingredients shops in Calabar and Owerri, respectively in Nigeria.

Experimental Bird/Design

A total of 126 Anak 2000 broiler chicks for the trial were purchased from a reputable local distributor at day-old. They were randomly divided into 6 groups of 21 birds each. At the starter phase of the experiment, the groups were assigned to 6 energy level diets (2742.43 ME (kcal/kg) - 3048.12 ME (Kcal/kg) and a single protein level diet (23.0% crude protein), in a 3X2 factorial arrangement involving three levels: 0, 25 and 50% of cassava peel meal/PKC mixture (in a ratio of 1:1) and two enzyme levels (0 and 0.25%). Each treatment was replicated three times with 7 birds per replicate and housed in a separate pen. A similar arrangement was used in the finisher phase of the experiment, during these period the diets were appropriately adjusted to suit the needs finisher broilers by reducing the protein level, and increasing the energy level.

Table 1: Gross Composition of Experimental Broiler Starter Diet (g/100g)

Ingredients	**Treatment/% Enzyme Level**					
	$T_{1,}$ 0.00E	$T_{1,}$ 0.25E	$T_{2,}$ 0.00E	$T_2$0.25E	$T_3$0.00E	$T_3$0.25E
Maize	50	50	25	25	-	-
CPM+PKC(1:1)	-	-	25	25	50	50
SBM	32.39	32.54	34.40	34.55	32.17	32.32
Wheat offal	9.17	9.31	0.70	0.30	2.93	2.55
Fish meal	3.5	3.5	3.5	3.5	3.5	3.5
Palm oil	-	-	7.0	7.0	7.0	7.0
Bone meal	3.0	3.0	3.0	3.0	3.0	3.0
NaCl	0.3	0.3	0.3	0.3	0.3	0.3
Methionine	0.3	0.3	0.3	0.3	0.3	0.3
Lysine	0.3	0.3	0.3	0.3	0.3	0.3
Nutrizyme	-	0.25	-	0.25	-	0.25
Vit./Tm premix*	0.5	0.5	0.5	0.5	0.5	0.5
TOTAL	100	100	100	100	100	100
Calculated Chemical Composition Crude protein (%)	23.00	23.00	23.00	23.00	23.00	23.00
Crude fibre (%)	3.97	3.94	5.52	5.49	7.75	7.73
ME (Kcal/kg)	2869.78	3048.12	3048.12	3044.69	2745.86	2742.43

* To provide the following per kg of diet: Vit. A, 10,000iu; Vit D_3 2,000 iu; Vit. E, 5ui; Vit K 2mg; Nicotinic acids, 20mg; Vit. B12, 10.01mg; Pantothenic acids, 56mg; Fe, 20mg; Cu, 10mg Zn, 50mg; Co, 125mg.

Table 2: Gross Composition of Experimental Broiler Finisher Diets (g/100g)

Ingredients	**Treatment/% Enzyme Level**					
	$T_1$0.00E	$T_1$0.25E	$T_2$0.00E	$T_2$0.25E	$T_3$0.00E	$T_3$0.25E
Maize	54.00	54.00	27.00	27.00	-	-
CPM+PKC(1:1)	-	-	27.00	27.00	54.00	54.00
SBM	23.56	23.72	25.61	25.68	22.99	23.09
Wheat offal	14.55	14.13	5.49	5.07	8.11	7.79
Fish meal	3.50	3.50	3.50	3.50	3.50	3.50
Palm oil	-	-	7.00	7.00	7.00	7.00
Bone meal	3.00	3.00	3.00	3.00	3.00	3.00

Na Cl	0.30	3.00	0.30	0.30	0.30	0.30
Methionine	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.30	0.30	0.30	0.30	0.30	0.30
Nutrizyme	-	0.25	-	0.25	0.25	-
Vit./Tm premix*	0.50	0.50	0.50	0.50	0.50	0.50
TOTAL	100	100	100	100	100	100
Calculated Chemical Composition Crude protein (%)	19.50	19.50	19.50	19.50	19.50	19.50
Crude fibre (%)	3.88	3.86	5.61	5.58	8.02	8.00
ME (Kcal/kg)	2862.67	2859.13	3016.97	3111.78	2778.78	2775.34

* To provide the following per kg of diet: Vit. A, 10,000 iu; Vit. D_3 2,000iu; Vit. E, 5iu; Vit. K 2mg; Riboflavin, 4.20mg; folic acid, 0.5mg; Choline, 3mg; Mg, 56mg; Fe, 20mg; Cu, 10mg; Zn, 50mg; Co, 125mg.

Management of Experimental birds

Normal brooding was carried out until the birds were three weeks old. Feed and water were provided *ad libitum*for all treatment groups throughout the experimental period. Also adequate prophylactic medications and vaccinations were carried out. The experiment lasted for a total of 8 weeks (56 days).

Data Collection

At the end of the feeding trial period of 56 days, six birds per treatment were selected for bleeding, this included two birds per replicate (the highest and lowest weighing bird in the replicate). Blood sample collection was carried out between 8.00am and 9.30am, this was sampled from the punctured webal sub-clavian vein with a 5ml scalp vein needle. A total blood volume of 7ml was aspirated from each bird, 2ml of which was discarded into ethylene di-amine tetra acetic acid (EDTA) treated Bijou bottles for hematological assay, while the remaining 5ml was allowed to coagulate in plain vial bottles (without anti-coagulant), to produce serum for blood chemistry evaluations.Accordingly, triplicate determinations were made per parameter, after which an average was taken.

Hematology

Un-coagulated blood samples were analyzed within three hours (3hours) of their collection for total erythrocyte (RBC) and leukocyte (WBC) counts, hematocrit (PCV), and hemoglobin concentration (HC).Other hematological indices (mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC)) were calculated from results obtained.

The concentration of blood hemoglobin in the test samples were estimated according to the cyanomethaemoglobin method of Alexander and Griffiths [42]. The packed cell volume (PCV) was measured by a micro hematocrit capillary tube using a Hematocrit reader. Erythrocyte concentration (RBC), and leucocyte concentration (White blood cell count), were measured using an automated cell counter within 24 hours after blood collection. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated according to the methods of [43].

Serum biochemical parameters

The samples separated for serum biochemistry were centrifuged at 3000 rpm for ten minutes to separate serum. Thereafter, the harvested sera were used for evaluation of total serum protein (TSP) and serum albumin (SA). Globulin was determined by calculation, which is by the difference between Total serum protein and serum Albumin. Cholesterol was determined using fresh serum (kits based).Other serum parameters investigated included aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP). The activities of these enzymes were determined using spectrophotometric methods. Total or unconjugated bilirubin (TB) and direct or conjugated bilirubin (DB), uric acid and serum lipids were determined by the use of automated chemistry analyser. All serum biochemical analyses were done according to standard methods described by [44].

Statistical Analyses

Data on hematology and serum biochemistry were subjected to factorial analysis of variance (ANOVA) as outlined by [45]. Where ANOVA detected significant treatment effects, means were separated using the Duncan's New Multiple Range Test (DNMRT) as outlined by [46]

III. Results

Hematology

The effect of enzyme supplementation of dried cassava peel meal and palm kernel cake based diets on the hematology of broiler chickens are presented on Table 3. The results show that White blood cell (WBC) was significantly ($P<0.05$) affected by the treatments, whereas packed cell volume (PCV), hemoglobin concentration

(Hb) and erythrocyte concentration (RBC), indicated no significant treatment effects (P>0.05). Other hematological indices that were not significantly (P>0.05) affected by treatments were the Mean cell volume (MCV), Mean cell hemoglobin (MCH) and Mean cell hemoglobin concentration (MCHC).

Table 3: Effect of enzyme supplementation of Dried Cassava peel meal and PKC base diets fortified with palm oil hematological indices of broiler chickens

	TREATMENT/%ENZYME LEVEL						
PARAMETERS	**T_1, 0.00E**	**T_1,0.25E**	**T_2,0.00E**	**T_2,0.025E**	**T_3,0.00E**	**T_3 0. 25E**	**SEM**
Hb (g/dl)	9.30^b	11.50^a	6.21^c	11.72^a	6.11^c	11.62^a	0.44
RBCX10^3/M	2.50^b	3.01^a	2.00^c	3.01^a	2.00^c	3.01^a	0.13
PCV(%)	41.10^b	44.53^a	31.78^c	44.68^a	30.50^c	44.11^a	0.41
WBCX10^3/M	10.50	10.51	10.49	10.53	10.50	10.52	0.45
MCV (FL)	100.35^b	108.01^a	50.02^c	110.15^a	48.99^c	108.48	2.04
MCH (PG)	34.58^b	43.28^a	25.14^c	44.39^a	24.82^c	43.75^a	1.03
MCHC (G/DL)	28.41^b	33.52^a	24.81^c	33.83^a	24.23^c	32.98	1.01

$^{abc\ldots}$**Means on the same row with different superscript are significantly different (P<0.05).**

Serum biochemical parameters

The effect enzyme supplementation of dried cassava peel meal (CPM) and Palm kernel cake(PKC) based diets on the blood chemistry of broiler chickens are reported in Table 4. The results show thatAlkaline phosphatase (ALP), Alanine transaminase (ALT), Aspartate transaminase (AST), Total bilirubin (TB), and Direct bilirubin (DB) were not significantly affected by treatments.

Table 4: Effects of enzyme supplementation of dried cassava peel meal and PKC based diets fortified with palm oil on serum biochemical indices of broiler chickens

	TREATMENT/%ENZYME LEVEL						
PARAMETERS	**T_1, 0.00E**	**T_1,0.25E**	**T_2,0.00E**	**T_2,0.025E**	**T_3,0.00E**	**T3, 0.25E**	**SEM**
ALP (iu/ul)	571.02	570.11	570.89	570.00	570.20	571.01	73.41
ALT (ui/ul)	31.12	31.26	32.01	31.43	32.01	31.14	1.61
AST (iu/l)	89.14	90.02	89.04	90.01	89.53	90.01	2.01
Total bilirubin (Mg/dl)	0.56	0.61	0.56	0.61	0.61	0.56	0.08
Direct Bilirubin (Mg/dl)	0.24	0.24	0.20	0.24	0.20	0.24	0.04
Total serum protein (g/dl)	3.15^b	4.90^a	3.01^b	4.91^a	3.00^b	4.87^a	0.19
Albumin (mg/dl)	1.79^b	2.58^a	1.70^b	2.58^a	1.70^b	2.55^a	0.14
Globulin (Mg/dl)	1.36^b	2.32^a	1.31^b	2.33^a	1.30^b	2.32^a	0.12
Triglycerides (Mg/dl)	68.72^c	69.15^c	79.48^b	95.17^a	79.76^b	94.68^a	9.25
Cholesterol (mg/dl)	105.82^c	106.23^c	115.68^b	134.84^a	115.20^b	133.28^a	40.14
Uric acid (mg/dl)	5.44	5.35	5.13	5.44	5.14	5.78	0.68

$^{abc\ldots}$ **Means on the same row with different superscripts are significantly different (P<0.05).**

Serum parameters that differed significantly (P<0.05) include Total serum protein (TP), Serum albumin (ALB) and serum globulin. Others were uric acid, triglycerides, and cholesterol.

IV. Discussion

Hematological indices

PCV was highest in enzyme supplemented treatment groups (T_1, 0.25% E, T_2, 0.25% E and T_3, 0.25%E). This was followed by the treatment group fed with maize based diet not supplemented with exogenous enzyme (T_1, 0.00E). The lowest PCV values were recorded in CPM/PKC based diets not supplemented with exogenous enzyme. A similar trend was recorded for hemoglobin concentration, RBC, MCV, MCH, and MCHC. However, the values of WBC was statistically similar among the treatment means (P>0.05).

Blood represents a means of assessing the clinical and nutritional health status of the animal in feeding trials [47]. It has been established that hematological parameters are useful in monitoring feed toxicity, especially with feed constituents that affect the blood [48, 49,50]. Nutrients absorbed after digestion of food/feed are carried by the blood to the different cells and tissues of the body, if such nutrients are toxic, the physiology of the animal subject will be affected [51].Indeed, hematological indices such as RBC, WBC, PCV, and hemoglobin concentration, have been found useful in disease prognosis and therapeutic regimen, as well as feed stress monitoring [52]. The RBC for instance functions primarily in the transport of oxygen from the lungs to body tissues, and removal of carbon dioxide from the tissues to the lungs through hemoglobin, in the course of the biological oxidation of nutrients known as tissue respiration. This process is crucial to the maintenance of other biological functions in vertebrate organisms.Reports by [53] indicated that Hb falls gradually in animals on low protein intake, parasitological infestation and/or liver damage or anemia. This is in agreement with the reports of [54], which posited that dietary protein may affect the physiological process involved in erythropoietin production. In the current investigation, there were no significant differences (P>0.05) among the treatment means in the value ofwhite blood cells (leucocytes). It could be that the experimental birds were not challenged disease infections by pathogenic organisms as a result of the high bio-security/hygiene protocols during period of study. On the other hand, it might be as a result of the lack of capacity of any of the treatment diets to equip the birds the ability to produce anti-bodies to fight against a local infection challenge in experimental birds. The later scenario is however very unlikely because the WBC count cross the treatments were within the normal range, and the birds were not sick. This pre supposes that immune system of the birds in the test and control groups were not compromised. Major functions of leucocytes and its differentials include, fighting infections, defense of the animal body against invasion by pathogenic organisms, by the process of phagocytosis; production, transport and distribution of antibodies by immune response. That is why animals with low white blood cells are generally more vulnerable to disease infection, while those with higher white blood cell status have a relatively greater degree of resistance against the same [28]. Animals with higher white blood cells count are also capable of enhanced adaptability to local environmental disease and disease prevalent conditions [55, 56].

The normal ranges of hematological parameters in chickens are, Hb: 7.0 – 13.0g/dl, PCV: 40.0 – 45.0%, RBC: 3.0 – 3.5 x10^6ul, and WBC: 9.0 – 31.0 (10^3ul) [57, 58]. Others are, MCV: 90.0 – 140.0 Fl, MCH: 33.0 – 47.0 pg/cell, and MCHC: 26.0 – 35.0 (g/dl) [59]. In the present study, all hematological parameters apart from WBC in treatment groups receiving exogenous enzyme supplemented diets (T_1, 0.25% E, T_2, 0.25% E and T_3, 0.25% E), were significantly (P<0.05) higher than the non-enzyme treated diet groups (T_1, 0.00 E, T_2, 0.00E and T_3, 0.00E). This suggests that the commercial feed enzyme supplemented diets provided a better blood building capacity than the non-enzyme treated diets (including the conventional diet, T_1, 0.00E). The result also indicates that even the conventional diet could supply more nutrients for better blood building if supplemented with feed enzyme as demonstrated by T1, 0.25 E. This is in agreement with the reports of [31, 32] which posited that the inclusion of exogenous enzymes in diets of animals also results in the reductions in pathogenic micro flora and the improvement in health and welfare of birds. The present study also shows that all hematological parameters of birds in treatment groups where feed enzyme treated diets were fed were within the normal ranges reported for chickens [57, 58,59].

Serum biochemical parameters

The liver enzymes which include Alkaline phosphatase (ALP), Alanine transaminase (ALT), and Aspartate transaminase (AST), were statistically similar across the treatments. The enzymes are important in monitoring the proper functioning of the liver [60].When the liver cell are damaged or diseased, the enzymes leak into the general blood circulation, thus an increased serum concentration of the enzymes indicates a compromised hepatocellular integrity and function [51].Functions of the liver include, filtering blood from the digestive tract, processing of waste products, and bioremediation. Others are, production of proteins, such as albumin, globulin and prothrombin (a factor that functions in blood clotting), production glucose (blood sugar), and lots more. These underscore the need for healthy hepatocytes [51]. Research reports [39, 40, 41] have also observed that a high value of ALP suggests increased activity of the liver due to presence of toxic substance [61, 62, 63]. Whilst low levels of the enzyme (below the normal range), can be a sign of protein deficiency arising from malnutrition or advanced liver disease. The liver enzymes in the present study were within the normal ranges reported by [64, 65]. This indicates that the experimental diets were nutritionally safe.

The values of total (unconjugated) bilirubin, and direct (conjugated) bilirubin were similar (P>0.05) across the treatments. Bilirubin is primarily a product of the breakdown of the hem moiety of hemoglobin. Elevated concentration of serum bilirubin is likely a result ofincreased destruction of the red blood cells or decreased removal of the compound (bilirubin) itself from the blood stream, occasioned by liver disease. The aspects of hematological and liver enzyme investigations of the current is study have shown that the red cell indices and hepatocellular integrity, respectively were not compromised. This suggests that the serum bilirubin values obtained were normal.

Serum total protein and serum albumin values obtained in the study were within the normal range for chicken reported by [64, 65], however, they were significant differences among the treatment means (P<0.05). The highest significant values were recorded by the enzyme supplemented treatment groups (T1, 0.25% E, T2, 0.25% E and T3, 0.25% E). Serum total protein is made up of albumin (ALB) and globulin (GLOB). Globulin was calculated as a difference between total protein and albumin. The calculated values of globulin also followed a trend similar to total protein and albumin. It has been observed that the value of serum total protein is a function of both the quality and quantity of dietary protein [66]. Increased serum total protein reflects the ability of the animal to retain protein when they have reached the maximum capacity for protein intake [67, 68]. However, excessively high serum total protein could be a sign of chronic infection or inflammation, it can also be an early sign of bone marrow disorder. On the other hand, low level of total protein is attributable to malnutrition, liver disease or even digestive disorder, in which case the system has an impairment in absorption of dietary protein [51].

According to [69], Albumin is responsible for transporting insoluble substances in the blood, and aids in the maintenance of oncotic pressure. Increased concentration of serum albumin may be a sign of dehydration, whilst a decreased concentration is attributable to sub-optimal hepatocellular functioning, traceable to such factors as malnutrition and infection. The combination of albumin with globulin in a normal ratio promotes normal water retention in the blood. [66] observed that globulin and albumin, as well as urea concentrations are indicators of the quality and quantity of protein supplied in the diet. Data generated in this study on serum total protein, albumin and globulin have shown that treatment diets supplemented with feed enzyme provided better/quantity for experimental birds.

The values of uric acid in the study were not significantly ($P>0.05$) affected by treatments. It was within the normal range reported by [70]. Uric acid is the major end-product of nitrogen metabolism, it is produced by the liver and constitutes the major proportion of total nitrogen excreted in avian urine by the renal tubules. High protein intake, increased protein metabolism, stress and dehydration influence the concentration of uric acid in the blood [71]. Measurement of serumuric acid is used as an index of renal function in birds. The serum uric acid concentrations of birds, either in test or control groups of the present study do not portray impairment in renal function of experimental birds.

The values of cholesterol showed significant treatment effect ($P<0.05$), however, they were within the normal range reported by [65]. Cholesterol is synthesized within the cell from dietary fat. In the present investigation, the highest concentration of serum cholesterol were recorded by CPM/PKC based diets which were fortified with palm oil. It may be that the dietary palm oil enhanced the synthesis of saturated fats that led to increased formation of cholesterol in the birds whose diets were fortified with palm oil. High level of cholesterol is a risk factor in cardiovascular disease. In the present study however, such condition would seem unlikely because the recorded cholesterol levels are within the normal reported ranges (87 – 192mg/dl) [65].

The triglyceride levels in the study differed significantly ($P<0.05$). Their concentrations followed a pattern similar to those of cholesterol. Triglycerides are synthesized in the liver from fatty acids, proteins and glucose, when these nutrients are above the body's current needs [72]. It is thought that palm oil used to increase the energy value test diets (CPM/PKC based diets), might have caused the significantly ($P<0.05$) increased concentration of triglycerides in experimental birds fed diets fortified with palm oil. High levels of triglycerides in combination with high level of cholesterol can increase the risk of heart disease. There have been conflicting reports on the impact of triglycerides inLDL-C, and HDL-C, the bad and good cholesterol, respectively. Since the levels of triglyceride in the current study were within normal limits reported by [65], it would seem that the test diets were nutritionally safe and healthy.

V. Conclusion

Findings of the investigation suggest that the use ofCassava peel meal (CPM) and palm Kernel cake (PKC) combo in the ratio of 1:1, as energy source, if supplemented with exogenous enzyme and fortified with palm oil, could successfully achieve up to 100% replacement of maize in the diets for rearing of broiler chickens without any health hazards. It is also concluded that supplementation of maize based diets with commercial feed enzyme can enhance its nutritive value and capacity in broiler chicken production.

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