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Haematological and Serum Biochemical Profiles of Broiler Chickens Fed Graded Levels of Fermented Palm Kernel Cake

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ABSTRACT

The study aimed to investigate the haematological and serum biochemical indices of broiler chickens fed graded levels of fermented palm kernel cake (FPKC). The birds were allotted into four dietary treatments in a completely randomized design. Each treatment was replicated four times and five birds were allocated into each replicate with twenty birds per treatment. The FPKC was included at 0%, 10%, 20%, and 30% levels in diets 1, 2, 3 diets and 4 respectively. At the end of the 56 days of feeding trial blood samples were collected from the birds and were subjected to haematological and serum biochemical analysis. The results revealed that the birds on control diet (0% FPKC) had significantly higher values of Hb, PCV, RBC, monocytes and granulocytes compared to other treatment groups, while the values of WBC, MCV, MCH, MCHC and lymphocytes were significantly higher in the birds fed diet 2 (10% FPKC) compared to the control and other FPKC groups. The biochemical parameters: total serum protein, globulin, ALT, ASP, ALP and cholesterol were significantly higher among the birds fed diet 2 (10% FPKC). The study concluded that FPKC did not pose a health challenge to broiler chickens, but improved health, immunity and performance can be achieved at the 10% inclusion level.

***Keywords*—Palm kernel, serum biochemical, haematological, mannanolytic bacteria, fermentation.**

I. INTRODUCTION

Palm kernel cake is an agro by-product derives from palm kernel, is among the most abundant and potentially cost effective agro by-products available locally in Nigeria. PKC supply moderate amount of nutrients which make it feasible as a partial substitute for soyabeans meal (SBM) and corn in poultry nutrition [1].

The major constraint to the use of PKC in the poultry's diet is the presence of the concentrated amount of non-starch polysaccharides (NSPs), such as Mannan, xylan and cellulose [2]. Additionally, palm kernel cake contains high level of crude fiber (CF), coarse texture and gritty appearance [3] all which resulted to

low digestibility of PKC most especially in monogastric animals because of its high non-starch polysaccharides (NSPs) content [4]. Due to these constraints, it appears necessary to improve its quality to make it more useful in poultry diet. Several attempts have been made to determine the influence of fermentation on the palm kernel cake in order to improve the quality of PKC using fungi in solid state fermentation (SSF) [5][6]. Although fungi have many characteristics and its produces higher enzyme activity than bacteria, but it was revealed that the fungi metabolites produced from the fungi may negatively affect the performance of the birds because the secondary products from fungi, such as mycotoxins, could depress the growth of animals. Replacing fungi with cellulolytic bacteria in SSF can be applied to overcome the mycotoxin problem [7]. Also, the amount of amino acids in the PKC increased as a result of SSF by cellulolytic bacteria [8].

Cellulolytic and mannanolytic bacteria such as Bacillus subtilis can be applied to reduce fibre content of PKC that are otherwise indigestible by the monogastrics through biodegradation process [9]. It improves the host nutrition and gut health through maintaining the balance of intestinal microbiota this makes it to function as probiotic microbes [10]. Bacillus subtilis can produce several enzymes such as protease, B-mannanase and cellulase that are useful in aiding digestion so that fibrous feed materials can be digested. The fermentation process makes PKC a good feed ingredient, promoting better overall health compared to raw PKC. Studies show that fermented PKC keeps hematological parameters within normal, healthy ranges for broilers, indicating its strong nutritional value on blood constituents [11].

Therefore, this study is geared at determining the influence of diets containing varying levels of fermented palm kernel cake on hematological and serum biochemical profiles of broiler chickens.

II. MATERIALS AND METHODS

A. Study Area

This research was carried out at the Poultry Research Unit of Teaching and Research Farm of Department of Agricultural Technology, Federal Polytechnic, Ado-Ekiti located in the southwestern zone of Nigeria.

B. Preparation of Test Ingredient

The Bacillus subtilis was cultured in the Microbiology laboratory of Department of Science Technology, Federal Polytechnic Ado-Ekiti. The palm kernel meal was obtained from feedstuff dealer in Ado Ekiti, it was sieved to separate the nutshell from it and was ground to fine particle size. The milled palm kernel cake was autoclaved for 20 min at 121^0C and then cooled to room temperature and was thoroughly mixed with sterile water. The 60 kg moist palm kernel cake was inoculated with 6g Bacillus subtilis and

incubated under anaerobic conditions at room temperature for 7days. The fermentation process was conducted according to methods developed by [12] and [13]. After the fermentation was terminated, the fermented palm kernel cake was oven-dried at 50^0C for 48 hours and was packed inside airtight containers prior to its use in feed formulation.

C. Experimental Design

The broiler chicks were randomly assigned to four (4) treatments in a completely randomized design (CRD). Each treatment was replicated four times with five (5) birds per replicate and 20 birds per treatment.

D. Management of Experimental Birds

A total of eighty (80) day old broiler chicks were weighed on arrival and were allocated to the four dietary treatments. Each treatment was replicated four times with twenty (20) birds per treatment and five (5) birds per replicate. Feed and water were supplied ad libitum throughout the period of the experiment. Standard management practices were strictly observed. The experiment lasted for 56 days.

E. Experimental Diet

Four diets were formulated to meet nutrient requirements of broiler chicks. A maize-soyabean diet served as the control diet. Fermented Palm Kernel Cake (FPKC) was included in the diets at varying levels of 0%, 10%, 20% and 30% at the expense of maize and soyabean meal in the control diet and were designated as diet 1, 2, 3 and 4 respectively. The experimental diet containing varying levels of fermented palm kernel cake as follows: Diets 1 - (control) 0% of FPKC, Diet 2 - 10% of FPKC, Diet 3 - 20% of FPKC, Diet 4 - 30% of FPKC. The feed composition for starter and finisher broiler diets are shown in Tables 1 and 2 respectively.

F. Collection of blood sample for haematological and serum biochemical analyses

At the end of the feeding trial (56th day), three birds were selected randomly from each treatment, blood samples were collected by severing their jugular veins. Blood samples were collected into sterile bottles containing anti-coagulant EDTA (ethylene diamine tetra-acetic acid) and were used to determine the haematological parameters using the standard procedures by [14]. Blood samples used for serum biochemical analysis were collected into plain sterile sample bottles without coagulant and these were used to determine the biochemical components according to the method of [15].

G. Statistical Analysis

The data collected was subjected to one-way analysis of variance (ANOVA) in a completely randomised design arrangement using the Statistical Package for Social Scientists [16]. Significant means were

separated using Duncan's Multiple Range Test.

Table 1: Composition of Broilers Starter Diet Containing Fermented Palm Kernel Cake (0 – 28 days)

Ingredients	Dietary Treatments			
	FPKC (0%)	FPKC (10%)	FPKC (20%)	FPKC (30%)
Maize	48	43.2	37.0	32.4
Soybean meal	36.1	33.9	32.1	28.7
Wheat offal	10	7	5	3
Vegetable oil	1.5	1.5	1.5	1.5
FPKC	-	10	20	30
Bone meal	2.0	2.0	2.0	2.0
Limestone	1.5	1.5	1.5	1.5
DL-Methionine	0.2	0.2	0.2	0.2
L-Lysine	0.2	0.2	0.2	0.2
Salt (NaCl)	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25
Total	100	100	100	100
Nutrient Analysis				
Crude protein (%)	22.54	22.45	22.12	22.00
Digestible energy (Kcal/kg)	2757.06	2709.9	2639.04	2579.86
Ether extract (%)	7.55	8.32	9.22	9.57
Crude fibre (%)	4.34	5.70	6.33	6.76

**Vitamin/Mineral Premix (Animal Care®) Vitamin A12000000IU, Vitamin D3 3000000IU, Vitamin E 30000mg, Vitamin K3 25000mg, Folic Acid 1000mg, Niacin 40000mg, Vitamin B2 5000mg, Vitamin B12 20mg, Vitamin B1 2000mg,Vitamin B6 3500mg, Biotin 80mg, Antoxidant 125000mg, Cobalt 250mg, Selenium 250mg, Iodine 1200mg, Iron 40000mg, Manganese 70000mg, Copper 8000mg, Zinc 60000mg Choline chloride 200000mg .*

Table 2: Composition of Broilers Starter Diet Containing Fermented Palm Kernel Cake (29 – 56 days)

Ingredients	Dietary Treatments			
	FPKC (0%)	FPKC (10%)	FPKC (20%)	FPKC (30%)
Maize	53.0	48.2	42.0	37.7
Soybean meal	29.6	27.4	25.6	22.9
Wheat offal	10	7	5	2
Vegetable oil	2	2	2	2
FPKC	-	10	20	30
Bone meal	3.0	3.0	3.0	3.0
Limestone	1.5	1.5	1.5	1.5
DL-Methionine	0.2	0.2	0.2	0.2
L-Lysine	0.2	0.2	0.2	0.2
Salt (NaCl)	0.25	0.25	0.25	0.25
*Premix	0.25	0.25	0.25	0.25
Total	**100**	**100**	**100**	**100**
Nutrient Analysis				
Crude protein (%)	20.12	20.00	20.07	20.01
Digestible Energy (Kcal/kg)	2753.16	2706	2635.14	2591.66
Ether extract	6.24	5.80	6.10	6.04
Crude fibre	5.66	6.78	7.20	7.48

**Vitamin/Mineral Premix (Animal Care®) Vitamin A12000000IU, Vitamin D3 3000000IU, Vitamin E 30000mg, Vitamin K3 25000mg, Folic Acid 1000mg, Niacin 40000mg, Vitamin B2 5000mg, Vitamin B12 20mg, Vitamin B1 2000mg,Vitamin B6 3500mg, Biotin 80mg, Antoxidant 125000mg, Cobalt 250mg, Selenium 250mg, Iodine 1200mg, Iron 40000mg, Manganese 70000mg, Copper 8000mg, Zinc 60000mg Choline chloride 200000mg .*

III. RESULTS

Table 3 below showed the result of haematological parameters of broiler chicken fed varying levels of bio-fermented PKC.

Table 3: Haematological parameters of broiler chicken fed varying levels of bio-fermented PKC

Parameters	Dietary Treatments				±SEM	*p*-Value
	FPKC (0%)	FPKC (10%)	FPKC (20%)	FPKC (30%)		
Haemoglobin (g/dl)	11.60^{a}	11.00^{ab}	10.60^{b}	10.00^{c}	0.21	0.03
Packed cell volume (%)	35.00^{a}	33.00^{b}	32.00^{c}	30.00^{d}	0.56	0.01
Red blood cell ($x10^{6}/mm^{3}$)	2.80^{a}	2.40^{b}	2.30^{b}	2.20^{b}	0.14	0.05
White blood cell ($x10^{3}/mm^{3}$)	4.00^{b}	6.00^{a}	3.40^{b}	3.20^{b}	0.36	0.04
MCV (fl)	125.00^{d}	137.50^{b}	139.13^{a}	136.36^{c}	2.00	0.02
MCH (pg)	41.67^{c}	45.83^{b}	46.09^{a}	44.45^{b}	0.67	0.05
MCHC (g/dl)	33.14	33.33	33.12	33.33	0.13	0.92
Monocytes (%)	4.00^{a}	0.00^{c}	0.00^{c}	2.00^{b}	0.51	0.01
Granulocytes (%)	30.00^{a}	22.00^{c}	18.00^{d}	28.00^{b}	1.44	0.03
Lymphocytes (%)	62.00^{d}	78.00^{b}	80.00^{a}	72.00^{c}	2.11	0.02

^{abcd}; Means in the same row with different superscripts are significantly ($p<0.05$) different.
SEM: Standard error of Mean, FPKC: Fermented Palm Kernel Cake. MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration.

A. Haematological parameters of broiler chicken fed varying levels of bio-fermented PKC

The haemoglobin concentration obtained in the birds fed control diet was significantly ($p<0.05$) similar to the values obtained in birds fed diet 2 but different from those values obtained in birds on diet 3 and 4. The packed cell volume of birds fed control diet was significantly ($p<0.05$) higher (35.00%) while the lowest value of 30.00% was obtained in diet 4. Red blood cell values recorded in diet 2, 3 and 4 were similar ($p<0.05$) and lower to value obtained in diet 1. The values of white blood cell obtained for birds fed diet 2 was significantly ($p<0.05$) different from birds fed diet 1, 3 and 4. Highest value of 6.00 x103/mm3 was recorded in diet 2 while the least value of 3.20 x103/mm3 was recorded in diet 4. The mean corpuscular volume obtained for birds on diet 3 was significantly ($p<0.05$) higher (139.13 fl) while birds fed control diet has the lowest value of 125.00 fl. The mean corpuscular haemoglobin of the birds on diet 2 and diet 4 were significantly ($p<0.05$) similar, but lower than value obtained for birds on diet 3. The value of mean corpuscular haemoglobin concentration obtained in all the birds fed experimental diets were significantly ($p>0.05$) similar.

The values of monocytes obtained in those birds fed diet 2 and 3 were significantly ($p<0.05$) similar, but lower than those values obtained in the birds fed control diet and diet 4. Highest value of granulocytes

(30.00%) was recorded in control diet while the least value of 18.00% was recorded in diet 3. The value of lymphocytes obtained for the birds on diet 3 (80.00%) was significantly ($p<0.05$) higher compared to values recorded in the birds fed diet 1, 2 and 4 (62.00%, 78.00% and 72.00%) respectively. Table 4 below showed the result of serum biochemical profiles of broiler chicken fed varying levels of bio-fermented PKC.

B. Serum biochemical profiles of broiler chicken fed varying levels of bio-fermented PKC

The value of total serum protein, albumin and globulin recorded in the birds fed diet 2 was significantly ($P<0.05$) higher than those fed diet 1, 3 and 4. There was significant ($P<0.05$) difference in the value of alanine aminotransferase (ALT) obtained in all the dietary treatment. The value of aspartate aminotransferase (AST) obtained showed significant ($P<0.05$) difference among the birds fed experimental diets, birds fed diet 2 had highest value of 98.35iu/l while the lowest value of 69.30iu/l was recorded in control diet. The value of alkaline phosphatase (ALP) recorded in all the birds fed experimental diets were significantly ($P<0.05$) influenced by the dietary treatments. Highest value was recorded among FPKC group while the lowest value was recorded in the birds fed control diet.

There was no significant ($P>0.05$) difference in the values of cholesterol recorded in the birds fed the experimental diets, highest numerical value of 5.24mmo/l was recorded in the birds fed diet 2 while the least value of 4.92mmo/l was recorded in the birds fed control diet.

Table 4: Serum biochemical indices of broiler chicken fed varying levels of bio-fermented PKC

Parameters	**Dietary Treatments**				±SEM	*p*-Value
	FPKC (0%)	**FPKC (10%)**	**FPKC (20%)**	**FPKC (30%)**		
Total serum protein (g/dl)	8.03^{b}	12.85^{a}	6.57^{c}	6.19^{c}	0.81	0.01
Albumin (g/dl)	1.99	2.64	1.89	1.84	0.16	0.25
Globulin (g/dl)	6.04^{b}	10.25^{a}	4.68^{c}	4.69^{c}	0.70	0.03
Alanine aminotransferase (iU/l)	4.96^{c}	12.72^{a}	8.03^{b}	3.68^{d}	2.30	0.04
Aspartate aminotransferase (iU/l)	69.30^{d}	98.35^{a}	77.00^{b}	73.85^{c}	3.36	0.02
Alkaline phosphatase (iU/l)	22.12^{d}	38.79^{a}	29.05^{b}	23.21^{c}	2.00	0.01
Cholesterol (mmo/l)	5.24	4.92	5.05	5.00	0.13	0.88

abcd; Means in the same row with different superscripts are significantly ($p<0.05$) different.
SEM: Standard error of Mean, FPKC: Fermented Palm Kernel Cake.

IV. DISCUSSION

A. Haematological parameters of broiler chicken fed varying levels of bio-fermented PKC.

Haemoglobin concentration obtained in this study were within reference values of 9.00 to 13.00 g/dl for chicken as reported by [17] this implies that the birds had no respiratory stress which can decrease oxygenated blood carrying capacity to the body's tissue.

The PCV values of (30 to 35.0%) observed in this study which are within the normal range of 24.9 to 45.2% for healthy chicken as reported by [18] and [19]. This is an indication that there was no symptoms of physiological anaemia in the experimental birds. PCV, RBC and Hb are interconnected markers of blood oxygen-carrying capacity, used to diagnose anaemia. The range of values of PCV, Hb, and RBC contents of birds fed the various treatments signified an improved oxygen carrying capacity of the cells which translated to a better availability of oxygen and nutrients to the birds thus affecting their well-being [20]. The WBC values obtained for different treatment groups in this study differed significantly the values obtained indicate that 10% inclusion of FPKC was the optimum level that promoted the highest level of WBC, because increase in inclusion level of FPKC led to a reduction of WBC count. The rise in WBC as the inclusion levels increased could suggest that PKC might have immune boosting properties up to a certain level in broilers. This result is supported by findings of [21] who revealed that the mannan contained in polysaccharides of PKC play vital biological functions in enhancing immune response and to control pathogens in poultry. The value of MCH and MCV varied significantly among the treatment group. Value obtained for MCH and MCHC were within reference values reported by [22] for broiler chicken on feeding wheat-based diets. Values obtained implies no nutritional deficiency was observed with inclusion of FPKC in all the dietary treatments, is also an indication that the birds did not suffer anaemia and that oxygen carriers in the blood are effective.

The result of monocytes and lymphocytes obtained in this study agreed with [23] who reported values of lymphocytes and monocytes for local chicken of Bangladesh fed Palm Kernel Cake. Monocytes of 10.85 to 10.90% were obtained for normal native chicken of Khashmir [24]. Monocyte is a white blood cell (leukocyte) that plays a crucial role in the immune system's defence against infections and foreign substances in the body [25].

Lymphocytes are implicated in antibody production, as they are reactive cells in inflammation and delayed hypersensitivity. The lymphocytes value increase when there is foreign body like pathogens in the body. Small amount of lymphocytes in the body may be responsible for the development of clones of

plasma cells while monocytes are phagocytic cells [26]. The results of white blood cells differential counts obtained in this study indicates that immune system of the birds was not compromised by the fermented PKC.

B. Serum biochemical profiles of broiler chicken fed varying levels of bio-fermented PKC

According to [27], serum proteins are synthesized in the liver to maintain blood volume by the colloidal osmotic effect, buffer blood pH, transport drugs and hormones, coagulate cells, catalyze enzymatic reactions, control hormones, and protect the body from foreign substances. The high value of total serum protein recorded in diet 2 resulted from high albumin and globulin levels, which sum up to give the total serum protein. The high total serum protein values in this study were consistent with the findings of [28] who indicated that high serum protein is a sign of healthy liver because severe liver damage or protein deficiency usually results to reduced plasma protein synthesis.

ALT, AST and ALP are enzymes in the liver and are released when the liver is damaged, in this study there was decrease in levels of these enzymes with increase in inclusion levels of FPKC, the values of ALT and AST obtained in the FPKC group fall within the reference range of 7 - 56 (u/L) and 70 - 220 (u/L) respectively, as reported by [29] The values of serum enzymes obtained in this study reflects normal liver function and this is an indication that FPKC fed to broiler improves liver health.

The lower values of cholesterol recorded in this study signify that there was an improvement in the blood circulatory system and general health of the birds which leads to production of lean broiler carcass [30]. Cholesterol blood levels for the birds were within normal standard range as reported by [31].

V. CONCLUSION AND RECOMMENDATION

Result of the of this study shows that FPKC at 10% inclusion level in the broiler diet maintain best overall blood health and immune response in broiler chickens. Further research is needed to better understand the mechanisms underlying the decrease in values of serum biochemical at the increase of FPKC in order to optimize PKC inclusion levels in broiler diets for improved health and performance.

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