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EFFECT OF DIFFERENT LEVELS OF INCLUSION OF CASSAVA PEEL MEAL BLENDED WITH PALM OIL SLUDGE ON THE HAEMATOLOGICAL PARAMETERS AND LIPID PROFILE OF BROILER CHICKENS

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ABSTRACT

The study examined the haematological parameters of broiler chicken fed graded level of cassava peel meal blended with palm oil sludge. This experiment was conducted at the Poultry Unit of Teaching and Research Farm Department of Animal science, Faculty of Agriculture Chukwuemeka Odumegwu Ojukwu University Igbariam Campus. One hundred and fifty broiler chickens were used for the experiment. The birds were randomly assigned to five different dietary treatments with fifty birds per treatment and ten birds per replicate. The experiment lasted for 2 months (56) days. At the end of the experiment, the blood was taken to a laboratory for haematology and lipid profile test. The results obtained in haematology showed that inclusion of cassava peel meal blended with palm oil sludge in broiler chicken diet does not have significant effect ($p>0.05$) on the monocytes, PCV, RBC, TWBC while the lipid profile also shows no significant effect ($p>0.05$) on Total cholesterol, Triglyceride, HDL, VLDL, LDL, Glucose. It can be concluded that up to 19% inclusion of cassava peel meal blended with palm oil sludge (CPMPOS) as a replacement to maize in broilers has no adverse effect on their health.

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Broilers, cassava peel meal, palm oil sludge, blood parameters, lipid profile

1.0: INTRODUCTION

The demand for poultry products has consistently increased over the years as a result of increase in world population, income rise and health attributes of poultry products among other reasons. According to Ravindran (2011), the highest increase in production occurs in developing countries. This increase in poultry production has a direct effect on the availability and price of feed. Maize, the traditional energy source of poultry, is increasingly competed for by the food and biofuel industries making it less available or at exorbitant prices for poultry feeding. According to USDA (2015), the price of maize increased by about 71% from September 2005 to September 2015. This scenario has compelled research into alternative energy source to replace part of maize in poultry diets. Nigeria is blessed with enough potential abound, and such potential is cassava peel meal and palm oil sludge which are by-products of different crops that thrive well on the good and healthy soil of the country. Poultry and livestock production may continue to be ulcerative if costly conventional feedstuffs are not replaced with cheaper and available feed stuffs, Cassava is an important food crop with estimated global annual production of 836 million tonnes (FAO, 2013). This trend in roots and tuber crop production is expected to increase due to several reasons including their adaptability to marginal environments, high yield per land area, flexibility in planting and harvesting time, ease of storage, nutrient density and cost advantage compared to cereal grains. Because of these attributes roots and tubers hold a key place in world food security. Large quantity of peels is produced from the processing of roots and tubers for food and industries uses. Peel weight may account for 1520% of the tuber weight (Oladunjoye, Ojebiyi, &Amao, 2010) but during manual peeling which is the commonest method the loss of tuber weight in the peels may be high as 25-30% (Bruinsma, Witsenburg, &Wurden, 1983). Peels and other forms of waste from roots, tubers and fruits are low valued by-products which are still underutilized and pose disposal problems in most developing countries (Babatunde, 2013) given the versatility and high starch content of these crops, they are increasingly being transformed into many important products such as chips, flour, starch, beverages and ethanol and this will further increase the production and availability of peels from these crops. Cassava processing for both household consumption and industrial use generates considerable quantities of cassava peel which are left in large heaps to rot or are set on fire. Rotting heaps release methane into the air and a stinking effluent pollutes nearby streams and underground water, while burning produces clouds of acrid smoke. Nigeria, which is the largest global producer of cassava, harvests about 59 million tonnes of cassava a year (20% of global production), resulting in about 15 million tonnes of wet peels. Transforming cassava peel has the potential to partially replace maize in animal feed while reducing environmental pollution and minimizing post-harvest losses. This crop-waste by-product could be a valuable feed alternative.

Materials and Methods

Experimental site

The experiment was carried out at the Poultry Unit of Teaching and Research farm of the Department of Animal Science, Faculty of Agriculture, Chukwuemeka Odumegwu Ojukwu University, Igbrariam Campus. Igbariam is located on the latitude $6^0$23'26.4 North and longitude $6^0$56'38.7 East with annual rainfall ranging from 1000mm – 1500mm.

Sources the test material

Cassava peels were gotten from Achalla cassava processing mill while the palm oil sludge was gotten from Achalla oil mill.

Cassava peel

The cassava peel when gotten was cleaned, grated into small pieces, the cassava peels was hung for at least 5 hours to allow excess moisture to drain away, the cassava peel will then be spread out on a tray until it is no thicker than a few millimeters, it was out to dry for a few days. As soon as the cassava is fully dried, it was ground to a fine powder, then put in a sack bag.

Palm oil sludge

The oil sludge was gotten from voluminous liquid waste that comes from the sterilization and clarification processes in milling oil palm.

Procurement of experimental birds

One hundred and fifty broiler chickens were used for the experiment. The broilers were purchased from Ibadan through a reputable source at Awka. They were transported to Igbariam in the cool hours of the morning to minimize heat stress and transit losses.

Experimental diet

Other ingredients as indicated in diet formulation table namely; maize, palm kernel cake, soybean meal, wheat offal, brewers grains, fish meal, bone meal, lysine, methionine, salt and vitamin premix was procured at Afor Nnobi market. They were used together with cassava peel meal blended with palm oil sludge to formulate three poultry diets at inclusion level of 0%, 12.5% and 15% respectively.

Data collection

After proper restraint, 3 ml of blood was collected from the right or left jugular vein of each bird using a 5 ml hypodermic needle and syringe and quickly and gently dispensed into sample bottles containing Na-EDTA. The sample bottles were gently rocked to mix the blood with Na-EDTA to prevent coagulation.

Haematological parameters

The following Haematological assays were analysed from the collected blood sample

PVC: The packed cell volume was determined by the microhematocrit method (Thrall and Weiser, 2002). A microcapillary tube was nearly filled with the anti-coagulated blood sample and sealed at one end with plasticine. It was centrifuged at 10,000 rpm for 5 minutes using a microhaematocrit centrifuge. The PCV was read after centrifugation as a percentage using a microhaematocrit reader.

Haemoglobin: The haemoglobin concentration of the blood samples was determined by the cyanomethaemoglobin method (Higgins *et al*., 2008). 3 ml of Drabkin'shaemoglobin reagent was added to a clean test tube. Then 0.02ml of the blood sample and standard (containing 16g/dl haemoglobin) was added to the reagent and mixed properly. The mixture was allowed to react for 20 minutes, and the haemoglobin concentration was read using a Diatek® Semi-automated Blood Biochemistry Analyzer (Diatek Instruments, Wuxi, China), set at the Haemoglobin Concentration Program Mode. The results (g/dl) were printed out.

Red blood cell: The erythrocyte count was done following the haemocytometer method (Campbell, 1994). Blood (0.02 ml) was pipetted from the blood sample and added to 4 ml of the Natt and Herrick's avian blood cell count fluid in a clean test tube to make a 1:200 dilution of the blood sample. The diluted sample was loaded onto a Neubauer counting chamber and red blood cells (erythrocytes) on the five central squares were counted using a light microscope at x40 objective. The number of cells counted for each blood sample was multiplied by 10,000 to obtain the red blood cell count per microlitre of blood.

Total White blood cell count: The total white blood cell count was determined by the haemocytometer method (Campbell, 1994). Blood (0.02 ml) was pipetted from the blood sample and added to 4 ml of the Natt and Herrick's avian blood cell count fluid in a clean test tube to make a 1:200 dilution of the blood sample. The diluted sample was loaded onto a Neubauer counting chamber and white blood cells on the four corner squares were counted using a light microscope at x10 objective. The number of cells counted for each blood sample was multiplied by 500 to obtain the total white blood cell count per microlitre of blood.

Differential White Blood Cell (Leukocyte) Count

A thin smear of the blood sample was made on a grease free slide and allowed to air-dry. The smear was later stained following the Leishman technique, using Leishman stain. The stained slides were examined under oil immersion at x100 objective of the light microscope using the meander counting method. Each cell type was identified and counted using the differential cell counter. The result of each cell was expressed as a percentage of the total count and converted to the absolute value per microlitre of blood (Campbell, 1994).

Total Serum Cholesterol

Name of method:

Enzymatic colorimetric method (Allain *et al*., 1974; Rifai *et al*., 2008).

Reagents:

i. Total Cholesterol Working Reagent composed of Cholesterol esterase, Cholesterol oxidase, Peroxidase, Pipes buffer pH 6.8, Phenol, 3,5-Dichlorophenol, 4Aminoantipyrine.
ii. Total Cholesterol Standard, equivalent to 200mg/dl Cholesterol

Procedure:

Clean test tubes were labeled according to the Sample designations, and two test tubes for the Standards (SD1 and SD2) and one for the Blank. Ten microliters of the Total Cholesterol Working Reagent was added to all the test tubes. Ten microliters of the serum sample was added to the test tubes labeled for the samples, according to their designations. Also, 10 microliters of the Standard was also added to the test tubes labeled for the Standards. The contents of the test tubes were mixed well by shaking, and allowed to stand at room temperature for 10 minutes. The Total Cholesterol levels was read against the Reagent Blank, using the Diatek Blood Biochemistry Analyzer (Wuxi HiwellDiatek Instruments Co. Ltd., China), set at the Total Cholesterol QCA Program Mode. The results were then printed out.

Glucose Determination (Glucose Oxidase / Perioxidase)

Name of Method:
Glucose oxidase method (Barham&Trinder, 1972; ADA, 2003).

Reagents:

i. Glucose Working Reagent, containing glucose oxidase, peroxidase, 4-aminoantipyrine, phenol and phosphate buffer (pH 7.5).
ii. Glucose Standard, containing 100 mg/dl (5.55 mmol/L) of Glucose.

Procedure:

Clean test tubes labeled for Samples, Standards and Blank were arranged. One mililitre of Glucose Working Reagent was added to all the labeled test tubes. Ten microliter of the serum sample was added into the test tubes labeled for the Samples, and also 10 µl of the Standard was added into the test tube labeled Standard. Ten microliter of distilled water was added to the test tube labeled Blank. The contents of all the test tubes well were mixed and were allowed to stand for 20 minutes at room temperature (20 – 25 °C). The plasma Glucose level of the

Samples was read against a Reagent blank on the DIALAB GLUCOSE Program Mode of the Diatek Biochemistry Analyzer (Wuxi HiwellDiatek Instruments Co. Ltd., China). The results were then printed out.

Triglyceride Determination.

Name of method:

The glycerol-phosphate oxidase method (Bucolo& David, 1973; Rifai*et al*., 2008).

Reagents:

i. Triglyceride Working Reagent composed of 4-chlorophenol, 4-aminoantipyrine, ATP, MgCl2, Glycerol kinase, Glycerol 3 phosphate oxidase, Peroxidase and Lipases.

ii. Triglyceride Standard, equivalent to 200mg/dl Triglyceride.

Procedure:

Clean test tubes for the Samples were labelled according to their designations, and two test tubes for the Standards, and one for the Blank. Ten microliters of the Triglyceride Working Reagent was added to all the labeled test tubes. To the test tubes labeled for the Samples, 0.01 ml of each of the serum sample was added. Also, to the ones labeled for Standards, 0.01 ml of the Triglyceride Standard was added. The contents of the test tubes were mixed well by shaking, and allowed to stand for 10 minutes at room temperature. The Triglyceride levels was read against the Reagent Blank, using the Diatek Biochemistry Analyzer (Wuxi Hiwell Diatek Instruments Co. Ltd., China), set at the Triglyceride QCA Program Mode. The results were then printed out.

High Density Lipoprotein (HDL) Concentration

Name of method:

Dextran sulphate-Mg (II) method (Albers *et al*., 1970; Rifai*et al*., 2008).

Reagents:

i. Precipitant solution containing dextran sulphate and magnesium acetate.

ii. Cholesterol Working Reagent composed of Cholesterol esterase, Cholesterol oxidase, Peroxidase, Pipes buffer pH 6.8, Phenol, 3,5-Dichlorophenol, 4Aminoantipyrine.

iii. Cholesterol Standard, equivalent to 200mg/dl HDL-Cholesterol

Method:

clean test tubes were labeled for the samples, according to their designations. To the labeled test tubes, 0.3ml of each of the serum samples was added. And to the serum samples in the test tube, 40µl of the precipitant solution was added. The contents of the test tubes were well mixed and allowed to stand for 15 minutes at

room temperature. Centrifuge at 3000 revolutions per minute for 10 minutes. Another set of test tubes for the Samples were re-labelled, for two Standards and one Blank. To all the labeled test tubes, 1.0 ml of the Cholesterol Working Reagent was added. Then 0.01 ml of the supernatant derived from centrifugation of the precipitant serum sample mixture was added to the appropriately labeled test tube. Also 0.01 ml of the Standard was added to the test tubes labeled Standard 1 and Standard 2. The contents of the test tubes were well Mixed by shaking, and allowed to stand for 10 minutes at room temperature. The HDL-Cholesterol level was read using the Diatek Biochemistry Analyzer (Wuxi HiwellDiatek Instruments Co. Ltd., China), set at the HDL-Chol QCA Program Mode. Then the results were printed out.

Low Density Lipo-Protein Concentration (L DL-C)

Very low density lipoprotein (VLDL) cholesterol is calculated by dividing the triglyceride concentration by five (VLDL = $^1/_5$ of Triglyceride), while the low density lipoprotein (LDL)-cholesterol is calculated by subtracting HDL and VLDL from total serum cholesterol (Friedewald *et al*., 1972; Warnick *et al*., 1990). **Statistical Analysis**

Data collected was analyzed using ANOVA for CRD while the mean was separated using least significant difference (LSD)

Results

Table 1: Effect of different levels of cassava peel meal blended with palm oil sludge on the haematological parameter of finisher broiler.

Parameters	T1	T2	T3	SEM
Lymphocytes	64.60^{a}	44.40^{b}	68.40^{a}	2.92
Heterophils	25.60^{b}	43.40^{a}	21.40^{c}	2.62
Eosinophil	6.40^{b}	8.00^{a}	6.60^{ab}	0.32
Monocytes	3.40	4.60	3.60	0.32
Haemoglobin(g/dl)	9.27^{ab}	10.98^{a}	9.01^{b}	0.38
PCV(%)	29.04	31.16	29.40	0.70
RBC(10^6/ul)	3.71	3.95	3.79	0.09
TWBC(10^3/ul)	56.60^{b}	53.10^{b}	76.00^{a}	2.78

^{a,b,c} means on the same row with different superscripts are significantly different ($P<0.05$).

PVC means Packed Cell Volume, RBC means Red Blood Cell, TWBC means Total White Blood Cell., SEM means Standard Error of Mean

Table 2: Serum lipid profile of broiler finishers fed processed cassava peel meal blended with palm oil sludge (CPMPOS).

Parameter	T1	T2	T3	SEM
Total cholesterol (mg/dl)	159.86	172.82	174.40	5.18
Triglyceride (mg/dl)	52.32	53.33	55.83	1.62
HDL (mg/dl)	109.28	115.19	114.51	3.71
VLDL (mg/dl)	10.46	10.66	11.16	0.32
LDL (mg/dl)	40.11	46.95	48.72	3.08
Glucose (mg/dl)	140.66^{b}	156.39^{ab}	161.51^{a}	3.67

^{a,b,c} means on the same row with different superscripts are significantly different ($P<0.05$). HDL means High Density Lipoprotein (HDL) Concentration

LDL means Low Density Lipo-Protein Concentration (L DL-C)

VLDL means Very Low Density Lipo-Protein Concentration

The results in Table 1 showed the effect of feeding finisher broilers with cassava peel meal blended with palm oil sludge on the hematological parameters. There were no significant difference on monocytes, PCV, and Red Blood Cell but lymphocytes, leterophil, Eosinophil, haemoglobin, and total white blood cell. There were significant difference ($P<0.05$) in haemoglobin with the treatment diet two (T2) showing the highest figure of 10.98 (g/dl), followed by diet 1 with the value of 9.27(g/dl) while the diet 3 showed the least value of 9.01(g/dl). Total white blood cell were significantly different ($P<0.05$) with the diet 3 scoring the highest value of 76.00(10^3/ul), then diet 1 and 2 were seen being similarity affected. There were significant difference among lymphocyte value ($P<0.05$) in which the diet 1 and 3 were similarly affected with the value 64.60 and 64.40 (g/dl) respectively.

The result in the Table 2 showed the influence of cassava peel meal mixed with palm oil sludge based diet on the lipid profile of broiler birds. There were no significant differences ($P>0.05$) on total cholesterol, triglyceride, HDL, VLDL and LDL. Only glucose was significantly difference ($p<0.05$). Diet 3 showed the highest value of 161.51 (mg/dl), followed by diet 2 which is slightly effected with the value of 156.39 (mg/dl) while treatment 1 and 2 were similarly effected with the values 140.66 (mg/dl) and 156.39 (mg/dl) respectively.

Discussions

Hematological and biochemical parameters

In the past, the use of plants in monogastric diet was restricted because of some negative effect on feed intake and nutrient utilization attributed to phyto-chemical composition that varies greatly due to variety, location and climate (Xiao, 2012). Collectively, energy between individuals bioactive compounds in cassava peel plants and palm tree may affect broad aspects of physiological activities in animals with the ultimate objective being positive interactions with the body (Mbikay, 2012).

Hematological and biochemical parameters are important indicator of the health status in animals and have been an indispensible tool in the diagnosis treatment and prognosis of many diseases. The blood in an animal serves as a transport medium. It transports food materials such as glucose, fatty acids, vitamins and electrolytes from the gastro intestinal tract to body tissues where they are utilized for body building and energy. Increase or decrease in body weight from the previous weight for a specific period in the principal measure by productivity in meat animal and it depends on the quantity of feed given (Blood, 1999). The following hematological parameters: lymphocytes, TWBC, haemoglobin, heterophil and eosinophils of broilers fed cassava peel meal blended with palm oil sludge (CPMBPOS) at levels 0%, 12.5% and 15% for control diet, diet 2 and 3 respectively were significantly ($P<0.05$) affected. Aderemi *et al.(*2020) reported that feed components affect blood constituents. The hematological parameters can thus be useful to assess the effects of the test ingredient. High hemoglobin and PCV observed in diet 2 could suggest that there is no anaemia since values obtained foe these parameters (PCV, Hb, and RBC) in treatment 2 fall within the normal range values for healthy birds as reported by Post (2007). The high RBC and PCV obtained in birds fed, 12.5% processed CPMPOS could also be due to low value of tannin an anti- nutritional factor because of the processing method (sundried) used and the addition of palm oil sludge. Tannins have been reported to negatively affect feed intake as well as dry matter and protein digestibility (Babatunde, 2013).

The RBC counts according to (Swenson, 1990) are influenced among other factors by nutrition and physical activities. White blood cell values also for the broilers were also within the normal range for healthy birds. Lymphocytes are imported in forming barriers against local diseases conditions and may be involved in anti-body formation (Ferdson ,1981) as cited in Aderemi (2020).

The high PCV, Hb, RBC obtained for birds fed 12.5% CPMPOS inclusion which was statistically similar with values obtained for birds fed control diet implied that birds on treatment diets had high oxygen carrying capacity (Bray, 1988). It is an indication that the nutritional profile of the diet was more enriched when supplemented with combined inclusion of cassava peel meal and palm oil sludge. Nutrition was reported to influence the haemoglobin level of the blood (Udo, 1987). Obadire *et al*. (2019) confirmed that haemoglobin levels are positively correlated with protein quality and level in the diets. This work did not agree with the report of Obadire *et al.* (2019) who reported decrease in RBC, Hb and PCV when fed solely graded levels of moringa and pumpkin leaves.

Serum lipid profile

The high value obtained in glucose of diets with test ingredients might be attributed to the fact that those diets contains fibrous substance which are found in cassava peels and also, the debris of cassava tuber that mixed with the peels helped in raising the glucose content of the treatment diets.

Conclusion

From the above feeding trail, it can be concluded that up to 19% inclusion of cassava peel meal blended with palm oil sludge (CPMPOS) as a replacement to maize in broilers has no adverse effect on their health.

Recommendation

This study in a way has recommended and also expanded the number of alternative feed ingredients for broiler production. Utilization of cassava peels should be encouraged among farmers to bridge the existing gap between the scarce and expensive conventional feed ingredients and the non-conventional.

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