HAEMATOLOGICAL PARAMETRES AND SERUM BIOCHEMICAL INDICES OF BROILER CHICKENS FED *Aspergillus niger* HYDROLYZED CASSAVA PEEL MEAL BASED DIET

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ABSTRACT

The effect of *Aspergillus niger* culture filtrate on hydrolyzed cassava peel meal based diet on the hematological parameters and serum biochemical indices of broiler chickens were studied. Delignified cassava peel was used as the carbon source in a composed medium under a pH of 5.0, concentration 3% and 35^{o}C determined as the optimal conditions for *Aspergillus niger* growth to grow a five milliliter developed inoculum of *Aspergillus niger* for five days. The culture filtrate obtained was then used to hydrolyze delignified cassava peel used as replacement for maize in broiler chicken feed at 25%, 50%, 75% and 100% respectively to meet the nutrient requirement of broilers. A total of thirty, two weeks old unsexed broiler chickens with average initial bodyweight of 0.685±0.0027g were divided into six groups of five chickens each allocated to six dietary treatments. With the increase of cassava peel in the diets, haematological indices like Haemoglobin (Hb), Red Blood Cell (RBC),White Blood Cell (WBC) and Packed Cell Volume (PCV) increased significantly ($P < 0.05$) . Differential leucocytes were used as indicators of stress response and sensitive biomarkers crucial to immune functions. The serum biochemical analysis of the blood samples showed that the serum urea differ significantly as the cassava inclusion level increases ($P<0.05$) while the serum total protein was stable across the groups level of cassava peel inclusion in feed notwithstanding. The marker enzymes; Aspartate aminotransferase (AST) and Alanine aminotransferase differ significantly as the level of cassava peel inclusion varies in feed.

keywords: *Culture filtrate, cassava peel, broiler, Aspergillus niger, hydrolysis.*

1. INTRODUCTION

Cassava is a major source of calories in developing tropical countries. In 1986, FAO estimated that 35% of the 137.4-million ton world production was produced in African countries. The first step in the processing of these tubers is the removal of the peels which are the two coverings of the tubers, these peels usually end up as waste or sometimes as feeds for ruminants. Cassava peels like most agricultural wastes are made up of mainly polysaccharides which are widespread in nature, they account for an estimated 66% of all global bound carbon (Gardnea, 1974). Monogastrics however do not have the wherewithal to digest cellulose but can be aided with enzymes to hydrolyze the cellulose. Microorganisms have been reported to have abilities to produce enzymes in large quantity (Raji *et.al*.,1998; Sani *et.al*.,1992; Belewu and Banjo, 1999).

The role of enzymes as additive in poultry diet is well established.(Atteh,2000; Abdulrashid *et.al*., 2007). Both observed that enzyme addition to monogastric feed reduced viscosity of the digester in the intestine as opposed to a situation of association with digestion of cereal grain and (by –products) and showed a marked improvement on the various morphological effect of feeding fibrous materials to non- ruminant. Dietary supplementation with microbial enzyme preparations are capable of hydrolyzing endosperm cellwalls and has increased performance of broiler chickens receiving cereal based diets (Abdulrashid *et al.*,2007; Atteh, 2000; Kayode , 2009.). Wheat, rye and triticale diets supplemented with a technical enzymes preparation containing pentosanase were fed to broiler chickens and it was evident that supplementation with enzymes preparation in pentonase activity can improve the nutritive value of diets based on cereal grains with Pentosarrich-endosperm cellwalls (Belewu and Banjo, 1999; Raji *et.al*., 1998). The effect of carbon source replacement of maize in broiler chicken feed is well documented. (Abdulrashid *et al.*,2007; Atteh, 2000; Kayode , 2009; Mohammad and Oloyede, 2009). However, information on the effect of hydrolyzed cassava peel as maize replacement on the haematological parametres and serum biochemical indices are very scanty in literatures. The blood contains several metabolites which provides useful information on nutritional status and clinical investigation of an individual hence WHO recommended the use of blood parameters for medical and nutritional assessments. (WHO,1963, Egbunike *et. al*.,2009)

The current study therefore investigated the effect of hydrolyzed cassava peel as main energy source in broiler chicken feeds on the haematology and serum biochemical parametres of the chickens after six weeks of feeding trials.

2. MATERIALS AND METHODS

Aspergillus niger was isolated from cassava peel collected from cassava peel dumpsite. A modified method of Ali *et.al.,* (1991) was used to delignify the cassava peel which involves alkaline and steam pretreatment of the substrates. The peel was autoclaved for one hour at 121 ^{0}C with 5% (w/v) NaOH in separate conical flasks for delignification. The autoclaved materials were filtered through muslin cloth, neutralized with dilute acids (0.1M H_2SO_4), and then washed with water. They were finally washed in distilled water and dried at 70^{0}C in a regulated oven (Gallenkamp). Each was then grinded with domestic blender (Nakai, Japan Mx- 736) for increased surface area. Mineral salts medium (MSM) was prepared for cultivation of the *Aspergillus niger* isolate using the compositions as shown below {g/l}.

KH_2PO_4 ,10g;$(NH4)_2SO4$,10.5 g ; $MgSO_4.7H_2O$, 0.3g; $CaCl_2$, 0.5 g; $FeSo_4$, 0.013g ; $MnSO_4.H_2O$ 0.04; $ZnSO_4.7H_2O$ 0.04; Yeast extract 0.5g; Cassava peel (40g). *Aspergillus niger* was grown in this composed under a pH of 5.0, concentration 3% and 35^{o}C for five days after which the culture filtrate was filtered with whitmann under sunction and the clear filtrate containing 100ppm of the cellulase enzyme was incorporated into cassava peel so as to hydrolyze it. The cassava peel was then air dried and then used to replace maize in broiler chicken feeds of groups A- E at 0% , 25% , 50% , 75% and 100% respectively. Also, unhydrolyzed cassava peel was used to replace maize 100% for group F as shown in Table 1 below.

2.1 Animal Management

The chickens were randomly allocated to six dietary treatment groups A - F using a completely randomized design. Each treatment group contained five chickens. Group A chickens were fed with the control diet (0% hydrolyzed cassava peel as main carbon source). Groups B-E were administered with experimental diets containing 25%, 50%, 75%, 100% of hydrolyzed cassava peels respectively replacing maize as energy source while group F was fed with diet containing 100% unhydrolyzed cassava peels replacing maize as the main carbon source. Feed and water were supplied ad-libitum for the six weeks feeding trial period. Vaccine and drugs were administered as at when due.

3. COLLECTION AND TREATMENT OF BLOOD SAMPLE

Haematological analysis was carried out using the blood collected from the experimental chickens at the end of the 3rd week of the feeding trials. The birds were starved for twelve hours and blood was collected from the wing vein of three selected birds per treatment group with the aid of needle and syringe. Twelve milliliter of blood was collected from each chicken and transferred immediately into a set of sterile plastic bottles with and without anti-coagulant for haematological and serum biochemical tests respectively. The direct measurements of erythrocytes values; {Haemoglobin (Hb), Packed cell volume (PCV), Red blood cells (RBC)}, Mean Corpuscular Haemoglobin (MCH), absolute erythrocyte indices; {Mean Corpuscular Haemoglobin Concentration (MCHC), White Blood Cells (WBC),Mean Corpuscular Volume (MCV)} were calculated. Platelet and differential counts (neutrophils and lymphocytes) were analyzed as described by Jains (1986); Davies ,1994; Chawla, 1999). For the relative microscopic differential count (M- diff),blood smear were stained with Giemsa stain, one hundred cells of neutrophils, eosnophils, monocytes Basophils and lymphocytes were counted, for the relative and absolute automated blood cell count (A-diff) , 500µL blood was analyzed with the automated haematology analyzer SYSMEX KX21-JAPAN at the university of Ilorin teaching Hospital. Frequencies of WBC, RBC and platelets were measured.

The serum biochemical assay was carried out using standard chemical procedures: Total serum protein by Golgberg refractometer method (Gornall *et.al*., 1949), urea nitrogen (Chawla,1999), serum enzymes (AST, ALT) by spectrophotometric method (Reitman and Frankel, 1957).

3.1 Statistical Analysis

SPSS 15.0 was used to perform the statistical analyses used in this study. Data were analyzed by ANOVA to determine the significance of the main effects and interactions. The data obtained were subjected to analysis of variance and the means were compared using the Duncan Multiple range test. A significant level of 0.05 was used. The experiments were all designed as a complete randomized design(CRD).

4. RESULTS AND DISCUSSIONS

The result of the haematological and serum biochemical parametres of the broiler chickens raised on hydrolyzed cassava peel is as shown on Table 2 and 3 respectively. All the chickens fed 100% unhydrolyzed cassava peel recorded 100% mortality within the first six days of the feeding trials while those on hydrolyzed cassava peel based meal recorded 0% mortality. With the increase of cassava peel in the diets, Haemoglobin (Hb), Red Blood Cell (RBC),White Blood Cell (WBC) and Packed Cell Volume (PCV) increased significantly ($P < 0.05$) and this agrees with the result of Tewe and Egbunike,2009. The slight variation in haemoglobin value measured tends to confirm

that diets affect the blood profile of chickens. The values obtained for PCV, MCV and MCH are significantly different as the amount of cassava peels replacement increases (P<0.05).. Packed cell volume (PCV), Haemoglobin (Hb), Red blood cell (RBC) , Mean cell volume (MCV), Mean cell Haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) fell within the values in literature (Muhammad and Oloyede, 2009; Tewe and Egbunike 2009; Post *et.al*.,2007). However, the value of the white blood cells is higher than most values in literatures for chickens fed 75% and 100% hydrolyzed cassava peels as energy source meaning that the chickens are immunologically challenged. Differential leucocytes were used as indicators of stress response and sensitive biomarkers crucial to immune functions. It has however been reported (Mohammad and Oloyede. 2009; Wendy and Jean, 1992; Tewe and Egbunike 2009; Post *et.al*.,2007) that bacterial and viral diseases affect the number of white corpuscles and the ratio between the different types of white corpuscles and the percentages of the various types in healthy animals vary slightly but are greatly modified in sick animals.

The results of neutrophils, basophils, eosnophils and monocytes show that the birds have no bacterial nor viral infection hence their non- significant values among the treatment group The values obtained for PCV, MCV and MCH are significantly different as the amount of cassava peels supplement increases (P<0.05), these also point to the fact that there is an indication of microcytic anaemia (Adebiyi,2007; Post *et.al*.,2007). The significant increase in Red and White blood cells agreed with the report of (Talebi *et. al.*,2005; Ogbuewu 2008; Emenalum *et.al*., 2009 and Ogbuewu *et.al*.,2008) showed that the number erythrocytes of animals in good health varies with species, age, sex, diets and clinical conditions of the animal. The elevated lymphocytes as cassava inclusion level increases could be a physiological adjustment against negative antigenic effects associated with the diet. The significant higher value in serum urea as cassava inclusion level increases suggest there is wasting or catabolism of muscle tissue as reported by Omole and Sonaiya (1981). This means the more cassava inclusion levels increase in food, the more the chickens survive at the expense of their body reserve. The stability in total protein values in the treatment groups irrespective of the cassava inclusion level suggest that the diet is adequate for the chickens (Egbunike *et.al*., 2009). The marker enzymes; Aspartate aminotransferase (AST) and Alanine aminotransferase differ significantly as the level of cassava peel inclusion varies in feed. The AST: ALT ratio is less than 1 in the control feed (A) and greater than 1 in other experimental feeds B to E (Table 3) indicating that the internal organs of the chickens might have been slightly distorted.

5. CONCLUSION

The close to normal of the Red blood cells in the various treatment groups and the zero mortality recorded in all the groups shows that hydrolysis of cassava peel by *Aspergillus niger* was able to reduce the toxic components of the cassava peel meal based diets. However , the increased serum urea as cassava inclusion level increases and the fact that the ALT: AST ratio is greater than one in all the experimental feed trial groups is a source of concern and should be given adequate attention while recommending cassava peel as a replacement for maize in broiler chickens feedstuffs.

6. REFERENCES

[1]. Abdulrashid , M., Agwuobi, L. N., Jokthan, G.E. and Abdul., S.B. (2007). Carcass quality characteristics of broiler finisher fed taro (*Colocasia esculanta*) cocoyam meal In : *Book of abstract of the 32nd annual conference* , NSAP, held at the university of Calabar, Calabar, Nigeria. 32: 457 – 460.

[2]. Adebiyi , O.A.(2007). Fungal degradation of cowpea seed hull for utilization by meaty type (broiler) chicken. Ph. D Thesis, university of Ibadan, Ibadan.

[3]. Ali, S., Sayed, A., Sarker, R.T., Alam, R. (1991) .Factors affecting cellulase production by *Aspergillu sterres* using water hyacinth. *World journal of microbiology and biotechnology*, 7: 62-66.

[4]. Atteh,J.O (2000). Use of enzyme to improve the nutritive value of wheat bran in poultry diets.

[5]. Belewu, G.I. and Banjo, N.O. (1999). Biodegradation of rice husk and sorghum stover by edible mushroom (*Pleurotussajo caju*). *Tropical journal of Animal science* 2: 137 -142.

[6]. Chawla, R. (1999). Kidney function test In : Practical Clinical Biochemistry: Methods and Interpretation .2nd edition.JAYPEE Brothers Medical Publishers (P) Ltd., New Delhi , India. Pp 90 - 92.

[7]. Davies, R.W. (1994). Heterologous gene expression and protein secretion in *Aspergillus* Program.*Industrial Microbioliology*. 29:527-560. *Nicotiana tabacum. Agricultural Biological Chemistry.* 40 : 359 -364

[8]. Egbunike, G.N., E.A. Agiang., A.O. Owosibo and A.A. Fafute. (2009) Effects of protein on performance and haematology of broilers fed cassava peel based diets. Archivos de zootecnia, Vol.58, number 224, p 656.

[9]. Emenalum, O.O., Esonu, B.O, Etuk, E.B and Anaba,C. (2009). Effect of *Mucuna pruriens* (Velvet Beans) leaf meal on performance and blood composition of finisher broiler chickens. Nigerian Journal of Animal Production. 36 (1) ; 52-60.

[10]. FAO (1986). Food and Agricultural Organization.World food production, Geneva FAO nutritional meeting. Report series NO. 52, pp210

[11]. Gardnea, K.H., and Blackwell, J.(1974). In: Microbiological Enzymes And Bioconversion, Economic Microbiology. Vol. 5(ed. A.H.Rose) Academic Press, London.pp 283-327.

[12]. Gornall , A.G., Bardawill , C.J. and David , M.M. (1949) .Determination of serum protein by means of the biuret reaction *Journal of BioChemistry*. 177 : 751 – 756.

[13]. Jain , N.C. (1986). Schalm veterinary Haematology 4th edition, Philadelphia, Lea and Febinger.

[14]. Kayode , R. M. (2009) . Suitability of Mango (*Mangiferaindica*) fermented kernel as animal feed supplement. . Ph. D Thesis, university of Ilorin, Nigeria

[15]. Muhammad, N.O. and O.B. Oloyede(2009). Haematological Parameters of Broiler Chicks Fed*Aspergillusniger* - Fermented *Terminalia catappa* Seed Meal-Based Diet. *Global Journal of Biotechnology & Biochemistry* 4 (2) : 179-183.

[16]. Ogbuewu ,I.P. (2008). Physiological responses of rabbits fed graded levels of Neem (*Azadiractha indica* A Juss) laef meal. M.Sc., Thesis, Department of Animal science and technology, federal university of technology, Owerri, pp 254.

[17]. Ogbuewu, I.P., M.C. Uchegbu., C.C. Ezuma and M.N. Opara. Physiological responses of finisher broilers to yam peel meal: Haematology and Serum Biochemistry. Electronic journal of Environmental, Agricultural and Food Chemistry. ISSN: 1579-1664.

[18]. Omole , T.A and Sonaiya, E.B. (1981). The effect of protein source and methionine supplementation of cassava peels meal supplementation by growing rabbits. Nutr. Reports intl. 23(4): 779-737.

[19]. Post, J.,1 J. M. J. Rebel, and A. A. H. M. terHuurne (2007).Automated Blood Cell Count: A Sensitive and Reliable Method to Study Corticosterone-Related Stress in Broilers.*ID-Lelystad, Institute for Animal Science and Health, Lelystad, The Netherlands.*pp: 777-781.

[20]. Raji, A.I., Ameh. J.B. and Ndukwe., M. (1988). Production of cellulase enzyme by *Aspergillus niger*– CS_{14} from delignified wheat straw and rice husk substrates..*Nigerain Journal of Technical Education*.15: 57-63.

[21]. Reitman , S. and Frankel , S. (1957) . A colometric method for the determination of glutamic oxaloacetic and glutamic pyruvic transaminase. *Animal .Journal of. Clinical Pathology.,* 28: 56 -66

[22]. Sani , A., Awe, F.A and Akinyanju J.A. (1992). Amylase synthesis in *Aspergillus niger* and *Aspergillus flavus* grown on cassava peel. *Journal of Industrial Microbio*logy 10: 55-59.

[23]. Talebi, A., Asri-Rezaei, S., Raszeh- chai, R and Sahraei, R (2005). Comparative studies on haematological values of broiler starins (Ross,Cobbs,Acres and Avian). Int. J.Poul. Sci. 4(80): 573-579.

[24]. Tewe .O.O, and Egbunike G.N. (1992). Utilization of cassava in non ruminant feeding.In : cassava as livestock feed in Africa. (S.K. Hahn., L. Reynolds and G.N. Egbunike (eds.). IITA Ibadan and ILCA, addisababa.Pp 28-38.

[25]. Wendy, B. Bollag and Jean-Marc Bollag (1992). *Encyclopedia of Microbiology*, volume 2, academic press inc., pp 269 – 274.

[26]. WHO (1963). Technical report series. No.842 (Expert committee on medical assessment and nutritional status). WHO, Geneva.

Table 1 : Composition of the Experimental Broiler Diet (%)

Ingredients	Control A(0%)	B(25%)Cpm	C(50%)Cpm	D(75%)Cpm	E(100%)Cpm	F(100%)Cpm
Maize	45.00	33.75	22.50	11.25	-	-
Delignified Hydrolyzed Cassava peels	-	11.25	22.50	33.75	45	45 (delignified unhydrolyzed cassava peel)
Soya bean meal	15.00	15.00	15.00	15.00	15.00	15.00
Groundnut cake	20.00	20.00	20.00	20.00	20.00	20.00
Brewers dried grain	10.00	10.00	10.00	10.00	10.00	10.00
Wheat offal	4.95	4.95	4.95	4.95	4.95	4.95
Fish meal(72%)	1.50	1.50	1.50	1.50	1.50	1.50
Bone meal	2.50	2.50	2.50	2.50	2.50	2.50
Oyster shell	0.30	0.30	0.30	0.30	0.30	0.30
Salt (Nacl)	0.30	0.30	0.30	0.30	0.30	0.30
*Vit/min premix	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Total	100	100	100	100	100	100

***** Vit. A,, 4,000,000 IU; Vit. D3, 800,000 IU; Tocopherols, 4,000 IU; Vit. K3, 800mg; Folacin, 200 mg; Thiamine, 600 mg; Riboflavin, 1,800 mg; Niacin,6,000 mg; Calcium Panthothenate, 2,000 mg; Pyridoxine, 600 mg; Cyanocobalamin,4 mg; Biotin, 8 mg; Manganese, 30,000 mg; Zinc, 20,000 mg; Iron,8,000 mg; Choline chloride, 80,000 mg; Copper, 2,000mg; Iodine, 480 mg; Cobalt, 80 mg; Selenium, 40 mg; BHT, 25,000; Anticaking agent, 6,000 mg. Cpm is cassava peel meal

Table 2 : Haematological Analysis of Blood Sample of the Chickens fed on Maize Replacement Diet by Hydrolyzed Cassava Peel as Main Carbon Source.

Chicken feeding groups / Blood Parametres	A (0%)	B(25%)	C(50%)	D (75%)	E (100%)	S.E.M
PCV (%)	28^{a}	33^{bc}	38^{c}	43^{d}	52^{cde}	1.26
Hb(g/l)	8.7^{a}	8.8^{a}	9.1^{a}	9.3^{a}	9.0^{a}	0.65
RBC($X10^{9}$/L)	2.51^{a}	2.97^{a}	3.21^{ab}	3.92^{b}	4.54^{cd}	0.85
WBC($X10^{9}$/L)	7.5^{a}	14.5^{b}	17.0^{c}	18.9^{cd}	23.95^{e}	1.82
Neutrophils	11	12	22	14	24	2.25
Lymphocytes	62^{a}	72^{b}	82^{c}	83^{c}	87^{d}	1.87
Eosnophils	05	04	03	08	04	0.75
Monocytes	-	-	-	-	-	
Basophils	03	02	02	01	-	0.04
MCV (fl)	128^{a}	131.8^{b}	135.3^{c}	128.2^{a}	132.1^{b}	0.89
MCH (pg)	47.9^{c}	45.7^{b}	45.3^{a}	44.8^{a}	44.7^{a}	0.67
MCHC (%)	35.7^{a}	34.6^{a}	34.2^{a}	33.8^{a}	33.5^{a}	0.35

^{abcde}Means with different superscripts in a row are significantly different ($p<0.05$) .RBC - Red Blood Cells; WBC- White Blood Cells; MCV - Mean Cell Volume; MCH - Mean Cell Haemoglobin; MCHC - Mean Cell Haemoglobin Concentration; Hb – haemoglobin, PCV – Packed Cell Volume, CPM - cassava peel meal

Table 3: Serum Biochemical Parameters of Broiler Chickens Fed On Maize Replacement Diets by Hydrolyzed Cassava Peel as Main Carbon Source.

Sample	A (0 %) Cpm	B (25%) Cpm	C (50%) Cpm	D (75%) Cpm	E (100%) Cpm	S.E.M
Serum urea(mg/dl)	19.82^{a}	24.65^{b}	35.90^{c}	37.85^{c}	54.8^{d}	8.55
Serum total protein(mg/dl)	2.9^{a}	2.8^{a}	3.1^{a}	3.2^{a}	2.9^{a}	0.74
ALT (µl)	6^{c}	15^{b}	25^{a}	27^{a}	29^{abc}	2.34
AST (µl)	10^{a}	12^{a}	19^{ab}	20^{ac}	26^{cd}	2.65
ALT : AST	0.60	1.25	1.32	1.35	1.11	-

^{a,b,c,d} Means within a row with different superscripts differ significantly ($p<0.05$); AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, Cpm – cassava peel meal, S.E.M -Standard error mean.