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Research Article
Histopathological Changes of Some Internal Organs and Brain Regions of Rabbits Fed Dietary Cassava Peel Meal as Replacement for Maize

[1]Emem Iboro Evans and [2]Pascal Ogar Ozung

[1]Department of Animal Science, Faculty of Agriculture, Akwa Ibom State University, Obio Akpa Campus, Akwa Ibom State, Nigeria
[2]Department of Animal Science, Faculty of Agriculture, Forestry and Wildlife Resources Management, University of Calabar, Calabar, Nigeria

Abstract

Background and Objective: Nutrition has a profound influence on the physiological responses of vital organs in the body of animals. Most of these unconventional feedstuffs have toxic effects on animals. This study investigated the histopathological changes of internal organs and brain regions of rabbits fed sun dried cassava peel meal as a replacement for maize. **Materials and Methods:** Sixty crossbred weaned rabbits of mixed sexes (30 bucks and 30 does) with an average initial body weight of 559.58±0.42 g/rabbit were used. Twelve rabbits were assigned per treatment in a Completely Randomized Design (CRD) experiment. Cassava peel meal replaced maize at 0, 25, 50, 75 and 100% levels for T_1, T_2, T_3, T_4 and T_5, respectively. The feeding trial lasted 25 weeks. At the end, the rabbits were slaughtered and internal and male gonads as well as the brain regions were harvested for histopathological assessment. **Results:** The histopathological changes of the kidney, testis, liver and brain regions revealed the normal histological arrangement of these organs in rabbits fed the control diet (T_1) and 25% (T_2) cassava peel meal. However, as the level of cassava peel meal replacement increases across treatments, there was a steady rise in tissue degeneration of all organs excluding the brain regions. Tissue abnormality was more pronounced in rabbits fed 75 and 100% cassava peel meal levels. **Conclusion:** The study concluded that higher replacement levels of cassava peel meal for maize beyond 50% could pose deleterious effects on internal organs of rabbits without any adverse effect on the brain regions.

Key words: Anti-nutrient, brain, cassava peel, tissue abnormality, histopathological changes

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Corresponding Author: Pascal Ogar Ozung, Department of Animal Science, Faculty of Agriculture, Forestry and Wildlife Resources Management, University of Calabar, Calabar, Nigeria Tel: +2348180802103 +2348062246745

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

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

INTRODUCTION

The quest for cheap and available feeding stuff has been well documented[1,2]. The nutrient level is essential and the key to existence; thus, it is, therefore, necessary to reduce the anti-nutritional levels of any nutrient to a safe limit or make it free from toxicants[3]. Nutritional toxicants are known to reduce the reproductive and productive performance of animals[4]. The alarming increase in the human population especially in developing countries has added to the strain in the use of cereal grains resulting in high prices. The problem is further compounded with the prevailing global feed crisis which further aggravates the incidence of hunger and malnutrition, especially in countries like Ethiopia and Nigeria[5]. Generally in developing countries, animal protein intake is about 3-5 kg/capita/year, which is ridiculously low compared to the world average of 38 and 124 kg/capita/year in the U.S.A[6]. There is a need to source for inexpensive animal protein sources. This could become a solution to the projected 56% growth in the demand for meat, especially in developing countries by 2020 and beyond[7]. The demand for meat may not be met by traditional livestock (cattle, sheep and goats) due to their long reproductive and productive cycles, thereby encouraging the emergence of micro livestock like snails, rabbits and quails. The rabbit with it numerous potentials can provide the much-needed animal protein for mankind.

According to FAO[8], animal feed is the pivot for the food industry globally. Therefore, for effective profit maximization; rabbit production in Nigeria and other developing countries should be geared towards exploring input resources which can reduce the cost of feeding and can also moderately improve growth rate and reproductive performance without negatively affecting the carcass characteristics[9,10]. A good portion of agro-industrial by-products can be utilized, minimally processed and integrated into farm animal diets. Good and cheap animal feeds make livestock production more viable throughout the world[11,12]. As availability is one of the major factors determining the suitability of any non-conventional feedstuff to avoid scarcity that may eventually increase the total cost of production. Most of the unconventional feedstuffs are plagued with dietary toxins which may pose danger to the general health of the livestock and eventually affect productive and reproductive performance.

Cassava peels are by-products of cassava processing. They constitute wastes in the cassava processing industry and accounts for 10-13% tuber by weight[11,12]. Cassava peels have 72.1% dry matter, 5.3% crude protein and 1.2% ether extract. The peels are relatively high in crude fiber (20.97%), ash (5.93%) and nitrogen-free extracts (66.60%)[13]. The only limiting factor to the use of cassava and its by-products is the presence of hydrocyanic acid or cyanide (HCN). Cyanide is a well-known toxin causing health problems in man and livestock especially rabbits and pigs[14,15]. Two types of cyanide toxicity have been identified: Acute and chronic toxicity[16]. The mechanism for acute cyanide toxicity has been well established. Cyanide reaction in the animal tissues results in the formation of a higher metal affinity with ferric ions, causing strong oxidation of this metal. This reaction lowers the ability of iron to carry electron in the electron transport chain; thereby, preventing tissue utilization of oxygen by the inhibition of the cellular respiratory enzyme, cytochrome oxidase. Acute cyanide toxicity is directly proportional to the amount of free cyanide that is released into the animal tissues. Inhalation or ingestion of cyanide produces a reaction in the cytochrome within seconds and death within minutes. Symptoms of acute cyanide toxicity are respiratory instability and acute animal deaths. Chronic cyanide toxicity only sets in with a consistent intake of a small quantity of cyanide in the diet; which overtime becomes pathogenic, causing different types of diseases[17,18]. Chronic cyanide intoxication has been implicated as the cause for degenerative neuropathy known as tropical ataxic neuropathy, renal disease and goiter[19].

Because of the residual cyanide content in cassava peel meal coupled with its availability at almost no cost to the rabbit rearers, it became imperative to investigate its utilization in animal diets in the tropics. This study was designed to determine the effect of replacing maize with varying levels of cassava peel meal on the histopathology of some organs and brain regions of rabbits.

MATERIALS AND METHODS

Location of the study: The study was carried out at the Rabbitry Unit of the Teaching and Research Farm, University of Calabar, Calabar, Cross River State, Nigeria. The feeding trial lasted for 25 weeks, while the laboratory evaluation lasted two weeks (i.e., 27 weeks, approximately 7 months) in all. The study started in July 2019 and ended in January 2020 accordingly. According to the GeoNames geographical database by Google earth[20]; Calabar is located at 4°57' latitude of the Equator and 8°19' longitude of the Greenwich meridian with an average elevation/altitude of 99 m above sea level. The average annual rainfall ranges between 3000 and 3500 mm and the average daily temperature is between 25°C (77°F) and 30°C (86°F). The relative humidity is between 70 and 80% while the wind speed/direction is 8.1 km h^{-1} west and the cloud is broken at 1000 ft with little cumulonimbus at 2200 ft. The time zone in Calabar is Africa/Lagos.

Table 1: Gross composition of experimental diets (%)

Ingredient	T_1 (0%)	T_2 (25%)	T_3 (50%)	T_4 (75%)	T_5 (100%)
Maize	30.00	22.50	15.00	7.50	0.00
Soybean meal	12.70	15.60	17.60	18.60	19.79
Crayfish dust	4.00	4.00	4.00	4.00	4.00
Palm kernel cake	7.00	11.10	12.10	12.80	12.91
Wheat offal	15.00	10.00	9.00	9.20	7.00
Rice husk	25.00	23.00	21.00	19.10	20.00
Cassava peel meal (CPM)	0.00	7.50	15.00	22.50	30.00
Bone meal	2.50	2.50	2.50	2.50	2.50
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Premix	0.20	0.20	0.20	0.20	0.20
Salt	0.10	0.10	0.10	0.10	010
Palm oil	3.00	3.00	3.00	3.00	3.00
Total (%)	100.00	100.00	100.00	100.00	100.00
Calculated analysis					
CP (%)	16.21	16.95	17.17	17.43	17.33
CF (%)	9.76	10.05	10.31	10.61	10.47
ME (Kcal kg^{-1})	2,482.05	2,391.53	2,279.28	2,155.90	2,022.02
Determined analysis of diets:					
CP (%)	18.91	17.81	17.37	16.88	16.83
CF (%)	10.27	11.38	11.58	11.87	11.96

Gross Composition of Bio –Super Premix per Kg; Vitamin A-1,500,000IU; Vitamin D3-300,000IU; Vitamin E-400mg; Vitamin B2-400mg; Vitamin B12-2,000mg; Nicotinamide-2,000mg; Calcium D Pantothenate-800mg; Choline Chloride-40,000mg; Ferrous Sulphate-2,000mg; Manganate Sulphate-5,000mg; Copper Sulphate-80mg; Zinc Oxide-3,000mg; Cobalt sulphate-10mg; Potassium Iodide-120mg; DL-Methionine-10,000mg and Antioxidant-18,000mg. The premix was manufactured by Bio-Phamachemic Company HCM city, CF: Crude fibre, CP: Crude protein, SCPM: Sun dried cassava peel meal, SBM: Soybean meal, PKC: Palm kernel cake, ME: Metabolizable energy. 0% CPM is the control diet

Processing of the test ingredient (Cassava peel meal, CPM): Fresh composite cassava peels were collected from cassava processing factories located at Odukpani and Akamkpa Local Government Areas of Cross River State, Nigeria. The peels were washed and sun-dried on concrete slabs to constant weight and milled to 3 mm sieve size with a hammer mill. The milled cassava peel samples were stored in sterile polythene bags at room temperature for used as a replacement for maize in the experimental diets. The proximate composition of sun-dried cassava peel meal was determined using the AOAC[21] methods.

Experimental diets: Five experimental diets were formulated each comprising the ratios of 100:0, 75:25, 50:50, 25:75 and 0:100 maize: sun-dried CPM for dietary treatments T_1, T_2, T_3, T_4 and T_5 respectively. Treatment T_1 served as the control diet. The proximate composition of the experimental diets was done based on the procedures outlined in AOAC[21]. The gross and proximate compositions of experimental diets and cassava peel meal are presented in Table 1.

Housing and equipment: The rabbits were housed in wooden double tier cages, with a wire mesh floor and measuring 65×65×65 cm and raised 25 cm from ground level. The cages and entire rabbitry unit were thoroughly washed with a strong disinfectant and allowed to dry for seven days before animals were brought in. A concrete watering trough and a fabricated feeding trough were placed in each hutch.

Experimental animals, design and management: A total of 60 (30 does and 30 bucks) crossed bred weaned rabbits aged between 5 and 6 weeks old with an average initial body weight of 559.58±0.42 g/rabbit were used for the study. The rabbits were obtained from standard rabbitries in Calabar and environs. Immediately on arrival, the rabbits were given anti-stress vitality via drinking water. The rabbits were thereafter randomly distributed into five dietary groups after equalizing for bodyweight using a Completely Randomized Design (CRD), with a stocking ratio of 6 bucks: 6 does per treatment. Each rabbit occupied a separate cage and served as a replicate. The rabbits were conditioned by screening against ecto and endo parasites using Ivomec sub cute injection. The rabbits were fed the control diet and a small quantity of *Tridax procumbens* during the two weeks adjustment /acclimatization period. The actual feeding trial with the respective dietary treatments proceeded thereafter for 25 weeks. Clean drinking water was provided *ad libitum*.

Histopathological examination: At the 25th week, six rabbits per treatment were slaughtered and internal organs and the entire brain harvested for gross pathological examination. The organs were carefully removed and fixed with 10% formalin solution. Heads of the rabbits were severed at the *Occipito-Atlantal* joint and immediately placed in ice containers. All the heads were then frozen for two weeks before dissection as recommended by Gatti *et al.*[22]. During dissection, each head was placed in a dorsoventral position on ice-cold porcelain tile for cutting as previously described in Egbunike[23] before the entire brain was carefully dug out by blunt dissection. The brain was then freed of adhering meninges and weighed. Gross histopathological examinations of the visceral organs such as kidney, liver and gonads (testes) and entire brain were carried out at the diagnostic laboratory of the University of Calabar Teaching Hospital. The histopathological examination was carried out using standard laboratory methods [24] and lasted for two weeks.

RESULTS

Histopathological lesions of organs and brain regions of rabbits fed cassava peel meal as a replacement for maize: The result of histopathological lesions of the kidneys, liver, testes and entire brain of rabbits fed cassava peel meal as a replacement for maize is summarized in Table 2. The histological arrangements of the kidneys, testes and liver as well as the entire brain region of rabbits fed 0, 25 and 50% (T_1, T_2 and T_3) cassava peel meal replacement for maize were normal without noticeable defects or abnormality. The respective photomicrographs are presented in Fig. 1-20. Specifically, Fig. 1-5 show the appearance of the general tissue structure of the testes for rabbits fed diets containing 0, 25, 50, 75 and 100% cassava peel meal replacement levels for maize. Rabbits fed diets with 0, 25 and 50% recorded normal testicular tissue structures while those fed 75 and 100 % levels

Fig. 1: Photomicrograph of testis in T_1 (0% CPM) section showing normal appearance of the general tissue structure

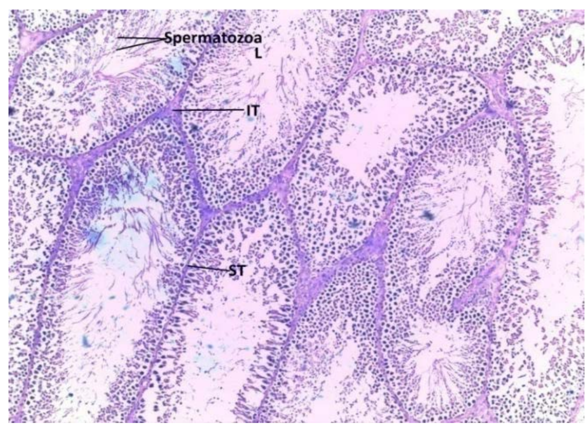

Fig. 2: Photomicrograph of testis in T_2 (25% CPM) section showing normal appearance of the general tissue structure

Table 2: Histopathological outcome of organs and the entire brain of rabbits fed cassava peel meal (CPM) as a replacement for maize

Dietary treatment	Kidney	Testis	Liver	Entire brain regions
0% CPM T_1	Normal appearance of the general structure of the kidney	Normal appearance of the general tissue structure of the testis	Normal appearance of the general tissue structure of the liver	Brain section showed the normal cellular distribution
25% CPM T_2	Section showed tissue with normal appearance of the glomerulus and normal architectural pattern of tissues	Normal appearance of the general tissue structure of testis	Normal appearance of the central vein, the sinusoids and hepatocytes. No structural defect was seen	Normal architectural and general tissue structure of the brain
50% CPM T_3	Structure of the kidney showed focal area of necrosis with no noticeable damage	Section of the testicular tissue with no visible damage on the general tissues	No visible effect noticed in the general tissue structure of the liver	Normal brain tissues with no lesions
75% CPM T_4	Visible degeneration, vacuolation and dilation of the kidney tubules	Affected testicular tubules with lumen showing degenerated spermatozoa	Slightly destroyed central vein with some enlarged sinusoids in the liver cells	Brain structure showed no obvious abnormality
100% CPM T_5	Extensive tissue degeneration and dilation of the kidney	System of testicular tissue showed perforated interstitial cells and enlarged lumen	Highly dilated and damaged sinusoids as well as enlarged hepatocytes	Normal appearance with no obvious abnormality

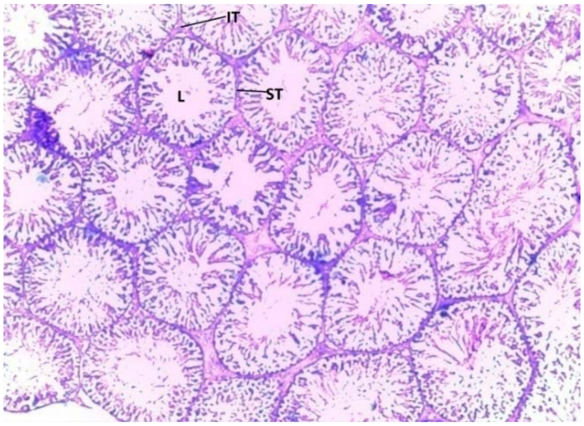

Fig. 3: Photomicrograph of testis in T_3 (50% CPM) section of testicular tissue showing no visible damage on the general tissue structure

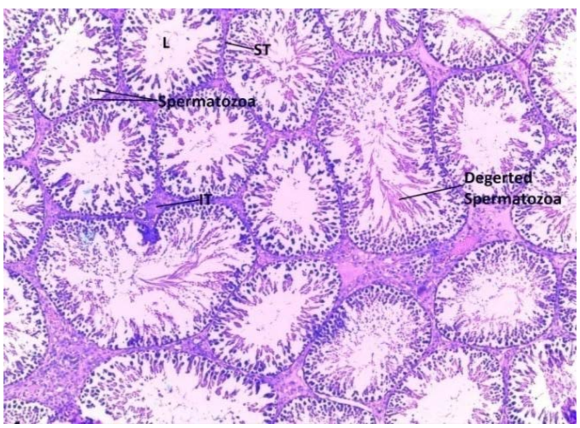

Fig. 4: Photomicrograph of testis in T_4 (75% CPM) section of testicular tissue with some lumen showing degenerated spermatozoa

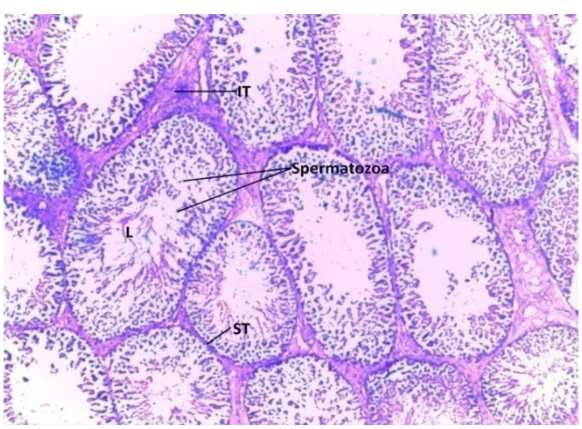

Fig. 5: Photomicrograph of testis in T_5 (100% CPM) section of testicular tissue showing perforated interstitial cells and enlarged lumen

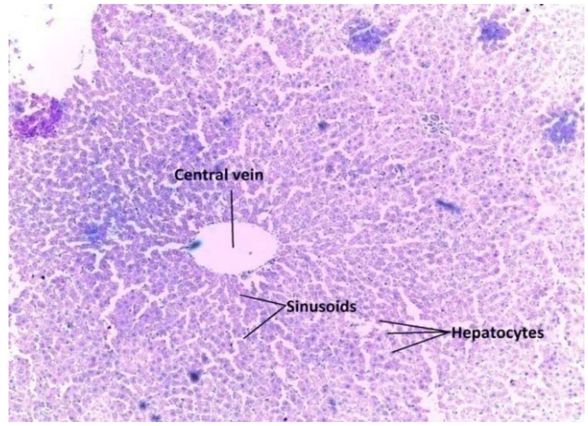

Fig. 6: Photomicrograph of Liver in T_1 (0% CPM) section showing normal appearance of the general tissue structure of liver section

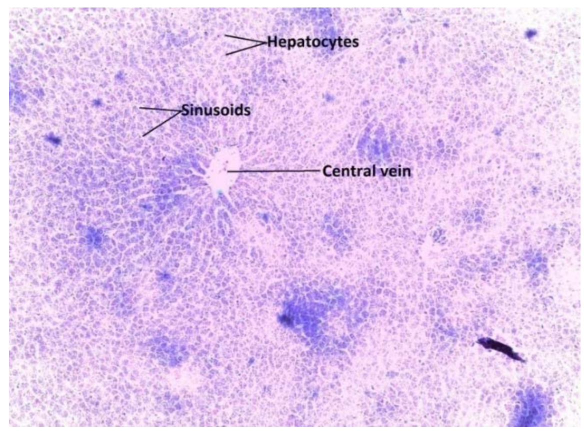

Fig. 7: Photomicrograph of Liver in T_2 (25% CPM) section showing liver tissue with normal appearance of the central vein, the sinusoids and hepatocytes, no structural defect seen

had different tissue alterations and abnormalities, due to the effect of residual cyanide in the cassava peels. Fig. 6-10 show the histological appearance of the liver of rabbits fed cassava peel meal-based-diets. Rabbits fed diets with 0, 25 and 50% recorded normal tissue structures of the liver while those fed 75 and 100% levels had different tissue alterations and abnormalities. Furthermore, Fig. 11-15 show the histological appearance of the kidney of rabbits fed cassava peel meal-based-diets. Rabbits fed diets with 0, 25 and 50% recorded normal tissue structures of the kidney while those fed 75 and 100 % levels had different tissue alterations and abnormalities. More so, Fig. 16-20 show the histological appearance of the brain regions of rabbits fed cassava peel meal-based-diets. Rabbits fed diets with 0, 25, 50, 75 and 100% cassava peel

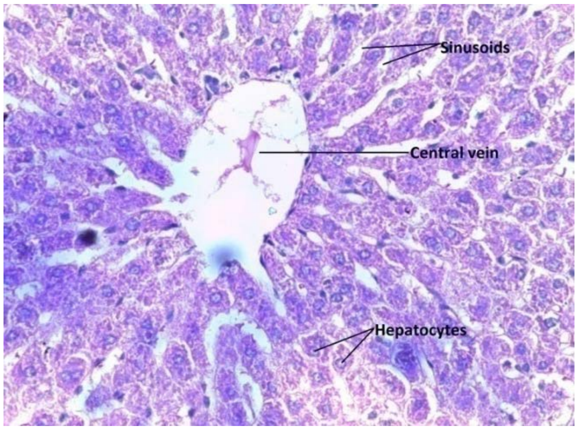

Fig. 8: Photomicrograph of Liver in T_3 (50% CPM) section showing no defect in the general tissue structural

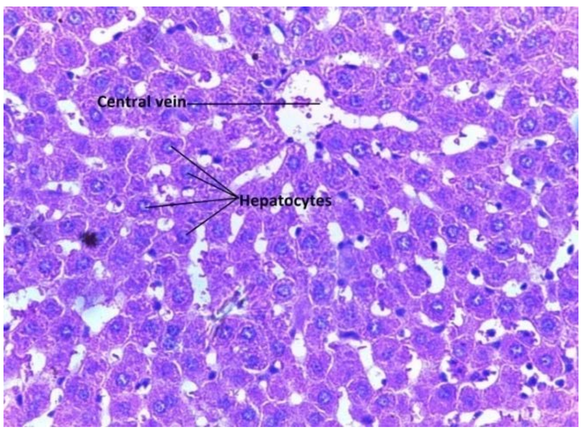

Fig. 9: Photomicrograph of Liver in T_4 (75% CPM) section showing a slightly destroyed central vein with some enlarged sinusoids

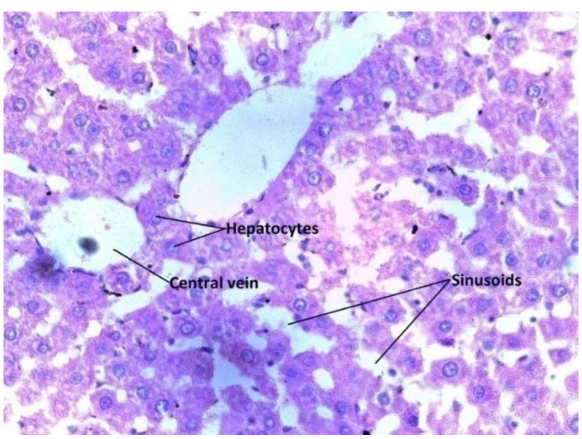

Fig. 10: Photomicrograph of Liver in T_5 (100% CPM) section showing highly dilated sinusoids and enlarged hepatocytes

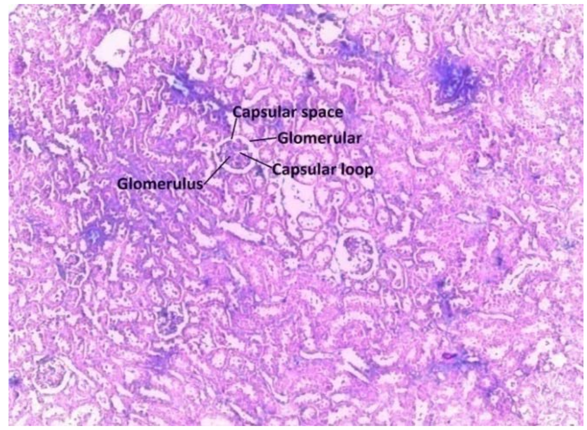

Fig. 11: Photomicrograph of kidney in T_1 (0% CPM) section showing normal appearance of the general tissue structure of a kidney section

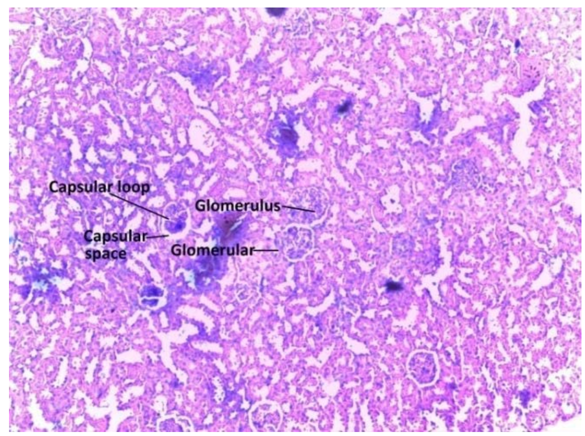

Fig. 12: Photomicrograph of kidney in T_2 (25% CPM) section showing kidney tissue with normal appearance of the glomeruli and normal architectural pattern of the tissue

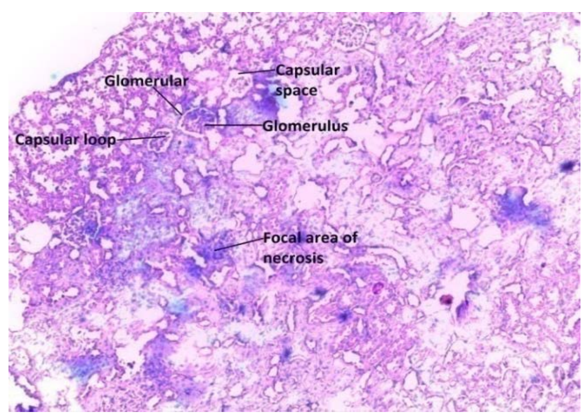

Fig. 13: Photomicrograph of kidney in K3 (50% CPM) section showing focal area of necrosis

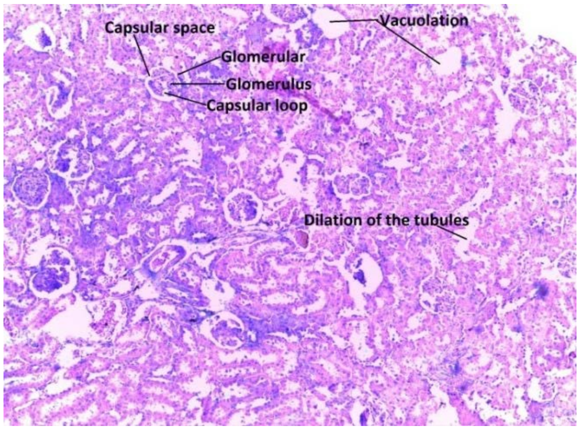

Fig. 14: Photomicrograph of kidney in T_4 (75% CPM) section showing kidney tissue dilation

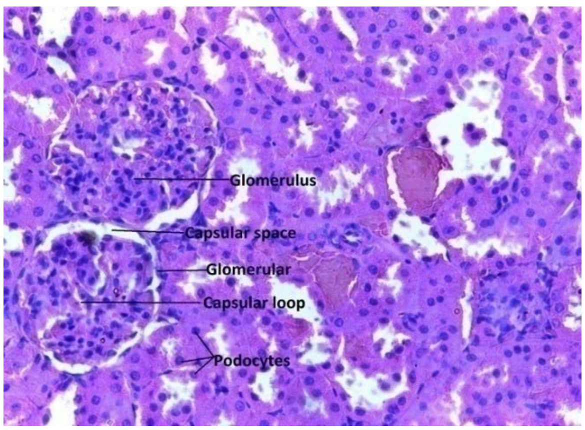

Fig. 15: Photomicrograph of kidney in T_5 (100% CPM) section showing kidney tissue dilation

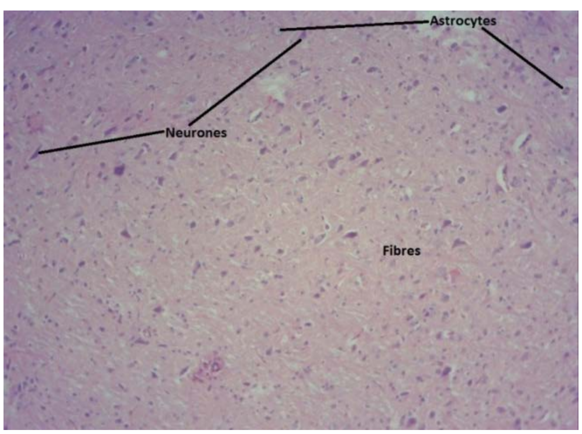

Fig. 16: Photomicrograph of the brain in T_1 (0% CPM) brain section showing normal architecture of the general tissue structure

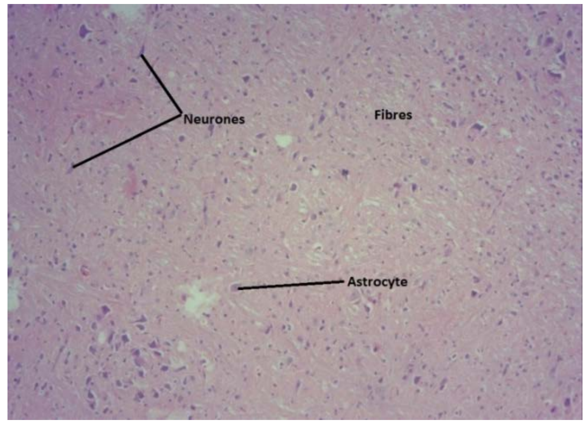

Fig. 17: Photomicrograph of the brain in T_2 (25% CPM) brain section showing normal distribution of cells

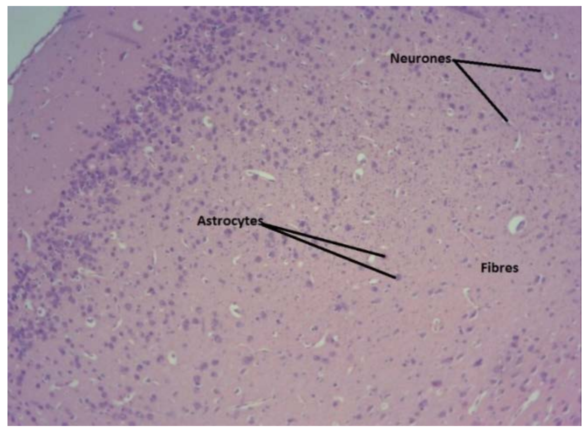

Fig. 18: Photomicrograph of the brain in T_3 (50% CPM) Normal appearance of the brain with no abnormal lesion seen

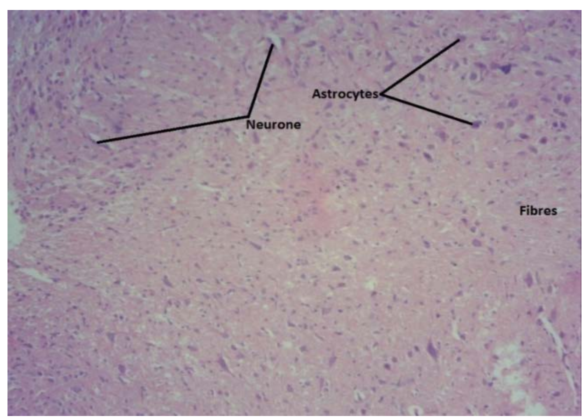

Fig. 19: Photomicrograph of the brain in T_4 (75% CPM) normal appearance of the brain cells with no abnormal seen

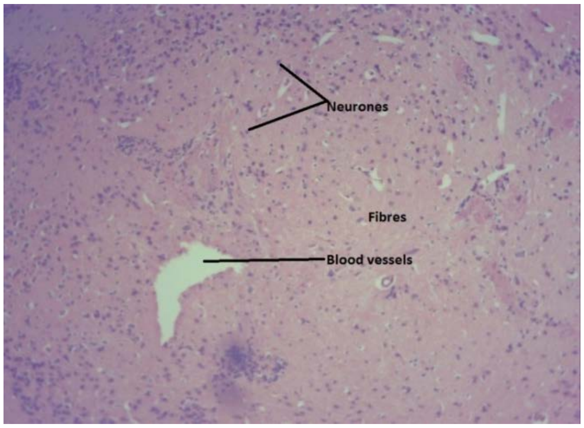

Fig. 20: Photomicrograph of the brain in T_5 (100% CPM) section showing brain tissue with no abnormality of destruction of tissue structure

meal recorded normal tissue structures of the brain regions without any noticeable alteration or abnormalities. However, as the levels of CPM replacement increases across dietary treatments, there was a steady rise in the level of tissue degeneration with the exception of the brain. Tissue abnormalities and damages were more pronounced in dietary treatments T_4 (75%) and T_5 (100%) cassava peel meal replacement levels.

DISCUSSION

The histopathological evaluation of the kidney showed extensive degeneration of tubular epithelial tissue and enlargement of the capsular loop with vacuolation as the level of cassava peel meal replacement increased beyond 50% across dietary treatments. Rabbits in the control diet T_1 (0%) up to T_2 (25%) however, showed normal appearance of the general tissue architecture without necrosis. The result corroborates the finding of Egbunike[23] and Drury *et al.*[24] who fed dried poultry litter, sword bean and cassava root meal to rabbits, broiler chickens and dogs respectively. The photomicrographs of the testes revealed that as the level of CPM replacement increases across dietary treatments there was a noticeable reduction in the volume of mature spermatozoa, degenerated spermatids and tubules with alteration in testicular morphometry, especially from treatments T_4-T_5 (75-00% replacement of CPM). The control diet (0%) and lower levels of 25-50% replacement showed normal testicular morphology and motile germ cells without any noticeable destruction of spermatids. The finding of this study concerning tissue degeneration of testes at 50% level of CPM and above agrees with the report of another study[25] that testicular gem cell sloughing, degeneration and lesions of testes occur in dogs fed higher levels cassava root diets. The histopathology of the liver of rabbits fed cassava peel meal as a replacement for maize revealed normal tissue structure without atrophy of hepatocytes for rabbits on the control diet (0%) and 25% replacement. The histological outcome at levels below 50% CPM showed that the liver was able to carry out its normal regulatory activity of detoxifying cyanide. This is in consonance with the report of Okolie and Osagie[26] who fed enzyme-treated cassava peel meal to pigs. However, 50% CPM replacement level and beyond revealed severe dilation of the sinusoids, with enlarged hepatocytes and damaged central vein. There is also extensive venous damage and congested blood vessels. This finding confirms the reports of Kamalu[27] and Unigwe *et al.*[28] who reported enlarged hepatocytes with vacuolation of cytoplasm and compressed sinusoids, focal necrosis in the liver of the rabbits, dogs and rats respectively exposed to dietary cassava peel meal. According to another study[25] intact cyanogenic glycosides absorbed from cassava root meal basically, linamarin which was not hydrolyzed to HCN is evident in liver inflammation, kidney and testicular lesions. Okolie and Osagie[26] reported an increase in Alanine Aminotransferase (ALT) in the blood of pigs fed fermented cassava peel meal which usually occurs when there is a leakage in the liver. The measurement of this enzyme in the blood is an indication of cell damage[26]. The histopathological result of the brain of the rabbits fed cassava peel meal as a replacement for maize revealed the normal architecture of tissue distribution with no deleterious lesions across all dietary treatments. This implies that residual cyanide in cassava peel meal may not have an adverse effect on the normal functioning of the brain; this may be attributed to the action of rhodanese enzyme in the liver that was able to detoxify the HCN (even with noticeable defects at CPM levels beyond 25%) before it could cross the blood-brain barrier of the rabbits to induce any adverse tissue alteration.

CONCLUSION

The study concluded that the optimum level for cassava peel meal replacement for maize is 25%. However, 50% CPM may replace maize with caution. Replacement levels beyond 50% are not advisable given the potential damage to internal organs of rabbits by the residual cyanide in cassava peel meal.

SIGNIFICANCE STATEMENT

This study discovered the beneficial effect of cassava peel meal in rabbit nutrition. The hitherto farm wastes can be beneficial to the agro-industrial companies via feed milling. Rabbits can survive on cassava peel meal based-diets without deleterious effects at levels not beyond 50% replacement for maize. This study will help researchers to uncover the critical areas of animal feed shortages that many researchers were not able to explore. Thus, a new theory on the utilization of non-conventional feedstuffs may be arrived at in rabbit production.

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