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Physiological response of mixed-breed rabbits fed varied replacement levels of maize with cassava root meal

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Abstract
The dietary protein of animal origin is in short supply for the populace and the available sources are too expensive for an average Nigerian family because of the high cost of production. This study was conducted to investigate the response of growing rabbits fed Cassava Root Meal (CRM) on growth performance, Nutrient digestibility, blood profile and organs parameters of mixed-breed rabbits. Twenty four rabbits (24) were used in completely randomized design (CRD) experiment with four treatments and six rabbits per treatment. Each rabbit serves as replicate and experiment lasted for eight weeks. Four diet were formulated T_1 (control- maize based diet) while T_2, T_3, and T_4 maize were replaced with cassava root meal at 33.33%, 66.67% and 100%, respectively. Growth parameters measured were weight gain, feed intake, final weight gain, average daily feed intake, feed conversion ratio, feed cost/kg and feed cost per kg/Weight Gain (WG) while blood parameter measured include all haematological parameters and some selected blood serum. Other parameters measured include nutrient digestibility and relative organs weight. Growth performance were significantly different ($p<0.05$) in weight gain, feed conversion ratio, cost/kg and cost/kg/WG across the treatments, while final weight gain, total feed intake and average daily feed intake were numerical different across the treatments. However, treatment T_3 with 66.67% inclusion of cassava root meal had the highest weight gain with lowest feed conversion ratio. The nutrient digestibility shows that crude protein, crude fibre, ash, ether extract and dry matter digestibilities were significantly different across the treatments while nitrogen free extract was numerically different across the treatments. However T_4 (100% CRM) provided high percentage crude protein, crude fibre, ash and dry matter digestibilities compared to other treatments which contain 0%, 33.33%, and 66.67% cassava root meal. The replacement of maize with *CRM at* 66.67% levels improved the growth performance, feed efficiency and blood parameters as compared to the control diet. All relative organs were statistically similar except abdominal fat. It was concluded from this study that 100% cassava root meal inclusion can be used to replace maize without any side effect in the rabbit's production, this help to reduce the cost of production especially during the time of maize scarcity.

Keywords: cassava, cassava root meal, rabbit, cassava flour/lafun

Introduction
Dietary protein of animal origin is in short supply and the available sources are too expensive for an average Nigerian family (Kperegbeji and Onwumere, 2007) [18]. This has made meeting the daily animal protein intake very difficult. The shortage of protein particularly of animal origin in human diets in all parts of Africa and most developing countries of the world has been well documented (Apata *et al*., 2000) [5]. The production of non–ruminant species and indeed rabbits represents the fastest means of correcting the shortage of animal protein in tropical Africa.
Mixed breed rabbits buck (*Oryctolagus cuniculus*) have potential as meat producing animals in tropics particularly on subsistent farm, have numbers of qualities that are advantageous to smallholder, subsistence farming system. The small body size, short generation interval, rapid growth rate, genetic diversity and high reproductive potential are characteristic which make rabbit suitable as meat producing small livestock in developing countries (Arijeniwa *et al*., 2000) [6]. Energy source for *Oryctolagus cuniculus* can be basically prepared from corn/maize meal. High cost of this ingredient and unavailability all year round may create a lot of problems in rabbit production. Cassava a staple food in the tropic can be a good source of alternative ingredient as it has a great potential as efficient and cheap source of dietary energy (Hew, 1995) [13]. It is the highest suppliers of carbohydrate among staple crops (FAO 1995) [12]. Its product has been in use for a long time as an energy source in place of cereals grains for livestock (Eruvbetine *et al*., 2003).
Cassava (*Manihot esculenta*) as a high energy crops is available throughout the year in Nigeria. Nigeria is known to be a major source of various varieties of cassava with different maturity age and usefulness. Nigeria was ranked as the world's largest producer of cassava with a production capacity of 40 million tones as far back as 2008 (Okoli, 2008) [23]. However, Oruwari *et al*., (2003) [25] stated that with proper protein balance, cassava meal could completely replace maize in poultry diet, therefore, the use of cassava as an alternative to conventional energy feedstuffs like maize could help to reduce feed costs (Ukachickwu, 2005). Cassava root meal is now widely accepted as alternative feed ingredients in every type of animal diet as reported by Juttopompong *et al*., (2004) [16]. Cassavas (*Manihot esculenta*) plant has many useful products and by-products such it's leaf, stem, tuber, peels etc while by-products such as cassava flour/lafun (which was used in this research work), starch, fufu, mingao, ampesi, macaroni, cassava pudding, gari etc (Balagopalan, 2002) [7].
Lafun is a dried white product of cassava tuber, it's obtained

by soaking fresh cassava tuber in a pot of clean water for four days to soften and ease the removal of peels, pressed to remove water and then sundry to remove the water content to about 10%. This product is very high in energy values of 3,200kcal/kg (Egena, 2006) [9] and low protein level of about 3.6% (Iyayi and Losel, 2001) [14]. Cassava tuber decay rapidly if not processed in good time. Oversupply during the peak of harvest or during period of abundant rainfall cause the price to fall. Channeling such excesses produce to animal feed or storing for animal feed will be an added advantage to the cassava producer and helps to improve the economy. Therefore this research was designed to evaluate physiological response of growing rabbits to varied inclusion levels of cassava root meal in the rabbit feed as a substitution for maize.

Materials and Methods

Experimental Site: The experiment was carried out at Rabbitry Unit, Teaching and Research Farm of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Nigeria, located in the derived Savanna zone of Nigeria.

Diet Preparation: Four experimental diets were formulated (Table 1) such that maize in treatment one serves as control, while maize in treatment two, three, four were replaced with 33.33% 66.67% and 100% of CRM, respectively.

Experimental Animal and Management

A total number of 24 growing buck rabbits were used for the experiment, the rabbits were randomly allocated to four treatments and each treatment consists of six rabbits, while each rabbit serves as replicate, in a completely randomized design (CRD). The experiment lasted for eight weeks and data were analysed using ANOVA at $\alpha_{0.05}$, using IBM SPSS version 21 and significant mean were separated using Duncan's multiple range test of the same software.

Data Collection

Feed samples and faeces were analysed for proximate composition using methods of AOAC (2001). Measurement of the average weekly feed intake and average weekly weight gain were carried out. Values obtained were used to calculate feed conversion ratio. Data were collected daily on the feed intake during the experimental period by subtracting the weight of the feed supplied from the weight of the left over feed of each animal.

Average Weight Gain: Live weight was taken every week throughout the study period, using a weighing scale. Weight gain determine by subtracting the final weight from initial weight. Feed Conversion Ratio was calculated by dividing the total feed consumed by total weight gain at the end of experiments

Digestibility Study

At the 8th weeks, the feacal samples were collected daily for 3 days, air dried and weight before been stored in air tight bags. The samples collected were bulked for each rabbit and sub-samples were taken for proximate composition.

Blood and Serum Analyses: This was done as reported by Shittu *et al*. (2017) [27].

Organs Analysis: At the end of the experiment, three animals selected from each treatment, starved overnight (12 hours) and weighed before slaughtered. The animals were stunned and slaughtered by cutting of the jugular vein. After bleeding, the animals were eviscerated. The organs were carefully dissected out for weighing. The organs such as liver, kidney, lungs, heart, were weighed using sensitive scale.

Results and Discussion

The result of the growth performance parameter of growing rabbit fed varied replacement levels of Cassava Root Meal (CRM) is shown in Table 2. Except final weight and average total feed intake other parameters were significantly ($P < 0.05$) affected. Weight gain of animal on T_3 (1,060.50g) had the highest value while the lowest weight gain was recorded in diet T_4 (892.05g). Feed conversion ratio had significantly similar values among rabbits fed control diet, T_2 (33.33%CRM) and T_3 (66.67% CRM). Cost per Kg feed decreased with increased replacement levels of CRM while cost per Kg weight gain was significantly higher under rabbits fed control diet. The result of the study revealed that increasing level of cassava root meal at 66.67% enhance growth performance of the rabbits for meat production, this can be deduced from the weight gained of rabbits on diet T_3 (1,060.50g). The final weight is heavier than the reported result of Amadi *et al* (2016) [2] when cassava root meal was included up to the 75% replacement of maize in the diet of growing rabbits. This results support the report of Ansah *et al*. (2012) [3] who reported a significant ($p<0.05$) variation in the weight gain of growing rabbit fed cassava root meal. The un-palatability nature of the diet did not affect the feed intake. This result negates the report of Amadi *et al*. (2016) [2] which identified reduction in the feed intake a result of the taste effect as the inclusion of cassava root meal increases in the diet of growing rabbits. The results agreed with the report of the British rabbit Council (2010) that concluded that bitter tastes are well tolerated by rabbits such as alfalfa leafs. Price is another major factor of production, the lower the cost of production the better. Rabbit on CRM based diet had the economically encouraging price per kilogram weight gain (N294.20 N276.57 and N309.06 for T_2, T_3 and T_4, respectively). This is better compared to the control diet that had N321.53. The performance of rabbis fed cassava root meal promoted better feed utilization for rabbits reared within the same study area (Amadi *et al*., 2016) [2] and it shows that rabbits can tolerate cassava root meal up to 100% replacement of maize.

Table 3 shows nutrient digestibility of growing rabbits fed varied replacement level of maize with cassava root meal. The result obtained shows that there were significant difference ($p<0.05$) in the crude protein, crude fibre, ash, ether extract and dry matter digestibility while nitrogen free extract were not significantly different ($p>0.05$) across the treatments. Crude protein digestibility significantly ($p<0.05$) increased with increased cassava root meal replacement levels. Out of all the affected nutrient digestibility parameters, growing rabbits on cassava root meal had the highest values compared to the control group. Apparent nutrient digestibility showed that the rabbits on diets T_2, T_3 and T_4 had better nutrient digestibilities than those on diets T_1 (control diet). Hundred percent CRM may be the optimum for efficient nutrient utilization. The significant improvement of crude protein (CP) and crude fibre (CF) digestibility in this study agreed with the previous report of Osakwe and Nwose (2008) [26] when feed intake and

nutrient digestibility of weaner rabbits fed cassava peel as replacement for maize was examined. The high protein digestibility observed in rabbits on CRM may be explained by the fact that the cyanide and fibre content of the diet may still be within the range that can be tolerated by the rabbits and/or the level of these compounds in the diets was not too high to affect protein intake and digestibility. Ether extract intake and digestibility followed a similar pattern as crude protein digestibility. Ether extract intake was significantly similar for rabbits fed the T_2, T_3 and T_4 but the T_1 fed rabbits had a depressed ether extract digestibility when compared to other groups. Table 4 shows the haematological parameters of rabbits fed varied replacement levels of cassava root meal (CRM) with maize. White blood cell, platelet, monocyte, eosinophil were significantly affected ($p<0.05$) by the varied replacement levels maize with cassava root meal. White blood cell, monocyte, eosinophil highest values were recorded among rabbit fed T_1 (control) and the lowest values were recorded on the rabbits fed varied level of CRM. The highest value of platelet was obtained from the rabbit fed T_3 (66.67%) while the lowest value was obtained on the rabbit fed T_4 (100% CRM). Blood is important and reliable medium for assessing the physiological and health status of individual animals (Egbe-nwiyi *et al*., 2000) [8]. According to James, (2004), the life of all flesh is in the blood. Blood is useful for assessing the health status, clinical evaluation for survey of physiological/pathological conditions and diagnostic and prognostic evaluation of various types of diseases in animal (Singh *et al.*, 2002 [29]; Obasoyo *et al*., 2005 [22] and Alade *et al*., 2005) [1]. The similar value of the neutrophil recorded across all the dietary treatments showed that the antnutrients present in cassava root meal had been removed or reduced by processing methods and it did not affect the blood quality/health status of the rabbits. The significant variation observed in the values of platelet with highest value recorded in rabbits fed 66.67% CRM could be due to the increased in the level of antibodies in the animal which in turn boost the ability of the animal to resist microbial infection and fight against foreign bodies. The value recorded fell between the normal range reported by Mitruka and Rawnsley (1977) [21] and Maxwell *et al*., (1990) [20]. Similarity in the lymphocytes indicates that the rabbits can survive in a stressful environment. It has been stressed by Mitruka and Rawnsley, (1977) [21] that the higher the lymphocyte and white blood cell are associated with the ability of the animal to perform well under a very stressful condition, on this fact is where the similarity lymphocyte obtained in rabbits study found its support. Table 5, shows the serum parameters of rabbits fed varied replacement levels of maize with cassava root meal. The varying replacement of cassava root meal had significant effect ($p<0.05$) on serum biochemical indices of growing rabbits except Alanine Transaminase (ALT), total protein and alanine phosphate. Aspartate aminotransferase (AST) had it highest value observed among rabbits fed CRM, and the lowest value was observed among rabbits fed T_1 (control diet). Cholesterol had it highest value among rabbit fed T_2 (33.33% CRM), T_3 (66.67% CRM) and T_4 (100.00% CRM) while it lowest value (50.00 g/dL) was observed on rabbits on diet T_1 (control diet). The use of serum indices as a pointer or indicator to conditions that cannot be readily noticed by performance and haematological indices cannot be over emphasized in animal science. This help to really understand the disposition of organs to the dietary treatments or variations. However, the numerical highest serum protein recorded in rabbits on 100% CRM (T_4), implies that the dietary treatment contains or influence the utilization of dietary protein. Albumin is the most abundant protein in blood plasma and it production was not affected by cassava root meal based diets. The significantly ($p<0.05$) highest albumin value in 100% (T_4) similar to those on 33.33% CRM (T_2) and T_3 (66.67% CRM) showed a synergetic effect on the protein quality of the test ingredients. Higher value of aspartate aminotransferase in this study when compared with the control birds may be as a result of the cyanide residue in the cassava root meal. Ewuola and Egbunike (2008) [11] observed that in their study that the highest significant value in aspartate aminotransferase (AST) is a clinical indication of diagnosing state of damage done to the organ producing it by toxic substance or infections. The increased in cholesterol as the replacement level of CRM increased and the highest value (62.69 mg/dL) obtained in the T_4 falls within the recommended level according to Mitruka and Rawsleys (1977). Although many factors has been suggested earlier to affect the cholesterol in broiler chickens. According to Maulana *et al*. (2019) [19], environmental factors, population density etc other than the nutritional factors can affect the cholesterol levels in broiler chickens. Table 6, shows the result of the relative organs weight of rabbits fed varied replacement level of maize with cassava root meal. Except abdominal fat other parameters such spleen, lung, liver, kidney and heart were not significantly ($P>0.05$) different across the treatments. The relative abdominal fat weight of rabbit fed diet T_2, T_3 and T_4- 2.80, 2.93 and 2.99%, respectively were similar. The result here helps to determine the response of various organs to the dietary variation. Similarities in the entire organs are an indication of similarity in the dietary activity of the various chickens and there was no enlargement or atrophy of the internal organs beyond normal when compare to the control animals thus indicating that the rabbits were able to tolerate the test ingredients. The relative weight of the liver and heart in this study were smaller compare to the report of Shittu *et al*. (2013) [28] and Orayaga *et al*. (2017) [24] when mango seed kernel meal were feed to the growing rabbits, this may be as a result of ant nutritional factor in the mango seed kernel meal used. One of the most important factors identified to affect organ growth or weight is the type of and amount of feed ingested by the animal (Kokoszynski *et al*., 2017).

Others include body size, species, breed, sex, age, health and physiological status of the birds, most of the changes occur in weight, length and histochanges. Kokoszynski *et al*. (2017) [17] stated that changes in digestive tract structure mainly concern the weight, length, and width of the different segments.

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

Ingredients (%)	T_1 (control)	T_2 (33.33% RM)	T_3 (66.67% CRM)	T_4 (100% CRM)
Maize	30	20	10	-
CRM	-	10	20	30
Fixed ingredients	70	70	70	70
Total	100	100	100	100
Calculated composition of the diet				
Metabolize energy	2666	2609.26	2565.00	2522.00
Crude protein (%)	16.17	16.72	16.07	15.50
Crude fiber (%)	6.70	7.10	7.20	7.40

CRM= Cassava Root Meal. Fixed ingredients were: soymeal (26%), Wheat offal (26%), corn bran (6%), fish meal (1%), Palm kernel cake (22.16%), Veg. Oil (2%), oyster shell (0.25%), bone meal (2%), salt 0.22%), Methionine (0.12%), grower premix (0.25).

Table 2: Growth Performance of rabbit fed varied replacement level of cassava root meal with maize

Parameters	T1 Control	T2 (33.33%CRM)	T3 (66.67%CRM)	T4 (100%CRM)	SEM
Initial Weight (g)	850.22	848.35	850.10	847.94	-
Final Weight (g)	1828.60	1898.80	1910.60	1743.00	16.96
Weight Gain (g)	978.38^{b}	1050.45^{ab}	1060.50^{a}	892.05^{c}	12.21
ADWG	17.38^{a}	18.76^{a}	18.94^{a}	15.92^{b}	2.09
TFI (g)	4232.00	4447.40	4547.10	4544.20	239.11
ADFI (g)	76.74	79.41	82.67	82.62	2.52
FCR	4.32^{b}	4.23^{b}	4.28^{b}	5.09^{a}	0.28
Cost/Kg feed (N)	74.43^{a}	69.55^{b}	64.62^{b}	60.72^{b}	0.28
Cost/Kg/(N)	321.53^{a}	294.20^{b}	276.57^{b}	309.06^{b}	4.85

^{abc} means having different superscript along the same row are significantly different ($p<0.05$), SEM = standard Error of Mean, TFI = Total Feed Intake, ADFI = Average Daily Feed Intake, FCR = Food Conversion Ratio, ADWG = Average daily weight gain, CRM = Cassava Root Meal

Table 3: Apparent Nutrients Digestibility of rabbits fed varied replacement levels of cassava root meal with maize

Parameters	T_1 Control	T_2 (33.33% CRM)	T_3 (66.67% CRM)	T_4 (100%CRM)	SEM
Crude Protein	73.51^{b}	84.43^{a}	86.14^{a}	87.33^{a}	1.51
Crude Fibre	33.15^{c}	$51.69b^{b}$	74.64^{a}	64.75^{a}	3.82
Ash	52.77^{c}	65.82^{b}	77.76^{a}	79.78^{a}	2.73
Ether extract	91.34^{b}	92.53^{a}	93.17^{a}	94.58^{a}	0.43
Nitrogen free extract	81.24	81.87	81.41	81.62	4.23
Dry matter	71.23^{b}	76.21^{b}	77.74^{ab}	83.51^{a}	1.39

^{a,b,c} means along the same row having different superscript are significantly different ($p<0.05$), SEM = Standard Error of mean.

Table 4: Haematological parameters of rabbit fed varied replacement levels of cassava root meal with maize

Parameters (%)	T_1 Control	T_2 (33.33% CRM)	T_3 (66.67% CRM)	T_4 (100%CRM)	SEM
Hb (g/Dl)	13.60	13.10	13.00	13.55	0.21
RBC ($x10^{6}$uL)	6.86	6.60	6.47	6.86	0.08
CRT (mg/Dl)	1..15	1.10	1.15	1.10	0.02
PCV (%)	41.50	40.00	39.00	41.00	0.57
WBC ($x10^{3}$uL)	70.00^{a}	42.00^{b}	51.25^{b}	38.50^{b}	38.86
Platelet ($x10^{3}$%)	8.75^{bc}	10.60^{b}	13.90^{a}	6.75^{c}	2.36
LYM (%)	66.50	69.00	69.00	67.30	1.02
NEUT (%)	27.50	26.50	27.00	28.50	1.17
MON (%)	2.50^{a}	1.50^{b}	1.50^{b}	2.00^{b}	0.24
EOS (%)	3.50^{a}	3.00^{b}	2.50^{b}	2.50^{b}	0.179

^{a,b,c} means along the same row having different superscript are significantly different ($p<0.05$), SEM = Standard Error of mean, MON = Monocyte, CR = Creatinine, RBC = Red Blood Cell, PCV = Pack Cell Volume, WBC = White Blood Cell, HB = Haemoglobin, LYM = Lymphocyte, LT = Platelet, EOS = Eosinophil.

Table 5: Shows the serum parameters of rabbits fed varied replacement levels of cassava root meal with maize

Parameters (%)	T_1 (Control)	T_2 (33.33% CRM)	T_3 (66.67% CRM)	T_4 (100%CRM)	SEM
CHOL (mg/dL)	50.00^{b}	59.61^{a}	63.08^{a}	62.69^{a}	2.09
AST (IU/L)	4.72^{b}	8.55^{a}	8.42^{a}	8.50^{a}	0.50
ALT (IU/L)	12.03	13.61	12.86	10.32	0.55
ALP (IU/L)	40.28	42.06	39.15	40.87	1.83
TP (g/dL)	6.41	6.29	6.85	6.55	0.08
ALB (g/dL)	2.99^{b}	3.69^{a}	3.48^{a}	3.70^{a}	0.08
Globulin (g/dL)	3.42	2.60	3.37	2.85	0.13

^{a,b} means among the same row having same superscript are significantly difference ($p<0.05$), CHOL = Cholesterol, SEM = Standard Error Mean, AST = Aspartate aminotransferase, TP = Total Protein, ALB = Albumin, ALT = Alanine Transaminase, ALP = Alanine Phosphate, CRM = Cassava Root Meal.

Table 6: Relative Organ Characteristics of Rabbit Fed Varied Replacement Levels of Cassava Root Meal with Maize

Parameters	T_1 (Control)	T_2 (33.32% CRM)	T_3 (66.67% CRM)	T_4 (100% RCM)	SEM
Abdominal Fat	2.45[b]	2.80[a]	2.93[a]	2.99[a]	1.08
Spleen	0.05	0.05	0.05	0.05	0.00
Lung	0.64	0.50	0.57	0.51	0.33
Liver	3.02	3.55	3.18	3.01	0.63
Kidney	0.64	0.67	0.81	0.83	0.14
Heart	0.33	0.32	0.28	0.30	0.08

[ab] means having different superscript along the same row are significantly different, CRM = Cassava Root Meal

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