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Whole cassava root meal from TME419 cassava variety can support performance and health of growing rabbits as dietary energy source

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Target Audience: *Animal nutritionists, Feed manufacturer and Rabbit farmers*

Abstract

A total of thirty-six (36) growing (6-8 weeks old) New Zealand and Haliquine rabbits of both sexes were used to evaluate performance and health status of rabbits fed fermented whole cassava root meal (WCRM, TME419 Cassava variety) with enzyme supplementation. The rabbits were allotted to experimental groups in a 3 x 2 factorial arrangement comprising three fermentation durations (0, 3 and 5days) and inclusion of enzyme (0, 0.5%) as factors in a randomized complete block design. The diets were designated as D_0^- (Basal diet (WCRM) without ensiling and no enzyme supplementation), D_0^+ (Basal diet (WCRM) without ensiling with enzyme supplementation), D_3^- (Basal diet (WCRM) with three days ensiling without enzyme supplementation), D_3^+ (Basal diet (WCRM) with three days ensiling with enzyme supplementation), D_5^- (Basal diet (WCRM) with five days ensiling without enzyme supplementation) and D_5^+ (Basal diet (WCRM) with five days ensiling with enzyme supplementation). The performance, hematological and serum biochemical indices were not significantly ($P>0.05$) influenced by fermentation and enzyme inclusion. It is therefore concluded that TME419 cassava variety can be fed to growing rabbits without fermentation or enzyme inclusion.

Keyword: *enzyme; growing rabbit; haematology; performance; serum chemistry; whole cassava root meal*

Description of Problem

Low protein in the diet leads to nutritional deficiencies, with greater severity observed in animal protein deficiency because of its high biological value (1). However, one of the solutions to address the issue of protein deficiency is the breeding of animals that have a short production cycle such as rabbit. The rabbit (*Oryctolagus cuniculus*) is a monogastric animal recognized as a livestock species that can provide a regular supply of high quality protein under sustainable systems that utilize renewable resources (2). Their digestive reservoir permits and increases the efficiency of utilization of fibrous diets (3) with the tendency of converting feed to meat and utilizes about 30% cassava products (4) due to the presence of large caecum.

Studies on production system showed that feeding is the major limiting factor in achieving optimal performance in rabbit. Feeding is a major part of management in non-ruminant animals and is estimated at about 60-75% of the total cost of production for intensively reared stocks (5, 6). Due to high cost of maize as major feed materials for rabbit diets have accelerated the demand for alternative which cut across accessibility and lower cost of production. Attention has been directed at cassava as cheaper, locally available and nutritionally viable alternative feedstuff that is drought resistant and produced all year round with lesser competition with humans, thereby limiting the dependence on maize as an energy source in rabbit diets (4). Cassava (*Manihot esculenta*) is a tuber crop with an

increased production stands over 63% of the 303 million tonnes produced globally in 2019 was from Africa (7). The whole cassava root meal composed almost exclusively of carbohydrate, as well as approximately 1% to 3% crude protein (8). The metabolizable energy (ME) of whole cassava root have been presented by various authors, with values ranging from 3,000 to 3,200 kcal/kg (9), 3,200 kcal/kg (10), 3145 kcal/kg (11) and 3,279 kcal/kg (12).

Despite the advantages of cassava to livestock, the restriction in the use is due to deficiencies in protein, high cyanide and fibre content. In order to inactivate the anti-nutritional factors and high fibre the process of fermentation and enzyme inclusion has been exploited (13, 14). This study evaluated whole cassava root meal subjected to fermentation and enzyme supplementation as dietary energy source in the diet of growing rabbits. The cassava variety TME419 was used for this experiment. According to (15) the cassava variety TME 419 has a dry content of cassava estimated as percentage (DM) of total fresh root weight ranging from 30.68 to 31.26%, the level of cyanide in the root is 6.33 ppm. This variety is mainly composed of starch but with a very low percentage of protein. The quantity of starch contained by percentage in this variety ranges from 63.08 to 73.93% while the quantity of protein ranges from 0.80 to 1.52% (16). Performance characteristics were evaluated as well as hematology and serum chemistry as indirect tools for the assessment of the health status of the rabbits. The importance of hematological and serum biochemical indices of animals cannot be over emphasized as it is well acknowledged (17).

Materials and Methods

Study location

The experiment was conducted at the Teaching and Research Farm, College of Agricultural Sciences, Olabisi Onabanjo University, Ayetoro Campus, Ogun State, Nigeria. The university campus is located in a deciduous/derived savannah zone of Nigeria at latitude $7^{0}15^{1}$N and longitude $3^{0}3^{1}$E. Climate is sub-humid tropical with an annual rainfall of 1,909.3mm. Rainy season is between early April and late October. Rainfall pattern is bimodial with two peaks in June and September. Maximum temperature varies between 29^{0}C during the peak of the wet season and 34^{0}C at the onset of the wet season and mean annual relative humidity is 81% (18).

Management of experimental animals

A total of thirty-six (36) growing (6-8 weeks old) with an average weight of 1.00±0.03kg, New Zealand White and Harlequin rabbits of both sexes were procured for the experiment. Animals were kept individually in cages (60cm x 30cm x50cm) in open sided housing under natural ventilation and lighting. During the period of acclimatization which lasted for 2weeks, animals were observed twice daily between 08:00 -9:00am and 3:00 -4:00pm. Feeding and watering was given without restriction.

Experimental diets

The cassava (variety TME419) was procured from the Crop Production Unit of the University Farm. The cassava tubers were washed with clean water to free them from dirt. They were milled with peel intact scooped into a sack, tighten to eliminate air and kept in a hydraulic compressor for a period of 3 or 5days. The compressed cassava root meal was sun dried until practical dryness was achieved after seven days. Other ingredients like soybean meal (SBM), fish meal (FM), wheat offal (WO), Brewer dry grain (BDG), bone meal (BM), enzyme, vitamins and mineral premixes, and salt were bought from feed millers. The enzyme used for the diet was cellulase (Fullzyme ® enzyme at rate of 50g to 100kg). The materials were stored in a cool dry place before mixing. The experimental diets were formulated to be iso-nitrogenous and iso-caloric. The experimental diets are presented in Table 1.

Table 1: Composition of experimental diets

Ingredients (%)	D_0^-	D_0^+	D_3^-	D_3^+	D_5^-	D_5^+
Whole Cassava Root Meal (WCRM)	30	30	30	30	30	30
Wheat Offal	25	25	25	25	25	25
Brewers Dry Grains	23	23	23	23	23	23
Palm Kernel Cake	9	9	9	9	9	9
Soybean meal	7	7	7	7	7	7
Fish meal	2	2	2	2	2	2
*Enzyme	0	0.5	0	0.5	0	0.5
Bone meal	2	2	2	2	2	2
Oyster shell	1	1	1	1	1	1
*Premix	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Total	100	100	100	100	100	100
Calculated Nutrient						
Crude Protein	15.3	15.3	15.3	15.3	15.3	15.3
Crude Fibre	11.7	11.7	11.7	11.7	11.7	11.7
Ether Extract	2.12	2.12	2.12	2.12	2.12	2.12
Calcium	1.05	1.05	1.05	1.05	1.05	1.05
Phosphorus	0.43	0.43	0.43	0.43	0.43	0.43
ME (Kcal/kg)	2293.7	2278.7	2263.7	2248.7	2278.7	2263.7

*Premix containing per kg diet: Vit. A 3.2×10^6 I.U; Vit.D 8.0×10^5 I.U; Vit. E 4×10^3 I.U; Vit. B1 400mg; Vit. B2 800mg; Vit. B_3 300mg; B_6 400mg; B_{12} 3mg; Niacin 600mg; Panthothenic acid1000mg; Folic acid 140mg; choline chloride 5.75×104 mg; Zinc 1.6×105 mg; Co 85mg; Mn 200mg; Ethoxyquine 650mg.
*enzyme (Fullzyme ® enzyme) contains Amylase, Protease, Cellulase, Lipase, Pectinase, Xylanase, Beta-Glucanase and Phytase

Experimentation

Experimental animals were allotted to treatments in a 3 x 2 factorial arrangement comprising three fermentation durations (0, 3 and 5days) and inclusion of enzyme (0, 0.5%) as factors in a randomized complete block design. The various experimental diets were fed to the rabbits for a period of eight weeks *ad libitum*. They are designated as follows:

D_0E^- – Basal diet (WCRM) without fermentation and no enzyme inclusion

D_0E^+ – Basal diet (WCRM) without fermentation with enzyme inclusion

D_3E^- – Basal diet (WCRM) with three days fermentation without enzyme inclusion

D_3E^+ – Basal diet (WCRM) with three days fermentation with enzyme inclusion

D_5E^- – Basal diet (WCRM) with five days fermentation without enzyme inclusion

D_5E^+ – Basal diet (WCRM) with five days fermentation with enzyme inclusion.

Data Collection

Daily feed intake

The feed was weighed to the nearest gram before offering to the animals. Daily feed consumed per head/animal was recorded as difference between feed offered and feed left over. Average feed intake per day was determined by dividing the total feed intake by the number of days of the experiment.

Live weight changes

Animals were weighed at the onset of the experiment and every week during the experimental period with top loading balance to the nearest grams. Weight gain was determined by subtracting the initial weight of the animal from the final weight.

Average Daily weight gain

This was determined as the difference between the final and the initial weights divided by number of days of the experiments.

$$\frac{\text{Final weight - Initial weight}}{\text{Number of days of experiment}}$$

Feed conversion ratio (feed/gain)

Feed to Gain ratio was estimated as the daily feed intake divided by the daily weight gain of each animal.

$$\text{FCR} = \frac{\text{Daily feed intake (g/day)}}{\text{Daily weight gain (g/day)}}$$

Collection of blood samples

At the end of the feeding trial, blood samples were obtained by marginal ear vein puncture and 10ml of blood was collected using hypodermic syringes, 5ml was poured into ethyl diamine tetra acetic acid (EDTA) bottle for haematological analysis while the remaining 5ml was poured into anticoagulant free plastic tubes and allowed to clot at room temperature for biochemical and enzymological analyses. The collection exercise was done at the end of the experiment.

Determination of haematological parameters

The Packed Cell Volume (PCV) was measured using the micro-haematocrit method. Haemoglobin (Hb) concentration was measured using Sahl's (acid haematin) method (19). Red Blood Cell (RBC), White Blood Cell (WBC) and the WBC differential counts i.e. the lymphocyte, neutrophils, basophils, eosinophils and monocytes were measured with the aid of Neubauer counting chamber (haemocytometer) after appropriate dilution (20). The Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean corpuscular Haemoglobin Concentration (MCHC) values were calculated from PCV, Hb and RBC values (21).

Determination of serum parameters

The plasma concentrations of glucose and cholesterol were determined using the glucose oxidase method described by (22). Biuret method of total serum protein determination was employed in this assay as described by (23). Albumin was determined using Bromocresol Green (BCG) method as described by (24). The globulin concentration was obtained by subtracting albumin from the total protein. Aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were determined using spectrophotometric methods as described by (25).

Statistical Analysis

Data collected were subjected to the analysis of variance (ANOVA). Differences between the treatment means were separated using the Duncan's Multiple Range Test (26). The linear statistical model:

$Yijk = M + Di + Ej + (DE)ij + Eijk$

Where Yij = individual observation

M = overall mean

Di = length of fermentation effect (D_0, D_3, D_5)

Ej = Enzyme inclusion effect (E^-, E^+)

(DE)ij = interactive effect of length of fermentation and enzyme inclusion

Eijk = random error effect.

Where:

D_0 - 0 day fermentation
D_3 - 3 days fermentation
D_5 - 5 days fermentation
E^- - without enzyme inclusion
E^+ - with enzyme inclusion

Results and Discussion

Tables 2 and 3 presented the main and interactive effects between period of fermentation and enzyme supplementation on performance characteristics of rabbit fed experimental diets. It was observed that all indices were neither significantly ($P>0.05$) influenced by duration of fermentation nor enzyme inclusion. The results obtained for rabbit fed with or without fermentation and enzyme inclusion agreed with the findings of (27, 28) who observed no beneficial effect on

growth performance of rabbit due to dietary enzyme supplementation. Also, (29, 30) who could not detect any significant effect of enzymes on rabbit's performance.

Table 2: The main effects of length of fermentation and enzyme inclusion on performance of growing rabbits fed WCRM based diets

Parameters	Days of fermentation			SEM	P-value	Enzyme inclusion		SEM	P-value
	D_0	D_3	D_5			E^-	E^+		
Initial weight (kg)	1.03	1.06	1.10	0.11	0.78	1.04	1.08	0.07	0.70
Final weight gain (kg)	1.58	1.82	1.80	0.17	0.74	1.70	1.77	0.12	0.67
Weight gain (kg)	0.55	0.76	0.70	0.10	0.60	0.66	0.69	0.07	0.50
Av. daily weight gain (g)	9.82	13.57	12.50	1.75	0.48	11.79	12.32	1.15	0.36
Feed Intake (g)	4375.04	4802.17	4905.21	400.79	0.45	4509.25	4879.03	264.61	0.33
Av. daily Feed intake (g)	78.13	85.75	87.59	7.16	0.45	80.52	87.13	4.73	0.33
Feed conversion ratio	7.96	6.32	7.01	1.86	0.87	6.83	7.07	1.31	0.84

Table 3: Interactive effect of fermentation and enzyme supplementation on the performance characteristics of growing rabbits fed WCRM based diets

Parameters	D_0		D_3		D_5		SEM	P-value
	E^-	E^+	E^-	E^+	E^-	E^+		
Initial weight (kg)	1.00	1.02	1.03	1.08	1.10	1.10	0.16	0.09
Final weight gain (kg)	1.62	1.55	1.73	1.90	1.74	1.87	0.25	0.58
Weight gain (kg)	0.62	0.53	0.70	0.82	0.64	0.77	0.15	0.86
Av. daily weight (g)	11.07	9.46	12.5	14.64	11.43	13.75	2.56	0.85
Feed intake (g)	4094.33	4655.75	4633.92	4970.42	4799.50	4810.92	402.80	0.64
Av. feed intake (g)	73.11	83.14	82.75	88.76	85.71	85.91	10.50	0.64
Feed conversion ratio	6.60	8.79	6.62	6.06	7.50	6.45	0.76	0.12

Tables 4 and 5 present the main and interactive effects of the length of fermentation and enzyme supplementation on hematological profile of rabbit fed experimental diets. It was observed that all indices were neither significantly ($P>0.05$) influenced by duration of fermentation nor enzyme inclusion. The findings on hematology in this research agreed with the findings by (31, 32, 33). The hematological values of rabbits in this research are within normal ranges for PCV, Hb and RBC (43-49%, 14.50-17.26g/dl and 7.80-8.60 10^{12}/l) as reported by (34, 35). This suggests that the experimental diets are nutritionally adequate and not physiologically detrimental. (36) described the red blood cell indices; Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) as important morphological characteristics of anaemia and the capacity of the bone marrow to produce RBC of normal size and metabolic capacity. The MCV, MCHC and MCH) values obtained were within normal range (60 – 73fl, 16.23-20 pg and 26–34%) for healthy rabbit (37, 38, 39). This is an indication that the rabbits were not anaemic.

The WBC values obtained in this study were within the normal range of 5 x 10^9/l to 13 x 10^9/l (37; 40). The white blood cell differentials were also within the normal range for healthy rabbit except for neutrophils which was lower than the values according to (34; 41, 35). However, (38) described that neutrophils and lymphocytes are the main variation in determining the normal or abnormal physiological status most especially in rabbit. The increase in neutrophils might be as reported by (42) who suggested that increase in white blood cell differentials is accompanied by an increase in plasma cortisol that is associated with stress. Also this agrees with (43) that stress alters the differential white blood cell count in any species most especially

rabbit which are particularly susceptible to the effects of stress. Moreover, the decreases in basophils and monocytes can be suggested that the experimental animals are in good health condition. As reported by (44) stated that increase in monocyte count can be associated with chronic bacterial infection. This indicated that in relation to the White cell count and its differential proves that the animals were healthy since no reduction in the number of WBC below the normal range which can result to leukocytopenia (an indication of allergic conditions.

Table 4: The main effect of length of fermentation and enzyme inclusion on hematological profile of rabbits fed WCRM based diets

Parameters	Days of fermentation			SEM	P-value	Enzyme inclusion		SEM	P-value
	D_0	D_3	D_5			E^-	E^+		
PCV (%)	49.00	45.67	47.67	2.46	0.64	49.44	45.44	1.53	0.07
Hb (g/dl)	16.48	15.30	15.90	0.85	0.62	16.58	15.21	0.53	0. 07
RBC (x 10^{12}/l)	8.23	7.68	7.97	0.43	0.67	8.28	7.64	0.29	0.08
MCV (pg)	51.25	59.42	59.65	4.74	0.39	54.19	59.36	3.17	0.12
MCH (fl)	20.03	19.92	19.95	0.08	0.58	20.03	19.90	0.05	0.11
MCHC (%)	33.65	33.52	33.48	0.12	0.60	33.54	33.56	0.08	0.10
WBC (x 10^9/l)	10.88	10.15	10.42	0.48	0.57	10.83	10.13	0.31	0.08
Neut (%)	29.67	28.67	29.50	1.02	0.76	30.00	28.56	0.64	0.06
Lym (%)	68.50	69.50	69.50	1.05	0.74	68.56	69.78	0.66	0.12
Eos (%)	0.67	0.33	0.17	0.20	0.22	0.33	0.44	0.14	0.07
Bas (%)	0.67	0.33	0.17	0.20	0.22	0.56	0.22	0.13	0.07
Mono (%)	0.67	1.00	0.67	0.17	0.12	0.44	0.67	0.14	0.07

PCV – Packed Cell Volume; Hb – Hemoglobin concentration; RBC – Red Blood Cell; MCV – Mean Corpuscular Volume; MCH – Mean Corpuscular; MCHC – Mean Corpuscular Hemoglobin Concentration; WBC – White Blood Cell; Neut – Neutrophil, Lym – Lymphocyte; Eos – Eosinophils, Bas – Basophils; Mono -Monocytes

Table 5: The interactive effect of length of fermentation and enzyme inclusion on the hematological profile of rabbits fed WCRM based diets

Parameters	Day 0		Days 3		Day 5		SEM	P-value
	E^-	E^+	E^-	E^+	E^-	E^+		
PCV (%)	49.69	46.66	48.00	43.33	49.00	46.00	3.60	0.19
Hb (g/dl)	17.26	15.70	16.10	14.50	16.36	15.43	1.24	0.20
RBC (x 10^{12}/l)	8.60	7.90	8.06	7.30	8.16	7.76	0.63	0.24
MCV (pg)	59.76	59.40	59.46	59.36	60.00	59.30	0.48	0.36
MCH (fl)	20.10	19.96	19.96	19.86	20.33	19.86	0.17	0.19
MCHC (%)	33.66	33.63	33.56	33.46	33.40	33.56	0.18	0.94
WBC (x 10^{12}/l)	11.16	10.60	10.56	9.73	10.76	10.06	0.72	0.26
Neut (%)	31.66	27.66	28.33	29.00	30.00	29.00	1.37	0.22
Lym (%)	66.33	70.66	70.33	68.67	69.00	70.00	1.34	0.29
Eos (%)	0.67	0.67	0.33	0.66	0.67	0.33	0.30	0.66
Bas (%)	1.00	0.33	0.33	0.33	0.33	0.33	0.27	0.16
Mono (%)	0.33	0.67	0.67	1.00	0.00	0.66	0.24	0.27

PCV – Packed Cell Volume; Hb – Hemoglobin concentration; RBC – Red Blood Cell; MCV – Mean Corpuscular Volume; MCH – Mean Corpuscular; MCHC – Mean Corpuscular Hemoglobin Concentration; WBC – White Blood Cell; Neut – Neutrophil, Lym – Lymphocyte; Eos – Eosinophils, Bas – Basophils; Mono -Monocytes

Tables 6 and 7 present the main and interactive effect on blood chemistry profile of rabbit fed experimental diet. All parameters measured were neither significantly (P>0.05) influenced by duration of fermentation nor enzyme inclusion. The value for total protein (57.60-

63.00g/l) compared favorably with the values (54-75 and 50-80g/l) reported for rabbits by (41, 37). This shows that the experimental diets met the rabbit's requirements. The values for albumin and globulin in the study (3.86-4.40 and 1.50-1.93) fall within the normal values (25-45.0 and 27-50g/l) for health rabbits (41, 35). It suffices to say that the nutrient profile of the diets was adequate to support the health status of the rabbits based on the comparable results obtained across the groups. The cholesterol level (55.33-71.30mg/dl) was not in the physiological range (11-54mg/dl) reported by (41). Moreover the result of increase in the cholesterol level was described by (41, 45) who stated that increase may result in anorexia, liver dysfunction, malabsorption of fat. Although, during the experiment, experimental animals did not show any sign of such.

The values for AST and ALT fall within the normal values (10-96u/l and 25-65u/l) for health rabbit as reported by (41). The monitored activities of the enzymes Aspartate Transaminase (AST) and Alanine Transaminase (ALT) plays vital role in processing proteins, reflection of a balance between synthesis and their release as a result of the different physiological processes in the body. Moreover, they are also responsible for the synthesis of non-essential amino acids through the process known as transamination according to (46). However, if the levels are higher than normal; it causes liver damage and gallbladder disease and also malfunctioning of the liver. The value obtained for thiocyanide falls within the recommended value by (35) for normal rabbit fed cyanide based diets. This suggested that whether fermented or not and with or without enzyme inclusion WCRM did not impart negatively on liver function.

Table 6: The main effect of length of fermentation and enzyme inclusion on blood chemistry profile of rabbits fed WCRM based diets

Parameters	Days of fermentation			SEM	P-value	Enzyme inclusion		SEM	P-value
	D_0	D_3	D_5			E^-	E^+		
Total Protein (g/dl)	6.1	5.77	6.08	0.19	0.33	6.03	5.96	0.13	0.67
Albumin (g/dl)	4.37	4.05	4.35	0.24	0.59	4.21	4.30	0.16	0.60
Globulin (g/dl)	1.78	1.68	1.72	0.27	0.97	1.81	1.64	0.18	0.06
Cholesterol (mg/dl)	57.83	59.20	71.30	14.73	0.78	64.69	60.87	9.65	0.81
AST (U/L)	62.50	49.50	51.00	6.39	0.32	55.89	52.78	4.43	0.70
ALT (U/L)	36.33	30.67	31.50	3.07	0.39	33.56	32.11	2.10	0.70
Thio (µg/ml)	3.27	4.03	4.07	0.82	0.74	4.47	3.11	0.50	0.62

AST – Aspartate Aminotransferase; ALT – Alanine Aminotransferase; Thio - Thiocyanide

Table 7: The interactive effect of length of fermentation and enzyme inclusion on the blood chemistry profile of rabbits fed WCRM based diets

Parameters	Day 0		Days 3		Day 5		SEM	P-value
	E^-	E^+	E^-	E^+	E^-	E^+		
Total Protein (g/dl)	6.00	6.30	5.77	5.77	6.33	5.80	0.27	0.73
Albumin (g/dl)	4.37	4.37	3.87	4.23	4.40	4.30	0.37	0.77
Globulin (g/dl)	1.63	1.93	1.87	1.50	1.93	1.50	0.41	0.63
Cholesterol (mg/dl)	60.33	55.33	62.43	55.97	71.30	71.30	23.22	0.84
Thio (µg/ml)	3.33	3.20	4.83	3.23	5.23	2.90	1.16	0.17
AST (U/L)	56.67	68.33	50.00	49.00	61.00	41.00	8.92	0.67
ALT (U/L)	33.33	39.33	31.67	29.67	35.67	27.33	4.35	0.69

AST – Aspartate Aminotransferase; ALT – Alanine Aminotransferase; Thio - Thiocyanide

Conclusion and Applications

This study revealed that:

1. Neither the performance nor health status of growing rabbits was compromised by the use of whole cassava root meal in this study.
2. It is therefore concluded that whole cassava root meal prepared from TME 419 cassava variety without fermentation or enzyme inclusion can be used as dietary energy source for growing rabbits up to 30% inclusion

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