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Nutritional Evaluation of Cassava Root Meal Fermented with Rumen Filtrate

[1]Adeyemi O.A.,[2]D. Eruvbetine, [2]T. Oguntona,[3]M. Dipeolu, [1]O.E. Fasina,
[1]H.A. Awojobi and [1]J.A. Adefowora
[1]Department of Animal Production, College of Agricultural Sciences,
Olabisi Onabanjo University, Yewa Campus, P.M.B, 0012 Ayetoro, Ogun State, Nigeria
[2]Department of Animal Nutrition, College of Animal and Livestock Production,
University of Agriculture, P.M.B, 2240 Abeokuta, Ogun State, Nigeria
[3]College of Veterinary Medicine, University of Agriculture,
P.M.B 2240 Abeokuta, Ogun State, Nigeria

Abstract: The study reported herein evaluates with rats the nutritional quality of cassava root meal (CRM) fermented with rumen filtrate using Caged Layer Waste (CLW), Pig Excreta (PE) and a 1:1 mix of CLW and PE respectively as sources of nitrogen. Some safety aspects regarding possible feed use were also investigated. Wistar rats were fed five purified diets viz: a basal diet (nitrogen free), a reference diet that contained casein and three test diets made of the enhanced CRM. Dietary treatments significantly influenced performance, biological indices and blood parameters ($p<0.05$). The performances of rats on the enhanced cassava diets were inferior to that of rats on casein diet. The level of serum urea and thiocyanate were significantly elevated for rats on the cassava-based diets compared to the casein diet. Among the enhanced cassava test protein, Protein Efficiency Ratio (PER) values of 0.88, 0.57 and 0.62; Net Protein Ratio (NPR) values of 0.27, -0.15 and –0.03 and Biological Value (BV) of 55.04, 39.96 and 52.27 were obtained respectively for cassava enhanced with Caged Layer Waste (CCLW), Cassava Enhanced with Pig Excreta (CPE) and cassava with 1.1 mix of caged layer waste and pig excreta (CCLPE). The result obtained from rats on CCLW was significantly better than the other enhanced cassava products.

Key wards: CRM, Caged Layer Waste (CLW), biological

INTRODUCTION

Cassava Root Meal (CRM) has potentials as an alternative energy source in monogastric feeding. The potentials are well pronounced in areas of heavy cassava production such as Nigeria (Agunbiade *et al*., 2002). Its usefulness is however beclouded by its low protein content (Eruvbetine *et al*., 2003). Use of CRM as replacement for cereal grains require the utilisation of substantial quantity of protein sources particularly fish meal and soybean meal which most times are too expensive for resource poor farmers. Thus the need to find cheaper means of upgrading its protein value. One of the means that had shown promise is the use of some select microorganisms to convert part of the starch in CRM to protein (Noomhorm *et al*., 1992). Adeyemi and (Sipe, 2004) observed significant improvement in crude protein content of CRM when fermented with rumen filtrate. (Adeyemi *et al*., 2004) obtained 237.8, 208.4 and 222.3% increases in crude protein content of CRM when fermented with rumen filtrate, using Caged Layer Waste (CLW), Pig Excreta (PE) and a 1:1 mix of CLW and PE respectively as sources of nitrogen.

Protein utilization is a good indication of proper feed utilization for growth. It is customary during the introduction of novel feed resources to assess the nutritive value of such material, especially their protein quality in order to ascertain their competence in meeting recommended allowance.

The purpose of this study was to evaluate with rats the nutritional quality of rumen filtrate fermented CRM and some safety aspects regarding possible feed use.

MATERIALS AND METHODS

Collection and preparation of rumen filtrate: Aliquots of rumen content of freshly slaughtered and eviscerated cattle were collected from the Ijebu-Igbo Central Abattoir, Ogun State. The mass of rumen content was squeezed and the liquid portion filtered through a sieve.

Collection and processing of nitrogenous sources: Caged Layer Waste (CLW): Droppings were collected from Black Olympia layers (50 weeks in lay) raised in battery cages by placing polythene sheets below the cage for a 24-h period. The birds were fed commercial layers

Crresponding Author: O.A. Adeyemi, Department of Animal Production, College of Agricultural Sciences, Olabisi Onabanjo University, Yewa Campus, P.M.B 0012 Ayetoro, Ogun State, Nigeria

Table 1. Composition of experimental diets fed to rats (%)

	Diets*				
Ingredient	1	2	3	4	5
Corn starc	82.0	70.52	8.36	1.7	32.63
Casein	-	11.48	-	-	-
Enhanced cassava	-	-	73.64	80.27	79.37
Non-nutritive cellulose	5.0	5.0	5.0	5.0	5.0
Bone-meal	2.0	2.0	2.0	2.0	2.0
Oyster-shell	0.5	0.5	0.5	0.5	0.5
Vit-min premix **	0.5	0.5	0.5	0.5	0.5
Sucrose	2.5	2.5	2.5	2.5	2.5
Glucose	2.3	2.3	2.3	2.3	2.3
Salt	0.2	0.2	0.2	0.2	0.2
Palm -oil	5.0	5.0	5.0	5.0	5.0
Total	100.0	100.0	100.0	100.0	100.0
Determined Analysis					
Crude Protein	0.00	10.10	9.99	10.26	10.17
Ether extract	5.05	5.12	6.28	6.45	6.32
Crude fibre	5.80	5.60	6.34	6.41	6.38
Ash	.4.11	425	6.10	6.18	6.15

* Diet 1. Basal (Nitrogen – free). 2. Reference diet (Casein). 3. Cassava + Caged layer waste. 4. Cassava + pig excreta. 5. Cassava + (1:1 mix caged layer waste + pig excreta) ** Vit. Min. Premix supplied the following per kg feed. Vit. A= 8000 iu; Vit D_3 = 1000 iu; Vit E= 40 mg; Vit. B_2 = 6 mg; Vit B_3 = 35 mg; Vit B_6 = 35 mg; Choline chloride 300 mg; Manganese = 100 mg; Iron= 50 mg; Copper = 10 mg; Iodine = 1.55 mg; Selenium = 0.10 mg; Vit. K_3 = 2.5 mg; Calcium pantothenate = 10 mg; Vit. B_{12} = 0.025 mg; Zinc = 45 mg; Cobalt = 0.225 mg Vit. B_1 = 2.0 mg; Biotin = 0.05 mg; Folic acid = 1.00mg

mash (17%CP) *ad libitum*. The droppings (weighing 7.5kg wet weight), devoid of feathers and broken shells were sun dried on a concrete platform to a moisture level of <6%, milled in a blender and stored in plastic container until time of use.

Pig Excreta (PE): Fresh pig excreta were collected from five finishing/growing pigs (60-80 kg live weights) raised in standard pig pens and fed wet brewers grains/palm kernel cake based diet. The excreta was bulked, sun dried on concrete platform to a moisture content of <6%, milled and stored till time of use.

Cassava root processing and fermentation: Fresh cassava roots (12 months old, variety TMS30572) were obtained from the Ago-Iwoye Farm settlement, washed to dislodge all adhering soil and mashed whole (unpeeled) using a petrol-operated grater. The cassava root meal was placed on a perforated tray, steam gelatinized over a water bath for 30 min and cooled. Five hundred grams (500 g) of the gelatinized cassava root meal were weighed into different 2litre capacity plastic containers mixed with the various nitrogen sources (caged layer droppings, pig excreta and 1:1 mix of caged layer droppings and pig excreta) at the rate of 75 g per kilograms of gelatinized cassava. Content of each plastic packs were sprayed with 100ml of rumen filtrate and made airtight with petroleum jelly and fermented for a duration of 72 h. The material from each pack was subsequently oven dried at 70°C for 72 h, milled and stored in airtight plastic bottles before chemical analysis and utilisation in diet formulation.

Experimental diet formulation: Five experimental diets were prepared (Table 1). The protein sources used for this experiment are the enhanced cassava root meal viz: CCLW, CPE, CCLPE and casein, used as a control. Protein contents of each of the samples were determined (AOAC, 1995), while the protein, content of casein is known.

Preparation of diets was done by incorporating each protein source separately at the expense of corn-starch to give 10% protein in each of the diets. All other ingredients were added at fixed amounts.

Biological assay of enhanced cassava root meal

Experimental animals and management: Forty growing albino rats of the Wistar strain weighing 50-60 g each and 25-30days old were obtained from the Department of Veterinary Pharmacology, University of Ibadan, Nigeria. No preference was made for sex since the sex of rat has been reported not to have significant effect on protein utilization by rats (Eggum and Pederson, 1983). Animals were housed individually per unit in wire screen metabolic cage with facilities for the supply of fresh clean water and feed as well as urine and faecal collection. The rats were assigned into 5 groups of eight rats each. Diet 1 was the basal diet while diet 2 served as the reference. The remaining three diets contained the enhanced cassava products.

The experiment lasted for 21 days. Feed and water were provided *ad-libitum*. Weight changes were measured weekly while feed consumption was taken daily. During the last 7 days, urine and faeces were collected daily from each replicate separately. The faecal matter was dried at 65°C in a Gallemhamp® oven, milled and kept in a tight Kilner bottles and frozen in deep freezer until required for analysis. The urine was collected into screw-capped plastic bottles containing a drop of Toluene and stored in the deep freezer until time of analysis. Storage of samples in the freezer before analysis did not last for more than 24 h. The concentration of nitrogen in urine and faeces was determined by the Kjeldlahl method (AOAC, 1995).

Toxicological evaluation of test ingredient: The test ingredients were subjected to toxicological evaluation. A twenty-eight day toxicological feeding trial was carried out with the Wistar rats whose management was described above.

The general appearance and reaction of the rats were observed. At the completion of the feeding period, the rats were killed by chloroform asphyxiation. Blood was allowed to flow freely into tubes. Blood samples from each group were pooled into different tubes. The blood

samples collected in EDTA was used for the determination of heamoglobin and Packed Cell Volume (PCV). The remaining blood sample was centrifuged and the serum dispensed into a clean tube for analysis. Immediately after decapitation, the rats were dissected and the livers, pancreas, small intestine and large intestine removed, rinsed with normal saline solution to remove adhering blood, wiped with paper and weighed in a pre-weighed petri dish. The organs were fixed in 10% formalin for subsequent histopathological examination.

Analysis of samples from biological and toxicological assays

Proximate analysis: Proximate composition of feed, feaces and urine were carried out according to procedure of AOAC (1995).

Analysis of blood parameters and serum samples: Blood collected in Tubes Containing EDTA were analysed for Haematological parameters including Haemoglobin (Hb), Packed Cell Volume (PCV), White Blood Cell (WBC) count and Red Blood Cell (RBC) count. The cell counts were carried out by the use of haemocytometer while Hb, PCV and serum chemistry indices were determined using standard methods (Baker and Silverton, 1985).

Statistical analysis: Data collected from the experiment were subjected to Analysis of Variance as discussed for a one-way classification of variance as described by Steel and Torrie (1980). Means were further separated by use of the Duncan's multiple range test (Duncan's 1955).

RESULTS

Performance of rats on experimental diets: The performance data obtained from rats fed experimental diets are given in Table 2. For all parameters measured the reference diet with casein as protein source was the most superior. Significant differences were seen for weight gain, feed intake and feed: gain ratio where among the enhanced cassava based diets, the highest body weight gain ($p<0.05$) was recorded in rats fed fermented cassava enhanced with Caged Layer Waste (CCLW) followed by rats on cassava fermented with 1:1 mix of caged layer waste and Pig Excreta (CCLPE). The least body weight gain was recorded for rats on enhanced Cassava Fermented with Pig Excreta (CPE). The reference diet elicited some 150% increase in the rate of body weight gain over the best-fermented cassava based diet. Feed intake and average daily protein intake were significantly influenced by dietary treatment ($p< 0.05$), with the same trend as average daily weight gain. Rats fed on the casein based reference diet consumed significantly ($p<0.05$) more feed compared with rats on the various cassava-based diets. The casein-based diet was also most efficient in terms of feed: gain. Rats on CCLW, CPE and CCLPE consumed approximately 15.50, 29.57 and 21.67% less feed respectively compared with rats on casein diet.

Table 2: Performance of rats on experimental diets

	Reference diet	CCLW diet	CPE diet	CCLPE diet	SEM
(Casein)					
Average initial weight (g)	57.60	56.37	57.84	56.96	0.85
Average final weight (g)8	1.90^{a}	66.03^{b}	62.75^{c}	63.48^{c}	1.45*
Weight gain (g/rat)24.30^{a}	9.76^{b}	5.43^{c}	6.52^{c}	0.88*	
Feed intake (g/rat/21 days)	131.37^{a}	110.93^{b}	92.52^{d}	102.90^{c}	3.12*
Feed: gain	5.41^{d}	11.37^{c}	17.04^{a}	15.78^{b}	0.60*

*Values in the same row followed by different superscripts are significantly different ($p<0.05$)

Evaluation of protein quality: The biological indices obtained for rats fed casein reference diet were significantly higher ($p<0.05$) than the corresponding values for the enhanced cassava test diets (Table 3).

The Protein Efficiency Ratio (PER) was similar for diets containing CPE and CCLPE but significantly lower than that of diet containing CCLW. The Net Protein Ratio (NPR) values of rats fed the different diets followed the pattern observed for PER. The rats on casein diet had a significantly higher ($p<0.05$) NPR value than the corresponding values for the enhanced cassava based diets. Rats on CCLW had a higher NPR value ($p<0.05$) compared to the rats on CPE and those on CCLPE. The NPR values for rats on these two diets were indeed negative.

Apparent Nitrogen Digestibility (AD), True Nitrogen Digestibility (TD) and Biological Value (BV) obtained on CPE and CCLPE diets are similar but lower ($p<0.05$) than values obtained on CCLW based diets. The values obtained for the Net Protein Utilization (NPU) were lower for all cassava-based diet compared to the casein diet ($p<0.05$). NPU value for CCLW was not different from the value obtained on CCLPE ($p<0.05$). The NPU values for the enhanced cassava based diets were however below 40%.

Effect of treatment on rat blood composition: The effect of the test ingredients on haematology and serum chemistry of rats is presented in Table 4.

Packed Cell Volume (PCV), total serum protein, serum albumin and serum globulin for the rats on the reference diet had the highest values ($p<0.05$) while the same set of rats had the least values for serum thiocyanate and urea respectively ($p<0.05$). Rats fed CCLW and CCLPE diets showed similar values for PCV and serum total protein ($p>0.05$), which were significantly higher when compared to the same indices in rats on CPE. Serum thiocyanate and serum urea values were higher and similar for rats on the cassava-based diets compared to rats on the control. The

Table 3: Biological evaluation of protein quality of enhanced cassava using rats

Parameter	Reference diet	CCLW diet	CPE diet	CCLPE diet	SEM
(Casein)					
Protein Intake (g)	13.07[a]	11.08[b]	9.49[d]	10.46[c]	0.25*
Protein Efficiency Ratio (Per)	1.86[a]	0.88[b]	0.57[c]	0.62[c]	0.11*
Net Protein Ratio (Npr)	1.34[a]	0.44[b]	-0.15[c]	-0.03[c]	0.08*
Apparent Nitrogen Digestibility (Ad)	78.32[a]	61.83[b]	52.42[c]	55.72[c]	1.75*
True Nitrogen Digestibility (Td)	79.46a	63.39[b]	54.00[c]	56.98[c]	1.58*
Biological Value (Bv)	79.47[a]	55.04[b]	39.37[c]	52.27[c]	1.25*
Net Protein Utilization (Npu)	63.28[a]	34.92[b]	21.47[c]	29.99[b]	2.60*

*Values in the same row followed by different superscripts are significantly different ($p<0.05$)

Table 4: Haematological parameters and serum metabolites of rats on experimental diets

	Reference diet	CCLW diet	CPE diet	CCLPE diet	SEM
(Casein)					
Packed Cell Volume (PCV)	42.0	35.78[b]	32.02[c]	34.17b	1.55*
Heamoglobin (Hb)	11.37	12.33	12.12	11.63	0.67
Total serum protein	7.23[a]	5.27[b]	4.27[c]	4.83b	0.26*
Serum albumin	4.10[a]	2.47[b]	2.33[b]	2.23[b]	0.19*
Serum globulin	3.13[a]	2.80[b]	1.94[c]	2.60b	0.15*
Serum thiocynate	21.70[b]	24.97[a]	24.70[a]	24.67[a]	0.80*
Serum urea	7.86[b]	14.83[a]	14.60[a]1	3.53[a]	0.92*

*Values in the same row followed by different superscripts are significantly different

Table 5: Relative organ weight of rats on enhanced cassava diets (% live weight)

	Dietary treatments				
	Ref. diet	CCLW diet	CPE diet	CCLPE diet	SEM
(Casein)					
Lung	1.52	1.52	1.51	1.53	0.06[NS]
Heart	1.10	1.09	1.07	1.12	0.03[NS]
Kidneys	1.40	1.41	1.38	1.40	0.02[NS]
Spleen	0.89	0.82	0.78	0.95	0.04[NS]
Liver	4.24	4.19	4.2	24.20	0.05[NS]

N.S – Figures in the same row are not significant different ($P>0.05$)

Table 6: Histopathological lesions of some organs of rats fed experimental diets

Dietary treatment	Liver	Pancreas	Small intestine	Large intestine
Ref (Casein)	Moderate to severe widespread sinusoidal congestion, vacuolar degeneration of hepatocytes, especially most severe in the centrilobular regions. Few focal areas of hepatic necrosis and mononuclear cell aggregations.	Normal	Moderately thickened villi with considerable number of mononuclear cells (80% lymphocytes 20% macrophages) in lamina propiae	Normal
CCLW	Normal	Normal	Many villi with swollen tips containing large number of lymphocytes and few macrophages in lamina propiae.	Normal
CPE	Normal except for few focal areas of mild peripheral mononuclear cell infiltration	Normal	Mild goblet cell hyperplasia, considerable number of mononuclear cells (70% lymphocytes 30% macrophages) in the lamina propiae.	Normal
CCLPE	Normal	Normal	Mild villous collapse, large number of lymphocytes and few macrophages in lamina propiae.	Normal

serum urea values for the enhanced cassava based diets are nearly twice the amount in the serum of rats on the control.

Effect of treatment on relative organ weights and histology of rats: Weights of the organs studied viz: lung, heart, kidney, spleen and liver did not vary significantly with dietary treatment ($p>0.05$, Table 5)

The result of histopathological examinations of rat organs is summarized in Table 6. Histopathological examinations indicate the normalcy of organs such as pancreas and the large intestine on all diets. The small intestine of all rats showed some lesions. However, it was on livers of rats on the casein based control diet and the CPE that lesions were observed.

DISCUSSION

The biological evaluation of the enhanced cassava samples showed that although approximately the same

amount of protein level was offered (9.99 – 10.26%) in the diets, the performance of the rat's varied considerably among the samples. The weight gains of rats on the cassava-based diets were significantly lower than the reference diets. The reduction in weight gain is thought to be associated with the reduction in feed intake of rats on the cassava-based diets.

Increase in feed intake will normally result in increased body weight gain (McDonalds *et al*., 1998). The reduced feed intake in this study may also be because of an aversion for the cassava-based diets based on palatability arising from odour and high ash content.

Aside from the differential feed intake, another reason for the differences in weight gain is the differences observed in protein intake. The weight gain followed the same trend as protein intake. The casein diets was also most efficient when measured in terms of feed: gain ratio. Fetuga (1972) reported higher weight gain for rats on animal protein sources compared to rats fed plant proteins.

The Protein Efficiency Ratio (PER), Net Protein Ratio (NPR) and Biological Value (BV) were related to the protein intake. Pallet and Young (1980) observed that the biological value of a protein is based on the quality of such protein that is retained, which can provide for growth and health maintenance. The reduction in the biological value of protein from the enhanced cassava is thus an indication of the inferiority of its protein compared to that from casein.

Oke *et al*. (2002) explained that Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) are indicators of protein quality based on weight gain and protein intake. Rats fed PE enhanced cassava diets and those fed CLW: PE enhanced cassava diets had negative NPR value. The results underscore the inability of the rats to properly utilize the enhanced cassava protein. The lower nutritional value exhibited by enhanced cassava as compared with casein may be attributed to high ash content which may have caused an imbalance in the levels of amino acids in the diets as reported by Aron (1985).

Protein sourced from enhanced Cassava Significantly Reduced the Packed Cell Volume (PCV), serum total protein, serum albumin and serum globulin while resulting in an increase in serum thiocyanate and serum urea. Since haematocrit and haemoglobin are known to be positively correlated with protein quality and protein level (Kirchgesserier *et al*., 1977), the reduced value of PCV of rats on the cassava based diets compared to those of rats on the reference diet is thought to be indicative of the poor quality of the protein supplied. Allison (1955) opined that total protein value is an indication of the protein reserve in an animal. In this study the significant decrease in serum total protein of rats on the enhanced cassava based diets is a reflection of the inadequacy of the dietary protein.

The significantly low serum urea value recorded on casein diet (7.8 mg dL^{-1}) compared to 14.83, 14.60 and 13.53 mg dL^{-1} for CCLW, CPE and CCLPE respectively signified no observable muscular wastage brought about by inadequacy of protein. Kumta and Harper (1961) demonstrated that an amino acid imbalance causes an increase in the blood urea concentration. Ranjhan (2001) explained that in a diet that is deficient in essential amino acids the amino acid present will be deaminated and hence result in an increase in the excretion of urea. Although no mortality was recorded in the course of the trials, histopathological examination revealed that some of the rats exhibited mononuclear cell proliferation. This proliferation is thought to be an indication of a viral infection. The cause can however not be directly linked with the enhanced cassava based diets since it was also observed on rats on the control diet.

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