

Nutritional Evaluation of Cassava (*Manihot esculenta*, Crantz) Leaf Protein Concentrates (CLPC) as Alternative Protein Sources in Rat Assay

Ayodeji O. Fasuyi

Department of Animal Production and Health, University of Ado-Ekiti, Ekiti-State, Nigeria

E-mail: dejifasuyi@yahoo.com

Abstract: The nutritional potential of cassava leaf protein concentrate (CLPC) as an alternative protein source was investigated using rat as the test animal. Data on the rat growth assay showed that the weight gain of rats fed protein quality trial diets containing CLPC supplemented and unsupplemented with DL-methionine were negative and significantly lower ($P<0.05$) than the reference diet (6.39 ± 0.11 g) that contained pure protein (casein) as the sole source of protein. The feed intake values for all experimental diets were similar ($P>0.05$). The nitrogen retention value was highest ($P<0.05$) for the reference diet containing casein with a value of 0.27 ± 0.09 g. Other test diets had similar ($P>0.05$) nitrogen retention values. The protein efficiency ratio (PER) value of the reference diet (1.49 ± 0.15) was also significantly different ($P<0.05$) from the other test diets which did not show any particular pattern with insignificant differences ($P<0.05$) across the various diets. The net protein ratio (NPR) was significantly higher ($P<0.05$) for the reference diet (casein diet). True digestibility (TD) value for the reference diet was distinctly higher ($P<0.05$) than that of any other diet. Other TD values were similar ($P>0.05$). The biological value (BV) of the reference diet (casein diet) was also significantly higher ($P<0.05$) than for other values. The other BV values showed a little variation but basically with insignificant differences ($P>0.05$). The net protein utilization (NPU) value for the reference casein diet was also significantly higher ($P<0.05$) than for the other experimental NPU values.

Key words: Cassava leaf protein concentrate, reference diet, rat, animal nutrition

Introduction

The major preoccupation of animal nutritionists has been to find alternative sources to feed ingredients that are in direct competition with human foods. These ingredients, particularly those of protein origin are becoming very expensive to incorporate into animal feed resulting in expensive finished feed with a concomitant rise in the unit cost of meat and meat products such as poultry meat, eggs, etc. To a reasonable extent, progress has been recorded in the quest by animal nutritionists to find alternative, less expensive and less competitive sources of ingredients both of energy and protein origins in animal ration formulation.

The recognition of protein from leaf sources is fast gaining prominence because of its ready availability and perhaps because it is the cheapest and the most abundant potential source of protein. The photosynthetic process of amino acids in leaves is a naturally simple process requiring unlimited and readily available primary materials, e.g energy from the sun, carbon dioxide, water, inorganic nitrogen (or atmospheric nitrogen in the case of legumes). The amino acids synthesized are polymerized into a less mobile forms and stored as such in the leaves.

However, the build-up of amino acids in leaves is also accompanied with other factors that render the amino acids less nutritious for consumptive purpose in man and animal. Such factors limiting the nutritive value of leaf protein are the high fibre content (Oke, 1973) and

other antinutrients (Liener, 1989; Huisman and Tolman, 1992; Aletor, 1993a; Jansman and Poel, 1993; Agbade and Aletor, 2003). The reduction or partial elimination of these antinutrients will go a long way in achieving a positive and increased incorporation of protein of plant origin into ration formulation and a consequent reduction in the overall cost of feed formulation especially in monogastric nutrition.

The fundamental measurement of protein quality for human use depends on growth and/or other metabolic balance evaluation procedures performed in suitable subject of the target population. Those procedures directly reflect the essential (indispensable) amino acid content, digestibility of the protein, and bioavailability of the amino acids in a food or food product (FAO/WHO, 1973). Such studies are easily carried out using animal assay techniques which correlate closely with data from human experiments. Rat growth assays as used in this research work have been widely used for predicting protein quality in foods and numerous workers have discussed the appropriateness of these methods. The present study has therefore been designed to assess the quality of leaf protein concentrate from different cassava varieties and to establish the influence of supplemental methionine on the protein quality indices.

Materials and Methods

Cassava Leaf Protein Production: Leaves of cassava (*Manihot esculenta*, crantz) were harvested in fresh

condition on the campus of College of Agriculture, Akure. The harvested leaves were weighed and washed prior to pulping with a leaf pulping machine. This process was followed by pressing with a screw-press as described by Fellows (1987). The separated leaf juice was heated in batches to 80 - 90°C for about 10 min to coagulate and pasteurize the leaf protein. The protein coagulum was thereafter separated from other fractions by filtering through pillow cases followed by pressing with screw-press as described for garri making (Aletor, 1993b). The leaf protein concentrate (LPC) was then pulverized and spread in the sun to dry. The dried LPC was then milled using laboratory hammer mill and kept in air tight container and deep frozen prior to chemical analysis and protein quality evaluation.

Determination of proximate constituents, mineral content and gross energy values: Proximate constituents of the leaves (previously sun-dried and milled to pass through 0.5mm sieve) and their corresponding LPCs were determined by the method of Association of Official Analytical chemist (AOAC, 1990). The Na and K were determined by flame photometry, and P by vanado-molybdate method (AOAC, 1990). The other nutritionally valuable minerals were determined after wet digestion with a mixture of nitric, sulphuric and hydrochloric acids, using Atomic Absorption Spectrophotometer (Buck Scientific 200A). Gross energy of the dried materials was determined against thermocouple grade benzoic acid using a Gallenkamp Adiabatic bomb calorimeter (Model CBB-330-01041).

Amino acids analysis: The amino acid composition of the LPCs were determined by hydrolysing 50-75mg sample with 5ml of 6N HCL in screw-capped glass hydrolysis tube. The tubes and content were refluxed for 24 hrs by placing tube in a heating block previously heated to 110±1°C. The hydrolysate was cooled and quantitatively transferred to a 50ml flask and diluted to volume with water. After filtration, a 10ml aliquot of the filtrate was heated in a rotary evaporator (40°C) to remove excess acid before analysis using HPLC Autosampler (Kontron 460). The operating systems of the HPLC were as follows:

Autosampler: Konton 460
Pump: Konton 420/525
Detector: Shimadzu RF-530 fluorescence HPLC monitor
Excitation 325mm
Emission 465
Sensitivity High
Data system: Konton 450/MT2
Flow rate: 1.5ml/min
Mobile phase: Na-acetate/ 1,4-dioxan/2-propanol
Column: Adsorbosphere OPA - HR 150 x 4.6mm
Injection: 50µl

Methionine was determined as methionine sulphone and cystine as cysteic acid after performing acid oxidation while tryptophan was determined chemically by basic hydrolysis as described by Miller (1967). To correct for slight fluctuations in amino acid peaks, DL-Amino-n-butyric acid was used as internal standard.

Site preparation: Before the arrival of the rats, the rat house and cages were properly cleaned and disinfected using izal solution. The cages were properly arranged and coupled making sure that the drinkers can comfortably drop water when nibbled by the rats and that the feeders are firmly in position to eliminate feed spillage.

Experimental animals: A total of 40 weanling albino rats of the Wistar strain were used for the experiment. These 40 weanling albino rats were obtained from the clinical rat colony of the School of Veterinary Medicine, University of Ibadan, Ibadan. The rats were weaned at about 14 days and reared on Standard Laboratory animals stock diets until they were about 21days old when they weighed between 29.2g and 29.7g. They were thereafter divided into 10groups of 4 rats each (2 males+2 females) on the basis of initial weight such that the mean group weights were identical (29.2-29.7g). The rats were housed in stainless steel individual metabolic cages with facilities for separate collection of faeces and urine.

Experimental diets: The composition of the basal diet is shown in Table 1. The Nitrogen free diet 1 was formulated such that there was no nitrogen furnished by any of the ingredients used. The reference diet 2 contained 10% crude protein on dry matter basis supplied by nutritional casein. Test diets 3-10 were formulated to furnish 10% crude protein using 4 sources of cassava leaf protein concentrates: MS6, TMS 30555, TMS 30572 and local variety. Each source of CLPC in the diets was duplicated with one having methionine supplementation while the other did not contain DL-methionine. The protein sources CLPC and CLM) to be evaluated were added at the expense of maize starch to give 10% crude protein on a dry matter proximate analysis basis. One group of four rats was given the N-free basal diet while the remaining nine groups were randomly allocated to the test and standard diets.

The experimental diets consisted of 4 major cassava varieties as the sole protein sources. These varieties (MS6, TMS 30555, TMS 30572 and a local variety referred to as Ege-Oda) have been previously evaluated for their proximate composition, amino acid profile, mineral content and gross energy. Each of these varieties was used in quantities adequate enough to supply 10% crude protein in the respective diet as determined from proximate analysis. Each variety was duplicated such that two identical diets were obtained

Table 1: Percentage Composition of the Nitrogen-free Diet 1

Ingredients	Percentages
Corn Starch	66.8
Glucose	5.0
Sucrose	10.0
Groundnut oil	10.0
Non-nutritive cellulose	5.0
Bone meal	2.0
Oyster Shell	0.5
Vit/Min premix*	0.5
Nacl	0.2
Total	100.0

contained vitamins A (10,000,000iu); D(2,000,000 iu); E (35000 iu); K (1900mg); B12 (19mg); Riboflavin (7,000mg); Pyridoxine (3800mg); Thiamine (2,200mg); D Pantothenic acid (11,000mg); Nicotinic acid (45,000mg); Folic acid (1400mg); Biotin (113mg); and Trace elements as Cu (8000mg); Mn (64,000mg); Zn (40,000mg); Fe (32,000mg) Se (160mg); I₂ (800mg) and other items as Co (400mg); Choline (475,000mg); Methionine (50,000mg); BHT (5,000mg) and Spiramycin (5,000mg) per 2.5kg.

and one of the two identical diets was supplemented with 0.2% DL - methionine.

All experimental diets were hand-mixed, starting with the smallest components to ensure uniform and proper blending of all ingredients. They were thereafter put into well-sealed plastic containers, labeled and stored at 4°C prior to use.

Management of experimental animals: Food and water were provided *ad libitum* to the rats for the 10-day experimental period. Records were kept of the weight changes and total feed intake. A 5-day (i.e., day 5 to day 10) faecal and urine collection was done for the rats during the trial. Collection of urine and faeces was done individually on a daily basis for each rat in each metabolic cage. The urine from each cubicle was collected into small urine container. About 1cm³ of concentrated sulphuric acid was added to each urine container as a preservative against fungal and other microbial growth. The daily faecal collection (day 5 - day 10) was undertaken and stored in screw-capped bottles. These bottles were stored at 4°C prior to chemical analysis. At the end of the rat trial, the faecal samples collected were bulked for each rat, weighed, dried and milled prior to laboratory analyses. Duplicate samples of urine, faeces and diets were taken for nitrogen determination. (AOAC, 1990)

Protein quality measurements: Following the determination of nitrogen in the feed, faeces and urine for the individual rat/treatment, the following protein quality indices were measured accordingly:

Nitrogen retention (NR): The nitrogen retained in the experimental rat trial calculated as the algebraic

difference between the feed and the sum of both the faecal and urinary nitrogen for the collection period.

$$NR = NI - (FN + UN)$$

NR, Nitrogen Retention; NI, Nitrogen Intake in feed; FN, Faecal Nitrogen; UN, Urinary Nitrogen.

Protein efficiency ratio (PER): The PER in the rat growth assay was determined by dividing the gain in body weight by the protein intake of each rat.

$$PER = \text{g gain in body weight} / \text{g protein intake}$$

Apparent nitrogen digestibility % (AND): The AND was determined by dividing the NR by the NI on a percentage basis.

$$AND = [NI - (FN + UN)/NI] \times 100$$

NI, Nitrogen Intake; FN, Faecal Nitrogen; UN, Urinary Nitrogen.

Net protein ratio (NPR): This was determined by finding the sum of weight gain of the test-protein group and the weight loss of the Nitrogen-free diet group and then dividing the value by the protein intake.

NPR = [weight gain of test - protein group + weight loss of the N - free diet group] / Protein intake

True Digestibility (TD):

The true digestibility of nitrogen (TD)

$$TD = [I - (F - M) \times 100] / I$$

Biological value (BV): The biological value of nitrogen in the diet was calculated thus:

$$BV = [I - (F - M) - (u - E)] / [I - (F - M)]$$

Net Protein Utilisation (NPU): The NPU was determined thus:

$$NPU = [I - (F - M) - (u - E) \times 100] / I$$

where

I = nitrogen intake (mg);

F = nitrogen excreted in faeces (mg)

M= metabolic faecal nitrogen (from basal diet) (mg);

u = nitrogen excreted in urine (mg)

E = endogenous urinary nitrogen (from basal diet) (mg).

Statistical analysis: All the data obtained were subjected to the principle of analysis of variance (Steel and Torrie, 1960) while using MINITAB computer package to carry out the analyses. Significant differences between treatment means were determined by the multiple range test of Duncan (1955). Also, mean values for all parameters between the leaf species were assigned coefficients of variation (CV).

Results and Discussion

Performance of experimental rats: Data on the rat growth assay is presented in Table 3. The mean weight gain of rats fed protein quality trial diets containing CLPC supplemented and unsupplemented with DL-

methionine were negative, and significantly lower ($P < 0.05$) than the reference (casein) diet 2. The feed intake values for all experimental diets were similar to the reference (casein) diet 2 without any significant difference. The highest mean weight gain at the end of the trial was recorded in the casein diet 1 (6.39 ± 3.11 g) lowest for diet 4 (-7.11 ± 0.87 g) that was not supplemented with DL - methionine.

The food consumption did not differ significantly ($P > 0.05$) between all the diets including the casein diet 1. This was also true for the DL - methionine supplementation which had no apparent effect on the food consumption.

The nitrogen retention value of the reference casein diet 2 varied significantly from the values obtained for other diets ($P < 0.05$). However, other experimental diets had similar mean nitrogen values ($P > 0.05$) apart from value obtained for diet 7 that was significantly different from values obtained for diets 3 and 5, respectively. Rats on reference diet 1 had the highest nitrogen retention value (0.27 ± 0.09 g) while rats on diet 6 had the least nitrogen retention value of 0.10 ± 0.04 g.

The protein efficiency ratio (PER) value of the reference diet 1 was also significantly different from all other PER values and this was the highest value at 1.49 ± 0.15 . The PER values for diets 3, 4, 5 and 9 were not significantly different ($P > 0.05$). Also PER values for diets 4, 5, 6 and 7 were not significantly different ($P > 0.05$). Diets 6, 7 and 8 also had similar PER values ($P > 0.05$). PER value obtained for diet 10 was also similar to values obtained for diets 4, 5, 6, 7, and 9.

The PER mean values for experimental diets 3, 4, 5 and 9 were similar ($P > 0.05$) while the mean values for experimental diets 4, 5, 6, 7, 9 and 10 were also similar with no significant difference ($P > 0.05$). The mean PER value for diet 8 was significantly lowest but was not significantly different from values obtained for experimental diets 6 and 7.

The net protein ratio (NPR) for the standard casein diet 2 was significantly higher ($P < 0.05$) than any other value. Experimental diets 3, 4, 5 and 9 had similar values ($P > 0.05$). This was also true for values obtained for experimental diets 6, 7 and 8. Diets 9 and 10 also had similar values ($P > 0.05$). Diet 8 with unsupplemented DL - methionine had the lowest NPR value (-5.23 ± 0.99).

The true digestibility (TD) for the standard casein diet 2 was distinctly higher ($P < 0.05$) than any other mean value. Experimental diets 3, 5, 7 and 9 had similar TD mean values ($P > 0.05$). Diets 4, 6, 8 and 10 also had similar TD mean values ($P > 0.05$). This was true for diets 6 and 7 ($P > 0.05$). Reference diet 1 had the highest true digestibility value (88.36 ± 7.61 g) while diet 8 (just as it had the lowest NPR value) also had the lowest TD value at 58.36 ± 3.29 g.

The mean biological value (BV) of the standard casein diet 2 was also significantly higher than any other values ($P < 0.05$). Experimental diets 3 and 9 had similar BV

mean values ($P > 0.05$). Diets 4, 6, 8 and 10 had similar mean TD values while diets 5, 7 and 9 also had similar TD values. Diet 8 also had the lowest BV value ($47.44 \pm 0.72\%$).

The net protein utilisation (NPU) mean value of the standard casein diet 2 was also significantly higher ($P < 0.05$) than all other values. Experimental diets 3, 5, 7 and 9 had similar NPU mean values. This was true for diets 4, 6, 8 and 10 ($P > 0.05$). Diets 6, 7 and 10 also had similar NPU mean values. Diet 8 also had the lowest NPU value ($27.70 \pm 1.86\%$).

DL-methionine supplementation in CLPC experimental

diets: The mean weight gain values of rats on diets supplemented with DL-methionine were consistently higher than the values for rats on unsupplemented diets for each cassava leaf protein source tested. Although these values were not statistically significant from each other ($P > 0.05$).

The observation above was also true for feed intake. The feed intake value for rats on each diet supplemented with DL-methionine were consistently higher than its counterparts on the unsupplemented diet. The nitrogen intake values which of course is directly related with the feed intake also followed the pattern above.

The faecal nitrogen value for each diet with DL-methionine supplementation was not significantly different ($P > 0.05$) from its duplicate diet that was not supplemented with DL-methionine.

The nitrogen retention value for each DL-methionine supplemented diet was not significantly different ($P > 0.05$) from its unsupplemented duplicate although the NR values of the DL-methionine supplemented diets were consistently higher than their corresponding unsupplemented duplicate diets.

The apparent nitrogen digestibility (AND) clearly reflected the observation that the DL-methionine supplementation may be a contributive factor in protein quality evaluation of the cassava leaf protein sources as indicated in the result. Apart from diets 3 and 4 (MS6 cassava LPC), where the AND values were not significantly different ($P > 0.05$) other duplicate diets (with and without DL-methionine supplementation) had significantly different values ($P < 0.05$). The DL-methionine supplemented diet 3 had an AND value of $50.39 \pm 2.91\%$ as against $45.25 \pm 3.49\%$ in the unsupplemented diet 4.

The protein efficiency ratio values also followed the same pattern with the diets with DL-methionine showing better (lower) PER values than their DL-methionine unsupplemented counterparts. Although diets 3 and 4 (-0.99 ± 0.74 and -1.80 ± 0.19); diets 5 and 6 (-1.55 ± 0.55 and -2.20 ± 0.20); diets 7 and 8 (-2.15 ± 0.80 and -2.45 ± 0.46); and diets 9 and 10 (-1.54 ± 0.34 and -1.83 ± 0.35) did not vary significantly statistically ($P > 0.05$), the consistently higher values in the DL-methionine unsupplemented diets over their supplemented

Table 2: Composition (g/100g) of experimental diets

Ingredients	N-free 1	Ref. 2	DIETS							
			3+	4-	5+	6-	7+	8-	9+	10-
Corn Starch	66.8	56.0	40.6	40.8	39.9	40.1	39.9	40.1	39.6	39.8
Casein (92.2%CP) -	10.8	-	-	-	-	-	-	-	-	-
CLPC MS6 (38.5%CP)	-	-	26.0	26.0	-	-	-	-	-	-
CLPC 30555 (37.5%CP)	-	-	-	-	26.7	26.7	-	-	-	-
CLPC 30572 (37.4%CP)	-	-	-	-	-	-	26.7	26.7	-	-
CLPC Local (37.11%CP)	-	-	-	-	-	-	-	-	27.0	27.0
DL – methionine	-	-	0.2	-	0.2	-	0.2	-	0.2	-
Glucose	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Sucrose	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Groundnut oil	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Bone meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Oyster shell	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vit/min Premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
NaCl	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Cal. Protein	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0

N-free = Nitrogen free basal diet; Ref. = Reference (control) diet; + = with methionine supplementation; - = without methionine supplementation

counterparts were very obvious.

The net protein ration was also in line with the previous observation. The NPR values for the DL-methionine supplemented diets were consistently higher than their unsupplemented duplicate diets and this was clearly seen between diets 5 and 6 (TMS 30555 cassava LPC source) that had significantly different ($P < 0.05$) NPR values.

Perhaps true digestibility (TD) was one protein index that clearly revealed the observation that DL-methionine supplementation actually had a role in cassava LPC utilisation. All the TD values for DL-methionine diets were significantly different ($P < 0.05$) from their duplicate counterparts without DL-methionine supplementation. The DL-methionine supplemented diets were all consistently higher than their unsupplemented duplicate diets.

The biological value (BV) ratio also reflected the observation with all the DL-methionine supplemented diets having significantly higher BV values over their unsupplemented duplicate diets except in diets 5 and 6 (TMS 30555 cassava LPC source) having values of $50.35 \pm 1.29\%$ and $48.50 \pm 0.97\%$ respectively.

The NPU values followed a similar trend as the BV data supplemented with or without methionine. All the DL-methionine supplemented diets had consistently higher values over their unsupplemented counterparts.

The results (Table 3) of the biological trial with rats showed that the cassava leaf protein concentrate (CLPC) of all the four cassava varieties (3 improved varieties - MS 6, TMS 30555, TMS 30572 and 1 local variety - Ege Oda) tested did not support growth and gave significantly ($P < 0.05$) lower nitrogen retention, protein efficiency ratio (PER), apparent nitrogen digestibility (AND), true digestibility (TD), biological value

(BV) and net protein utilisation (NPU) values than the standard (reference) diet containing casein as the protein source.

Interestingly, feed intake and thus the nitrogen intake/protein intake values of the experimental diets did not vary significantly ($P > 0.05$) from the standard casein diet showing a fairly normal protein intake by the experimental animals. This suggests the presence of certain inherent factors in the CLPC that are militating against its usefulness as a protein source in animal feed.

The presence of some antinutritional factors in cassava leaves have been examined in previous research works by the same author. Of prominence was the presence of cyanide, phytin and tannin which have nutritional implication, particularly in protein utilisation (Aletor and Fasuyi, 1997). The mean values for the 4 varieties of cassava leaves examined averaged $52.9 \pm 8.9\text{mg}/100\text{g}$ for hydrocyanic acid (HCN), $9.7 \pm 3.6\text{g}/100\text{g}$ for tannin and $192.0 \pm 60.4\text{mg}/100\text{g}$ for phytin. The nutritional significance of dietary cyanide derives from several observations (Frake and Sharma, 1986; Aletor and Fetuga, 1988; Aletor, 1993) that cyanide, either in synthetic or organic form can cause marked changes in weight gain, nutrient utilisation, liver enzyme activities and thiocyanate concentrations in serum and urine of rats and hamsters. Although a cyanide - thiocyanate sulphur - transferase (rhodanase) pathway is a suggested route of cyanide detoxification (Maner and Gomez, 1973), this pathway requires organic sulphur donors in form of sulphur-containing amino acids such as methionine and cystine. The requirement for sulphur in rhodanase pathway naturally precipitates the deficiency of methionine in an otherwise balanced diet. Mehansho *et al.* (1987) opined that tannins bring about

Table 3: Nutritive Value of Cassava Leaf Protein Concentrate (CLPC) Supplemented and Unsupplemented with DL-methionine

Parameter	Reference (Casein) diet 2	DIETS			
		MS 6		TMS 30565	
		3+	4-	5+	
Weight gain in 10days (g)	6.39 ± 3.11	-3.90 ± 3.11	-7.11 ± 0.87	-5.51 ± 2.01	
Feed Intake in 10days (g)	44.30 ± 4.62	44.78 ± 3.51	44.28 ± 1.90	41.30 ± 3.16	
Nitrogen Intake in 10days (g)	0.70 ± 0.07	0.72 ± 0.06	0.38 ± 0.01	0.66 ± 0.05	
Faecal Nitrogen in 10days (g)	0.18 ^a ± 0.02	0.36 ^{bc} ± 0.03	0.38 ^b ± 0.01	0.34 ^c ± 0.02	
Nitrogen Retention (g)	0.27 ^a ± 0.09	0.18 ^b ± 0.04	0.16 ^{bc} ± 0.03	0.17 ^b ± 0.03	
Apparent Nitrogen Digestibility (%)	73.82 ^a ± 10.35	50.39 ^b ± 2.91	45.25 ^{bc} ± 3.49	51.03 ^b ± 1.24	
Protein Efficiency Ratio	1.49 ^a ± 0.15	-0.99 ^b ± 0.74	-1.80 ^{bc} ± 0.19	-1.55 ^{bc} ± 0.55	
Net Protein Ratio	-0.61 ^a ± 0.43	-2.91 ^b ± 0.86	-3.58 ^b ± 0.22	-3.52 ^b ± 0.83	
True Digestibility (%)	88.36 ^a ± 7.61	65.24 ^{bd} ± 0.75	60.04 ^c ± 1.58	66.02 ^b ± 1.40	
Biological Value (%)	72.73 ^a ± 2.64	52.57 ^b ± 0.82	47.68 ^c ± 1.22	50.35 ^{de} ± 1.29	
Net Protein Utilisation (%)	64.41 ^a ± 7.81	34.18 ^b ± 0.85	28.62 ^c ± 0.57	33.23 ^b ± 0.99	

Parameter	DIETS				
	TMS 30565		TMS 30572		Local
	6-	7+	8-	9+	10-
Weight gain in 10days (g)	-6.91 ± 1.22	-6.91 ± 1.27	-6.79 ± 1.13	-5.56 ± 1.81	-6.13 ± 0.28
Feed Intake in 10days (g)	34.66 ± 4.71	36.94 ± 3.43	30.78 ± 4.08	39.54 ± 3.10	38.16 ± 4.77
Nitrogen Intake in 10days (g)	0.56 ± 0.08	0.60 ± 0.05	0.50 ± 0.06	0.64 ± 0.05	0.62 ± 0.08
Faecal Nitrogen in 10days (g)	0.32 ^c ± 0.03	0.30 ^c ± 0.02	0.30 ^c ± 0.02	0.32 ^c ± 0.02	0.36 ^c ± 0.03
Nitrogen Retention (g)	0.12 ^{bc} ± 0.05	0.15 ^{bc} ± 0.04	0.10 ^c ± 0.04	0.16 ^{bc} ± 0.03	0.13 ^{bc} ± 0.05
Apparent Nitrogen Digestibility (%)	40.91 ^{cd} ± 7.87	48.34 ^{bd} ± 2.99	37.01 ^c ± 7.28	50.08 ^b ± 2.83	41.12 ^c ± 6.07
Protein Efficiency Ratio	-2.20 ^{cd} ± 0.20	-2.15 ^{cd} ± 0.80	-2.45 ^d ± 0.46	-1.54 ^{bc} ± 0.34	-1.83 ^c ± 0.35
Net Protein Ratio	-4.73 ^{cd} ± 0.91	-4.71 ^{cd} ± 0.95	-5.23 ^d ± 0.99	-3.64 ^{bc} ± 0.12	-4.11 ^c ± 1.09
True Digestibility (%)	60.87 ^{cd} ± 2.26	65.54 ^{bd} ± 0.48	58.36 ^c ± 3.29	65.90 ^b ± 0.48	59.34 ^c ± 1.29
Biological Value (%)	48.50 ^{cd} ± 0.97	50.33 ^{de} ± 1.06	47.44 ^c ± 0.72	52.04 ^{bc} ± 1.23	48.61 ^{cd} ± 2.20
Net Protein Utilisation (%)	29.52 ^{cd} ± 1.20	32.98 ^{bd} ± 1.20	27.70 ^c ± 1.86	33.39 ^b ± 1.54	28.86 ^{cd} ± 1.85

Means with different superscripts in the same horizontal row are significantly different (P < 0.05).

(+) = with methionine supplementation; (-) = without methionine supplementation

their antinutritional influences largely by binding dietary proteins and digestive enzymes into complexes that are not readily digestible. Tannins are also associated with poor palatability in diets containing them in high levels as a result of its astringent property which is a consequence of its ability to bind with proteins of saliva and mucosal membranes. This property probably influenced the amino acid assimilation in diets containing cassava leaf protein concentrate (CLPC) as the major protein sources thereby rendering the amino acids partially unavailable and thus the reflection in the poor growth parameters.

Phytin is another notably present antinutrient in cassava leaves. Phytins are known to chelate certain mineral elements especially Ca, Mg, Fe and Zn and thereby causing the inability of these minerals to be absorbed during metabolism (Forbes and Erdman, 1983). This problem is further compounded in non-ruminants (including man) where phytase is absent to break down phytins to release phosphorus for metabolism. Phytate is a strong acid that forms a wide variety of salts with several heavy metals depending on the pH medium as well as the presence of secondary cations among which calcium has been most prominently mentioned. It is a known fact that an important factor in the

precipitation of the phytate is the synergistic effect of two or more cations, which, when present simultaneously, may act together to increase the quantity of metallic phytate precipitated. This phenomenon is common for zinc and calcium and for copper and calcium. Unfortunately, the pH of 6 which is the approximate pH of the duodenum, where most of the absorption of these divalent cations occur is best suited for the maximum precipitation of zinc phytate or of zinc-calcium phytate. This same relationship although to a lesser extent was observed for copper and calcium.

The combined effect of the antinutrients discussed above could generally have a significantly negative nutritional effect on the proper utilisation of cassava leaf as a protein source even though it appears as if the amino acid profile is in order except in methionine, lysine and perhaps isoleucine which can easily be supplemented by synthetic sources.

The result of the biological evaluation suggests that when cassava leaf protein concentrate (CLPC) was fed as the sole protein source, the CLPCs did not support the growth of rats. Even when the CLPCs sources were supplemented with DL-methionine, there was no appreciable improvement in growth rate. However, there were indications that the DL-methionine

supplementation brought about a little improvement over the unsupplemented diets in protein evaluation indices such as apparent nitrogen digestibility (AND), protein efficiency ratio (PER), net protein ratio (NPR), true digestibility (TD), biological value (BV) and net protein utilisation (NPU).

The supplementation of CLPC diets with DL-methionine was apparently not enough to augment the methionine deficiency inherent in cassava leaf protein concentrate as a sole protein sources in the animal diets. This is in agreement with the work of Akinrele (1963) who reported that leaf protein is a poor source of methionine, and ideally needs to be supplemented with another protein source relatively rich in this amino acid. Rogers and Milner (1963) also reported that amino acid composition of all Brazilian manioc leaf varieties indicated that the protein in cassava leaves is deficient in methionine, and possibly marginal in tryptophan, in relation to the FAO reference protein.

Conclusion: The result obtained from the analytical study of cassava leaf protein concentrate (CLPC) especially in the crude protein, crude fat, gross energy and amino acids revealed its potential as an alternative protein resource for humans and animals. However, when fed as the only protein source to rats during bioassay, the CLPC did not support growth even when supplemented with DL-methionine up to 0.2% in the diet. This is an indication that CLPC may not solely be fed as a protein source in human or livestock food. CLPC must be supplemented with another viable protein sources in human and livestock diets. These research and development (R&D) needs arising from this study call for further studies which had been carried out by the same author.

Acknowledgement

I am indebted to my academic mentor, Professor V. A. Aletor who designed this project work and Mr. M. O. Oguntokun who carried out the laboratory analyses.

References

Akinrele, I.A. 1963. *Jl W. Afr. Sci. Ass.*, 8: 74.
Agbede, J.O. and V.A. Aletor, 2003. Studies of the chemical composition and protein quality evaluation of differently processed *Canavalia ensiformis* and *Mucuna pruriens* seed flours. *J. Food Comp. Analy.* Elsevier Publisher, London, UK. In press.
Aletor, V.A., 1993a. Cyanide in garri. 1. Distribution of total, free and bound hydrocyanic acid (HCN) in commercial garri, and the effect of fermentation time on its residual cyanide content. *Int. J. Food Sci. and Nutr.*, 44: 281-287.
Aletor, V.A., 1993b. Cyanide in garri. 2. An Assessment of some aspects of the nutrition biochemistry and haematology of rats fed garri containing varying residual cyanide levels. *Int. J. Food Sci. Nutr.*, 44: 289-295.

Aletor, V.A. and A.O. Fasuyi, 1997. Nutrient composition and processing effects on cassava leaf (*Manihot esculenta*, Crantz) anti-nutrients. *Proc. Of the 2nd Ann. Conf. Of Anim. Sci. Ass. Of Nigeria (ASAN)*, Airport Hotel, Lagos, Sept. 15-17, pp: 231-242.
Aletor, V.A. and B.L. Fetuga, 1988. Dietary interaction of lima bean (*Phaseolus lunatus*) trypsin inhibitor, haemagglutinin and cyanide: I. Effect on growth performance, nitrogen utilization and physiopathology in growing rats. *J. Anim. Physiol. Anim. Nutr.*, 60: 113-122.
AOAC, 1990. Association of official analytical chemists. Official method of analysis (13th edition), Washington D.C., U.S.A.
Duncan, D.B., 1955. Multiple range and multiple F-test. *Biometrics* 11: 1-42.
FAO/WHO, 1973. Energy and Protein Requirements. Technical Report Series No 52, WHO, Geneva, Switzerland, pp:1-118.
Fellows, P., 1987. Village - scale Leaf Fractionation in Ghana. *Trop. Sci.*, 27: 77-84.
Forbes, R.M and J.W. Erdman, 1983. Bioavailability of tree minerals elements. *Am. Rev. Nutr.*, 3: 213-231.
Frake, R.A. and E.P. Sharma, 1986. Comparative metabolism of linamarin and amygdalin in harvester, *Food Chem. Toxicol.*, 24: 417-420.
Huisman, J. and G.H. Tolman, 1992. Antinutritional factors in the plant proteins of diets for Non-ruminants. In P.C. Garnsworthy, W. Haresign & D.J.A. Cole (Eds), recent Advances in Animal Nutrition. Butterworth-Heinemann Ltd., Oxford (UK), pp: 3-31.
Jansman, A.J.M. and A.F.B. Poel, 1993. Anti-nutritional factors in legume seeds. Nutritional effects and (Bio-) technological inactivation. Seventh forum for Applied Biotechnology, Gent (Belgium), 30 September-1 October 1993, Mededelingen Facultiet Landbouwkundige en Toegepaste Biologische Wetenschappen. Univ. of Gent, 58: 1657-1668.
Liener, I.E., 1989. Antinutritional factors in legume seeds: State of the art. In J. Huisman, T.F.B. Van der Poel and I.E. Liener (Eds), Recent advances of research in antinutritional factors in legume seeds. Pudoc, Wageningen, pp: 6-13.
Maner, H.J. and G. Gomez, 1973. Chronic cassava toxicity. M proceedings of an interdisciplinary Workshop, London Jan 29-30.
Mehansho, A., L.G. Butler and D.M. Carbon, 1987. Dietary tannin and salivary prolinerich proteins: interaction, induction and defence mechanism. *Am. Rev. Bnutr.*, 7: 423-430.
Miller, E.L., 1967. Determination of the tryptophan content of feedingstuff with particular reference to cereals. *J. Sci. Food Agri.*, 18: 381-390.
Oke, O.L., 1973. Leaf protein research in Nigeria: A Review. *Trop. Sci.*, 15: 139-155.
Steel, R.G.D. and J.H. Torrie, 1960. Principles and procedures of statistics 1st Edition. McGraw - Hill, New York, pp: 107-9.