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Bio-nutritional evaluations of three tropical leaf vegetables (*Telfairia occidentalis*, *Amaranthus cruentus* and *Talinum triangulare*) as sole dietary protein sources in rat assay

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Abstract

The bio-nutritional potentials of three tropical vegetable leaf meals (*Telfairia occidentalis* leaf meal, TOLM; *Talinum triangulare* leaf meal, TTLM and *Amaranthus cruentus* leaf meal, ACLM) were investigated using albino rat as the test animal. Some protein quality evaluation indices were measured when the three vegetable leaf meals (VLMs) were used as sole protein sources in diets fed to the experimental animals and results were compared with data obtained for a basal nitrogen free diet (diet 1) and another reference diet (diet 2) in which the protein was solely supplied by nutritional casein (pure protein). The weight gain value recorded over a 10 day experimental period for the test animals on the reference (casein) diet 2 was consistently higher ($P < 0.05$) than the weight gain value obtained for the animals on the 3 VLMs diets (diets 3–5). Feed intake values recorded for the rats on the VLMs were similar ($P > 0.05$) and significantly higher ($P < 0.05$) than the value obtained for rats on the reference (casein) diet. Nitrogen excreted in faeces (feacal nitrogen) was lowest ($P < 0.05$) for the animals in reference diet 2. However, the nitrogen excreted in urine (urinary nitrogen) was highest ($P < 0.05$) for the animals on reference diet 2 and lowest for animals on diet 3 (TOLM diet) ($P < 0.05$). The nitrogen retention (NR) value obtained for the test animals on the reference diet 2 was similar ($P > 0.05$) to the value obtained for animals on diet 3 (TOLM). These values were significantly higher ($P > 0.05$) than NR values obtained for animals on diets 4 (TTLM) and 5 (ACLM). Expectedly, the apparent nitrogen digestibility (AND), protein efficiency ratio (PER), net protein ratio (NPR), true digestibility (TD), biological value (BV) and net protein utilization (NPU) all indicated higher and better values ($P < 0.05$) than the corresponding values obtained for the 3 VLMs diets (diets 3–5).

Keywords: Rat bioassay; Protein quality evaluation; Reference diet; Nitrogen free diet

1. Introduction

The world shortage of animal protein, particularly in developing countries of Africa, has necessitated investigations of several novel alternative feeding materials for possible incorporation into human/animal diets (Fasuyi, 2005). Recent studies have shown that malnutrition among children in developing countries is mainly due to the consumption of cereal based porridge which is bulky, low in energy and density and high in antinutrients (Michaelsen & Henrik, 1998). A growing interest in the use of unconventional sources of protein and energy in animal feed especially poultry has gained prominence (Eggum, 1970; Ravindran & Ravindran, 1998; Siddhuraju, Becker, & Makkar, 2001).

Amaranthus cruentus, *Telfairia occidentalis* and *Talinum triangulare* are three known vegetable crops grown in tropical regions of the world including Africa, India, Bangladesh, Sri Lanka and the Caribbean. They are also grown as leaf vegetables through South-East Asia and Latin America. The economic and nutritional advantages of these vegetable crops are further accentuated by their agro-

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nomic superiority over many other plant protein sources. For instance, harvesting is done 20–30 days after planting. Thereafter, shoots can be harvested at 1–4 weeks intervals for a period of two months depending on the vegetable type. On the average, farmers can harvest four times from a plant before its growth starts to decline for *Amaranthus* and *Talinum* plants (Akachuku & Fawusi, 1995). Another potential nutritional advantage is the chemical composition of the vegetable leaf meals which is highly skewed in favour of the leaves as rich sources of plant protein (Aletor & Adeogun, 1995; Fasuyi, 2006; Leung, Busson, & Jardin, 1968) and their rich sources of vitamins and minerals. *Telfairia* leaves are remarkably rich in Fe, Mg and K, carotene and vitamin C. It is also rich in essential fatty acids making it desirable as cooking oil (Oderinde, 1990). Fasuyi (2006) quoted protein values of 19.9, 23.0 and 35.1 g kg^{-1} DM for *Talinum triangulare*, *Amaranthus cruets* and *Telfairia occidentalis*, respectively.

Such factors limiting the nutritive value of leaf protein are the high fibre content (Oke, 1973). The build-up of the amino acids in plant leaves is also accompanied with other toxic factors and antinutritional components. These are variables affecting the nutritional value of foods and feeds (Aletor & Adeogun, 1995). The vegetable leaves are known to be considerably high in oxalates, phytins, tannins and to lesser extent saponins (Kohda & Yamaoka, 1992). However, processing effects such as shredding and sun drying have been suggested to reduce/eliminate some of the antinutritional factors (ANFs) inherent in plant leaves (Fasuyi, 2005).

The present study is therefore aimed at investigating the bio-nutritional potentials of some selected commonly grown tropical leaf vegetables as a prelude to incorporating them substantially into food/feed systems.

2. Materials and methods

2.1. Production of vegetable leaf meals

Leaves of the three vegetable crops (*Telfairia occidentalis*, *Amaranthus cruentus* and *Talinum triangulare*) were purchased fresh from local farmers at various locations across Akure, a city in the South Western part of Nigeria. The purchased leaves were weighed and exposed to sunlight for gradual drying for a period of three days. Sun drying is a popular means of drying most agricultural products in tropical Africa where there is an average of 12 h of sunlight per day. Ambient temperature, 22–37 °C; relative humidity, 70%; wind, SSW at 11 mph (18 km/h); barometric pressure, 29.68′ Hg (F). The drying process was monitored to be gradual with adequate manual turning by raking the spread leaves to facilitate uniform drying and eliminate mould. This process was followed by milling the brittle dried leaves using a laboratory hammer mill (Dietz, Dettingen-Teck, West Germany) and passed through 0.5 mm sieve to reduce the fibrous stems and leaf veins. The prepared leaf meals were then kept in air tight containers and deep frozen at −20°C prior to chemical analyses and protein quality evaluation.

2.2. Determination of proximate constituents, amino acids, mineral content and gross energy values

Proximate constituents of the leaves (previously sun-dried and milled to pass through 0.5 mm sieve) were determined by the method of Association of Official Analytical Chemists (AOAC, 1995). The amino acids were determined using amino acid analyzer model 80-2107-07 Auto Loader. The Na and K were determined by flame photometry, and P by vanado–molybdate method (AOAC, 1995). The other nutritionally valuable minerals were determined after wet digestion with a mixture of nitric, sulphuric and hydrochloric acids, using an 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).

2.3. Experimental animals

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 fitted with feeders and drinkers that can comfortably drop water when nibbled by the rats. The feeders were firmly placed in positions to eliminate feed spillage. 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, Nigeria. The rats were weaned at about 14 days and reared on standard laboratory animals stock diets until they were about 21 days old when they weighed between 29.2 g and 29.7 g. They were thereafter divided into five groups of eight rats each (4 males + 4 females) on the basis of initial weight such that the mean group weights were identical (29.2–29.7 g). The rats were housed in stainless steel individual metabolic cages with facilities for separate collection of feaces and urine.

2.3.1. 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–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 1 cm^3 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 bulked 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 bulked faecal samples for each rat were weighed, dried and milled

prior to laboratory analyses. Duplicate samples of urine, faeces and diets were taken for nitrogen determination. (AOAC, 1995).

2.4. Experimental diets

The compositions of the basal diet 1 (nitrogen free diet), reference (casein) diet 2 and the other diets containing the 3 test ingredients (diets 3–5) are shown in Table 4. 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 3–5 were formulated to furnish 10% crude protein using the three VLMs (*Telfairia occidentalis*, *Amaranthus cruentus* and *Talinum triangulare*) now referred to as *Telfairia occidentalis* leaf meal, TOLM; *Amaranthus cruentus* leaf meal, *ACLM* and *Talinum triangulare* leaf meal, (TTLM). The three VLMs were the major protein sources to be evaluated and were added at the expense of maize starch to give 10% crude protein on a dry matter proximate analysis basis. One group of eight rats was given the N-free basal diet 1, another group of eight was kept on the reference diet 2 while the remaining three groups were randomly allocated to the diets containing the test ingredients (TOLM, ACLM and TTLM).

All experimental diets were hand-mixed, starting with the minute 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.

2.5. Protein quality measurements

Following the determination of nitrogen in the feed, faeces and urine for the individual rat per treatment, the following protein quality indices were measured according to described procedures (FAO/WHO, 1989).

(i) *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.

$$\mathrm{NR} = \mathrm{NI} - (\mathrm{FN} + \mathrm{UN})$$

NR, nitrogen retention; NI, nitrogen intake in feed; FN, faecal nitrogen; UN, urinary nitrogen.

(ii) *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.

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

$$\mathrm{NPR} = \frac{\text{weight gain of test} - \text{protein group} + \text{weight loss of the N} - \text{free diet group}}{\text{Protein intake}}$$

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

$$\mathrm{AND} = \frac{\mathrm{NI} - (\mathrm{FN} + \mathrm{UN})}{\mathrm{NI}} \times 100$$

NI NI, nitrogen intake; FN, feacal nitrogen; UN, urinary nitrogen.

(iv) *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.

(v) *True digestibility (TD)*:The true digestibility of nitrogen (TD)

$$TD = \frac{I - (F - M) \times 100}{I}$$

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

$$\mathrm{BV} = \frac{I - (F - M) - (u - E)}{I - (F - M)}$$

(vii) *Net protein utilisation (NPU):* The NPU was determined thus:

$$\mathrm{NPU} = \frac{I - (F - M) - (u - E) \times 100}{I}$$

where I = nitrogen intake (mg); F = nitrogen excreted in feaces (mg) M = metabolic faecal nitrogen (from basal diet) (mg); u = nitrogen excreted in urine (mg)'E = endogenous urinary nitrogen (from basal diet) (mg).

2.6. Statistical analysis

Data were expressed as means ± standard deviation of two measurements. One way ANOVA (SPSS 11.0 for Windows, SPSS Inc., Chicago IL, USA) was used to analyse the mean differences between the dietary treatments. A significant difference was considered at a level of $p < 0.05$.

3. Results

The proximate composition and gross energy values of the three VLMs are presented in Table 1 while the mineral compositions are presented in Table 2. The phytic acid, phytin phosphorous, oxalate, tannic acid and cyanide contents of the tropical VLMs as cited by Aletor and Adeogun (1995) and Fasuyi (2006) are presented in Table 3. The amino acid profiles are shown in Table 4. The compositions of the experimental diets are presented in Table 5 while

Table 1
Proximate composition (g kg^{-1} DM) and gross energy values (MJ kg^{-1}) of vegetable leaf meals (means, $n = 2$)

Vegetable leaf meal	Dry matter (%) (g kg^{-1} DM)	Crude protein (g kg^{-1} DM)	Crude fibre (g kg^{-1} DM)	Ether extract (g kg^{-1} DM)	Ash(g kg^{-1} DM)	N-free extract (g kg^{-1} DM)	Gross energy (MJ kg^{-1})
Talinum triangulare	89.6 ± 1.2	19.9 ± 1.8	11.9 ± 2.3	29.2 ± 2.1	19.4 ± 3.0	19.7 ± 0.3	383.2
Amaranthus cruentus	88.6 ± 2.2	23.0 ± 1.3	8.8 ± 3.1	5.4 ± 3.2	19.3 ± 5.7	43.5 ± 0.7	251.5
Telfairia occidentalis	91.0 ± 2.0	35.1 ± 1.7	12.7 ± 4.2	9.6 ± 4.1	10.9 ± 6.2	31.7 ± 0.8	341.3

Table 2
Mineral contents of air-dried vegetable leaf meals (means, n=2)

Vegetable leaf meal	Ca (g kg^{-1} DM)	Mg (g kg^{-1} DM)	Zn (g kg^{-1} DM)	Ni (g kg^{-1} DM)	Na (g kg^{-1} DM)	K (g kg^{-1} DM)	P (g kg^{-1} DM)	Fe (g kg^{-1} DM)
Talinum triangulare	0.8	0.7	0.5	1.3	3.8	2.7	0.8	0.4
Amaranthus cruentus	2.0	2.5	0.9	1.2	7.1	4.8	0.9	1.1
Telfairia occidentalis	1.8	1.2	0.7	0.8	8.2	3.7	1.0	0.9

Table 3
Phytic acid, phytin-P, oxalate, tannic acid and cyanide contents of tropical leaf vegetables (means, $n = 2$)

VLMs	Phytic acid (mg/100 g)	Phytin-P (mg/100 g)	Phytin-P as % of total P	Oxalate (mg/100 g)	Tannin (mg/100 g)	HCN (mg/100 g)
Telfairia occidentalis	189.2	120.1	8.1	470	43.0	61.2
Talinum triangulare	250.0	70.0	7.8	110.0	62.0	12.7
Amaranthus cruentus	580.0	160.0	12.2	620.0	72.1	42.0
Amaranthus hybridus	660.0	140.0	15.5	500.0	68.0	47.3

Sources: Aletor and Adeogun (1995), Fasuyi (2006).

data on the rat growth assay and protein quality evaluation is presented in Table 6.

The VLMs are potential sources of protein at 19.9%, 23.0% and 35.1% for *Talinum triangulare, Amaranthus cruentus* and *Telfairia occidentalis* leaf meals, respectively (Tables 1 and 2). The mineral compositions also reflect abundant levels of Ca, Na, K, Mg and Fe except for Zn that appears deficient particularly for *Talinum triangulare.* The presence of phytin–phosphorous, oxalates and tannins are most noticeable in *Amaranthus cruentus* leaf meal while traces of hydrocyanic acid are found in all the VLMs. Amino acid profiles indicate that the VLMs are rich in some essential amino acids particularly alanine, aspartate, glutamate, isoleucine, leucine, valine and glycine. However,

Table 4
Amino acid profile (g kg^{-1}) of vegetable leaf meals

Amino acids	*Talinum triangulare*	*Amaranthus cruentus*	*Telfairia occidentalis*	FAO/WHO (1973) recommended pattern	Whole egg[a]
Alanine	382.5	396.3	406.9		
Aspartic acid	438.1	320.0	388.1		
Arginine	372.5	375.6	313.8		381.3
Glycine	350.6	251.3	381.3		
Glutamic acid	586.3	644.4	688.1		
Histidine	125.6	131.9	86.3		150.0
Isoleucine	351.3	300.6	318.8	250.0	350.0
Lysine	167.5	111.9	131.3	343.7	393.8
Methionine	131.3	86.3	155.0		200.0
Cystine	81.3	81.9	67.5		112.5
Meth. + Cys.	212.5	275.6	285.0	218.8	312.5
Leucine	563.8	529.4	473.8	437.5	518.8
Serine	251.3	273.1	244.4		
Threonine	256.3	196.9	238.1	250.0	318.8
Phenylalanine	388.1	363.8	303.1		318.8
Valine	381.3	326.9	387.5	312.5	475.0
Tyrosine	294.4	312.5	351.3	375.0	250.0
Tryptophan	113.8	147.5	195.0	62.5	112.5

[a] Cited by Robinson (1987). FAO/WHO and whole egg amino acid profiles were initially in g 16 g^{-1} N unit before conversion into g kg^{-1}.

Table 5
Composition (g/100 g) of experimental diets

Ingredients	Basal diet	Reference diet	Test ingredient diets		
	N-free 1	2	3	4	5
Corn starch	66.8	56.0	38.0	20.1	28.1
Casein (92.2%CP)	–	10.8	–	–	–
TOLM (35.14%CP)	–	–	28.6	–	–
TTLM (19.85%CP)	–	–	–	51.5	–
ACLM (23.00%CP)	–	–	–	–	43.5
Non-nutritive cellulose	5.0	5.0	5.0	–	–
DL – methionine	–	–	0.2	0.2	0.2
Glucose	5.0	5.0	5.0	5.0	5.0
Sucrose	10.0	10.0	10.0	10.0	10.0
Groundnut oil	10.0	10.0	10.0	10.0	10.0
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[a]	0.5	0.5	0.5	0.5	0.5
Nacl	0.2	0.2	0.2	0.2	0.2
Total	100.0	100.0	100.0	100.0	100.0
Cal. Protein	10.0	10.0	10.0	10.0	10.0

N-free = nitrogen free (basal diet); Reference diet = reference (control) diet;TOLM, *Telfairia occidentalis* leaf meal; ACLM, *Amaranthus cruentus* leaf meal; *Talinum triangulare* leaf meal, TTLM.

[a] Contained vitamins A (10,000,000 IU); D(2,000,000 IU); E (35000 IU); K (1900 mg); B12 (19 mg); riboflavin (7000 mg); pyridoxine (3800 mg); Thiamine (2200 mg); D Pantothenic acid (11,000 mg); nicotinic acid (45,000 mg); folic acid (1400 mg); biotin (113 mg); and trace elements as Cu (8000 mg); Mn (64,000 mg); Zn (40,000 mg); Fe (32,000 mg); Se (160 mg); I_2 (800 mg) and other items as Co (400 mg); choline (475,000 mg); methionine (50,000 mg); BHT (5000 mg) and spiramycin (5000 mg) per 2.5 kg.

Table 6
Rat growth assay and protein evaluation values of vegetable leaf meals

Parameters	Reference (casein) diet		Test ingredient diets	
	2	3	4	5
Weight gain in 10 days (g)	$6.41^{a} \pm 3.11$	$3.57^{b} \pm 2.45$	$2.49^{c} \pm 2.87$	$2.52^{c} \pm 2.01$
Feed intake in 10 days (g)	$43.75^{a} \pm 4.52$	$54.31^{b} \pm 5.32$	$52.28^{b} \pm 3.90$	$51.73^{b} \pm 3.16$
Nitrogen intake in 10days (g)	$0.70^{a} \pm 0.07$	$0.87^{b} \pm 0.06$	$0.84^{bc} \pm 0.01$	$0.83^{c} \pm 0.05$
Faecal nitrogen in 10days (g)	$0.18^{a} \pm 0.02$	$0.51^{b} \pm 0.03$	$0.51^{b} \pm 0.01$	$0.46^{c} \pm 0.02$
Urinary nitrogen in 10days (g)	$0.25^{a} \pm 0.22$	$0.11^{b} \pm 1.34$	$0.17^{c} \pm 1.20$	$0.20^{c} \pm 2.32$
Nitrogen retention (g)	$0.27^{a} \pm 0.09$	$0.25^{a} \pm 0.04$	$0.16^{b} \pm 0.03$	$0.17^{b} \pm 0.03$
Apparent nitrogen digestibility (%)	$75.72^{a} \pm 8.22$	$64.39^{b} \pm 2.91$	$57.25^{c} \pm 3.49$	$58.17^{c} \pm 4.35$
Protein efficiency ratio	$1.52^{a} \pm 0.27$	$1.20^{b} \pm 0.64$	$0.86^{c} \pm 0.29$	$0.99^{bc} \pm 0.67$
Net protein ratio	$-0.61^{a} \pm 0.56$	$-1.24^{b} \pm 0.86$	$-2.54^{c} \pm 0.47$	$-2.51^{c} \pm 0.83$
True digestibility (%)	$88.40^{a} \pm 7.61$	$65.81^{b} \pm 0.75$	$64.04^{b} \pm 1.58$	$63.02^{b} \pm 1.40$
Biological value (%)	$74.73^{a} \pm 3.64$	$55.63^{b} \pm 3.82$	$54.14^{b} \pm 2.83$	$53.28^{b} \pm 2.29$
Net protein utilisation (%)	$64.41^{a} \pm 3.52$	$55.32^{b} \pm 2.10$	$42.35^{c} \pm 1.87$	$49.43^{bc} \pm 2.99$

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

there seems to be a deficiency in the quantities of methionine and lysine. The mean weight gain of rats over a period of 10 days fed the reference diet containing pure protein (casein) was highest at 6.41 ± 3.11 g and significantly different ($P < 0.05$) from other values obtained for rats on the three VLM based diets. This is further highlighted in Fig. 1 where the growth patterns of rats fed the VLMs were compared against the rats fed the reference diet in a column chart. The feed intake (FI) pattern indicated that rats on the VLM based diets had similar intake values ($P > 0.05$) which were significantly higher ($P < 0.05$) than the FI value obtained for the rats put on the reference diet.

The nitrogen retention (NR) value was highest ($0.27 + 0.09$) for rats on the reference diet 2 but similar to the value ($0.25 + 0.04$) obtained for rats on the TOLM based diet (diet 3). The NR values obtained for rats on diets 4 and 5 of TTLM and ACLM, respectively were similar ($P > 0.05$) and lower than the others.

The apparent nitrogen digestibility (AND), protein efficiency ratio (PER), net protein ratio (NPR), true digestibility (TD), biological value (BV) and net protein utilization (NPU) all followed a predictable pattern. This is graphically depicted in a column chart in Fig. 2 showing some investigated protein quality parameters of rats fed the VLM based diets against the reference diet. All these values were significantly higher ($P < 0.05$) for rats placed on the reference (casein) diet. While the AND value obtained for rats on reference diet was an impres-

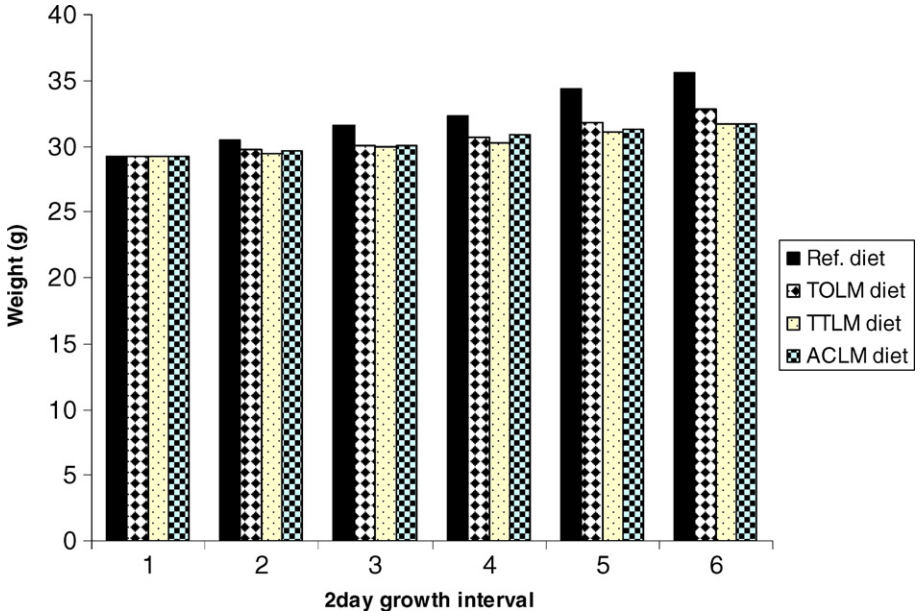

Fig. 1. Growth pattern of rat fed vegetable leaf meal diets against reference (casein) diet reference diet, reference (casein) diet; TOLM, *Telfairia occidentalis* leaf meal based diet; TTLM, *Talinum occidentalis* leaf meal based diet; ACLM, *Amaranthus cruentus* leaf meal based diet.

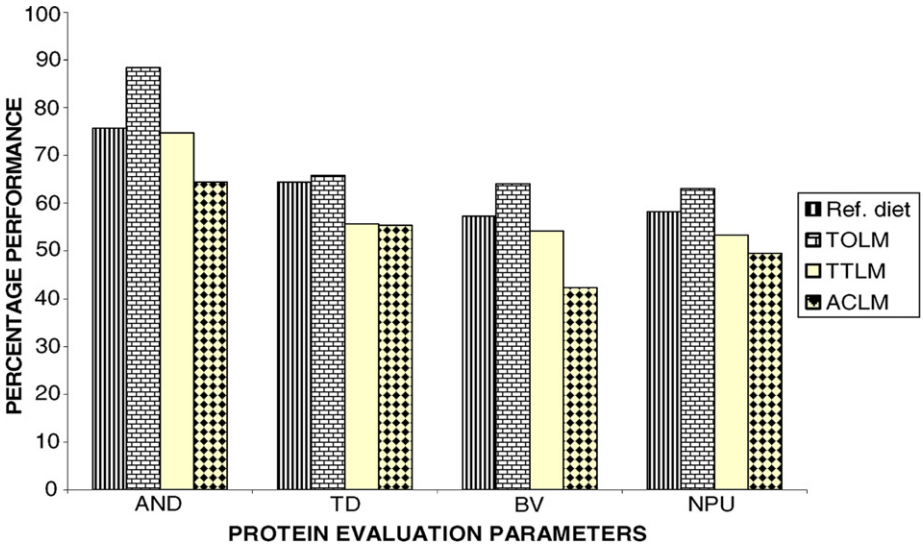

Fig. 2. Column chart of some investigated protein quality parameters of vegetable leaf meals against reference diet AND, apparent nitrogen digestibilty; TD, true digestibility; BV, biological value; NPU, net protein ratio. reference diet, reference (casein) diet; TOLM, *Telfairia occidentalis* leaf meal based diet; TTLM, *Talinum occidentalis* leaf meal based diet; ACLM, *Amaranthus cruentus* leaf meal based diet.

sive 75.7% (±) 8.22, rats on TOLM based diet (diet 3) had an AND value of 64.4% (±) 2.91. This value was significantly higher ($P < 0.05$) than the other 2 AND values (57.3% (±) 3.49 and 58.2% (±) 4.35) obtained for rats on TTLM and ACLM based diets (diets 4 and 5, respectively).

The protein efficiency ratio value of 1.52 (±) 0.27 was obtained for rats on the reference diet 2 and significantly higher ($P < 0.05$) than the PER values obtained for rats on the VLM based diets (diets 3–5). The net protein ratio (NPR) values were all negative for all experimental rats. However, the rats on the reference diet had the highest ($P < 0.05$) NPR value of −0.61 (±) 0.56. The NPR value for rats on the TOLM based diet ranked second (−1.24 (±) 0.86) and significantly higher ($P < 0.05$) than the NPR values obtained for the rats on the TTLM and ACLM based diets which were similar ($P > 0.05$).

The true digestibility (TD) value obtained for rats on the reference diet (88.4 ± 7.61%) was remarkably higher ($P < 0.05$) than the values obtained for the three VLM based diets. The rats on the VLM based diets had TD values ranging from 63.0 ± 1.40% in ACLM based diet to

65.8 ± 0.75% in TOLM based diet. These three TD values were similar ($P > 0.05$).

The biological value (BV) obtained for rats on the reference diet was 74.7 ± 3.64% and distinctly higher ($P < 0.05$) than the other three VLM based diets. Rats on the three VLM based diets had similar ($P > 0.05$) TD values ranging from 53.3% ± 2.29 in ACLM based diet to 55.6 ± 3.82% in TOLM based diet. The net protein utilization (NPU) also showed the same pattern with NPU value obtained for the rats on the reference diet (64.4 ± 3.52%) clearly higher ($P < 0.05$) than the other VLM based diets. There were similarities ($P > 0.05$) in the TD values obtained for rats on TOLM and ACLM based diets on one hand and TTLM and ACLM based diets on the other hand.

4. Discussion

The data on the proximate and mineral constituents of the VLMs indicate clearly their potential as food/feed resources. For example, the crude protein content of the VLMs with a range of 19.9–35.1% compared favourably with, and in some cases, surpassed those reported for legumes grown in West Africa (Oke, Tewe, & Fetuga, 1995; Ologhobo, 1980). The VLMs amino acid profiles also conformed to the FAO/WHO recommended values for some essential amino acids (FAO/WHO, 1973) and compared favourably with the values reported for whole egg (Robinson, 1987). However, supplementation with synthetic amino acids or complementary feeding with other ingredients high in these limiting amino acids (especially methionine and lysine) may be necessary to augment the deficiency in amino acid profile of the VLMs. The mineral compositions seem adequate especially for Ca, Na, K, Mg and Fe except for Zn that appear deficient particularly for *Talinum triangulare* leaf meal. The mineral levels were comparable and in some cases higher than the reported values for other plant sources that are already in conventional use in human/animal diets (Eggum, 1970; Fasuyi, 2005).

In this study, the growth rate indicated that the reference diet with casein as the sole protein source was significantly better than the three VLM based diets. This was expected since casein is a pure protein source with well balanced amino acid profile and hence the choice as a reference or standard diet with which other VLM based diets can be compared. Diet 3 (TOLM based diet) had the next WG value for the weanling albino rats while the WG values obtained for the TTLM and ACLM based diets (diets 4 and 5) were similar. It has long been established that green leaves are excellent sources of β-carotene and protein (Barbeau, 1989; Fasuyi & Aletor, 2005). Oke (1973) also confirmed that the incorporation of leaf protein concentrate obtained from further processing of leaves (Fellows, 1987) in the diet is as good as milk powder for rats and that it enhances weight gain in children. The growth rate pattern of weanling rats reared on the VLM based diets were comparable with results obtained when soyabean was solely used as the protein source in rat diets (Agbede & Aletor, 2003). The incorporation of the three VLMs (TOLM, TTLM and ACLM) has the potential to enhance the utilization of nutrients such as provitamins, which are growth promoters. The favourable amino acid profiles of the VLMs (Fasuyi, 2005) may have accounted in part for the relatively remarkable weight gain and food intake. The increased feed intake among the rats on the VLM based diets could be due to the increased level of dietary fibre (Fasuyi, 2005). It has earlier been recognized that the major drawbacks to the use of vegetable materials as major sources of nutrients by monogastrics (including man) are their high fibre and bulkiness which call for large quantities to be consumed to provide adequate levels of nutrients needed (Aletor & Adeogun, 1995).

The NR results from the experiment indicated that the rats retained the same amount of nitrogen in the reference (casein) diet and the TOLM diet but these NR values were significantly higher than the NR values obtained for rats on the TTLM and ACLM based diets (diets 4 and 5). However, the utilization of the nitrogen retained varied considerably as was evident from the values obtained for apparent nitrogen digestibility (AND), protein efficiency ratio (PER), net protein ratio (NPR), true digestibility (TD), biological value (BV) and net protein utilization (NPU). Nevertheless, the above parameters had significantly higher and better values for rats on the reference casein diet, rats on the 3 VLM based diets had values comparable with results obtained for soyabean based diet (Agbede & Aletor, 2003). These results agreed with the report of Oke (1973) that leaf protein is highly digestible and may in fact be better than fishmeal as a protein supplement.

The presence of some anitnutritional factors (ANFs) in VLMs (Fasuyi, 2005) is of negative nutritional relevance. The presence of ANFs (phytins, oxalates, tannins and to a lesser extent saponins) in VLMs was a probable factor that militated against the digestibility of crude protein (CP) and amino acids (AAs) in VLM based diets. Higher contents of tannins in the VLMs could have contributed to the poor digestibility of their CP and AAs in the VLM based diets compared to the reference (casein) diet that had little or none of these ANFs. Phenolic compounds like tannins exert their influence by binding with various compounds, including protein and making them less available to the animal (Bell & Janzen, 1971) because as dietary tannin content increases, the digestibility of energy and protein in the diet also decreases. Low apparent digestibility of CP and AAs in diets with high tannins as reported in faba beans is due to the effects of the condensed tannins in the hulls (Jansman, Versetegen, & Huisman, 1993). Other studies with pigs (Jansman, Verstegen, Huisman, & Van Den Berg, 1995), chickens (Longstaff & Mcnab, 1991) and White Pekin ducks (King, Fan, Ejeta, Asem, & Adeola, 2000) have shown the negative effects of high tannins on CP and AA digestibility. Phytic acid levels in the VLMs were high and similar to those earlier reported (Proll, Petzke, Ezeagu, & Metges, 1998). Phytic acid can

bind with proteins to form phytate protein complexes (Saio, Koyama, & Watanabe, 1967). This complex can adversely affect the digestibility of proteins (Reddy, Sathe, & Salunkhe, 1982) by inhibiting a number of digestive enzymes in the gastrointestinal tract such as pepsin (Camus & Laporte, 1976), trypsin (Cadwell, 1992) and chymotrypsin (Singh & Krikorian, 1982) thereby reducing the digestibility of proteins and AAs as indicated in the protein evaluation indices (Table 5). ANFs have been reported as having inhibiting effects on the digestive enzyme activity in chickens and rats (Longstaff & Mcnab, 1991; Welsch, Lachance, & Wasseman, 1989). The relative better protein evaluation indices recorded for animals on TOLM based diet can be attributed to the well balanced amino acid profile and its rich contents of minerals (particularly Fe), vitamins and essential fatty acids. Oleic and linoleic acids constitute over 63% of the fatty acid composition of *Telfairia* leaf (Asiegbu, 1988). Akoroda (1990) further confirmed the superiority of the fatty acid content when compared with other commonly consumed leafy vegetables in Nigeria.

However, it is conceivable that an improved growth rate may still be obtained in rats if the formulated diets are complementarily fed with ingredients high in amino acids found deficient in the VLMs or further supplemented with synthetic sources of the amino acids and relevant enzymes (e.g. cellulases and phytases) to facilitate the breakdown of fibre and phytins.

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