

# NUTRITIONAL QUALITY OF FOODS FORMULATED FROM COWPEA-MILLET-CRAYFISH MIXTURES

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## ABSTRACT

Six different formulations of supplementary foods (FS) based on cowpea, millet and crayfish were prepared. Three of the FS were produced with raw cowpea, millet and crayfish, and the other three prepared with combinations of cooked cowpea, millet and crayfish. These blends were fortified with vitamins and minerals. The FS prepared based on cowpea and millet in combination with crayfish were evaluated for proximate composition. One hundred gram portions of these foods provided a range of 11.86 g (Diet 4) to 16.32 g protein (Diet 3) and a range of energy value (k cal) of 369.4 (Diet 3) to 383.4 (Diet 4). Proximate composition of all the developed FS were within the range prescribed by FAO for processed supplementary foods and could satisfactorily meet one third of the recommended daily allowance (RDA) of these nutrients per day for growing children (except fat content). Further, these FS were evaluated among others for feed utilization (FU), protein efficiency ration (PER), net protein utilization (NPU), true digestibility (TD), biological value (BV) and organ weight using 48 growing albino rats of the Wistar strain. Ranges were for FU (196.2-315.5) mg/g, PER (1.48-2.22), NPU (69.0-78.6), TD (93.4-97.5), and BV (71.8-82.4). The nutritional parameters slightly differed among the FS groups but each was significantly lower ( $p < 0.05$ ) than FU (426.5 mg/g), PER (2.62), NPU (85.8) and BV (92.2) of positive control group (Diet 8). Heat processing improved organ weights of rats. Visual inspection at autopsy did not reveal pathological changes (pancreatic enlargement or hypertrophy) showing that there were physiologically tolerable levels of toxic factors in the FS formulated. Diets 6, 4 and 5 are the best of all the FS evaluated with Diet 6 having comparable nutritional quality with Diet 8. It is evident from the results that formulations prepared with cooked cowpea were nutritionally and biologically as good as the positive control than those with raw cowpea with regard to nutritional aspect of the formulations.

**KEYWORDS:** Cowpea-millet-crayfish, formulated supplementary foods, nutritional quality.

## INTRODUCTION

In the developing countries like Nigeria, the search for protein and energy foods will continue to be an on-going process as long as human civilization persists, coupled with dearth in the availability of foods with good nutrient sources. Animal protein is expensive and hence, dependency on plant protein would ever remain high (Addy et al, 1995). It has been estimated that in Nigeria alone, 8000 children might die annually before the age of four from malnutrition and that mortality rate due to protein-energy malnutrition (PEM) most critically shown by growth retardation was about ten to twenty times higher in the developing countries compared to developed nations (Goldsmith, 1975). Ossai and Malomo (1988) reported PEM to be one of the most important nutritional deficiency problems in developing countries of the world.

Grain legumes are rich, less expensive and important sources of B-complex vitamins, minerals and carbohydrates that contribute significantly to the protein content of the diets of people in India and other developing countries (Duhan et al, 2002). In such developing countries, most traditional weaning foods are formulated from cereals, starchy roots and tubers, but such foods are often of poor protein quality and have high pasting properties (Desikachar, 1979). Grain legumes show promise in the supply of vegetable proteins that the world would need in the near future (National Academy of Sciences, 1979). Legume fortified weaning foods have been shown to be of good nutritive value and prevent PEM (Ojefeitimi et al, 1984; Abiodun, 1991). Weaning foods formulated from African yam bean (a legume) – rice – prawn mixtures have been reported to be of good nutritional quality (Onyeike and Morris, 1996).

Whole cereal grains (rice, wheat, sorghum and millet) are an important component of diets for the majority of population in Asia and Africa (Jood, 1995). They are used together with legumes and vegetables in vegetarian meals

consumed in the tropics (Cunnane, 1988). Pearl millet is rich in carbohydrate, B – complex vitamins, trace minerals and contains two to ten times higher levels of zinc, copper and iron than rice (Göplan et al, 1993; Agte et al, 1995). It was in an attempt to combat persistent malnutrition disorders mainly in children of 0-5 years that diets which could be beneficial as weaning foods were formulated from cowpea, millet and crayfish in the present study.

This paper reports on the chemical composition and nutritional quality of eight diets, six based on an alternative protein source from crayfish powder, one on corn flour (negative control) and a positive control diet (Nutrend).

## MATERIALS AND METHODS

### Sample preparation:-

The pearl millet seeds (*Pennisetum typhoidemum*), cowpea seeds (*Vigna unguiculata*) and dried crayfish were purchased from Mile 3 market in Diobu, Port Harcourt. The millet seeds were sorted, washed to remove dirt particles, boiled (100 °C) over a kerosene stove to tenderness and sun – dried for 48 hr. The cowpea was divided into two lots of 1kg each. The first lot served as raw sample. The second lot was cooked in boiling water over a kerosene stove for 120 min to tenderness as in domestic practice.

Each of processed millet and cowpea as well as raw cowpea sample and crayfish was separately ground into flour using a food grinder (Model MX 491N, National, Sheffield, UK) to pass through a 30 – mesh sieve, wrapped in polyethylene bags, sealed in clean dry air – tight containers and stored in a refrigerator (4°C) for 3 days prior to analysis and use in compounding the experimental diets. Diets were compounded using the ingredients as shown in Table 1a. Non-nutritive cellulose was sieved fine wood shavings added to provide roughage while red palm oil was used to provide dietary fat. Sucrose was added at the expense of cowpea and millet flours

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Table 1a. Composition of formulated diets (g/kg) assigned to the eight groups of rats

Components	Diets							
	1	2	3	4	5	6	7	8
Raw cowpea flour	162.5	325	487.5	-	-	-	-	-
Cooked cowpea flour	-	-	-	162.5	325	487.5	-	-
Cooked millet flour	412.5	250	87.5	412.5	250	87.5	-	-
Dried crayfish flour	92.4	92.4	92.4	92.4	92.4	92.4	-	-
Corn flour	-	-	-	-	-	-	667.4	-
Vitamin and mineral mixture	50.0	50.0	50.0	50.0	50.0	50.0	50.0	-
Non-nutritive cellulose	25.5	25.5	25.5	25.5	25.5	25.5	25.5	-
Red palm oil	18.6	18.6	18.6	18.6	18.6	18.6	18.6	-
Sucrose	238.5	238.5	238.5	238.5	238.5	238.5	238.5	-
Nutrend	-	-	-	-	-	-	-	1000
Total (kg)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

Table 1b. Average composition of Nutrend Diet (Manufacturer's Specification)

Component	Value/100 g	Component	Value/100g
Protein (g)	16.0	Vitamin E (IU)	3.00
Fat (g)	9.0	Vitamin C (mg)	35.0
Carbohydrate (g)	64.0	Folic acid (mcg)	22.5
(Sucrose, starch and oligosaccharide) g	19.0, 39.6	Thiamine (Vit B <sub>1</sub> ) mg	0.80
Dietary fibre (g)	5.40		
Mineral (ash) (g)	5.00	Riboflavin (Vit B <sub>2</sub> ) mg	0.30
Moisture (g)	2.00	Niacin (mg)	4.00
Energy value (kcal)	4.00	Pyridoxine (Vit B <sub>6</sub> ) mg	0.30
Energy value (kj)	401.	Vitamin B <sub>12</sub> (mcg)	0.75
Linoleate (g)	1678.	Biotin (mcg)	25.0
Vitamin A (IU)	3.60	Panthenic acid (mg)	1.50
Vitamin D (IU)	1030	Calcium (mg)	310
	200	Phosphorous (mg)	200

Sources: Food Specialities (Nig.) PLC (1994), and Onyeike et al (1999).

to make the diets palatable. Animal protein is expensive and it was deemed economical to add crayfish as one of the supplements to increase the protein content of the formulated foods. Diet 7 was meant to be a non-protein control diet. The 7.57% protein in Diet 7 may have been contributed by corn flour. The diet was not considered as basal for calculating NPU values but as negative control while Nutrend was positive control and its composition is as shown in Table 1b.

#### Proximate analysis.

The methods of the Association of Official Analytical Chemists (AOAC, 1984) were used to determine moisture, ash, crude fat and crude protein. The AOAC (1984) method numbers were 14.004, 14.006, 7.062 and 2.057 for the determinations of moisture, ash, crude fat and crude protein respectively. Moisture was obtained by heating three 2.0 g of each sample in a crucible placed in a hot air-circulating oven (Plus 11 Sanyo, Gallenkamp PLC, UK) at 105°C for 3 hr to a constant weight. Ash was determined by the incineration of three 1.0g samples in a muffle furnace (LMF4 Carbolite, Bamford, Sheffield, UK) at 630 °C for 3hr. Crude protein (% N x 6.25) was determined by the Kjeldahl method using three 1.0g samples. Crude fat was obtained by exhaustively extracting three 5.0g samples in a Soxhlet apparatus using petroleum ether (b.p 40 – 60 °C) as the extractant (Onyeike et al, 2005).

Total carbohydrate was determined by difference as reported (Onyeike and Onwuka, 1999) while the Clegg Anthrone method (Southgate, 1969; Nwinuka et al, 1997) was used to determine available carbohydrate as percent glucose. The energy content was calculated as reported by Onyeike et al, (2005).

#### Composition of vitamin and mineral mixture used

The vitamin mixture used in Glaxo Laboratories (Cuthbertson, 1957) and reported by Onyeike et al (1999) used in this study composed of the following per kilogram of the semi-synthetic ration: vitamin A 4000 I.U, vitamin D

(calciferol) 2000 I.U., vitamin E ( $\alpha$ -tocopherol) acetate 280 mg, vitamin k 2.00 mg, vitamin B<sub>1</sub> (thiamine) 30.0 mg, vitamin B<sub>2</sub> (riboflavin) 30.0 mg, vitamin B<sub>6</sub> (Pyridoxine) 8.00 mg, calcium panthothenate 100 mg, nicotinic acid 100 mg, vitamin B<sub>12</sub> (Cobalamine) 50.0 mg, choline 1000 mg, pteroylglutamic acid (folic acid) 1.00 mg, biotin 0.20 mg, inositol 220 mg and p-aminobenzoic acid 75.0 mg).

The mineral mixture from Sigma Chemical Company, Poole, England contained the following in gram: sodium chloride 139, potassium hydrogen sulphate 389, magnesium sulphate 53.7, calcium phosphate 38.4, ferrous sulphate 27.0, manganous sulphate 4.00, zinc sulphate 0.50, potassium iodide 0.50, copper sulphate 0.50 and sodium fluoride 0.04.

#### Animal feeding study

A total of 48 weanling male albino rats of the wistar strain, weaned at 3 weeks of age weighing 61.7 to 64.2g were used in a feeding study that lasted 28 days. The animals were ethically cleared to use them for experimental purpose. The rats after four days acclimatization on conventional feed were randomly distributed to the groups based on initial body weights and litter origin. The six rats per group were equalized as nearly as possible with respect to body weight and individually housed in perforated Perspex wire bottom cages with facilities for food, water, urine and faecal collection. Feed and water were given to the rats *ad libitum* for the period of study.

The diets were offered in stainless steel feeders with anti-spill devices. The rats were not trained to consume their respective experimental diets between 07.00 and 15.00 hours daily for six days with the feeder being placed in the metabolic cage for 15min at intervals of one hour. This was to avoid significant stress effects on the rats particularly those on Diet 7, and to ensure no influence on the outcome of the study. At the end of the feeding period, the animals were weighed and sacrificed by exposing them to chloroform in a closed container. Each carcass was labeled and the abdomen

opened by an incision along the mid ventral line. The skin and musculature were folded back to expose the viscera.

The organs (lungs, kidneys, pancreas, liver, spleen and heart) were excised, inspected for pathological changes, weighed and returned into the individual carcasses. Relative organ weights were calculated. Each carcass was dried in an oven, weighed, ground and analyzed for nitrogen content. Faecal droppings which were collected daily for individual rats per group and kept in an oven were bulked up, further oven-dried at 80°C for 24 hours, weighed, ground and nitrogen content determined and nutritional parameters calculated.

Feed utilization was calculated as reported by Onyeike and Morris (1996) while PER was obtained by the method of Bender and Doell (1957). NPU was determined by the method of Bender and Miller (1953) while TD and BV were calculated as reported by Miller and Bender (1955).

**Statistical analyses**

Means ± standard deviations of triplicate determinations were computed. Data were analyzed by a one-way analysis of variance (ANOVA). Differences between

means were compared using Duncan's (1955) multiple range tests. Significance was accepted at a p-value of 0.05.

**RESULTS AND DISCUSSION**

The proximate composition and energy values of samples used in formulating the diets are presented in Table 2. Crayfish was the highest source of protein (62.7± 0.33%) while cooked millet was the lowest (11.4±0.55%). Ash ranged from 1.99± 0.02% in millet to 9.03±0.37% in crayfish while crude fat range from 2.95±0.70% in raw cowpea to 5.15±0.20% in millet. Cooked millet was the major source of total carbohydrate with a value of 76.6± 0.57%. The energy contents (kcal/100 g) ranged from 346.3 in crayfish to 398.3 in cooked millet. The high-energy value of samples used may be attributable to the high values of crude protein and total carbohydrate. The crude protein, crude fat and total carbohydrate values obtained for these samples which were similar to the values reported by Oyenuga (1968) suggested that the samples were adequate for use in formulating the experimental diets.

Table 2: Proximate composition (%)\* of samples used in formulating the experimental diets

Constituents	Samples			
	Raw Cowpea	Cooked Cowpea	Cooked Millet	Crayfish
Moisture	11.4±0.65 <sup>a</sup>	8.77±0.13 <sup>b</sup>	4.87±0.25 <sup>c</sup>	9.08±0.17 <sup>b</sup>
Crude protein	24.1±0.57 <sup>b</sup>	18.4±1.08 <sup>c</sup>	11.4±0.55 <sup>d</sup>	62.7±0.33 <sup>a</sup>
Ash	3.50±0.55 <sup>b</sup>	2.63±0.18 <sup>c</sup>	1.99±0.02 <sup>c</sup>	9.03 ± 37 <sup>a</sup>
Crude fat	2.95 ±0.70 <sup>c</sup>	4.10±0.32 <sup>b</sup>	5.15±0.20 <sup>a</sup>	3.74±0.21 <sup>b,c</sup>
Total carbohydrate (by difference)	58.1±0.21 <sup>c</sup>	66.2±0.51 <sup>b</sup>	76.6±0.57 <sup>a</sup>	15.5±0.16 <sup>d</sup>
Carbohydrate (by Anthrone method)	57.7±0.10 <sup>c</sup>	65.7±0.34 <sup>b</sup>	76.9±0.70 <sup>a</sup>	14.9±0.14 <sup>d</sup>
Energy value (kcal/100g sample)	355.4	354.4	398.3	346.3

\* Values are means ± standard deviations of triplicate determinations. Values in the same row bearing the same superscript letters are not significantly different at the 5% level.

The proximate composition and energy values of the FS are shown in Table 3. The moisture contents were low (below 10%) in all the diets. Protein ranged from 7.57% in Diet 7 to 16.3% in Diets 3 and 8 while ash was highest (3.85%) in Diet 2 and lowest in Diet 7 (1.35%). Crude fat was generally low with the highest value of 3.96% in Diet 4. Total carbohydrate was highest (81.1%) in Diet 7 and lowest in Diet 3 (69.3%). The energy values were high in all the diets

and the order was Diet 8 > Diet 4 > Diet 1 > Diet 5 > Diet 6 > Diet 2 > Diet 7 > Diet 3. The low moisture levels would indicate no storage problems or fungal growth (Badamosi et al, 1995), and deterioration by other microbes since high moisture level may increase microbial action due to high water activity resulting in food spoilage. Each of the percent crude protein values was slightly lower but compared well with the 20% protein level recommended by Protein Advisory Group for

Table 3. Proximate composition (%)\*and energy values of the experimental diets

	1	2	3	4	5	6	7	8
Moisture	5.44±0.13 <sup>a</sup>	6.39±0.31 <sup>d</sup>	8.04±0.02 <sup>a</sup>	6.44±0.00 <sup>d</sup>	6.96. ±0.02 <sup>c</sup>	7.23±0.01 <sup>b</sup>	7.84±0.03 <sup>a</sup>	3.03±0.01 <sup>f</sup>
Crude protein	13.30±0.02 <sup>e</sup>	14.70±0.01 <sup>d</sup>	16.32±0.01 <sup>a</sup>	11.86±0.01 <sup>f</sup>	15.30±0.01 <sup>c</sup>	16.11±0.01 <sup>b</sup>	7.57±0.01 <sup>g</sup>	16.27±0.09 <sup>a</sup>
Ash	3.13±0.02 <sup>e</sup>	3.85±0.03 <sup>a</sup>	3.35±0.01 <sup>b</sup>	2.67±0.01 <sup>a</sup>	2.58±0.01 <sup>e</sup>	2.64±0.02 <sup>d</sup>	1.35±.02 <sup>g</sup>	2.40±0.01 <sup>f</sup>
Crude fat	3.50±0.05 <sup>e</sup>	3.72±0.02 <sup>d</sup>	2.98±0.01 <sup>f</sup>	3.96±0.02 <sup>b</sup>	3.78±0.01 <sup>c</sup>	3.80±0.01 <sup>c</sup>	2.11±.01 <sup>g</sup>	7.39±0.01 <sup>a</sup>
Total carbohydrate (by-difference)	74.63±0.16 <sup>c</sup>	71.34±0.32 <sup>d</sup>	69.32±0.02 <sup>e</sup>	75.07±0.03 <sup>b</sup>	71.37±0.02 <sup>d</sup>	70.21±0.02 <sup>e</sup>	81.13±0.05 <sup>a</sup>	70.65±0.10 <sup>e</sup>
Total carbohydrate (by Anthrone method)	74.47±0.15 <sup>c</sup>	71.20±0.28 <sup>d</sup>	69.25±0.04 <sup>e</sup>	75.06±0.04 <sup>b</sup>	71.34±0.04 <sup>d</sup>	70.20±.06 <sup>e</sup>	81.09±1.02 <sup>a</sup>	69.62±0.24 <sup>e</sup>
Energy value(kcal/100 g sample)	383.2	377.6	369.4	383.4	380.7	379.5	373.8	414.2
kJ/100 g sample	1609.4	1585.9	1551.5	1610.3	1598.9	1593.9	1570.0	1739.6

Constituents Diets

\* Values are means ± standard deviations of triplicate determinations. Values in the same row bearing the same superscript letters are not significantly different at the 5% level.

weaning foods in developing countries (Ketiku and Smith, 1984). The protein content of all the FS appeared adequate to meet the requirements of children as the values were within the range of 11.0 to 14.0% recommended by Beaton and Swiss (1974). The adequacy of the protein in the FS should therefore not be a matter of concern since the recommended daily allowance (RDA) of protein for a 6-month old infant is 13 g per day (National Research Council, 1989). The ash levels of the experimental diets were found adequate to support the growth of rats. It has been reported that when vegetable leaves are to be used as human food, they should contain about 3.0% ash (Pivie and Butler, 1977).

Feeding at 100 g FS per day gave energy values in kilocalories (kcal) of 383.2, 377.6, 369.4, 383.4, 380.7, 379.5, 373.8 and 414.2 for Diets 1-8 respectively. The calorific values (kcal/100 g diet) indicate that 678.5 g, 688.6 g, 703.8 g, 678.1 g, 683.0 g, 685.1 g, 695.6 g and 627.7 g of Diets 1-8 would respectively provide 2600 kcal. This energy value (2600kcal) is within the range of 2500 to 3000 kcal which Bingham (1978) reported as the daily energy requirement for adults. It also follows that if the FS are consumed at 220 g daily, they would provide energy values (kcal) of 843.0, 830.7, 812.7, 843.5, 837.5, 834.9, 822.4 and 911.2 for Diets 1-8 respectively. It is therefore evident from this work that the energy content of all the FS fed to rats met the FAO (1973) recommended range of 800-1200 kcal for adults if the Diets are consumed at only 220 g daily.

The performance characteristics of rats fed Diets 1-8 are presented in Table 4. Body weight gain was highest (128.7

g) in Nutrend-fed rats (Diet 8) followed by rats fed Diet 6 (82.3 g) and was lowest (10.3 g) in rats adapted to Diet 7. As the incorporation of cooked cowpea flour increased from Diets 4-6, body weight gain increased as well over values for Diets 1-3 which contained increasing levels of raw cowpea flour (Table 4). Average food intake was in the order Diet 8 > Diet 4 > Diet 1 > Diet 6 > Diet 3 > Diet 5 > Diet 2 > Diet 7. Mean protein intake was highest (49.2 g) in Diet 8 and lowest in Diet 7 (11.7 g). Feed utilization (FU) was highest in rats fed Diet 8 and lowest in rats adapted to Diet 7. The highest PER value was obtained for rats fed Diet 8 (2.62) followed by rats fed Diet 4 (2.22) and Diet 6 (1.96) which contained cooked cowpea. These values were generally higher than those of rats fed Diets 1-3 which contained raw cowpea flour. Rats that consumed Diet 7 had the lowest PER value of 0.89. The NPU ranged from 69.0±3.56 in Diet 3 to 85.8±4.38 in Diet 8. Values for Diets 4 and 6 which did not differ significantly at the 5% level followed that of Diet 8 closely but were significantly ( $p<0.05$ ) higher than those for Diets 2, 3 and 5. True digestibility (TD) ranged from 93.1 in Diet 8 to 97.5 in Diet 1. All the diets showed high apparent biological values. The highest was obtained for rats adapted to Diet 8 (92.2) followed by rats fed Diet 6 (82.4) and lowest value of 71.7 was obtained for rats that consumed Diet 2. (Table 4). The highest values of body weight gain, food and protein intakes for Nutrend fed rats may be due to better balanced nutrient patterns in the commercial weaning diet. The better performance of rats fed diets containing cooked samples over those that consumed diets containing raw samples could be explained on the basis of heat destruction of the toxic factors in the food materials investigated.

Table 4. Performance characteristics of weanling albino rats fed formulated diets for 28 days

Parameters	Diets							
	1	2	3	4	5	6	7	8
Initial body weight (g)	62.6±0.67	63.6±0.98	61.7±0.70	63.0±0.32	62.5±0.76	64.2±0.54	63.0±0.94b	63.7±1.34
Final body weight (g)	116.0±2.50 <sup>a</sup>	118.0±9.38 <sup>a</sup>	131.7±8.67 <sup>a</sup>	143.3±7.56 <sup>b</sup>	122.9±9.07 <sup>a</sup>	146.5±2.38 <sup>b</sup>	73.4±1.13 <sup>a</sup>	193.2±7.68 <sup>b</sup>
Body weight gain (g)	53.4±2.32 <sup>a</sup>	54.4±9.74 <sup>a</sup>	71.0±6.92 <sup>a</sup>	80.3±7.32 <sup>b</sup>	60.4±9.32 <sup>a</sup>	82.3±2.49 <sup>b</sup>	10.3±0.30 <sup>a</sup>	128.7±6.84 <sup>b</sup>
Average food intake (g per 28 days)	272.4±3.75 <sup>b</sup>	245.47±0.15 <sup>a</sup>	254.1±10.46 <sup>a</sup>	305.4±7.83 <sup>b</sup>	246.4±6.59 <sup>a</sup>	262.3±17.80 <sup>ab</sup>	154.4±17.44 <sup>a</sup>	307.4±4.73 <sup>b</sup>
Mean protein intake (g per 28 days)	36.2±0.49 <sup>a</sup>	36.1±1.81 <sup>a</sup>	41.5±1.70 <sup>a</sup>	36.2±0.92 <sup>a</sup>	37.7±1.01 <sup>a</sup>	42.2±2.88 <sup>b</sup>	11.7±1.34 <sup>a</sup>	49.2±0.76 <sup>a</sup>
Feed utilization (mg/g)	196.2±9.73 <sup>a</sup>	222.4±43.10 <sup>ab</sup>	279.3±23.23 <sup>c</sup>	262.8±21.36 <sup>c</sup>	245.4±39.72 <sup>bc</sup>	315.5±29.95 <sup>b</sup>	67.3±8.34 <sup>a</sup>	426.5±27.8 <sup>b</sup>
Protein efficiency ratio	1.48±0.07 <sup>a</sup>	1.51±0.29 <sup>a</sup>	1.71±0.14 <sup>a</sup>	2.22±0.18 <sup>b</sup>	1.60±0.25 <sup>a</sup>	1.96±0.18 <sup>b</sup>	0.89±0.11 <sup>a</sup>	2.62±0.17 <sup>b</sup>
Net protein utilization	76.5±4.89 <sup>a</sup>	69.0±3.80 <sup>a</sup>	69.0±3.56 <sup>a</sup>	76.8±3.68 <sup>a</sup>	70.7±5.14 <sup>a</sup>	73.6±4.94 <sup>a</sup>		85.8±4.38 <sup>b</sup>
True digestibility	97.5±1.95 <sup>a</sup>	96.1±1.24 <sup>ab</sup>	96.0±1.01 <sup>ab</sup>	94.3±2.06 <sup>bc</sup>	93.4±1.29 <sup>bc</sup>	95.61±2.34 <sup>ab</sup>		93.1±4.38 <sup>b</sup>
Biological value	78.7±3.34 <sup>bc</sup>	71.8±3.24 <sup>a</sup>	71.8±0.43 <sup>a</sup>	81.5±5.29 <sup>bc</sup>	75.7±8.09 <sup>bc</sup>	82.4±5.46 <sup>b</sup>		92.2±4.98 <sup>b</sup>

Values are means ± standard deviations for 6 rats per group (n=6). Values in the same row having the same superscript letters are not significantly different at the 5% level.

The relatively low body weight gain and FU for rats fed diets containing raw cowpea compared to those that consumed cooked cowpea diets may be due to the presence of higher levels of toxicants in the raw samples as earlier reported for raw African yam bean (*Sphenostylis stenocarpa*) diet (Onyeike et al, 1995). Most raw legumes have been reported to be poorly utilized and cooking improved their utilization (Sri Kantha and Erdman, 1988). A Kinetic approach has been used to establish the heat inactivation of African yam bean trypsin inhibitors (Onyeike et al, 1991). Values of PER for rats fed cooked cowpea diets (Diets 4-6) were lower than the value for rats fed Nutrend (Diet 8), but generally higher than for rats adapted to raw cowpea (Diets 1-3). African locust bean samples (seed and seed with pulp) were shown to be detrimental to the growth of rats which lost weight giving negative PER values of -3.19 and -2.54 respectively (Fetuga et al, 1974). PER values calculated at protein intake ranging between 9.0 and 12% of other commonly consumed legumes apart from African locust bean was found to range from 0.10 to 2.00 (Narayana-Rao and Swaminathan, 1969). Recent report on diets based on larvae of raphia palm beetle and weevil (emergency food protein sources) showed PER values to be 0.30 and 0.35 as against -2.50 for rats fed basal diets,

while PER for rats fed Nutrend was 1.67 (Ayalogu et al, 2003). It can be seen that in the present study, the FS especially those containing heat-processed samples supported the growth of rats since no rat group lost weight and each gave positive PER value. The PER of 2.62 obtained in this work for rats fed Nutrend was comparable to that of 2.60 for the control casein diet reported by Badamosi et al (1995) who also calculated a PER value of 2.25 for a locally formulated weaning food JUTH-PAP (a multimix weaning food formulated by the Dietetics and Rehabilitation Unit in the Jos University Teaching Hospital, Nigeria). So far, the most widely used method for determining protein quality in foodstuffs useful in human nutrition is the PER test.

For animal proteins, some of the NPU values earlier reported were for fish cod (83.0), egg albumin (82.5), dried milk (75.0), beef liver (65.0), crude casein (60.0) and pancreas residues (38.5), while for plant proteins, values reported were wheat germ (67.0), soya flour (56.0), maize (55.0), groundnut meal (42.8) and rice gluten (36.0) (Miller and Bender 1955). A recent report has shown NPU values of 49.45 ± 0.48 and 59.11 ± 0.43 for rats fed diets based on raphia palm beetle and weevil respectively (Ayalogu et al, 2003). The high NPU values obtained in this study may be

due to lower urinary nitrogen excretion and better balanced amino acid pattern in the diets evaluated.

High digestibility among rat groups may suggest that there were very low levels of the antinutritional factors in the diets and that heat processing may have resulted in the desirable pattern of essential amino acids in the FS. The nutritional value of protein depends on the total essential amino acid content of the protein and protein digestibility, which is an indicator of the availability of essential and non-essential amino acids as earlier reported (Ihekoronye, 1988). The biological values (BV) for all groups of rats calculated were apparent since the values were not corrected for endogenous nitrogen losses in faeces and urine. It can be stated that rats fed Nutrend and diets containing cooked cowpea had the lowest urinary nitrogen and highest nitrogen retention compared to rats fed raw cowpea diets and hence, had higher biological values. It was expected that as the level of incorporation of cooked cowpea flour increased (Diets 4-6) the nutritional parameters would also increase in that order. It is not certain why the weight gain, PER, NPU, TD and BV of rats fed Diet 5 was low compared with those fed Diet 4. This may be due to relatively lower average food intake and ability to utilize food arising from individual biological variations of the

animals. The indigestible protein that was undoubtedly present in Diet 7 may have affected the faecal excretion of nitrogen by the rats and hence the NPU, TD and BV calculations.

The absolute and relative organ weights of rats fed Diets 1-8 are presented in Table 5. The absolute weight of the organs of rats fed Diet 8 was generally significantly ( $p < 0.05$ ) higher than for rats fed Diets 4-6 (cooked cowpea diets) and the latter were also generally significantly higher than for rats that consumed raw cowpea (Diets 1-3). At autopsy, the organs did not show any pathological disorder. Specifically, there was no pancreatic enlargement or hypertrophy, no hepatomegaly and no renal dystrophy.

The organ weights were improved by heat processing. Even in rats that consumed diets containing raw cowpea, visual inspection at autopsy did not show for the organs change in appearance, abnormal pancreatic enlargement and decrease in liver weight. The feeding of raw soya flour had earlier been shown to rapidly induce pancreatic hypertrophy and hyperplasia (adenomas and carcinomas) in rats (Mc Guinness et al, 1984) and in the mouse (Schingoethe et al 1974). The diets evaluated in this

Table 5: Absolute and relative organ weights of rats at the end of the feeding studies

	Diets							
	1	2	3	4	5	6	7	8
Final body weight (g)	116.0±2.50 <sup>a</sup>	118.0±3.38 <sup>a</sup>	131.7±8.67 <sup>c</sup>	143.3±7.56 <sup>b</sup>	122.9±9.07 <sup>a</sup>	146.5±2.38 <sup>b</sup>	73.4±1.13 <sup>a</sup>	193.2±7.68 <sup>a</sup>
LUNGS								
AOW	0.653±0.146 <sup>b</sup>	0.520±0.070 <sup>c</sup>	0.510±0.039 <sup>c</sup>	0.625±0.102 <sup>b</sup>	0.796±0.070 <sup>a</sup>	0.698±0.058 <sup>b</sup>	0.519±0.022 <sup>c</sup>	0.793±0.034 <sup>a</sup>
ROW	0.561±0.119 <sup>b</sup>	0.442±0.053 <sup>cd</sup>	0.389±0.045 <sup>d</sup>	0.437±0.075 <sup>cd</sup>	0.694±0.056 <sup>a</sup>	0.477±0.043 <sup>c</sup>	0.706±0.023 <sup>a</sup>	0.413±0.029 <sup>d</sup>
KIDNEYS								
AOW	0.611±0.07 <sup>d</sup>	0.652±0.024 <sup>d</sup>	0.758±0.051 <sup>c</sup>	0.785±0.071 <sup>c</sup>	0.759±0.021 <sup>cd</sup>	0.968±0.124 <sup>b</sup>	0.605±0.023 <sup>d</sup>	1.223±0.133 <sup>a</sup>
ROW	0.527±0.05 <sup>e</sup>	0.557±0.055 <sup>cd</sup>	0.578±0.050 <sup>cd</sup>	0.549±0.058 <sup>cd</sup>	0.621±0.043 <sup>cd</sup>	0.661±0.084 <sup>b</sup>	0.825±0.038 <sup>a</sup>	0.635±0.059 <sup>cd</sup>
PANCREAS								
AOW	0.281±0.09 <sup>d</sup>	0.291±0.033 <sup>cd</sup>	0.347±0.010 <sup>c</sup>	0.357±0.35 <sup>c</sup>	0.359±0.069 <sup>c</sup>	0.451±0.089 <sup>b</sup>	0.301±0.012 <sup>cd</sup>	0.599±0.017 <sup>a</sup>
ROW	0.243±0.007 <sup>c</sup>	0.248±0.031 <sup>c</sup>	0.265±0.021 <sup>b</sup>	0.251±0.030 <sup>c</sup>	0.291±0.043 <sup>c</sup>	0.309±0.062 <sup>b</sup>	0.411±0.017 <sup>a</sup>	0.312±0.016 <sup>b</sup>
LIVER								
AOW	4.003±0.636 <sup>d</sup>	4.317±0.158 <sup>c</sup>	4.476±0.184 <sup>c</sup>	4.704±0.34 <sup>c</sup>	4.475±0.374 <sup>c</sup>	5.466±0.478 <sup>b</sup>	2.147±0.103 <sup>c</sup>	6.156±0.344 <sup>a</sup>
ROW	3.449±0.529 <sup>abc</sup>	3.693±0.444 <sup>a</sup>	3.412±0.241 <sup>abc</sup>	3.296±0.36 <sup>cd</sup>	3.647±0.238 <sup>b</sup>	3.733±0.342 <sup>a</sup>	2.925±0.113 <sup>d</sup>	3.209±0.257 <sup>cd</sup>
SPLEEN								
AOW	0.203±0.006 <sup>b</sup>	0.196±0.012 <sup>a</sup>	0.232±0.019 <sup>d</sup>	0.229±0.33 <sup>d</sup>	0.277±0.008 <sup>b</sup>	0.257±0.005 <sup>c</sup>	0.252±0.010 <sup>c</sup>	0.311±0.006 <sup>a</sup>
ROW	0.175±0.008 <sup>c</sup>	0.167±0.016 <sup>c</sup>	0.177±0.019 <sup>c</sup>	0.161±0.030 <sup>c</sup>	0.226±0.015 <sup>a</sup>	0.176±0.003 <sup>c</sup>	0.343±0.010 <sup>a</sup>	0.162±0.006 <sup>c</sup>
HEART								
AOW	0.319±0.049 <sup>a</sup>	0.379±0.017 <sup>cd</sup>	0.347±0.031 <sup>cd</sup>	0.402±0.047 <sup>c</sup>	0.397±0.052 <sup>cd</sup>	0.471±0.058 <sup>b</sup>	0.363±0.026 <sup>cd</sup>	0.541±0.028 <sup>a</sup>
ROW	0.275±0.042 <sup>c</sup>	0.323±0.030 <sup>b</sup>	0.260±0.031 <sup>c</sup>	0.282±0.041 <sup>cd</sup>	0.323±0.030 <sup>b</sup>	0.322±0.040 <sup>b</sup>	0.494±0.033 <sup>a</sup>	0.282±0.017 <sup>cd</sup>

Values are means ± standard deviation of six determinations (n=6). Values in the same row bearing the same superscript letters are not significantly different at the 5% level (p=0.05). AOW = absolute organ weight (g of organ per final body weight) ROW = relative organ weight (g of organ per 100 g body weight)

study are thus of good nutritional quality as they also improved organ weights of rats.

**Conclusion:** The FS are adjudged to be of good nutritional value. Heat processing improved the indices of nutritional quality and organ weights of rats. Of all the foods evaluated, Diet 6 gave the best overall result and could be adapted as weaning food formulae for infants and other vulnerable groups. It compared highly with Nutrend with respect to nutritional quality and can be used in communities with high infant PEM. However, for such to be adopted, further tests like sensory evaluation on humans, shelf life and microbiological studies should be carried out.

REFERENCES

Abiodun, P.O. 1991. Use of Soyabean for the dietary prevention and management of malnutrition in Nigeria. *Acta Pediatrica Scandinavica Supplement* 374:175-185.

Addy, E.O.H, Salami, L.I., Igboeli, L.C. and Remawa, H.S. 1995. Effect of Processing on nutrient composition and anti nutritive substances of African locust bean (*Parkia filicoidea*) and baobab seed (*Adansonia digitata*). *Plant Foods for Human Nutrition* 48: 113 - 117.

Agte, V.V, Gokhale, M.K., Paknikar, K.M. and Chiplonkar, S.A. 1995. Assessment of pearl millet versus rice - based diets for bioavailability of four trace metals. *Plant Foods for Human Nutrition* 48: 149 - 158.

AOAC (1984). *Official Methods of Analysis* (14<sup>th</sup> edn.) Arlington, Virginia, Association of Official Analytical Chemists.

Ayalogu, E.O., Onyeike, E.N. and Okaraonye, C.C. 2003. Evaluation of the nutritional status of rats fed diets formulated from larvae of raphia palm beetle (*Oryctesrhinoceros*) and weevil (*Rhynchophorus phoenicis*). *Nigerian Journal of Biochemistry and Molecular Biology* 18(1): 47 -51.

- Badamosi, E.J., Ibrahim, L.M. and Temple, V.J. 1995. Nutritional evaluation of a locally formulated weaning food, JuTH – PAP. *West African Journal of Biological Sciences* 3(1 and 2): 5 – 93.
- Beaton, G.H. and Swiss, L.D. (1974). Estimation of Protein – energy ratio. *American Journal of Clinical Nutrition* 27: 485 – 489.
- Bender, A.E. and Doell, B.H. 1957. Biological evaluation of proteins: A new aspect. *British Journal of Nutrition* 11:140-148.
- Bender, A.E. and Miller, D.S. 1953. Calculation of the net protein utilization. *Biochemical Journal* 53:7-10
- Bingham, S. 1978. Nutrition: A Consumer's Guide to Good Eating, *Transworld*, London, pp. 123 – 127.
- Cunnane, S.C. 1988. Zinc: Clinical and Biochemical Significance, CRC Press Inc. Boca Rotan, Florida, USA.
- Cuthbertson, W.J.F. 1957. Nutrient requirements of rat and mice. *Proceedings of Nutrition Society* 16:90-95.
- Desikachar, H.S.R. 1979. Development of weaning food with high caloric density and low hot paste viscosity using traditional technologies. *Food Nutrition Bulletin* 2: 21 –33.
- Duhan, A., Khetarpaule, N. and Bishnoi, S. 2002. Changes in phytate and hydrochloric acid extractability of calcium, phosphorus and iron of soaked, dehulled, cooked and sprouted pigeon pea cultivar (UPAS – 120). *Plant Foods for Human Nutrition* 57:275 – 284.
- Duncan, D.B. 1955. Multiple range and multiple F-tests. *Biometrics* 11: 1-42.
- Fetuga, B.L., Babatunde, G.M. and Oyenuga, V.A. 1974. Protein quality of some unusual protein footstuffs: Studies on the African locust bean seed (*Parkia filicoidea* Welw.). *British Journal of Nutrition* 32: 27-34.
- Food and Agriculture Organisation 1973. Energy and Protein Requirement. World Health Organisation, Geneva, Switzerland.
- Goldsmith, G.A. 1975. Food and Population. *American Journal of Clinical Nutrition* 78: 934 – 940.
- Gopalan, C., Ramasastri, B.V., Balasubramanian, S.C. 1993. Revised and updated by Rao, B.S.N., Deosthale, Y.G. and Plant, K.C, Nutritive Value of Indian Foods, 2<sup>nd</sup> edn. Hyderabad: National Institute of Nutrition, ICMR, India.
- Ihekoronye, A.I. 1988. Estimation of the biological value of food proteins by a modified equation of the essential amino acid index and the chemical score. *Die Nahrung* 32(8): 783 – 788.
- Jood, S., Kapoor, A.C and Singh, R. 1995. Amino acid composition and chemical evaluation of protein quality of cereals as affected by insect infestation. *Plant Foods for Human Nutrition* 48(2): 159 – 167.
- Ketiku, A. and Smith, A. 1984. Nutritional studies of a Nigerian multimix weaning food – Apapa multimix. *Nigerian Journal of Nutritional Sciences* 5: 39 –44.
- McGuinness, E.E., Morgan, R.J.H and Wormsley, K.G. 1984. Effects of soyabean flour on the pancreas of rats. *Environmental Health Perspective* 56: 205 – 210.
- Miller, D.S. and Bender, A.E. 1955. The determination of the net utilization of proteins by a shortened method. *British Journal of Nutrition* 9: 382 – 388.
- National Academy of Sciences 1979. Tropical Legumes: Resources for the Future, USA p. 331.
- National Research Council 1959. Recommended Dietary Allowances, 10th edn. Washington DC, National Academy Press.
- Narayana – Rao, M. and Swaminathan, M. 1969. Plant proteins in the amelioration of protein deficiency states. *World Review of Nutrition and Dietetics* 11: 106-110.
- Nwinuka, N.M., Abbey, B.W. and Ayalogu, E.O. 1997. Effect of processing on flatus – producing oligosaccharides in cowpea (*Vigna unguiculata*) and the tropical African yam bean (*Sphenostylis stenocarpa*). *Plant Foods for Human Nutrition* 51: 209 – 218.
- Ojofeitimi, E.O., Afolabi, O.A., Fapoluno, O.O., Grisson, F.E and Oke, O.L. 1984. The use of black –eyed cowpea – maize gruel mixture, "Ewa – Ogi" in the treatment and prevention of infantile protein malnutrition. *Nutrition Reports International* 30: 841 – 852.
- Onyeike, E.N., Abbey, B.W. and Anosike, E.O. 1991. Kinetics of heat inactivation of trypsin inhibitors from the African yam bean (*Sphenostylis stenocarpa*). *Food Chemistry* 40: 9 – 23.
- Onyeike, E.N, Ayalogu, E.O. and Abbey, B.W. 1999. Nutritional status of rats fed diets based on different treated African yam bean seed flours. *Global Journal of Pure and Applied Sciences* 5(2): 207-214.
- Onyeike, E.N, Olungwe, T. and Uwakwe, A.A. 1995. Effect of heat treatment and defatting on the proximate composition of some Nigerian local soup thickeners. *Food Chemistry* 53: 173-175.
- Onyeike, E.N. and Morris, P.I. 1996. Evaluation of the nutritional quality of a weaning food from African yam bean – rice – prawn mixture. *Delta Agriculturist* 3: 134 – 147.
- Onyeike, E.N. and Onwuka, O. 1999. Chemical composition of some fermented vegetable seeds used as soup condiments in Nigeria East of the Niger. *Global Journal of Pure and Applied Sciences* 5(3): 337 – 342.
- Onyeike, E.N. Ayalogu, E.O. and Okaraonye, C.C. 2005. Nutritive value of the larvae of raphia palm beetle (*Oryctes rhinoceros*) and weevil (*Rhynchophorus phoenicis*). *Journal of the Science of Food and Agriculture* 85 (11): 1822-1828.
- Ossai, G. E. A and Malomo, O., 1988. Nutritional and sensory evaluation of a new cereal-legume weaning food. *Nigerian Food Journal* 6: 23 – 34.
- Oyenuga, V.A. 1968. Nigeria's Foods and Feeding stuffs, 3<sup>rd</sup> ed. Ibadan, Ibadan University Press pp. 79 – 83.
- Pivie, N.W. and Butler, J.B., 1977. A simple unit leaf. *Proceedings of the Nutrition Society* 36: 136.

Schingoethe, D.J., Tideman, I.J., Uckert, J.R., 1974. Studies in mice on the isolation and characterization of growth inhibitors from soyabeans. *Journal of Nutrition* 104: 1304 - 1309.

Southgate, D.T.A., 1969. Determination of carbohydrates in foods, I: Available carbohydrates. *Journal of the Science of Food and Agriculture* 20:326 -330.

Sri Kantha, S. and Erdman Jr, J.W. 1988. Effects of different heat treatments on winged bean seed flour on the nutritional status of rats. *Nutrition Reports International* 38 (2): 423 - 435.