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Protein and hematological evaluations of infant formulated from cooking banana fruits (Musa spp, ABB genome) and fermented bambara groundnut (*Vigna subterranean L. Verdc*) seeds

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Abstract

Protein-energy malnutrition is regarded as one of the public health problems in developing countries as a result of poor feeding practices due to poverty. This study, therefore, aimed at evaluating nutritional quality of a potential weaning food formulated from locally available food materials. The cooking banana fruit (CB) and bambara groundnut seeds (BG) were purchased from local market in Akure, Ondo State, Nigeria. The CB and BG were processed into flours, mixed in ratios of 90:10, 80:20, 70:30 and 60:40 and subjected into proximate, sensory and biochemical analyses using standard procedures. Nutrend (a commercial formula) and ogi (corn gruel, a traditional weaning food) were used as control. The nutrient composition (g/100 g) of the food samples were ranged as follows: moisture 2.94-6.94, protein 7.02-16.0, ash 1.76-2.99, fat 0.76-8.45, fibre 1.52-3.75, carbohydrate 63.84-88.43 and energy 1569.8-1665.7 kcal. The biological value (BV), net protein retention (NPR), protein efficiency ratio (PER) and feed efficiency ratio (FER) of the experimental food samples were significantly ($p<0.05$) lower than nutrend, but higher than ogi. The haematological variables of rats fed with formulated food samples, commercial formula (nutrend) and traditional weaning food (ogi) were not significantly ($p>0.05$) influenced by the dietary treatment. However, the values obtained for red blood cell (RBC), white blood cell (WBC), pack cell volume (PCV) and erythrocyte sedimentation rate (ESR) were higher in the experimental food samples than the commercial food. The growth rate of animals fed with experimental food samples were lower than those fed with the nutrend, but higher than those fed with ogi. In conclusion, the nutritional quality of CB and fermented BG mix of 60:40 ratio was better than ogi; and comparable to the nutrend. This implies that it can be used to replace low quality traditional weaning food and the expensive commercial weaning formula.

Key Words: Nutritional quality, cooking banana, bambara groundnut, infant diets

Introduction

Protein-energy malnutrition continues to be a major public health problem among children throughout the developing world, Nigeria inclusive (Brabin & Coulter, 2003; FAO, 2004; Schofield & Ashworth, 1996). Poverty and poor feeding practices have been attributed as the major factors responsible for this nutrition problem (Duncan, 2001; Sachs & McArthur, 2005). Evidence has shown that the real income of a household is indeed an important determinant of its access to food which, in turn, is a major determinant of the nutritional status of its members (Khan, 1997; Osmani, 1997).

A number of studies have reported that the nutritional qualities of traditional weaning foods in developing countries, particularly in Nigeria, are low in protein content and also devoid of vital nutrients that required for normal child growth and development (FAO, 2004). For instance, the corn gruel (ogi) is the traditional weaning food of infants in many parts of West Africa countries. Quite a number of studies have shown that corn gruel (ogi) is bulky and devoid of essential nutrients, such as protein and vital micronutrients that is needed for normal child growth and development (Levin *et al.*, 1993; Millward & Jackson, 2004; Pinstrup-Andersen *et al.*, 1993)

In order to alleviate the problem of protein-energy malnutrition (PEM) among the infants, several studies have been carried out on the formulation and processing of weaning foods, such as soy-ogi (maize and soybean), crayfish-ogi (maize and crayfish), maize-cowpea (maize and cowpea), etc., from locally available food materials (Akpapunam & Sefa-Dedeh 1995; Ijarotimi, 2006; Ijarotimi & Aroge 2005; Ijarotimi & Bakare, 2006; Ikujenlola & Fashakin, 2005; Onilude *et al.*, 1999). However, it is evident that most of these formulated complementary foods are still not accessible to many nursing mothers, as a result of the high cost of food materials and production processes. In view of this, this study is aimed at producing weaning foods from cooking banana fruits and bambara groundnut seeds that are locally available.

Cooking banana (*Musa aab* species) plays a dual role as an important commercial crop and a starchy food in the West and

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Central Africa where 50% of the world's plantain crop is produced. Cooking bananas are consumed in a wide variety of manners in Africa; and the fruits are consumed raw as snacks or desserts. Cooking banana is high in carbohydrate and some vital minerals, but low in protein and fat (Swennen, 1990). Bambara groundnut is essentially grown for human consumption. The seed makes a complete food, as it contains 14-24% protein, 60% carbohydrate and 6-12% oil, which is less than half the amount found in peanuts, making them not useful as an oilseed crop (Mbata *et al.*, 2007). The protein is reported to be higher in the essential amino acid methionine than other grain legumes (Oliveira 1976; Oluyemi *et al*. 1976). The seeds are used to prepare various fried or steamed products, such as 'akara', 'moin-moin' and 'milk' in Nigeria (Brough *et al*., 1993; Obizoba, 1983).

Methods and Materials

Materials

The materials, that is, cooking banana (Musa spp, ABB genome), Bambara groundnut (*Vigna subterranean L. Verdc*), nutrend (commercial weaning food) and ogi (a corn gruel, a traditional weaning food), were purchased from a reputable local market and supermarket in Akure town, Ondo State, Nigeria. The nutrend was purposely selected as the control, because it was formulated from maize and soybean a plant based food materials.

Preparation of materials

Cooking banana fruit flour: The cooking bananas were peeled manually and sliced into pieces lengthwise. The sliced chips were oven-dried at 60℃ for 24 hours. The dried product was milled and sieved through 0.4 mm wire mesh screen. The cooking banana flour was stored in sealed cellophane bag pending the time of analysis.

Fermented bambara groundnut flour: The seeds were sorted, cleaned and soaked for 48 hours in hot water. The soaked seeds were drained, hulled manually by hand rubbing, boiled for one hour, cooled and tightly wrapped in a plantain leaf for four days to ferment. The fermented seeds were oven dried, milled, sieved through 0.4 mm wire mesh screen and stored in a cellophane bag for future used.

Food Formulation: The cooking banana fruit flour and fermented Bambara groundnut flour were blended in ratios of 90:10, 80:20, 70:30 and 60:40 respectively.

Chemical analyses

Nutritional composition: The nutritional composition of the food samples was determined using the standard procedures of Association of Official Analytical Chemists (1990). Triplicate samples of each sample were determined for moisture content in a hot-air circulating oven (Galenkamp, size 3, hot box, London, UK). Ash was determined by incineration of known weights of the samples in a muffle furnace (Gallenkamp, size 3, hot box, London, UK). Crude fat was determined by exhaustively extracting a known weight of sample in petroleum ether (boiling point, 40℃ to 60℃) in a soxhlet extractor. The ether was volatilized and the dried residue was quantified gravimetrically and calculated as percentage of fat. Protein ($N \times 6.25$) was determined by the Kjeldahl method. Crude fiber was determined after digesting a known weight of fat-free sample in refluxing 1.25% sulfuric acid and 1.25% sodium hydroxide. The carbohydrate content was determined by subtracting the total crude protein, crude fiber, ash, and fat from the total dry weight (100 g) of the food sample differences. The gross energy was determined using a Gallenkamp, Autobomb automatic adiabatic bomb calorimeter (London, UK).

Experimental design

The experimental layout was of completely randomized design. Twenty-five (15 males and 10 females) weanling albino rats of the Wistar strain at approximately 4 weeks of age of an average weight of 50.5 g were used for the study. The albino rats were divided into five groups of five rats per group. The rats were individually housed in separate cubicles in a metabolic cage with facilities for separate collection of urine and feacal. The animals were subjected to five days acclimatization. The animals were administered with the experimental diets (containing 70:30 and 60:40 of cooking banana and bambara groundnut flour respectively; based on nutritional composition and overall acceptablity, control diets (nutrend and ogi, a commercial and traditional weaning food respectively) and water *ad libitum* for 21 days. The body weights of the animals were measured at two days intervals. The total feaces and urine voided during the last 5 days of experiment were collected, weighed and preserved. The urine collected was preserved by adding a few drops of H_2S0_4 to prevent any loss of ammonia and also to serve as a preservative agent, while the corresponding feed consumed was also recorded for nitrogen determination.

Hematological analysis and organ measurements

At the end of the experiment, all the rats were starved for 3 hours and weighed. Before sacrifice, each rat was anaesthetized with chloroform inside a desiccator. Blood samples from each rat were collected into sample bottles containing a few milligrams of EDTA prior to hematological analysis. The packed cell volume (PCV) was estimated by spinning about 75 μl of each blood sample in heparinised capillary tubes in a haematocrit microcentrifuge for 5 min, and the total red blood cell (RBC) and white blood cell (WBC) counts were determined (Lamb, 1981). The heart, lungs, kidneys, liver, intestine and carcass were separated, blotted free of blood oven dried and weighed. The values were subsequently expressed in g/kg body weight.

Nutritional evaluations

The nutritional values of the diets in rats were evaluated using various parameters. For protein, the following parameters were computed:

$$\text{Protein Efficiency Ratio (PER)} = \frac{\text{Gain in body weight (g)}}{\text{Protein intake (g)}}$$

Net protein Ratio (NPR) =

$$\frac{\text{Weight gain of test protein group} + \text{Weight loss of N-Free N-diet group}}{\text{Protein intake}}$$

$$\text{True Digestibility (TD)} = \frac{Ni-(NF_1-NF_2) \times 100}{Ni}$$

$$\text{Biological value (BV)} = \frac{Ni-(NF_1-NF_2)-(NV_1-NV_2) \times 100}{Ni-(NF_1-NF_2)}$$

Net protein Utilization (NPU) = BV × TD/ 100

Nitrogen Retention (NR) = Ni − (F + NU)

$$\text{Feed Efficiency Ratio} = \frac{\text{Gain in body weight (g)}}{\text{Food intake of each rat (g)}}$$

Where

A = Desired % protein level
Y = Weight of the sample to produced
w = % protein level of the sample
X = Expected weigh to be mixed with band diet
Ni = Nitrogen of animal feed with test diet
NF_1 = Nitrogen executed in faeces of animal fed -test diet
NF_2 = Nitrogen excreted in faeces of animal fed protein free diet
NU_1 Nitrogen excreted in urine of animal fed test diet
NU_2 = Nitrogen excreted in urine of fed-free diet

Sensory evaluation

The food samples obtained from the different fractions of cooking banana and bambara groundnut mixes were made into light gruels, using about 20 g and 60 ml of water. The reconstituted blends were then evaluated along with two references infant food (i.e nutrend, a commercial weaning food and ogi, a traditional weaning food) for their sensory characteristics. Sensory evaluation was conducted on the reconstituted samples which were coded and presented to 10 panelist members familiar with the product. The samples were rated on the following attributes namely: color, aroma, taste, mouth feel and overall acceptability using 9 point hedonic scale ranked between dislike extremely (1) and like extremely (9).

Statistical analysis

The data were analysed using SPSS version 13.0. The mean and standard deviations of the triplicate analyses were calculated. The analysis of variance (ANOVA) was performed to determine significant differences between the means using Duncan.

Results

The nutritional composition of cooking banana fruit and fermented bambara groundnut seed flour (CBG) blend are presented in Table 1. The energy and nutrient values of the formulated and control food samples ranged as follows: energy value 1569.82-1665.68 kcal., moisture content 2.94-6.94 g; protein 7.02-16.0 g, ash content 1.76-2.99 g, fat 0.76-8.45 g, while the remaining nutrient composition, that is, fibre and carbohydrate ranged between 1.52-3.75 g and 63.84 -88.43 g respectively.

The sensory attributes of the formulated weaning foods, ogi (corn gruel, a traditional weaning food) and nutrend (a commercial weaning food) are presented in Table 2. The commercial formula was significantly rated high in terms of color, aroma, taste, mouth feel and overall acceptability compared to ogi and formulated food samples ($p<0.05$). Of all the formulated food samples CBF_3 (i.e., 70% cooking banana and 30% bambara groundnut flour) was rated highest compared to others food samples.

The nutritional quality of the food samples are presented in

Table 1. Proximate composition of formulated weaning foods, ogi (a traditional weaning food) and nutrend (a commercial weaning food) (g/100 g)

Samples	Moisture	Ash	Protein	Fat	Fibre	Carbohydrate	Energy
CBF_1	6.94^a	2.99^{abc}	7.02^d	6.38^c	3.75^b	73.16	1599.12
CBF_2	6.44^{ab}	2.61^{cd}	9.69^c	6.64^b	3.51^{cd}	70.67	1569.81
CBF_3	6.88^a	2.84^c	12.06^b	6.83^b	2.57^d	68.82	1610.67
CBF_4	6.17^{ab}	2.91^{bc}	15.60^a	6.93^b	1.52^e	67.87	1658.40
OGI	2.94^c	1.76^e	5.62^e	0.76^d	0.49^f	88.43	1626.97
NUTREND	4.83^b	2.65^{cd}	16.00^a	8.45^a	4.48^a	63.84	1665.68

Mean values followed by different superscript within column are significantly different at $p<0.05$.
CBF_1-Cooking Banana + Fermentation B.G (70:30)
CBF_2-Cooking Banana + Fermentation B.G (60:40)
Nutrend-Commercial Diet (Nutrend)
BD-Basal Diet
Casein-Casein

Table 2. Sensory attributes of reconstituted formulated weaning foods (cooking banana and bambara groundnut flour mixed), ogi (traditional weaning food) and nutrend (commercial weaning food)

Samples	Colour	Aroma	Taste	Mouth Feel	Overall Acceptability
CBF_1	6.33 ± 0.56^{bc}	6.17 ± 0.29^b	6.33 ± 0.56^{bc}	6.50 ± 0.31^b	6.17 ± 0.70^b
CBF_2	5.83 ± 0.65^c	5.83 ± 0.42^b	5.83 ± 0.65^b	6.17 ± 0.31^b	6.17 ± 0.60^b
CBF_3	6.83 ± 0.48^{bc}	6.33 ± 0.78^b	6.83 ± 0.48^c	5.83 ± 0.75^b	6.50 ± 0.43^b
CBF_4	5.83 ± 0.84^c	6.00 ± 0.34^b	5.33 ± 0.84^b	5.50 ± 0.67^b	6.17 ± 0.83^b
OGI	7.67 ± 0.21^{ab}	6.83 ± 0.31^b	7.67 ± 0.21^{ab}	7.00 ± 0.37^b	7.33 ± 0.33^{ab}
NUTREND	9.00 ± 0.00^a	8.83 ± 0.17^a	9.00 ± 0.00^a	9.00 ± 0.00^a	8.83 ± 0.17^a

Means with similar alphabets belong to the same homogenous subset are not significantly different from each other at the 5% statistical level.

Table 3. Nutritional Quality of Cooking Banana and fermented Bambara groundnut blend

Parameters	CBF_3	CBF_4	Ogi	Nutrend	Casein
BV%	72.3^{b}	74.3^{b}	52.1^{c}	93.8^{a}	97.4^{a}
TD %	59.3^{a}	62.0^{a}	41.7^{c}	41.9^{c}	54.5^{b}
NPU%	55.4^{a}	45.0^{b}	31.3^{c}	42.9^{b}	53.5^{a}
NPR	-0.83^{b}	-0.70^{b}	-0.68^{b}	1.29^{a}	1.94^{a}
PER	0.18^{c}	0.15^{c}	0.52^{b}	2.09^{a}	2.58^{a}
FER	0.02^{bc}	0.02^{bc}	0.08^{b}	0.21^{a}	0.26^{a}
NR	0.98^{ab}	1.03^{ab}	0.09^{c}	1.03^{ab}	1.56^{a}

Mean values followed by different superscript within column are significantly different at $p<0.05$.

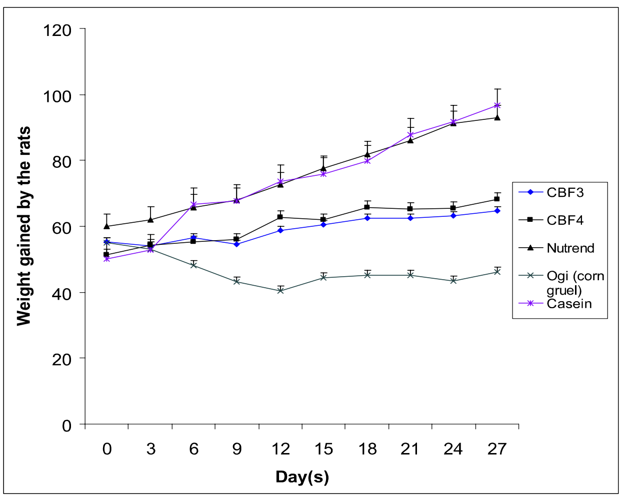

Fig. 1. Growth rate of albino rats fed with cooking banana-bambara groundnut mixed compared to nutrend, ogi and casein diets

Table 3. The biological value of the experimental food samples, that is, CBF_3, (72.3%) and CBF_4, (74.3%), were significantly higher than the ogi (52.1%), but lower than that of the nutrend (93.8%) ($p<0.05$). For the true digestibility (CBF_3, 59.3%; CBF_4, 62.0%), net protein utilization (CBF_3, 55.4%; CBF_4, 45.0%) and nitrogen retention (CBF_3, 098; CBF_4, 1.03) of the experimental food samples were also significantly higher compared with ogi (that is, BV 52.1%, TD 41.7%, NPU 31.3% and NR 0.09) and also that of the nutrend (that is, TD 41.9%, NPU 42.9% and NR 1.03) respectively ($p<0.05$). For the net protein ratio (NPR), protein efficiency ratio (PER) and feed efficiency ratio (FER) the values for the experimental food samples were lower compared with the ogi and nutrend. The growth patterns of animals fed with the formulated food samples and control food samples (nutrend and ogi) are shown in Fig. 1. The result showed that the growth rate of animals fed with commercial weaning formula (nutrend) was significantly higher compared to the formulated food samples. But the growth rates of formulated food samples were higher than those of animals fed with ogi.

Table 4 shows various organ weights of rats fed with formulated food, nutrend and ogi food samples. The weight range of the animal organs were as follows: heart was between 0.95 g of animals fed with CBF_1 and 1.35 g of ogi; liver, 2.75 g of CBF_1 and 5.23 g of commercial formula; kidney, 1.53 g of CBF_1 and 2.1 g of ogi; intestine, 8.13 g of ogi and 13.93 g of CBF_1 and carcass, 48.4 g of ogi and 60.17 g of commercial formula.

Table 4. Relative Organ Measurement of albino rats fed with experimental and control diets

Parameters	CBF_3	CBF_4	Nutrend	Ogi	Casein
Heart	0.95^{b}	1.00^{b}	1.08^{ab}	1.35^{a}	1.03^{b}
Liver	2.75^{c}	3.35^{bc}	5.23^{a}	4.60^{ab}	4.850^{a}
Kidney	1.53^{c}	1.45^{c}	1.78^{bc}	2.10^{b}	2.52^{a}
Intestine	13.93^{b}	12.62^{b}	13.42^{b}	8.13^{b}	10.95^{b}
Carcass	51.90^{b}	54.12^{b}	60.17^{a}	48.40^{c}	57.80^{ab}

Mean values followed by different superscript within column are significantly different at $p<0.05$.

Table 5. Hematological variables of rats fed with cooking banana-bambara groundnuts mixed, nutrend, casein and basal diet

Parameters	CBF_3	CBF_4	Nutrend	Ogi	Casein
RBC (10^6)	2.75^{a}	2.20^{a}	3.033^{a}	2.65^{a}	1.77^{a}
WBC (mm^3)	3560.00^{a}	2950.00^{a}	3633.33^{a}	2650.00^{a}	2466.67^{a}
PCV %	31.00^{a}	24.50^{a}	33.67^{a}	30.50^{a}	31.33^{a}
ESR (mm/hr)	0.500^{c}	2.50^{a}	0.001^{d}	2.00^{b}	0.001^{d}

Mean values followed by different superscript within column are significantly different at $p<0.05$.

The hematological variables of rats fed with formulated food samples, commercial formula and traditional weaning food sample are presented in Table 5. The values of red blood cell (RBC) (3.033), white blood cell (WBC) (3633.33) and pack cell volume (PCV) (33.67) of commercial formula were not significantly high compared to 2.20, 2950.0 and 33.67 of red blood cells, white blood cells and pack cell volumes for CBF_2 and 2.75, 3560.0 and 31.0 were for RBC, WBC and PCV for CBF_1 respectively.

Discussion

Infants after 6 months of life need to consume adequate energy and nutrient densities weaning foods to supplement breast milk (Dewey & Brown, 2003; WHO/UNICEF, 1998). However, in many parts of developing countries scientific studies have reported that most of the traditional weaning foods were formulated from cereals and tubers that are low in protein and other essential micronutrients which are important for the normal physical growth and cognitive development of a child (Dewey & Brown, 2003). In this present study, the protein content of the formulated food samples, particularly CBF_3 and CBF_4, were two- to three-fold higher than that of the traditional weaning foods (ogi); but there were no significant differences between the protein contents of the experimental food samples compared with that of commercial weaning formula (nutrend) ($p<0.05$). The energy values of the formulated food samples (CBF_4) were almost the same with that of the ogi and commercial weaning formula.

It is evident that the nutritional qualities of most plant based food materials improved particularly when combined (Ijarotimi & Aroge, 2005; Ijarotimi & Ashipa, 2006; Ijarotimi & Bakare, 2006; Mensa-Wilmot & Sefa-Dedeh, 2001; Uwaegbute & Nnanyelugo, 1987). The significant difference between the nutritive values of the formulated food samples in this present study and that of commercial formula and the traditional weaning food (ogi) had been early on reported by several researchers (Guiro *et al.*, 1987; Ikujenlola & Fashakin, 2005). For instance, studies have shown that the ogi is bulky and devoid of many vital nutrients; hence, it has been implicated in the eatiology of protein-energy malnutrition in children, particularly in the areas where it is being used as sole infant diets (Guiro *et al.*, 1987; Ikujenlola & Fashakin, 2005).

The overall acceptability of the formulated food samples was significantly rated low compared with commercial formula ($p<0.05$); however, there was no significant difference between the overall acceptability of the formula and that of traditional weaning food (ogi). The rating of commercial formula (nutrend) above the formulated food samples in term of color, aroma, taste, mouth feel and overall acceptability could be attributed to the incorporation of flavoring, sweetening and other sensory enhancing agents to the formula during its formulation.

The biological value, net protein retention, protein efficiency ratio and feed efficiency ratio of the formulated food samples were significantly higher than the traditional weaning food (ogi); but lower compared to the commercial weaning formula. This observation may be as a result of the fortification of the commercial formula with quality protein like casein or other growth promoting nutrients. The growth pattern of animals fed with experimental food samples was higher compared to those fed with ogi, but lower when compared with the animals fed with the nutrend. Several findings have established that traditional infant foods made of cereals or tubers were low in several nutrients including protein, vitamin A, zinc and iron; these nutrients are of special importance due to their impact on physical and cognitive development (Krebs & Westcott, 2002; Levin *et al.*, 1993; Michaelsen & Friis, 1998; Millward & Jackson, 2004; Neumann *et al.*, 2002; Pinstrup-Andersen *et al.*, 1993). The poor nutritive value of traditional weaning foods and improper feeding practices have implicated as the main factors that are responsible for the growth faltering in children belonging to low-income families (WHO, 2001).

It was observed in this study that there were no significant differences ($p>0.05$) between the weight of heart, kidney and intestine of the rats fed with formulated diets compared to those fed with nutrend and ogi; however, the weight of the liver of animals fed with formulated diets were significantly ($p<0.05$) lower than those fed with the nutrend and ogi. This showed that the formulated food samples did not influence the weight of these organs. Also, the haematological variables of rats fed with formulated food samples, commercial formula and the traditional weaning food sample were not significantly ($p>0.05$) influenced by the dietary treatment. However, the values obtained for red blood cell (RBC), white blood cell (WBC), pack cell volume (PCV) and erythrocyte sedimentation rate (ESR) were generally high and comparable to the commercial food, thus indicating the adequacy of the diets in blood formation.

In conclussion, the results of this study established that the food sample containing 60% cooking banana and 40% bambara groundnut, contains an appreciable amount of proteins of comparable biological value with the commercial weaning formula; therefore, it may be used as a substitute for traditional weaning food (ogi) and commercial weaning fromula, particularly for the children belonging to low-income families. However, further study is needed in the area of improving the sensory attribute of the diet and also biological evaluation using human subjects.

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