

Formulation and Nutritional Evaluation of Maize, Bambara Groundnut and Cowpea Seeds Blends Complementary Food

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Abstract Complementary diets were prepared from flour blends of maize, bambara groundnut, and cowpea seed and compared with Nutrend, a vegetable based protein commercial food manufactured by Nestle plc. The formulated diets are corn flour which was mixed with flours from bambara groundnut and cowpea at a ratio maize-bambara (80:20), maize-cowpea (80:20) and maize-bambara-cowpea (60:20:20). Thirty (30) weaning rats were grouped into six groups of five rats each and fed with five dietary samples for 28days. The suggested diets especially those of maize-bambara-cowpea supplied adequate amounts of most minerals. Physical and biochemical parameters established no significant difference in the analytical data for formulated and control diets. The assessment results showed that the formulated diets were comparable nutritionally to control diet in supporting animal growth without any significant organ impairment as indicated in the liver and kidney function tests. The diets formulated from maize, bambara groundnut and cowpea seeds blends complementary food were well accepted as shown by the amounts consumed by the rats hence were acceptable, readily available, affordable and nutritionally adequate, animal growth.

Keywords: *vegetable based protein, malnutrition*

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1. Introduction

The United Nations Standing Committee on Nutrition [UNSCN] pointed out that malnutrition is directly and indirectly associated with more than 50% of all children mortality, and is the contributor to disease in developing world [1]. Malnutrition is thus associated with more than half of all deaths of children worldwide. In Nigeria, and indeed most developing countries, the underlying problems have been identified to include poverty, high cost of first class protein, inadequate nutrient intake particularly during pregnancy, period of rapid growth and complementary feeding in infants, ignorance about nutrient values of foodstuff and parasitic infections [1,2]. This problem is exacerbated by the faltering economy, depreciating buying power of the Naira and the declining import of costly protein-rich foods. The use of cereal-legume based food is therefore advocated as alternative protein and energy source for infant and adult food products. In order to help alleviate the ever-increasing problems of malnutrition in developing countries, the need for fortification of popularly consumed low protein staple foods with inexpensive sources of plant proteins that would give weaning foods that are low in viscosity, high in caloric density and with adequate necessary nutrients cannot be overemphasized. Several other locally available

species like the bambara groundnut, kidney beans, lima beans and jack beans which show remarkable adaptation to tropical conditions are less commonly used by the people [3,4]. Bambara groundnut (*Vigna subterranea*) is among the legumes that are widely cultivated in Nigeria and it is underutilized. It has not been adequately exploited, as human food because of constraints like hard to cook phenomenon, strong beany flavour, presence of anti-nutrients and poor dehulling and milling characteristics. Bambara groundnut is essentially grown for human consumption. The seed makes a complete food, as it contains sufficient quantities of protein, carbohydrate and fat [3,4]. The seed is regarded as a balanced food because when compared to most food legumes, it is rich in iron and the protein contains high lysine and methionine amino acids. In addition, bambara groundnut is known to contain 63% carbohydrates, 18% oil and the fatty acid content is predominantly linoleic, palmitic and linolenic acids [3,4]. The seed grain has a good balance of essential amino acids with a relatively high proportion of lysine (6.6%) . Many studies have reported earlier that Bambara groundnut protein contain higher essential amino acid, methionine than other grain legumes, while the oil content is less than half of the amount found in legumes like peanuts. Since their lysine content is very high, they allow to efficiently complementing cereals where lysine is the limiting amino acid [4,5]. Research has also shown that no single plant protein source on its own can supply all the essential

amino acid needed for growth compared to animal protein sources such as milk-based diets. To this end, this study will be evaluating mixture of maize, cowpea and bambara groundnut. It is in this view that we have decided to look into the nutrient composition of some low cost locally formulated weaning diets in a bid to use them for the alleviation of malnutrition [5,6].

2. Materials and Methods

2.1. The Materials for the Formulations

Yellow maize (*Zea mays* L), cream coat bambara groundnut (*Vigna subterranean* L) and Cowpea (*Vigna unguiculata*) of the black eye specie seeds were bought from a Oja Oba, Akure, Ondo state, Nigeria. Maize, bambara groundnut and cowpea grains were sorted and cleaned. Maize was steeped in water for 72 hours at room temperature ($28 \pm 2^{\circ}\text{C}$) and oven dried at 60°C for 24 hours, bambara groundnut seeds were soaked in water for 1 hour and boiled at a temperature of 100°C for 45 minutes. The seeds were then dehulled manually and oven dried at 60°C for 24 hours. The dried maize and bambara groundnut seeds were cleaned by winnowing and milled using attrition mill (Hunt No 2 premier mill, Hunt and Co UK) to average particle size of less than 0.3mm. The milled grains were sieved through a fine mesh (0.5mm) to obtain maize and bambara groundnut flour. While cowpea seeds were soaked in water for 30 minutes and manually dehulled after which the seeds were boiled at 100°C for 30 minutes, the boiled seeds were oven dried at 60°C for 24 hours. The dried cowpea seeds were grinded into flour using attrition mill (Hunt No 2 premier mill, Hunt and Co UK) to obtain cowpea flour. The flours were packed in air tight containers and kept in the refrigerator until needed. The flours were used as supplements either sole source or mixed source of protein. Mixing of the flour with the vitamin mix containing multivitamin. Multivitamin consists of vitamin A 2250IU, vitamin B₁ 1.0mg, vitamin B₂ 1.0mg, vitamin B₆ 0.5mg, vitamin B₁₂ 0.75mcg, vitamin E 5IU, vitamin C 25.0mg, nicotinamide 15mg, vitamin D₃ 150IU, calcium 75mg, folic acid 125mcg, phosphorus 58mg, iodine 0.075mg, potassium 2.0mg, iron 14mg, copper 1mg, Magnesium 3.0mg, manganese 1.0mg, molybdenum 0.1mg, zinc 15.0mg. The basal diet is 100 per cent carbohydrate, having no protein [7,8].

2.2. Chemical Analysis

Chemical analysis included protein (nitrogen $\times 6.25$), moisture, fat, crude fibre carbohydrate, and vitamins of the ingredients and formulated diets were determined according to AOAC, carbohydrate was determined by difference, energy was determined using combustion calorimeter, model e2K [7]. Biological assay such as biological value, (BV)% true digestibility (TD%) protein efficiency ratio (PER), feed efficiency ratio (FER) and net protein ratio (NPR) net protein utilization (NPU%) of the experimental animals were determined by the method of Ibrionke, *et al* 2012 [8].

2.3. The Mixture

Corn flour was mixed with flours from bambara groundnut and cowpea at a ratio maize bambara groundnut

and cowpea at a ratio maize bambara (80:20), maize cowpea (80:20) and maize-bambara-cowpea (60:20:20) and labeled MAB, MAC and MABC respectively to meet the targeted protein of a minimum of 15% and 19% for the combination of two and three samples respectively [9].

2.4. Experimental Animal Procedure

This study was approved by the ethical review committee of the Obafemi Awolowo University, Osun state, Ile-Ife, Nigeria. Thirty male albino weanling rats of the Wistar strain at approximately 4 weeks of age with an average weight of 76.30g at the beginning of the experiment were obtained from the Faculty of Health Sciences, Obafemi Awolowo University, Ile-Ife, Nigeria for the study. They were randomly distributed in metabolic cages and fed on normal (pellet) diets for a period of 7 days for acclimatization to the environment before the commencement of the experiments. After this period the animals were re-weighed and re-grouped so that the average weight per group was approximately the same and divided into five groups. 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 fecal matter. One group of five animals served as base line control for the experimental groups, was sacrificed and tissues from liver, kidney and plantaris muscle of the hind leg were removed, weighed and frozen until nitrogen was determined. The remaining grouped animals were placed on experimental diets (Diets MAB, MAC, and MABC). The remaining two groups of animals were administered with Nutrend (a commercial weaning food), Ogi (corn gruel), which is a traditional weaning food. The groups of animals were fed with the food samples and water *ad libitum* for 28 days. During this period dietary intake was weighed at three-day intervals and growth of the animals was recorded. The total feces and urine voided during the last 7 days of the experiment were collected, weighed and preserved. The urine collected was preserved by adding a few drops of dilute sulphuric acid to prevent any loss of ammonia and was kept in a frozen condition while the corresponding feed consumed was also recorded for nitrogen determination. Pooled samples of feces were dried in an oven at 80°C for 12h, cooled and weighed. Nitrogen in the urine and feces were determined by micro-kjeldahl method. At the end of the experiment, the animals were anaesthetized and sacrificed. Tissue samples from liver, kidney and plantaris muscle were obtained, weighed and frozen until nitrogen was determined [10].

3. Results and Discussion

Figure 1 shows the rate and pattern of growth as well as absence of illness and mortality could also be used to assess the adequacy of a diet. At the end of the feeding period, the formulated diets were found to compare well with the proprietary diets in terms of food intake, weight gain, growth rate and PER, and no mortality was recorded among the animals fed on the formulated and the proprietary diets. Average weight changes in body weight during the experimental period may be influenced by the source of nitrogen which agrees with the findings that food intake and changes in body weights were influenced

by the difference in protein/ nitrogen source. Rats fed on the basal diet lost weight and their body weights were lower than rats fed on the formulated diets. reported similar findings. There was a progressive weight increase

from MAB fed rats to MABC fed rats, which corresponded to increase in protein ingested from MABC. This implies that MABC diet promoted growth better than other MAB and MAC [11].

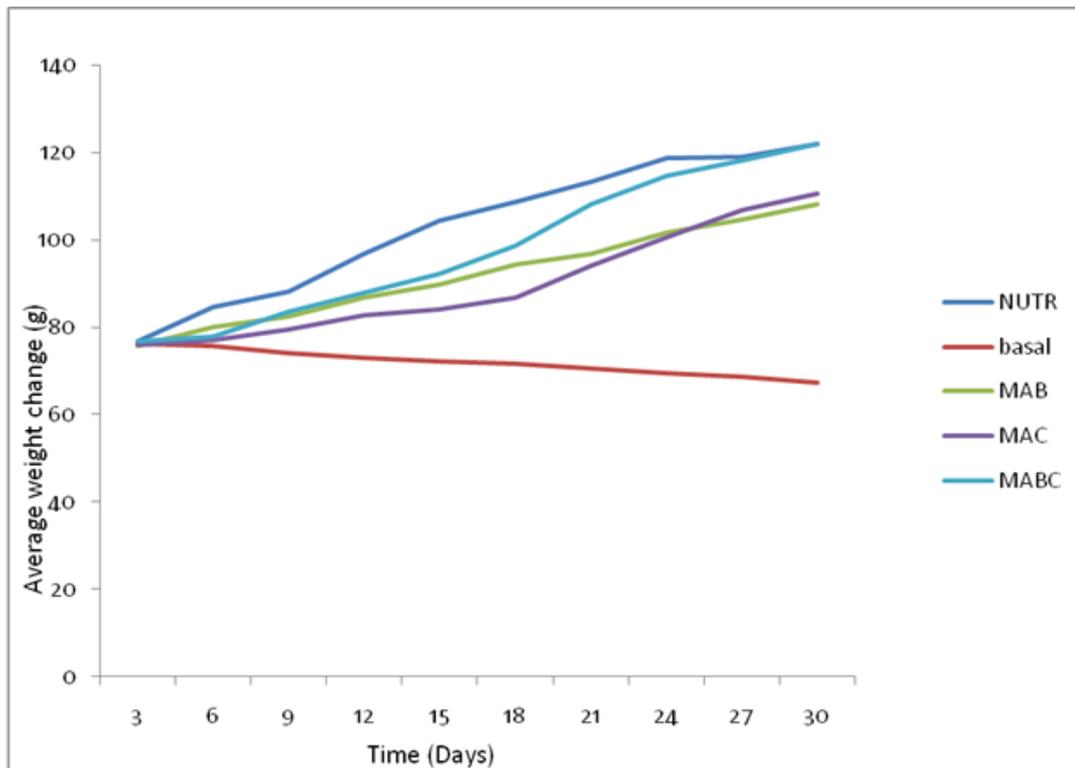


Figure 1. Average Changes in Weight of the Animals During the Experimental Period

MAB = maize–bambara (80:20), MAC = maize–cowpea (80:20)MABC = maize–bambara–cowpea (60:20:20), NUTR = nutrend.

Table 1. Proximate Composition of the Formulated Diets on Dry Weight Basis.

Sample	Crude protein (%)	Lipid (%)	Total ash (%)	Crude fibre (%)	Carbohydrate (%)	Energy (Kcal)
MAB	15.46 ^b ±2.97	7.58 ^a ±0.29	1.36 ^b ±0.28	0.74 ^{ab} ±0.31	74.93 ^a ±2.87	429.82 ^{ac} ±3.07
MAC	17.04 ^{ab} ±1.55	6.54 ^b ±0.18	1.61 ^b ±0.37	0.57 ^{ab} ±0.38	71.85 ^{ab} ±1.92	422.55 ^b ±1.56
MABC	19.90 ^a ±0.93	6.88 ^{ab} ±0.70	1.74 ^a ±0.33	0.81 ^a ±0.24	70.18 ^b ±2.08	422.28 ^b ±1.90
NUTR	19.11 ^a ±2.08	6.47 ^b ±0.21	2.47 ^a ±0.20	0.26 ^a ±0.06	71.29 ^{ab} ±0.54	419.84 ^{ab} ±0.10

Each value represents the mean value of three replicates ±SD. Values with different superscripts in the same column are significantly different at p<0.05. MAB = maize–bambara (80:20), MAC = maize–cowpea (80:20)MABC = maize–bambara–cowpea (60:20:20), NUTR = nutrend.

Table 1 shows the proximate composition of the formulated diets. All the diets (MAB, MAC and MABC) had protein contents which met the standard given by that protein should not be less than 15%. Apart from diet MAB that has a protein level of 15.55%, protein and energy contents of all the legume-fortified weaning foods developed were above the recommended levels for weaning foods[15g/100g]. However, the values of the fat contents did not meet up with recommended 10% in weaning diet formulation [12].

The tissues from liver, kidney and plantaris muscle of the animals fed on MABC were bigger than the ones from other formulated diets (MAB, MAC) even the proprietary diet (Nutrend). There was increase in tissue nitrogen of the animals that ate the formulated and the proprietary diets and this was in agreement with the findings that the increase in tissue nitrogen contents of the group fed on the formulated and proprietary diet are similar. In this study organ impairment was not noticed during physical examination of the organs. The liver is one of the major important organs of the body that has enzyme to

metabolize amino acids, and it acts as the body's chemical factory. It regulates the levels of most of the main blood chemicals and acts with the kidney to clear the blood of drugs and toxic substances. Therefore, it can be said that the weight and general appearance of the liver and other body organs could tell a lot on the health status of the experimental animals fed on the formulated diets. The results obtained in this study agree with the findings [12].

Table 2. Weight of Selected Tissues of Experimental Animals [g]

Samples	Liver	Kidney	Muscle
NUTR	4.83 ^a ±0.35	0.70 ^b ±0.10	0.40 ^b ±0.10
BASAL	2.27 ^d ±0.32	0.60 ^b ±0.17	0.40 ^b ±0.17
MAB	2.67 ^{cd} ±0.41	0.60 ^b ±0.10	0.33 ^b ±0.10
MAC	3.37 ^{bc} ±0.55	0.70 ^b ±0.10	0.50 ^b ±0.07
MABC	5.10 ^a ±0.36	1.07 ^a ±0.23	0.83 ^a ±0.15

Each value represents the mean value of three replicates ±SD. Values with different superscripts in the same column are significantly different at p<0.05. MAB = maize–bambara (80:20), MAC = maize–cowpea (80:20) MABC = maize–bambara–cowpea (60:20:20), NUTR = nutrend.

Table 3. Total nitrogen in the selected tissues of the experimental animals (mg)

Diets	Liver	Kidney	Plantaris Tissue
Basal	27.22 ^c ±1.07	14.89 ^f ±1.64	33.29 ^f ±0.60
MAB	105.24 ^a ±1.77	48.84 ^c ±0.81	46.37 ^b ±0.52
MAC	109.024 ^c ±1.45	37.73 ^d ±1.02	37.01 ^c ±0.58
MABC	123.99 ^a ±2.65	58.09 ^a ±1.22	53.09 ^a ±1.57
NUTR	112.47 ^b ±2.76	51.44 ^b ±1.25	47.44 ^b ±1.91

Each value represents the mean value of three replicates ±SD. Values with different superscripts in the same column are significantly different at $p < 0.05$. MAB = maize–bambara (80:20), MAC = maize–cowpea (80:20) MABC = maize–bambara–cowpea (60:20:20), NUTR = nutrend.

The highest nitrogen content was observed for the rats fed with MABC followed by nutrend. This might be due to the relative proportion of protein in these diets. This is similar to the result reported where the rats fed with high concentration of protein had the highest nitrogen concentration [12].

Table 4. The Protein Efficiency Ratio (PER), Feed Efficiency Ratio (FER) and Net Protein Ratio (NPR) of the Experimental Animal

Sample	Protein Efficiency Ratio [PER]	Feed Efficiency Ratio [FER]	Net Protein Ratio [NPR]
MAB	2.15 ^a ±0.21	0.29 ^a ±0.06	1.60 ^a ±0.15
MAC	2.30 ^a ±0.50	0.13 ^b ±0.15	1.68 ^a ±0.38
MABC	2.42 ^a ±0.11	0.14 ^a ±0.01	1.94 ^a ±0.09
NUTR	2.57 ^b ±0.35	0.15 ^b ±0.01	2.01 ^b ±0.30

Each value represents the mean value of three replicates ±SD. Values with different superscripts in the same column are significantly different at $p < 0.05$. MAB = maize–bambara (80:20), MAC = maize–cowpea (80:20) MABC = maize–bambara–cowpea (60:20:20), NUTR = nutrend.

Protein efficiency ratio (PER) is the weight gained per gram protein consumed. The PER of NUTR the commercial weaning food was higher ($P < 0.05$) compared to the formulated diets although there was no significant difference. The PER values obtained in this study were all above the 2.1 minimum PER value recommended for such flours by Protein Advisory Group. PER value below 1.5 describes a protein of poor quality; between 1.5 and 2.0 an intermediate quality and above 2.0, good quality. Therefore, NUTR, MABC, MAC and MAB respectively possessed good protein quality. Other researchers have also suggested the inclusion of milk and egg in infant diets to improve the protein quality. This is because proteins of plant origin have low biological value. The comparable food intake, weight gain and growth rate among the animals fed with the formulated and proprietary diets could also be partly responsible for the higher PER values observed for these diets. The PER of the formulated diets compared well with the values earlier reported for casein (2.5), whole egg (3.8) and cow's milk (2.0). NPR (Net Protein Ratio) provides information on the ability of proteins to support both maintenance and growth. It is an improvement of PER. The result for the NPR was in accordance that the highest value was apparent in the proprietary diet. The higher value NPR for proprietary diets could be due to its quality of protein not its amount [12,13].

Biological value [BV] gives information on how much of the absorbed nitrogen is actually retained or utilized by the body. The BV of rats fed on MABC was significantly higher [$P < 0.05$] than for rats fed on other diets. This indicates that rats fed on MABC had higher nitrogen retention than those on other diets (as was also reflected

by high NPR value]. This also suggested that the essential amino acids in the product were present in sufficient quantity to meet the needs for growth; the nutritional composition of the foods indicates their suitability for young children. The result obtained in this study is in agreements with reports of earlier workers [12,13]. The consumption of processed legume seed proteins was reported to decrease the endogenous nitrogen loss through the shedding of intestinal mucosa, an effect that increase the biological value of raw legume seed proteins [14]. The presence of various anti-nutritional substances in the raw materials, including trypsin inhibitors, which inhibits the complete digestion of proteins and increases the excretion of endogenous fecal nitrogen which had been removed in the process of soaking, cooking, and dehulling of the legume seeds. This observation is similar to other studies that reported on the nutritional qualities of food products produced from a cereal and legume combination [14]. The true digestibility [TD] values gives information on the percentage of nitrogen intake absorbed by the body. The TD values of the diet MABC was higher than that of the commercial weaning food manufactured by Nestle plc, although there was no significant difference [$P < 0.05$]. However, TD values of all the formulated diets were higher than the [85%] values recommended for children. NPU is the percentage of nitrogen intake retained as body nitrogen. Net protein utilization is similar to the biological value except that it involves a direct measure of retention of absorbed nitrogen. Net protein utilization and biological value both measure the same parameter of nitrogen retention; however, the difference lies in that the biological value is calculated from nitrogen absorbed whereas net protein utilization is from nitrogen ingested [15]. The group of rats fed on diet MABC blend had the highest NPU value [90.48]. The higher NPU value for the group fed the Diet MABC could be due to lower fecal and urinary Nitrogen excretion and higher Nitrogen retention, which implies that it has better quality protein source compared to other formulated diets [MAB and MAC]. Values obtained in this study compares well with values reported [15].

Table 5. Protein Quality of the Formulated Diets.

Sample	Biological value (BV%)	True digestibility (TD%)	Net protein utilization (NPU%)
MAB	96.22 ^c ±0.72	87.83 ^c ±0.34	84.80 ^d ±0.74
MAC	97.18 ^{bc} ±0.79	89.59 ^b ±0.31	86.44 ^c ±0.43
MABC	98.55 ^a ±0.32	92.15 ^a ±0.40	90.48 ^a ±0.50
NUTR	97.49 ^{ab} ±0.44	91.52 ^a ±0.45	89.51 ^b ±0.50

Each value represents the mean value of three replicates ±SD. Values with different superscripts in the same column are significantly different at $p < 0.05$. MAB = maize–bambara [80:20], MAC = maize–cowpea (80:20) MABC = maize–bambara–cowpea (60:20:20), NUTR = Nutriend.

4. Conclusion

In conclusion, fortified weaning foods were prepared from bambara-nut, cowpea and maize seeds. It was highly digestible, has better nitrogen retention with excellent biological value comparable with control samples. Bambara-nut based weaning foods contain adequate nutrient, quality and quantity protein, amino acid that meet recommended daily requirement for infants. Hence could

be applied to combat protein energy malnutrition in developing countries.

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