Nutritional Evaluation of Baobab Seed Protein Extract and its Potential as a Component of Weaning Food

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Abstract

Cereals form the primary foundation for most of the local weaning foods in Nigeria and they are inadequate in some essential amino acids needed for weaning infants. Baobab seed, an under-utilized crop is rich in protein but the knowledge of the utilization potential of the protein extract is yet to be readily available. Baobab seeds were sourced from Ahmadu Bello University Teaching and Research Farm, Zaria and its protein was extracted by defatting the baobab seed flour and precipitating the protein using a centrifuge. The maize meal was blended with extracted protein at 90:10, 80:20, 70:30 and 100:0 w/w, respectively and fed to 20 male albino rats for 28 days. The baobab seed flour was subjected to proximate analysis and the blood of the rats fed with the crude protein extract was evaluated for some hematological parameters [Serum Glutamate Oxaloacetate Transaminase (SGOT), Alkaline Phosphatase (ALP) and Total Protein (TP)]. The histopathology of the liver and kidney was carried out to determine the effect of the residual concentration of anti-nutrients in the protein extract and the Protein Efficiency Ratio (PER) was calculated. Data obtained were subjected to Analysis of Variance and the means were separated using Duncan multiple range test at 95% probability level.

The proximate values for the baobab seed flour were 8.42, 27.58, 11.87, 16.88, 9.53 and 25.72% for moisture, protein, ash, fat, crude fibre and carbohydrate contents, respectively. The range of values obtained for the SGOT, ALP and TP for test groups of 90:10, 80:20, 70:30 and 100:0 were 338 - 467.2, 130.8 - 146.6 U/L and 20 - 49 g/L, respectively. Mild inflammatory cell infiltrations were recorded in the histopathology of the kidney and liver of the experimental animals. The average PER of the test groups were 1.13, 1.60 and 1.84, compared with a value of 2.2 which is the standard value of casein protein for 90:10, 80:20 and 70:30, respectively. Baobab seed protein extract was well tolerated by the experimental rats. It was concluded that baobab seed protein extract could be a potential supplement for weaning foods.

Keywords

Baobab, Protein extract, Histopathology, Hematological parameters

Introduction

Several research works have reported that most of the weaning foods eaten by the children in many parts of developing nations are inadequate in essential macronutrients and micronutrients [1, 2]. In the light of this nutritional problem, various techniques have been used to produce weaning food, by merging locally obtainable foods that complement each other in such a way as to create a new pattern of amino acids that provide the recommended daily allowance for children, [3-5]. The common first weaning food in Nigeria is called ‘pap’, ‘akamu’,
‘ogi’, or ‘koko’ made from maize, millet, or guinea corn [6]. After pap has been successfully introduced, other basic foods eaten in a household are given to the child. Maize products are poor nutritionally like other cereal products and this may be due to their deficiency in tryptophan, lysine, threonine, and the presence of anti-nutritional factors [7] such as tannins and phytates that binds with proteins, vitamins and minerals, making their nutrients unavailable fully for the body [8, 9]. Baobab tree is one of the common trees growing in African continent. The fruit of baobab tree consists of large seeds surrounded with a sour acidic pulp and shell, and is commonly found in the Northern states of Nigeria. Igboeli [10] and Gebauer et al. [11] reported that each part of baobab tree is useful. The seeds, leaves, and the fruit pulp of baobab seed contain protein [12-14]. Assogbadjo et al. stated that baobab seeds contain higher protein and crude lipid than all the remaining parts of the tree [15]. High protein content was reported in baobab seeds; 28.7% - 36.3% [16-18], while lower values of protein 15.12% - 18.4% were reported by Lockett et al. and Osman respectively [19, 20]. Generally, weaning foods eaten in developing nations contain high levels of carbohydrate with very low proteins due to expensive cost of animal protein foods. Baobab seed, an under-utilized crop is rich in protein but the knowledge of the utilization potential of the protein extract is yet to be readily available. The objective of this study is to investigate the nutritional quality of the protein extract obtained from Baobab seeds and its potential as a component of weaning food.

Materials and Methods

Source of test ingredient

Baobab seed (*Adansonia digitata*) was sourced from Ahmadu Bello University Teaching and Research Farm Zaria while maize (*Zea mays*) were obtained from Ladoke Akintola University of Technology Teaching and Research Farm. Other equipment including Centrifuge and Rotatory Shaker used for the extraction of protein were from Nigeria Stored Product Research Institute (NSPRI) at Ilorin, Kwara state.

Preparation of baobab seed flour

Baobab seeds were soaked in water for 72 hours to dissolve some whitish pulp surrounding it and to reduce the quantity of some anti-nutritional compounds found in the seed [21]. The seeds were sunried and roasted at 70 °C for 30 min and stirred continuously to ensure uniform roasting. The golden brown material, which marked the state of proper roasting was cooled, milled and sieved to discard the shaft. The resultant flour was used for protein extraction. Other laboratories wares were obtained from the Department of Food Science and Engineering, LAUTECH, Ogbomoso.

Preparation of maize flour

The maize grain was winnowed, cleaned to take away stones, dirt, and other foreign materials that may affect the attribute of the final product. It was milled and sieved to remove the shaft. The resultant flour was used in flour mixture.

Proximate composition of the baobab seed flour

Moisture, ash, crude fiber, fat and protein content were determined by standard methods [22]. Carbohydrate content in each sample was determined by difference i.e. subtraction of addition of % moisture content, % protein, % fat, % ash, and % crude fiber from 100.

Baobab seed protein extraction

Baobab seed protein was extracted using the method described in AOAC [22]. Fifty (50) g of different fraction of defatted baobab seed flour and 1 litre of distilled water was used along with NaOH (0.2 M). The mixture was stirred at 1200 rpm for 1 h at 30 °C and subsequently centrifuged at 3000 rpm for 20 mins to remove the insoluble carbohydrate residues. The supernatant was collected and the pH was adjusted to 4.5 with 1N H₂SO₄ to precipitate the proteins. The precipitate was creamy white in color. It was further centrifuged at 5000 rpm for 15 mins to recover the protein and was washed repeatedly with distilled water to free it from acid tinge (residues). It was later neutralized to pH 7 using sodium salt. The protein extract was air dried and weighed.

Residual concentration of antinutrients in the protein extract

Alkaloid, Tannin, Trypsin Inhibitor, Oxalate, Phytic acid, Protease Inhibitor, Phytates, Amylase inhibitors were determined by standard methods [22].

Feed preparation

Four experimental diets were formulated. The control diet (Diet I) was basically maize meal, and the other three diets (Diet II-IV) were then formulated with maize meal to contain 10 %, 20 % and 30 % of the baobab seed protein extract [23, 24]. Vitamin and mineral premixes were sufficiently supplied. Each group was placed on each of the diets I-IV.

Formulation of feed and feeding regime

Vitamin and mineral premixes was sufficiently supplied in the feed. The experimental animals received their respective diets and water for 4 weeks. The body weights were measured weekly and food intake were measured daily. The levels were standardized at 10% protein levels to facilitate subsequent studies with regards to Protein Efficiency Ratio (PER). After composition, each diet was thoroughly mixed inside a vortex mixer to ensure homogeneity. Each diet was packed, labeled and sealed to prevent insect or mould infestation. Animals were divided into four groups of five animals each. Each group was placed on each of the diets I-IV [23, 24].

Experimental animals and management

Twenty male albino rats weighing between 40-45 g (3 weeks old) were divided into four groups. Each group contained five animals with each housed individually in stainless mesh cage with free access to food and water. The rats were weighed and acclimatized for seven days during which the animals were placed on normal rat feeds and re-weighed. The animals now commence feeding on experimental diet.
I-IV for 28 days. The body weights were measured weekly and physical observations were made daily [23, 24].

**Feed intake**

Feed intake was determined by subtracting the left-over of the feed supplied the previous day from the quantity given. Feed Intake = Feed supplied - Leftover.

**Weight gain**

Each rat was weighed at the beginning of the experiment and thereafter weekly using a digital weighing scale. Weight gain was obtained weekly by subtracting weights obtained in a new week from the previous week.

**Histopathology**

The experimental animals were sacrificed and the kidney and liver from each animal were removed and fixed in 10% buffered formalin (pH 7.4). Blood was separately collected, and the serum was allowed to separate and maintained at refrigeration temperature. Blood was included as sample material because of the circulatory functions it performs in the body. Liver was added to the selection, as it is a chief site of metabolism and excretion of toxicants/drugs from the body. It is also the first organ to be exposed to these substances following absorption from the gastro intestinal tract. The kidney also plays an essential role in the excretion of body wastes and ingested toxic materials, and has been found to serve as an important indicator of various forms of ingested chemicals or toxicants as deposits of certain chemicals tend to bioaccumulate in the adrenal cortex [23, 25].

**Hematological parameters**

At the completion of the 4 weeks feeding period animals were sacrificed by cervical dislocation. About 5 cm$^3$ of blood was let into lithium heparin tube to prevent coagulation of the blood. This was later centrifuged at 3000rpm for 5 minutes to obtain the serum. The serum obtained was used for the analysis of the following hematological parameters: total protein, serum glutamate oxaloacetate transaminase, and alkaline phosphate [26].

**Determination hematological parameters**

Serum glutamate oxaloacetate transaminase and alkaline phosphate activity was determined using colorimetric method as previously carried out by Adelakun et al. [27]. Total protein was determined by standard [22].

**Protein efficiency ratio (PER)**

The Protein Efficiency Ratio was calculated using the following formulae [22].

**Statistical analysis**

Data were assessed by analysis of variance (ANOVA) using a generalized linear model. Results were expressed as the mean of three replicates. The means were separated using Duncan’s multiple range tests using SPSS (Statistical Package for Social Scientist) version 16.0.

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**Results and Discussion**

The results of the proximate composition were as presented in table 1. The result showed baobab seed contained 27.58% protein. This value is higher than the value of 16.6% reported by Nanji et al [24] and that of Anene et al. [28] who reported 18.4%. Comparing the content of the protein with soybean, baobab seed has lower value in protein than soybean (36.70 g/100 g) but higher than maize (8.90 g/100 g) as reported by Proll et al. [29]. This showed that baobab seed is a competent source of protein. The Crude fat, 16.88% is within the range obtained by Osman [20] and Ajayi et al. [30] which are 12.25% and 33.00% respectively indicating that baobab seeds could be a competent source of edible oil. The crude fiber obtained 9.53%, is lower than that obtained by Lockett et al. [19] and higher than that obtained by Nkafamiya et al. [31] which are 49.72% and 6.71% respectively. The Carbohydrate content of the seed was found to be 25.72% which falls within the range obtained by Murray et al. and Proll et al. which are 11.2% and 56.75% respectively [16, 29]. This indicates that baobab seeds could be a good source of energy. The ash content, 11.87% was higher than the result obtained from Oladunjoye et al. [32] which is 4.5%. The ash content can be compared with the results obtained by Ajayi et al. which is 7.61%. This showed that baobab seed may have a satisfactory quantity of mineral elements for building healthy body and proper maintenance of body tissues. The total moisture content of the seed however was 8.42% which is higher than 5.02% and 4.80% as obtained by Ajayi et al. and Murray et al. respectively [16, 30]. The results on anti-nutritional components in table 2 showed the absence of trypsin inhibitor, which could be considered as a nutritional advantage. Tannin (0.02 mg/g) and Alkaloid (0.03 mg/g) appeared to be very low when compared with 1.22% and 2.05% reported by Adubiaro et al. [33] for tannin and alkaloid in baobab seed flour, respectively. Tannins have commonly been regarded as anti-nutritional factors but it is now known that their helpful properties rely upon their chemical structure and dosage and the total recommended tannin daily intake for a man is 560 mg. [34]. Tannins in legumes are known to hinder the actions of digestive enzymes [35] and nutritional effects are mainly connected to their interaction with protein and minerals. Tannins are supplemented to diverse manufactured foods, including ice caramel. They are also used as purifying materials to precipitate proteins in wines and beers. Recent studies have showed that products containing chestnut tannins.

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**Table 1: Proximate composition of baobab seed flour.**

| Nutrients       | Composition (%) |
|-----------------|-----------------|
| Moisture Content| 8.42 ± 0.34     |
| Protein Content | 27.58 ± 0.13    |
| Ash Content     | 11.87 ± 0.26    |
| Fat Content     | 16.88 ± 0.24    |
| Crude Fibre     | 9.53 ± 0.35     |
| Carbohydrate    | 25.72 ± 0.32    |

N.B: Carbohydrate was calculated by difference. Carbohydrate content (%) = 100 – (moisture + protein + fat + ash + crude fibre). Means are values of 3 replicates ± S.D.
The concentration of Total Protein increased in the test group reliably index for curative effects of various compounds [41]. The differences between the test animals were significant when compared with the control (35 ± 0.38, 47.1 ± 0.40, 49 ± 0.41 and 20 ± 0.13) (p < 0.05) as shown in table 3 and this may indicate that baobab seed is a good source of protein [42]. The concentration of total protein in the serum may indicate the synthetic function of the liver [42] and the nutritional status. Protein Efficiency Ratio (PER) has been the method most widely used to evaluate the quality of protein because of its simplicity. The PER ascertains the efficiency of a protein through the measurement of animal growth. This method requires feeding rats a test protein and then measuring the weight gain in grams per gram of protein consumed. However, this calculation provides a measure of growth in rats and does not provide a strong correlation to the growth needs of humans. PER is compared to a standard value of 2.2, which is the standard value of casein protein [43]. Any value that exceeds 2.2 is considered to be an excellent protein source. It is known that the PER for any protein is dependent upon the amount of protein incorporated in the test diet. PER is not proportional to the nutritive quality of the proteins tested but is related to the weight gain, the amount of food consumed, the amount of protein in the diet, and the nutritive quality of the protein in the diet [44]. From table 4, the PER for the entire test groups were not significant (p > 0.05) with average PER of 1.13 ± 0.13, 1.60 ± 0.20 and 1.84 ± 0.30 and it did not exceed 2.2. However, there was an increase in the weight of all the test animals when compared with the weight of the animals in the control group which indicated that baobab seed is needed for maintenance and growth and that baobab seed is a good source of protein.

### Table 2: Residual concentration of anti-nutrients in the protein extract.

| Anti-nutrient       | Concentration |
|---------------------|---------------|
| Alkaloid            | 0.03 mg/g     |
| Oxalate             | ND            |
| Phytic Acid         | 0.02 mg/g     |
| Protease Inhibitors | ND            |
| Phytate             | ND            |
| Amylase Inhibitors  | ND            |
| Trypsin Inhibitor   | ND            |
| Tannin              | 0.02 mg/g     |

ND: Not Detected.

### Table 3: Hematological parameters.

| Groups                  | ALP  | SGOT  | TP  |
|-------------------------|------|-------|-----|
| Diet I (Control)        | U/L  | U/L   | g/L |
| Diet II                 | 130.8 ± 0.30 a | 376.4 ± 0.36 a | 20 ± 0.13 a |
| Diet III                | 134.2 ± 0.31 a | 359.8 ± 0.35 a | 35.5 ± 0.38 b |
| Diet IV                 | 143.2 ± 0.33 a | 467.2 ± 0.37 a | 47.1 ± 0.40 a |
| Diet V                  | 146.6 ± 0.34 a | 338 ± 0.34 a | 49 ± 0.41 b |

*(Means not followed by the same superscript on the same column are significantly different from the control diet (P < 0.05). Mean ± SD (Standard Deviation) of three replicates. ALP = Alkaline Phosphate; SGOT = Serum Glutamate Oxaloacetate Transaminase; TP = Total Protein.)*

### Table 4: Protein efficiency ratio (PER) for the experimental groups.

| Animals | Group 2 (10% Protein Extract) | Group 3 (20% Protein Extract) | Group 4 (30% Protein Extract) |
|---------|-------------------------------|-------------------------------|-------------------------------|
| A       | 1.12 ± 0.11                   | 1.60 ± 0.23                  | 1.81 ± 0.34                  |
| B       | 1.13 ± 0.14                   | 1.55 ± 0.21                  | 1.85 ± 0.36                  |
| C       | 1.15 ± 0.13                   | 1.69 ± 0.20                  | 1.82 ± 0.00                  |
| D       | 1.10 ± 0.15                   | 1.40 ± 0.00                  | 1.84 ± 0.35                  |
| E       | 1.14 ± 0.10                   | 1.61 ± 0.24                  | 1.9 ± 0.37                   |
| Average | 1.13 ± 0.13                   | 1.60 ± 0.20                  | 1.84 ± 0.30                  |

Means within the same column with different superscripts are significantly different (p < 0.05).

The different ratios of the diet were well tolerated by all the animals as there were no toxic effects observed by gross
visual observation of the animals throughout the experiment. There was no death and apparent behavioral changes recorded during the course of the experiment in all treatment groups and the control group. The liver in the control groups showed normal hepatocytes and mild congestion of the central and portal veins respectively while the liver from the experimental groups showed moderate hepatocytes with moderate congestion of the central vein and portal triad as shown in Table 5 respectively. The kidney from the control group showed normal podocytes, glomeruli, and renal venules while the experimental groups showed the extension of the renal glomerular space with scanty inflammatory cells. Also, the podocytes from the experimental groups are normal and there was no abnormal proliferation of the epithelial cells. This might suggest the non-toxic effect of the protein extract.

Table 5: Observed changes in the internal organs of the animals.

| Diet | Organ | Observed Changes                  |
|------|-------|-----------------------------------|
| I    | Kidney| Normal Cells. Empty tubules and glomeruli. |
| II   | Kidney| Extension of renal glomerular space with mild inflammatory cells. |
| III  | Kidney| Extension of renal glomerular space with mild inflammatory cells. |
| IV   | Kidney| Extension of renal glomerular space with mild inflammatory cells. |
| I    | Liver | Normal Cells. Empty tubules and glomeruli. |
| II   | Liver | Extension of renal glomerular space with mild inflammatory cells. |
| III  | Liver | Moderate hepatocytes with moderate congestion of the central vein and portal triad. |
| IV   | Liver | Normal Cells. Empty tubules and glomeruli. |

Conclusion

Baobab seed could be used as protein supplement when mixed with low protein foods such as cereals grains for both animals and human. The seeds could also serve as a good source of carbohydrate for human and all classes of livestock since it is found to contain a high percentage of carbohydrate.

The protein extract showed some mild changes in the histopathological findings especially for kidney and liver of the animals in the test group which may be as a result of the residual concentration of anti-nutrients in the protein extract.

On the bases of the present investigation, baobab seed protein extract was well tolerated by experimental rats and thus could be recommended as a potential protein source. Further investigation on its potential as a source of supplement for weaning foods can be further investigated.

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