BAOBAB (*Adansonia digitata* L.) SEED PROTEIN UTILIZATION IN YOUNG ALBINO RATS I: BIOCHEMICAL INGREDIENTS AND PERFORMANCE CHARACTERISTICS

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ABSTRACT

Raw, cooked and HCl-extracted baobab, Adansonia digitata seed meals were used for biological and nutritional evaluation studies. The seed is low in protein (16.60g/100g DM) but could be a good source of oil (17.50g/100g) and minerals, particularly sodium, potassium and phosphorus, which contained 228.0, 1429.0 and 924.5 mg/100gDM respectively. Low levels of antinutritional factors such as tannin, phytate, cyanide, oxalate, nitrate/nitrite and absence of trypsin inhibitors were observed. Seed protein is high in sulfur-amino acid, with a chemical score (CS) of 126.80, but marginally limiting in lysine and threonine, with CS of 64.31 and 85.59 respectively based on the preschool age (2-5yrs) reference protein requirement. The seed oil contain appreciable level of unsaturated fatty acids with oleic and linoleic acids making up 66.32% of total fatty acids. The raw diet was similar to the casein diet in weight gain, feed intake, net protein retention (NPR) and true digestibility (TD) but significantly inferior in protein efficiency ratio (PER). Cooking did not have any significant effect on feed intake but significantly lowered the weight gain relative to the raw and casein diets. HCl-extracted meal exerted significantly lower weight gain compared to the raw, cooked and casein diets. It is concluded that the raw seed showed promise as a source of food supplement and is likely to be satisfactory in supporting growth and maintenance in livestock feeding.

Keywords: Adansonia digitata, Baobab seed protein, Biochemical ingredients, Performance, Rats

INTRODUCTION

The majority of sub-Saharan African countries including Nigeria are faced with acute food shortages. The solution to the food problem must be sought through a combination of all available sources. Food and agricultural scientists are beginning to screen wild and under-exploited native plants for possible potential sources of food in an attempt to widen the narrow food base (Vietmeyer and Janick 1996; Oelke *et al.,* 1997). Several reports have also indicated that lots of lesser-known native crop species are high in nutrients and could possibly relieve critical food shortages if given adequate promotion and research attention (Madubuike *et al.,* 1994; Murray *et al.,* 2001). Working on the prospects of utilizing such lesser known and neglected plants, research reports have revealed that quite a large number have useful qualities – either for direct use as animal feed ingredient or as a raw material for seed protein extraction (Ezeagu *et al.,* 2000, 2003). However, prior to utilization of such unconventional resources data indicating the nutrient composition and toxic factors should be available. Toxicological evaluation of possible epidemiological response to the ingestion of novel food sources and the methods of processing that will enhance their utility as food or feed ingredient are all necessary in order to achieve optimal utilization (Longvah *et al.,* 2000).

Baobab is a well-adapted deciduous tree native to the arid parts of Central Africa and widely spread in the savannah regions in Nigeria (Wickens, 1980; FAO, 1988). Its leaves, bark and fruit are used as food and for medicinal purposes in many parts of Africa. In the Sahel, for example, baobab leaf is a staple the Hausas used to make "*miyan kuka",* a soup prepared by boiling the leaf in salt water and reported to be a rich source of Vitamin C. During acute seasonal food supply fluctuations or famine periods, the leaves and fruit of baobab are of particular importance as supplementary and emergency food (Humphrey et al. 1993). The seed has a relatively thick shell, which is not readily separated from the kernel. The kernel is edible but the difficulties of decorticating seem to have limited its use as food/feed and consequently large quantities go into waste. But the increasing pressure of population and predictable food shortages are creating a demand for new food sources of human nutrition. Few reports have indicated the composition of the baobab fruit pulp and leaves (Nour *et al.,* 1980;

Yazzie *et al.,* 1994; Obizoba and Anyika, 1994), but reports on the nutritional and/or biochemical evaluation of the whole seed are scarcely available. An earlier report had indicated the potential of its use as food component or feed supplement (Proll *et al.,* 1998). This study seeks to verify further the nutritional qualities of baobab seed as a protein source and the effect of processing on the nutritional quality in albino rats

MATERIALS AND METHODS

Treatment of Sample: About 2 kg of the matured fruits were harvested from different locations around the city of Ibadan, Nigeria and the seeds were manually separated from the pods. Cooking was done by immersing in boiling water and allowing boiling for 30 min. For acid-extraction, 500g of seeds were immersed in 0.25 M HCl at 60 ^{o}C for 4 h according to the method of Tasneem *et al.* (1982). The acid extract was decanted and the residue washed free of acid using tap water and then dried. Raw and treated samples (350 g each) were ground to flour using a Wiley Mill with the 1 mm mesh sieve and stored in plastic bags at -4^{o} C until analysis.

Proximate Analysis: Nitrogen, fat, ash, micro- and macro-minerals were determined by standard methods (AOAC, 1990). Crude proteins (CP) and total carbohydrates were calculated by N x 6.25 and difference respectively. Total soluble sugars and starch were determined by the combined methods of Duboise *et al.* (1956) and Kalenga *et al.* (1981). Soluble sugars were extracted with ethanol (95 %) and residual starch was then hydrolysed with perchloric acid into monosaccharides. The sugars were then colourimetrically determined with phenol-sulphuric acid. Gross energy was calculated from the Atwater conversion system (FAO, 1982).

Analysis of Antinutritional Factors: Tannin was determined by the Folin-Denis method (AOAC, 1990); Phytic acid by the method of Wheeler and Ferrel (1971); trypsin inhibitor activity by the method of Kakade *et al.* (1974) using benzoyl-DL-arginine-p-nitroanilide (BAPNA) as substrate. Phytohaemagglutinating activity was determined by the serial dilution method of Liener and Hill (1953) using trypsinized rabbit erythrocytes and expressed as haemagglutinating unit (HU)/mg sample. Cyanide was extracted with 0.1 M ortho-H_3PO_4 acid and estimated using an auto analyzer according to the method of Rao and Hahn (1984). Nitrate and nitrite were determined as previously described (Ezeagu and Fafunso, 1995) and oxalate was determined by the method of Baker (1952).

Amino Acid Analysis: Amino acids were determined according to the recommendations of FAO/WHO (1991) by triple hydrolysis (Pellet and Young, 1980) as previously described (Petzke *et al.,* 1997).

Fatty Acid Analysis: For fatty acid analysis the lipid extract was transmethylated with trimethylsulfonium-hydroxide (TMSH) as described by Schulte and Weber (1989). Aliquots (10 mg) of fat were dissolved in 250 µl trichloromethane, followed by addition of 250 µl of the internal standard and 250 µl of TMSH solutions. The fatty acid methyl esters were analyzed using a GLC (model 5890 series II, Hewlett Packard Co., Palo Alto, CA.) equipped with a flame-ionization detector and a 30 m capillary column (DB-Wax, id 0.32mm). The initial oven temperature was 140 ^{o}C followed by temperature programming in three steps: a first rate of 4^{o}C/min until 170^{o}C, followed by a second rate of 1.5^{o}C/min until 185^{o}C and a third rate of 4^{o}C/min until 220 ^{o}C. The final temperature was maintained for 33 min. The injection temperature was 225 ^{o}C and the detector temperature was 250 ^{o}C. Helium was used as the carrier gas. Peak areas were integrated using Hewlett-Packard 3365 Series II ChemStation software, and the fatty acids were expressed as percentage of total fatty acid pool. Fatty acids were identified by comparison of their retention time with those of known standards. Quantitative data were obtained using tricosanoate ($C_{23:0}$) as an internal standard.

Animals and Diets: Experimental diets were prepared according to the method of Chapman et al. (1959) with adequate provision of vitamins and minerals (Miller, 1963). Twenty weanling male albino rats, about 24 days old with mean weights of 26-28 g were obtained from the Preclinical Laboratories of the University of Ibadan, Ibadan. The animals were housed individually in all-aluminum screen metabolic cages with provision for urine, faecal collection and unrestricted access to water and food. The rats were assigned four per group, equalized for body weight in a randomized block design. One group was fed the basal protein-free diet, another group was given a 10% protein diet based on casein, the other three groups were assigned to diets with 10% protein supplied by the raw, cooked or acid-extracted meals, respectively. Weighed amounts of diets were daily offered to the animals for 21 days. The food residues were collected, dried and weighed. The faeces were oven-dried at 60 ^{o}C and stored in plastic containers until analyzed. A drop of dilute H_2SO_4 was added to urine samples to prevent any loss of nitrogen. The rats were weighed weekly and protein efficiency ratio (PER), net protein retention (NPR) and true digestibility (TD) were computed from total feed intake, total faeces voided, as well as the nitrogen determination.

Table 1: Proximate composition and mineral components of baobab seed meal compared to soybean, cowpea and maize

	Baobab	Soybean*	Cowpea*	Maize*
Proximate Composition				
Crude protein	16.60	36.70	23.1	8.9
Crude fat	17.50	20.10	15.0	3.9
Ash	5.50	4.60	3.4	1.2
Carbohydrates	60.40	33.95	67.8	74.2
Total sugars	2.52	-	-	-
Starch	22.60	-	-	-
Crude fibre	14.94	-	-	-
Energy, kJ (kcal)/100g	1883 (450)	1816 (434)	2016 (482)	1490 (356)
Minerals (mg/100g)				
Sodium	228.0	10.0	20.0	
Potassium	1429.0	192.0	96.0	
Calcium	212.0	260.0	130.0	
Magnesium	353.0	320.0		
Phosphorus	924.5	750.0	430.0	
Iron	11.13	-	-	
Copper	2.55	-	-	
Zinc	8.41	-	-	
Manganese	2.10	-	-	

** FAO 1982.[1] Mean of two independent analyses, - Not available*

All analysis was done in duplicate. Data were analyzed by one-way analysis of variance. Treatment means were compared by the Duncan's (1955) multiple range tests.

RESULTS AND DISCUSSION

Chemical analysis of the whole baobab seed is presented and compared to some common staples in Table 1. The results seem to be on the same level with the previous report (Proll et al., 1998). Comparing protein contents, baobab seed is lower in protein (16.60g/100g) than soybean (36.70 g/100g) and cowpea (23.10g/100g) but higher than maize (8.90g/100g). Total sugar is low (2.52g/100g) but starch content of (22.60 g/100g) is higher than the 18.44 g/100g reported for soybean (Ezeagu et al., 2000). With a total fat and carbohydrate contents of 17.50 and 60.40 g/100g respectively, baobab seeds could be a good source of energy and edible oil, and thus a useful supplement in animal feed formulation. There are appreciable levels of minerals, potassium (1429.0) and phosphorus (924.5mg/100g) being the most abundant. The seed meal seems to be higher in iron (11.13), copper (2.55) and zinc (8.41mg/100g) than conventional staples and will easily satisfy animal needs, assuming that they occur in readily available forms. About 34% of total P occurred as phytate-P, which is lower than 80% value reported for most legumes (Rackis and Anderson, 1977). Gross energy (1883.28) was higher than that of maize (1490.22) but comparable to those of soybean (1815.89) and common beans (2016.40 kJ/100g).

The results on antinutritional components (Table 2) showed absence of trypsin inhibitor, which could be considered as a nutritional advantage. Tannin (0.29 mg/g), phytate (1.20 g/100 g), total oxalate (42.0 mg/100 g) and cyanide (0.25mg/100g) appeared low and in reasonable agreement with values reported for commonly consumed food articles.

Table 2: Antinutritional components of baobab seed

Parameters	Baobab* seed
Tannin, mg/g	0.29
Phytate, g/100g	1.20
Phytate-phosphorus	0.34
Phytate-P as % total P	1.0
Trypsin inhibitor, TIU/mg	ND
Haemagglutinins, HU/mg	0.250
Cyanide, mg/100g	0.25
Total oxalate, mg/100g	42.0
Water soluble Oxalate	26.0
Soluble oxalate as % of total oxalate	61.9
Nitrate, mg/g	19.45
Nitrite, mg/g	0.104

*ND: Not Detected, *Means of two independent analyses*

Amino acid profile as shown in Table 3 indicates a fair complement of essential amino acids. Using the FAO/WHO/UNU (1985) preschool age (2-5yrs) reference amino acid requirement as a guide in calculating the chemical score (CS) of amino acids, the seed seems marginally limiting in lysine and threonine (CS 64.31 and 85.59% respectively) but

Table 3: Essential amino acid profile of baobab seed (g/100g Protein)

	Baobab	Chemical score	FAO/WHO/UNU 1985 (reference pattern)	
			Child* 2-5yr	Adult
Lysine	3.73	64.31	5.80	1.60
Methionine	1.25			
Cystein	1.92			
Total S-Amino Acids	**3.17**	**126.80**	**2.50**	**1.70**
Isoleucine	3.54	126.43	2.80	1.30
Leucine	6.54	99.09	6.60	1.60
Phenylanine	4.54			
Tyrosine	2.72			
Total Aromatic Amino Acids	**7.26**	**115.24**	**6.30**	**1.90**
Threonine	2.91	85.59	3.40	0.90
Tryptophan	1.38	125.45	1.10	0.5
Valine	4.99	142.57	3.50	1.30
Histidine	1.98	104.21	1.90	1.60
[1]**Total EAAs**	**33.52**	**43.30**	**33.90**	**12.70**

**For calculating the chemical score of amino acids, the FAO/WHO/UNU (1985) reference pattern for children 2-5 years old was used, Total EAAs: Total essential amino acids*

high in sulphur-amino acids (CS 126.8%). Acceptable CS is considered to be in the order of 60 and above (Nordeide *et al.,* 1994). However, the seed protein was quite adequate in total essential amino acids (EAAs) and compared favorably to the reference protein in total EAAs and in meeting the recommended adult requirements.

Oleic and linoleic acids are the most abundant unsaturated fatty acids (Table 4). With low polyunsaturated/saturated ratio (P/S) (1.1) compared to soybean (3.6) and other high linoleic sources (Sinclair, 1964), the baobab seed oil may not be considered a good source of essential fatty acids.

Table 4: Fatty acid composition of oil (Area %)*

Fatty acids	Baobab
Lauric C_{12}	-
Myristic $C_{14:0}$	0.25
Palmitic $C_{16:0}$	22.06
Palmitoleic $C_{16:1n-7}$	0.27
Hexadecadienic $C_{16:2n-4}$	0.95
Stearic $C_{18:0}$	4.02
Oleic $C_{18:1n-9}$	34.97
Oleic (isomer) $C_{18:1n-7}$	1.00
Linoleic $C_{18:2n-6}$	26.14
Linolenic $C_{18:3n-6\ \gamma}$	0.49
Linolenic $C18:3n-3\ \alpha$	2.00
Arachidic $C_{20:0}$	0.86
Gadoleic $C_{20:1n-9}$	0.22
Benhenic $C_{22:0}$	0.42
Lignoceric $C_{24:0}$	-
Sum	93.65
Sat[a]	27.61
P/S ratio[b]	1.07

*[a]Sum of $C_{14:0}$ +$C_{16:0}$+ $C_{18:0}$ +$C_{20:0}$+$C_{22:0}$, [b]Polyunsaturated ($C_{16:2}$+$C_{18:2}$+$C_{18:3}$ / Saturated ($C_{16:0}$+$C_{18:0}$+$C_{20:0}$+$C_{22:0}$+$C_{24:0}$), *Mean of two independent analyses*

Results of the feeding experiment (Table 5) showed total weight gain, feed and protein intakes (13.45, 70.40 and 8.42 g respectively) of rats maintained on the raw meal were statistically similar ($P < 0.01$) to those on the casein control diet (17.70, 71.53 and 7.15 g respectively). Rats on the raw meal recorded a significantly ($P < 0.01$) superior weight gain (13.45) compared to those on the cooked (9.80 g) and HCl-extracted (3.53 g) meals. Cooking did not have significant effect on feed and protein intakes but significantly ($P < 0.01$) lowered the weight gain compared to the raw and casein diets. It is possible heat damage may have occurred, even though, prolonged cooking was avoided in this experiment (Amadi and Hewilt, 1975). Heat is reported to enhance the nutritive value of proteins by making the sulfur-containing amino acids more available to the animal (Hayward et al., 1936). It was also observed that animals on HCl-extracted meal have significantly ($P < 0.01$) lower feed intake relative to those on the raw, cooked and control diets. PER values of 1.63, 1.57 and 0.49 obtained for raw, cooked and HCl-extracted meals respectively differed significantly ($p<0.01$) compared to the casein diet (2.47). PER for the raw and cooked meals seems to be on the same level with 1.96 previously reported for autoclaved baobab meal (Proll *et al.,* 1998).

While cooking did not improve the PER over the raw meal, acid-extraction significantly lowered the PER relative to the raw and cooked meals. Kawatara *et al.* (1969) has reported low growth of rats fed HCl-extracted meals. Acid–

Table 5: Protein quality indices of raw and processed baobab seed meal

Diets	Weight gain (g)	Feed intake (g)	Protein intake (g)	PER	NPR	TD %
Casein	17.70 ±1.71[a]	71.53±4.71[a]	7.15±0.47[ab]	2.47±0.08[a]	3.07±2.34[a]	93.17±5.37[a]
Raw	13.45 ±2.64[a]	70.40±4.14[a]	8.42±1.26[a]	1.63±0.29[b]	2.18±2.96[a]	80.64±15.23[a]
Cooked	9.80 ± 1.27[b]	62.90±4.98[a]	6.29±0.47[b]	1.57±0.21[b]	2.28±2.48[a]	85.53±1.49[a]
HCl-Extracted	3.53 ± 3.07[c]	59.88±10.09[b]	6.35±0.99[b]	0.49±0.62[c]	0.69±7.90[b]	79.91±1.69[b]

abc (Means not followed by the same subscript on the same column are significantly different (P<0.05) Mean ±SD (Standard Deviation)

extraction may have affected palatability negatively and/or caused loss of nutritional components resulting to poor quality meal. But this observation however, contradicts the report of Tasneem and Subramanian (1986) that acid extraction leached out antinutritional substances from guar seed and significantly improved growth parameters of experimental animals. Effect of acid extraction may therefore depend on the nature of the food substrate. NPR and TD values were lower, but only significantly ($P < 0.05$) for the acid-extracted meal, compared to casein. The fiber content of baobab seed and residual antinutritional factors may have hindered attack of proteins by digestive enzymes, thus reducing digestibility.

It may be inferred from this study that uncooked baobab seed is well tolerated by experimental rats and thus could be recommended as a potential protein source. However, factors of amino acid digestibility and/or availability may need further investigation.

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