BAOBAB (*Adansonia digitata* L.) SEED PROTEIN UTILIZATION IN YOUNG ALBINO RATS. II. HAEMATOCRIT, PLASMA AND HEPATIC BIOCHEMICAL METABOLITES

EZEAGU, Ikechukwu Edwin
Nutrition Unit, Department of Medical Biochemistry, University of Nigeria, Enugu Campus, POBox 15676, Enugu, Nigerian. Email: ikezeagu@yahoo.co.uk

ABSTRACT

The effect of differently processed baobab (Adansonia digitata L.) seed meals on haematocrit and some plasma and liver biochemical parameters in albino rats was investigated. While the animals placed on the raw seed meal recorded comparable growth and liver and plasma biochemical indices to the casein control diet, the cooked and HCl-extracted meals induced significantly (p<0.05) slower rates of growth than the control. Also raw and acid-extracted meals induced significant hyperglycemic effect. Serum and liver total lipids were elevated but non-significantly in the animals fed the raw and acid-extracted meals relative to the controls. There was no consistent pattern in the serum and liver cholesterol and the haematocrit trend, but red blood cell (RBC), packed cell volume (PCV) and haemoglobin (Hb) were decreased significantly in rats fed the acid-extracted meal relative to the control. The possible reasons and nutritional implications of these observations were briefly highlighted. It was concluded that the raw seed has a better 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, Protein utilization, Haematocrit, Plasma, Hepatic, Biochemical metabolites, Rats

INTRODUCTION

As population increase in the tropical regions continue to outstrip food production, it is becoming increasingly necessary to explore and possibly exploit scores of little-known wild crops for protein and energy as supplements to traditional crops and staples (Wickens *et al.* 1989, Oelke *et al.*, 1997, Ezeagu *et al.*, 2002). The exploitation of inexpensive alternative sources of protein and energy for man and/or animal could measurably reduce malnutrition. Unconventional and lesser-known plants could contain useful amounts of nutrients as indicated by some research (Eromosele *et al.*, 1994, Murray *et al.*, 2001; El-Adawy and Taha, 2001). However, prior to the utilization of such novel sources, either in human food or feed, thorough toxicological evaluation of possible biochemical, haematological or epidemiological response to their ingestion is necessary (Wolf *et al.* 1975). Many plants are endowed with the ability to synthesize a wide variety of chemical substances, which, under practical circumstances, can impair some aspects of animal metabolism when ingested by man or animals (Cheeke and Shull, 1985).

Studies have shown that malnutrition in the form of specific nutrient deficiency results in general stunting and reduced organ size and marked changes in some biochemical parameters. Also ingestion of certain toxic factors or chemicals have manifested in distorted haemato-biochemical metabolites (Geol and Sharma, 1988). Baobab is a common multipurpose tropical fruit tree wildly consumed in savannah region in Northern Nigeria. A previous study (Ezeagu, 2005) indicated the protein marginally limiting in lysine and threonine and that weight gain was retarded in animals fed both the raw, cooked and acid-extracted baobab meals relative to casein. Growth retardation was, however, only significant in rats fed the cooked and acid extracted diets. But whether the body composition was altered was an unanswered question. Therefore, it was thought to be of interest to further investigate possible toxicological effects of ingestion of both raw and processed baobab seed meal with respect to some body systems from medical standpoint.

MATERIALS AND METHODS

The collection, processing, formulation of diets and feeding protocols were carried out as previously described (Ezeagu, 2005). At the end of the 21 days experimental period, the rats were weighed, anaesthetized with chloroform and dissected. Blood was collected from the heart by cardiac puncture using a syringe and needle and deposited in heparinised tubes. Haematocrit was determined immediately (Jain, 1986). The whole blood was kept in the refrigerator and later centrifuged and separated at 3,000g for 10 minutes to obtain a clear serum. The livers were excised, weighed and homogenized with 5 ml. phosphate buffer (pH 7.5) using a hand homogenizer. Serum and liver proteins, lipids and cholesterol were determined according to the methods outlined by Lynch *et al.* (1969). Food efficiency ratio (FER) and protein efficiency ratio (PER) were calculated by the following formulae: FER = Gain in body weight (g) / Food consumed (g); PER = Gain in body weight (g) / Protein consumed (g). All analyses were done in triplicate.

Table 1: Nutritional indices of rats fed baobab meals*

	Casein	Raw	Cooked	HCl-extracted
Weight gain (g/day)	0.84 ± 0.08[a]	0.60 ± 0.17[b]	0.47 ± 0.03[b]	0.02 ± 0.01[c]
Food Consumption (g/day)	3.41 ± 0.22[a]	5.51 ± 0.25[a]	3.13 ± 0.13[a]	2.79 ± 0.17[b]
FER	0.25 ± 0.03[a]	0.19 ± 0.04[b]	0.16 ± 0.02[b]	0.02 ± 0.01[c]
PER	2.47 ± 0.08[a]	1.46 ± 0.35[b]	1.62 ± 0.21[b]	0.18 ± 0.04[c]

*abc (Means not followed by the same superscript on the same row are significantly different (P<0.05), *Mean± SD (Standard Deviation)*

Table 2: Effect of baobab seed meal on plasma and hepatic biochemical parameters

	Plasma (mg/100 ml)				Liver (mg/g)				
Diet	**Sugar**	**Protein**	**Lipid**	**Cholesterol**	**Protein**	**Lipids**	**Cholesterol**	**NPR**	**TD %**
Casein	37.58 ±3.95[b]	4.20 ±0.38[b]	374.48 ±61.17[a]	55.23 ±2.7[a]	41.23 ±10.33[b]	68.10 ±22.64[a]	6.10 ±5.13[a]	3.07 ±2.34[a]	93.17 ±5.32[a]
Raw	66.23 ±20.92[a]	3.54 ±0.08[a]	388.94 ±12.90[a]	60.96 ±4.05[a]	30.24 ±2.91[a]	91.70 ±25.27[a]	4.47 ±6.51[a]	-	-
Cooked	42.00 ±11.02[b]	3.86 ±0.42[a]	363.81 ±73.3[a]	48.38 ±5.02[a]	33.84 ±1.84[a]	273.69 ±33.11[a]	4.57 ±9.81[a]	0.19 ±1.53[b]	89.57 ±2.0[a]
HCl-extracted	66.97 ±18.88[a]	3.78 ±0.22[a]	380.29 ±3.73[a]	62.67 ±5.80[a]	32.38 ±2.22[a]	278.5 ±53.39[a]	4.83 ±6.71[a]	0.74 ±1.18[b]	82.03 ±5.32[b]

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

Table 3: Effect of baobab seed meal on haematocrit*

	Casein	Raw	Cooked	HCl-extracted
Hb g/100 ml	14.95±0.94[a]	9.98±2.58[a]	10.68±1.29[a]	8.00±2.61[b]
RBC x 10^6 mm^{-3}	5.97±0.64[a]	4.64±1.44[a]	5.40±0.63[a]	3.25±0.73[b]
WBC x10^3 mm^{-3}	10.33±0.88[a]	9.85±2.77[a]	6.00±2.41[a]	10.40±2.47[a]
PCV %	40.0±6.38[a]	29.5±8.35[a]	33.5±3.70[a]	28.25±10.28[b]

*abc (Means not followed by the same superscript on the same row are significantly different (P<0.05).*Mean± SD (Standard Deviation).Hb: Haemoglobin, RBC: Red blood cell, WBC: White blood cell, PCV: Packed cell volume*

The data were subjected to analysis of variance (ANOVA) and treatment means were compared by the Duncan's (1955) multiple range test.

RESULTS AND DISCUSSION

Nutritional indices, presented in Table 1, followed the same trend as obtained in the previous report (Ezeagu, 2005). However, rats on the cooked meal recorded relatively better indices in this study. The raw seed meal seems to be more palatable than the cooked and acid-extracted meals, hence the higher feed intake. Palatability may have been affected by acid treatment as indicated by the poor feed intake. This could be due to the effect of possible residual acid or, perhaps, some reaction products. Kawatara *et al.* (1969) has reported similar poor growth in rats fed acid-extracted meals. Nutrient losses during acid-extraction and the cooking process may have contributed to the poor performance of the animals on treated baobab meals (Anthoni Raj and Singaravadivel, 1980).

Blood sugar levels were elevated only significantly ($p < 0.05$) in rats fed raw and acid-extracted meals. Elevated blood sugar may be due to inhibition of glycolysis by the presence of glycoproteins and saponins, which may have adversely effected regulation of insulin from pancreatic B-cells and blood sugar, as suggested by Mandal *et al.* (1982). According to Yadav *et al.* (1973), it seems some plant proteins exhibits hypoglycemic effect and some hyperglycemic in experimental animals. In this regard baobab seeds may not be useful in the management of diabetes.

Serum and liver biochemical parameters are shown in Table 2. Liver protein values show same trend as serum proteins, being related physiologically. Rats on the casein diet recorded significantly ($p<0.05$) higher total plasma and liver proteins than those on the raw, cooked and acid-extracted meals. Among the three baobab test meal groups, plasma and liver protein levels were comparable and non-significantly different, though animals on the acid-extracted meal recorded the lowest levels. Rats on the test diets could be consuming adequate protein but absorption from the alimentary tract may be defective. Loss of protein in the urine and into alimentary tract and increased catabolism of proteins may also be responsible for lower serum and liver proteins of the animals on the baobab meals. However, the low body weight gain and reduced serum total protein as observed for the test diets also reflect the low quality of the baobab protein relative to casein. Plasma total protein is regarded as good indices of the quality of dietary proteins (Lewis *et al.*, 1977; Babatunde and Pond, 1988).

Serum total lipids and cholesterol were elevated, but not significantly ($p>0.05$), in rats fed the raw and HCl-extracted meals relative to casein (Table 2). But rats on the cooked meal had lower serum lipid and cholesterol levels. Serum lipids were highest in rats placed on raw diet (388.94 mg/100 ml) and least in those on cooked meal (368.81 mg/100 ml) groups. Balogun *et al.* (1982)

reported significantly higher levels of serum lipids in rats fed plant proteins as compared with rats fed similar diets containing casein as the only protein source. However, other workers observed no consistent differences in serum lipids in rats fed diets containing either plant or animal proteins (Okita and Sugano, 1981; Adeyeye *et al.*, 1989; Dong *et al.*, 1990).

While liver lipids were elevated, the cholesterol levels were lowered in rats fed the test diets relative to the control but non-significantly (P > 0.01). Also the mean serum cholesterol of rats on raw and acid-extracted meals were elevated, while that of those on cooked meal were non-significantly (P < 0.01) lowered relative to the controls. These observations seem to be in conflict with the reported hypocholesterolemic effect of plant protein compared with casein diet (Mokady and Liener 1982; Potter, 1995). However, findings by Leelamma *et al.* (1978) have shown that the effect of dietary proteins on lipid levels depends upon the nature of particular protein rather than its source. This may be related to the amino acid composition and digestibility of different proteins and also the experimental animal used (Hassan and Rashwan, 1986; Sugano and Koba, 1993). It is also thought that arginine/lysine (Arg/Lys) ratio in seed proteins, not investigated in this study, may be relevant to the plasma cholesterol lowering effect of plant proteins (Kritchevsky, 1979). Balogun *et al.* (1982) observed that rats fed diets with low Arg/Lys ratios tend to be less hypocholesterolemic than diets having higher ratios. Hence Yadav *et al.* (1973) observed that some plant proteins were hypocholesterolemic while some others were hypercholesterolemic in experimental animals. The processing treatments may have altered the Arg/Lys ratios of the test diets resulting in differing cholesterolemic effects.

Also, since undigested protein is able to bind bile acids, reabsorption of bile acids in the intestine is reduced (Woodward and West, 1984). Recycling of bile acid is thus diminished and consequently synthesis of bile acids from cholesterol is stimulated which results in lower levels of serum cholesterol (Beynen *et al.*, 1986). In the light of the fairly high true digestibility (TD) value of the test meals (79.91 - 85.53 %) (Ezeagu, 2005), protein digestibility may not be responsible for the differing cholestrolemic effects of the test meals. Kuyvenhoven *et al.* (1987), after testing four different proteins in rats, reported that there was no conformity between digestibility and serum and liver cholesterol levels. On the other hand, studies in experimental animals have also demonstrated that presence of certain plant fibers in the diet is accompanied by significant lowering of serum and tissue cholesterol levels. Dietary fiber adsorbs bile salts and faecal excretion of bile acids is thus enhanced. Consequently synthesis of bile acids from body cholesterol is stimulated thereby reducing the blood cholesterol concentrations also (Potter, 1995; Oakenful and Fenwick, 1978; Uberoi *et al.*, 1992). Fiber content of 14.94g/100g was reported (Ezeagu, 2005) for raw baobab seed meal. Boiling may have increased the fiber components (Vidal-Valverde *et al.*, 1992) resulting in the hypocholesterolemic effect of the cooked meal. The higher the lignin component in fibers, the better the hypocholesterolemi effect (Uberoi *et al.*, 1992). However, in view of the non-significant results, it is not possible to definitive on the effect of baobab seed meal on serum cholesterol.

Haematological changes are presented in Table 3. Significant (P < 0.05) decrease in Hb, RBC and PCV were exhibited only in the HCl-extracted test diet. A significant fall in RBC reflects erythropenia (Geol and Sharma, 1988). Poor feed intake and/or residual acid may be implicated in the poor negative effect of acid-extracted meal. On the other hand acid treatment may have leached out nutritive factors. There was no consistent pattern in white blood cells (WBC) trend, which seems to be of no physiological significance. A significant increase in WBC would indicate toxicity or poisoning.

It is evident therefore that only the HCl-extracted meal exerted a significant negative effect on the body weight and the dynamic equilibrium of blood protein. Except for the higher volume of faecal matter voided by the rats on the test diets, neither the control nor the treated animals showed any sign of behavioral abnormality, side effect or any toxic reaction throughout the experimental period. The negative effect on weight gain could therefore, be due to nutrient loss during processing and/or amino acid imbalance and may not be a result of any toxic insult. Dong *et al.* (1990) reported that lower weight gain in rats fed poor sources of protein could be due mainly to essential amino acid deficiency and/or lower *in vivo* apparent protein digestibility and subsequent supplementation of protein with limiting amino acids restored normal growth response in the animals. It is possibly that normal growth rate would be restored by amino acid supplementation in the experimental animals.

Conclusions: On the bases of the present investigation, the raw baobab seed meal exerted little or no negative effect on the dynamic equilibrium of the blood and liver metabolites. Therefore, it may be concluded that baobab seed meal would be safe for edible use and has a better promise as a source of food supplement and likely to be satisfactory in supporting growth and maintenance in livestock feeding. Further studies on the improvement level of animal performance by amino acid supplementation will continue.

REFERENCES

ANTHONI RAJ, S. and SINGARAVADIVEL, K. (1980). Influence of soaking and steaming on loss of similar constituents in paddy. *Journal of Food and Nutrition Sciences, 5(3):* 63 - 71.

ADEYEYE, S. O., OYENUGA, V. A. and FETUGA, B. L. (1989). Serum cholesterol and triacylglycerol in rats fed different dietary protein sources. *Nutrition Report International, 4*: 323 - 334.

BABATUNDE, G. M. and POND, W. G. (1988). Nutritive value of Nigeria rubber seed meal and oil III. Performance characteristics, relative organ weights, haematocrit and plasma metabolites of growing female rats fed corn diets, containing rubber seed meal, soya bean meal or casein. *Animal Feed Science and Technology 20*: 125 - 133.

BALOGUN, E. A., BALOGUN, O. O. and ODUTUGA, A. A. (1982). Arginine, Lysine ratio as a contributive factor to hypocholesterolmic effect of plant protein sources. *IRCS Medical Sciences, 10*: 643 - 645.

BEYNEN, A. C., VAN DER MEER, R and WEST C. E (1986). Mechanism of casein-induced hypercholesterolemia: Primary and secondary features. *Arteriosclerosis, 60*: 291-299.

CHEEKE, P. R. and SHULL, L. R. (1985). *Natural Toxicants in Feeds and Poisonous Plants*. The AVI Publishing Company, Incorporated, Westport, Connecticut, USA.

DONG, F. M., RASCO, B. A., GAZZAZ, S., S., BUENAVENTURE M. L. S and HOLCOMB, L. M. (1990). Body composition and serum and liver lipids in rats fed distillers dried grains. *Journal of Science of Food and Agriculture, 51*: 299 - 308.

DUNCAN, D. B. (1955). Multiple range and multiple F tests. *Biometrics, 11*: 1 – 42.

EL-ADAWY, T. A. and TAHA, K. M. (2001). Characteristics and composition of different seed oils and flours. *Food Chemistry, 74*: 47 - 54.

EROMOSELE, I. C., EROMOSELE, C. O., AKINTOYE, A. O. and KOMOLAFE, T. O. (1994). Characterization of oils and chemical analysis of the seeds of wild plants. *Plant Foods for Human Nutrition, 46*: 361 - 365.

EZEAGU, I. E. (2005). Baobab (*Adansonia digitata* L) seed protein utilization in young albino rats. I. Biochemical ingredients and performance characteristics. *Animal Research International, 2(1)*: 240 – 245.

EZEAGU, I. E., PETZKE, J. K., METGES, C. C., AKINSOYINU, A. O. and OLOGHOBO, A. D. (2002). Seed protein contents and nitrogen-to-protein conversion factors for some uncultivated tropical plant seeds. *Food Chemistry, 78*: 105 - 109.

GOEL, K. A. and SHARMA, S. D. (1988). Haematological and seroenzymological characteristics of albino rat induced by diphenyl-bis-azo binaphthionic acid (Congo red). *Journal of Food Science and Technology, 25:* 97 - 98.

HASSAN, H. A. and RASHWAN, S. (1986). The effect of high and low dietary proteins on growth and serum biochemical changes in rats. *Nutrition Report International, 33*: 851 - 859.

JAIN, N. C. (1986). *Schalman's Veterinary Haematology*, 4th edition. Lea and Fabinger, Philadelphia, USA.

KAWATARA, B. L., GARCHA, J. S. and WAGLE, B. S. (1969). Studies on Guar (*Cyamopsis tetragoloba*). *Journal of Nutrition and Dietetics, 6:* 91- 97.

KRITCHEVSKY, D. (1979). Vegetable proteins and atherosclerosis. *Journal of American Oil Chemists Society, 56*: 135 - 137.

KUYVENHOVEN M. W., WEST, C. E, HAKKERT, B. C., MENSINK G. B. M. and BEYNEN A. C. (1987). Digestibility of dietary proteins and serum cholesterol in rats. *Nutrition Reports International, 36(3)*: 537 – 549.

LEELAMMA, S., MENON, P. V. G. and KURUP, P. A. (1978). Nature and quality of dietary protein and metabolism of lipids in rats fed normal and artherogenic diet. *Indian Journal of Experimental Biology, 16*: 29 – 33.

LEWIS, A. J., PEO, E. R. JR., CUNNINGHAM, P. J. and MOSER, B. D. (1977). Determination of the optimum dietary proportions of lysine and tryptophan for growing rats based on growth, food intake and plasma metabolites. *Journal of Nutrition, 107*: 1361 - 1368.

LYNCH, M. J., RAPHAEL, S. S., MELLOR, L. D., SPARE, P. D. and INWOOD, M. J. H. (1969). *Medical Laboratory Technology and Clinical Pathology*. 2nd Edition, W. B. Saunders Company.

MANDAL, B., MAJUMDAR, S. G., and MAITY, C. R. (1982). Nutritional evaluation of the protein isolate obtained from seed of *Eucalyptus kirtoniana* in albino rats - A comparative study. *Proceedings of the Indian National Science Academy, B48*: 596 - 602.

MOKARDY, S. and LIENER, I. E. (1982). Effect of plant proteins on cholesterol metabolism in growing rats fed artherogenic diets. *Annals of Nutrition and Metabolism, 26*: 138 -144.

MURRAY, S. S., SCHOENINGER, M. J., BUNN, H. T., PICKERING, T. R. and MARLETT, J. A. (2001). Nutritional composition of some wild plant foods and honey used by Hadza foragers of Tanzania. *Journal of Food Composition and Analysis, 14*: 3 - 13.

OAKENFULL, D. G. and FENWICK, E. D. (1978). Adsorption of bile salts from aqueous solution by plant fibre and cholestyramine. *British Journal of Nutrition, 40:* 299 – 309.

OELKE, E. A., PORTER, R. A., GROMBACHER, A. W. and ADDIS, P. B. (1997). Wild rice – New interest in an old crop. *Cereal Foods World, 42(4)*: 234 - 247.

OKITA, T. and SUGANO, M. (1981). Effects of dietary soybean globulins on plasma and liver lipids and on faecal excretion of neutral sterols in rats. *Journal of*

Nutritional Sciences and Vitaminology, 27: 379 - 388.

POTTER, S. M. (1995). Overview of proposed mechanisms for the hypocholesterolemic effect of soy. *Journal of Nutrition, 125: 606S - 611S.*

SUGANO, M. and KOBA, K. (1993). Dietary protein and lipid metabolism: a multifunctional effect. *Annual New York Academy of Science, 676*: 215 - 222.

UBEROI, S. K., VADHERA, S. and SONI, G. L. (1992). Role of dietary fibre from pulses and cereals as hypocholestrolemic and hypolipidemic agent. *Journal of Food Science and Technology, 29*: 281 - 283.

VIDAL-VALVERDE, C., FRIAS J. and ESTEBAN R. (1992). Dietary fiber in processed lentils. *Journal of Food Science, 57(5):* 1161 – 1163.

WICKENS, G. E, NAQ, N. and DAY, P. (eds.) 1989). *New Crops for Food and Industry.* Chapman and Hall, New York.

WOLF, A., HRIVNAK, D., MARESOVA, P. and SVABOVA, M. (1975). The evaluation of new protein sources from health point of view. *Nahrung, 19(18):* 657 - 667.

WOODWARD, C. J. H. and WEST, C. E. (1984). Bile acids binding and the cholesterolemic effect of dietary proteins. *Proceedings of the Nutrition Society, 43:* 63 - 68.

YADAV, N. R., BHARADWAJ, U. R., PANDE, S. R. and RAJWANSI, V. S. (1973). Biochemical studies on Indian wild legumes 10: Effect of *Acacia melanoxylon, Albizzia moluccana* and *Leucaena glauca* seed diets on blood sugar and total cholesterol levels in normal young rats. *Indian Journal of Medical Sciences 26*: 729 - 732.