

Biochemical Characteristics of Rats Fed on Shea Nuts (*BUTYROSPERMUM PARKII*) Meal

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ABSTRACT

There is current interest in the search and use of agro-waste in preference to conventional ingredients in agriculture as animal feed hence the need to investigate the potentials of these wastes and their effects on animals. This study was designed to investigate the biochemical characteristics of rats fed on shea nuts (*Butyrospermum parkii*) meal. Defatted shea nut seed (exfactory) and whole shea nut seed (raw) meals were fed as whole food to male albino rats for twenty eight (28) days. Biochemical parameters in the blood serum, urine, faeces and tissues were studied to assess the adequacy of the shea nut meals for food value. There were significant decreases in body weight as well as organ weights of liver, spleen and heart for the rats on the shea nut meals as compared to the control. Total protein, albumin, non protein nitrogen, urea, as well as glycogen in the liver and serum fell significantly ($p > 0.05$) with the two experimental meals, with the effect showing more in the raw shea nut meal. Serum minerals, phospholipids in liver and kidney tissues were not affected. However, transaminases (GOT and GPT) and alkaline phosphatase were elevated in both serum and liver, indicating insufficient calorie and reduced quality of food value (especially protein) in the meals. A high percentage nitrogen loss was observed in rats on the shea nut meals compared to the control at a significant difference ($p < 0.05$). The excessive loss of hair observed would account for a negative nitrogen balance in the experimental rats, thus explaining the weight loss. The observed biochemical indices depict a poor response of the rats to both the exfactory and raw shea nut diets as food.

KEY WORDS: Waste; Meal; Tissues; Transaminases; and Livestock.

INTRODUCTION

Protein deficiency is a serious cause of ill health and death in developing countries (WHO and FAO, 2007). It is particularly very deficient as it became increasingly difficult to maintain farm livestock, which serves as a main source of protein. Thus new sources of edible protein should therefore be investigated directly for human consumption or indirectly as food for animals.

Shea nut, (*Butyrospermum parkii*) is a traditional food plant in Africa. This vegetable has potential to improve nutrition, boost food security, foster rural development and support sustainable land care (National Research Council, 2007). Shea trees take approximately 31 years to reach maturity.

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Shea nut (*Butyrospermum parkii*) meal, a solid residue from the shea fat industry, is available in large quantities in West Africa (Dei, *et al.*, 2007). The meal is now receiving increased attention as a potential feed ingredient for poultry due to the increased amounts that are available due to high demand for shea fat in cosmetics and as a cocoa butter substitute in chocolate. However, very few reports exist on the effect of the shea nut or defatted shea nut diet on biochemical characteristics of animals fed the diet.

This study aims at evaluating the effect of feeding the whole shea nut meal as well as the exfactory shea nut waste (defatted meal) as food on the biochemical characteristics necessary for metabolism in male albino rats.

MATERIALS AND METHODS

Preparation of shea nut meal

Exfactory shea nuts meal (already milled and defatted industrially by steam extraction) was purchased as waste from Vegetable Oil Nigeria Ltd., Lagos, Nigeria. Raw shea nut seeds were obtained from the local market, cleaned and milled into a meal. Laboratory chow was purchased at Pfizer Nigeria Ltd., Lagos, Nigeria. Proximate analysis was out on all shea nut meals in triplicates using the AOAC method (AOAC, 1997). This study was carried out at the Biochemistry department, Lagos State University, Lagos, Nigeria from February 2007 to September 2007.

Experimental animals

Thirty male albino rats with an average weight of 85.7 ± 2.82 g were used. All animals were fed a pelleted commercial laboratory chow for 4 days, before they were divided into experimental groups. They were divided into three feeding groups of 10 rats each as shown below:

GROUP 1= Exfactory defatted shea nut seed diet

GROUP 2 = Whole (raw) shea nut seed diet

GROUP 3 (Control) = Laboratory chow

The grouping was done in a random fashion such that the weight difference between each group was < 0.1 g. Diets and water were administered *ad libitum*.

The rats were housed in a double compartment wire cages with 5 rats in each compartment in a room with controlled temperature ($25 \pm 2^{\circ}\text{C}$) and lightening (alternative 12 hours periods of light and darkness). The study lasted for 28 days.

Prior to daily feeding of the animals, diet intakes were recorded each day and animals were weighed every three days. Urine and faeces were collected on a daily basis.

At the end of the feeding trial, animals were lightly anaesthetized with chloroform. The serum was separated and frozen until analysis. The livers, kidney and spleen were excised, weighed and frozen at -20°C until latter analysis.

Analytical procedure

Serum was analyzed for urea, non protein nitrogen, total protein, albumin, total glycogen, cholesterol, phospholipids, calcium, sodium and potassium, Transaminases, Glutamic Oxaloacetic Transaminase (GOT), and Glutamic Pyruvic Transaminase and Alkaline Phosphatase (Harris and Demets, 1972).

Urine samples were analyzed for urea, creatinine, non-protein nitrogen and total nitrogen (Eggum, 1970).

Total nitrogen content of the faecal sample was determined. Weighed liver tissue was homogenized in distilled water and further dilutions were assayed for total glycogen, Glutamic Oxaloacetic and Glutamic Pyruvic transaminases (Reitman and Frankel, 1957). Homogenized kidney and spleen tissues were assayed for cholesterol and phospholipids (Zlatkis, *et al.*, 1953).

Statistical Analysis

Statistical significance was established using One-Way Analysis of variance (ANOVA) and data were reported as mean \pm standard deviation. Statistical analyses were carried out using SPSS for Windows, version 14.0 (SPSS Inc. Chicago, IL, USA).

RESULTS

Food intake, Body weight gain and Organ weights

There was a general reduction in food intake (table 2) among the animals on exfactory shea nut and raw shea nut diets as compared to the control on laboratory chow ($p < 0.05$) being 42.9 and 37.1% of control food respectively.

Rats consuming the raw shea nut diet and the exfactory shea nut diet exhibited significantly ($p < 0.05$) reduced weight gain than the control animals on laboratory chow, however, the difference between the two groups was not statically significant. Also, significant decreases ($p < 0.05$) were observed in liver, spleen and heart weights on rats fed the two experimental diets as compared to control, though interaction did not show significant differences between the two groups ($p > 0.05$). However, the diets did not seem to affect the kidney and brain weights ($p > 0.05$).

Table 1: Proximate Composition (g/100g sample) of Shea Nut Meal (Exfactory and the Raw Shea Nut Meal)

Parameters	Exfactory Shea Nut Meal (group 1)	Raw Shea Nut Meal (group 2)
Moisture	5.30	5.89
Protein	11.1	9.41
Fat	22.4	53.0
Ash	5.70	12.0
Crude Fiber	7.40	9.60
Carbohydrate	48.1	48.1

All determinations are in triplicate

Table 2: Effect of Diet on Food Intake, Weight Gain, and Organ Weights

Parameters	^a Control	^a Exfactory Shea Nut Meal (group 1)	^a Raw Shea Nut Meal (group 2)
Daily food intake (g/rat/day)	7.0 \pm 1.3	3.0 \pm 1.05	2.6 \pm 1.26 be
Average daily weight gain (g/rat/day)	3.73 \pm 6.8	2.77 \pm 10.4	3.8 \pm 7.4 be
Liver weight (g)	3.96 \pm 1.3	2.13 \pm 1.42	1.91 \pm 1.15 bc
Body weight (%)	3.63 \pm 1.26	2.46 \pm 1.34	2.25 \pm 1.12 bc
Kidney weight (g)	0.73 \pm 0.56	0.59 \pm 0.09	0.62 \pm 0.12 de
Spleen weight (g)	0.17 \pm 0.05	0.12 \pm 0.03	0.10 \pm 0.04 be
Brain weight (g)	1.36 \pm 1.16	1.36 \pm 1.10	1.36 \pm 1.12 de
Heart weight (g)	0.38 \pm 0.01	0.23 \pm 0.04	0.27 \pm 0.03 bc

a =value are expressed as mean \pm SD of 10 rats

b = Significant difference between all groups $p < 0.05$ (one way analysis of Variance ANOVA)

c =Significant difference between groups 1 and 2 $p < 0.05$ (t-test)

d = No significant difference between all groups $p < 0.05$ (one way analysis of Variance ANOVA)

e =No Significant difference between groups 1 and 2 $p < 0.05$ (t-test)

Blood values

The results for serum biochemical indices in rats fed exfactory shea nut and raw shea nut diets are shown in table 3. The values for total serum protein, albumin and non-protein nitrogen were lower in the rats fed exfactory and shea nut diets as compared the control group though the difference in value between the two feeds was not significant.

Control > Exfactory = Raw

The creatinine levels were reduced in rats fed the shea nuts diets but did not differ significantly ($p > 0.05$) among all feeding levels. Serum glycogen was reduced significantly than that observed with exfactory shea nut diet (Control > Exfactory > Raw). Serum cholesterol was observed to be raised in the two experimental diets ($p < 0.05$), however, the value of serum cholesterol in rats fed raw shea nut diet was significantly higher than rats on exfactory shea nut diets (Control > Exfactory > Raw).

The experimental diets had no effect on phospholipids. All the serum minerals assayed were not affected by the diets and no significant differences were observed, except for potassium which was significantly reduced with the exfactory nut meal.

Glutamic Oxaloacetic transaminase (GOT) as well as Alkaline phosphatase were significantly elevated with the diets but no significant difference was observed between groups (control < exfactory = raw shea nut).

However, the Glutamic pyruvic transaminase on the other hand was not significantly affected ($p > 0.05$) by the experimental diets.

Table 3: Some Biochemical Indices in Rats Fed Raw Shea Nuts Meal, Exfactory Shea Nut Meal and Control Diet

Measurements	Control	Exfactory Shea Nut Meal (group 1)	Raw Shea Nut Meal (group 2)
Total protein (g/l)	42.5 \pm 1.93	30.0 \pm 3.4	32.5 \pm 3.8 be
Albumin (g/l)	19.6 \pm 1.32	16.8 \pm 2.5	16.9 \pm 2.6 be
Non-Protein N (mg/dl)	44.54 \pm 6.4	35.08 \pm 7.3	38.46 \pm 9.7 be
Urea (mg/dl)	38.7 \pm 5.4	31.67 \pm 4.3	31.25 \pm 1.3 be
Creatinine (mg/dl)	1.57 \pm 0.94	1.47 \pm 0.67	1.44 \pm 0.73 bc
Glycogen (mg/dl)	52.0 \pm 4.3	32.0 \pm 3.8	29.0 \pm 3.4 bc
Cholesterol (mg/dl)	113.3 \pm 8.7	136.6 \pm 8.7	159.9 \pm 9.0 de
Phospholipids (mg/dl)	11.8 \pm 1.7	10.6 \pm 2.1	10.8 \pm 1.37 de
Calcium (mg/dl)	1.2 \pm 0.8	0.96 \pm 0.64	1.22 \pm 0.72 de
Sodium (nmol/dl)	134.0 \pm 13.3	124.0 \pm 11.8	126.0 \pm 11.5 de
Potassium Glutamic Oxaloacetic (nmol/dl)	4.10 \pm 0.34	3.10 \pm 0.6	4.30 \pm 0.84 bc
Transamine Glutamic Pyruvic	28.5 \pm 0.7	6.20 \pm 1.3	6.0 \pm 1.05 be
Transaminase (u/l)	6.80 \pm 1.25	7.15 \pm 1.4	7.35 \pm 1.5 de
Alkaline Phosphatase (u/l)	24.0 \pm 8.3	130.0 \pm 38.4	120.0 \pm 23.9 be

a = Value are expressed as mean \pm SD; n = 10 rats

b = Significant difference between all groups $p < 0.05$ (one way analysis of Variance ANOVA)

c = Significant difference between groups 1 and 2 $p < 0.05$ (t-test)

d = No significant difference between all groups $p < 0.05$ (one way analysis of Variance ANOVA)

e = No Significant difference between groups 1 and 2 $p < 0.05$ (t-test)

Faecal Nitrogen and Nitrogenous Compounds in Urine

Table 4 shows the effect of diet on faecal nitrogen and the nitrogenous compounds in urine. Faecal nitrogen as well as the percentage of nitrogen absorbed was found to be significantly lower ($p < 0.05$) in rats fed the exfactory shea nut and the raw shea nut diets. However, there was no significant difference ($p > 0.05$) in the faecal nitrogen and digestibility of rats fed the exfactory diet or raw shea nut diets.

Total urinary nitrogen was lower ($p < 0.05$) among rats on the exfactory and raw shea nut meals. Urea excretion accounted for 83.1%, 76.9% and 74.07% of total urinary nitrogen in rats fed the control, exfactory meal and shea nut diets.

Creatinine excretion was found to be lower but not significant ($p > 0.05$) in rats fed the exfactory shea nut and raw shea nut diets. The values between groups were not significant. No significant difference was observed between the various groups for non-protein nitrogen. However, the urinary non-protein nitrogen of the rats on raw shea nut was significantly lower than the control.

Table 4: Effect of Diet on Faecal Nitrogen and Nitrogen Compounds in Urine

Parameters	Control	Exfactory Shea Nut Meal (group 1)	Raw Shea Nut Meal (group 2)
Nitrogen Intake (g/day)	4.5 ± 0.94	1.8 ± 0.71	1.76 ± 0.68 ad
Faecal total N ₂ (g/day)	0.76 ± 0.07	0.57 ± 0.05	0.59 ± 0.04 ad
*Nitrogen digestibility (%)	82.7 ± 7.8	68.3 ± 0.3	65.0 ± 6.7 ad
Urine total N ₂ (g/day)	2.71 ± 0.8	1.56 ± 0.2	1.54 ± 0.15 ad
Urine total Urea (g/day)	2.25 ± 0.4	1.20 ± 0.7	1.14 ± 0.9 ad
Urine Creatinine (g/day)	0.19 ± 0.06	0.16 ± 0.06	0.15 ± 0.05 cd
Non Protein N ₂ (g/day)	0.12 ± 0.04	0.09 ± 0.04	0.08 ± 0.03 cd

a = Value are expressed as mean ± SD; n= 10 rats

b = Significant difference between all groups p<0.05 (one way analysis of Variance ANOVA)

c = Significant difference between groups 1 and 2 p<0.05 (t-test)

d = No significant difference between all groups p<0.05 (one way analysis of Variance ANOVA)

e = No Significant difference between groups 1 and 2 p<0.05 (t-test)

* Nitrogen digestibility % = $\frac{\text{Nitrogen Intake (g/day)} - \text{Faecal N}_2 \text{ (g/day)}}{\text{Nitrogen Intake (g/day)}}$

Liver Total Glycogen, Transaminase and Lipids in Kidneys and Spleen

Table 5 shows the effect of feeding raw shea nut diet and exfactory shea nut diet on liver total glycogen and transaminase as well as lipids in the kidney and spleen. Liver total glycogen (mg/g) was significantly different (p<0.05) at all levels between groups. Liver aspartate transaminase was significantly raised in rats fed the shea nut diet but the difference between the two groups on shea nut was not significant (p>0.05). Similarly, liver Alanine-Amino Transferase levels were raised in the exfactory shea nut diet, and no significant difference observed between the groups. Phospholipids in the kidneys were significantly lowered with feeding of the exfactory and raw shea nut meal but no cholesterol in the kidneys were raised with the shea nut meals. Levels of phospholipids and cholesterol of the shea nut were not different (p>0.05) between the two different groups studied.

Spleen phospholipids was also significantly reduced (p>0.05) in the rats fed on shea nut diet but there was no difference between the groups. The cholesterol level of the spleen was significantly higher in rats fed raw shea nut than the exfactory rats which did not differ significantly (p>0.05) with the control.

Table 5: Effect of Feeding Raw Shea Nut Meal and Exfactory Shea Nut Meal on Liver Total Glycogen, and Transaminases and Lipids in the Kidney and Spleen

Parameters	Control	Exfactory Shea Nut Meal (group 1)	Raw Shea Nut Meal (group 2)
Liver total Glycogen mg/g	34.0 ± 5.3	29.0 ± 4.7	12.0 ± 4.4 ab
Liver Aspartate	26.8 ± 3.4	47.2 ± 4.4	47.4 ± 3.9 ad
Transaminase U/g*	8.8 ± 1.2	12.1 ± 2.6 a	9.25 ± 1.2 cd
Kidney phospholipids mg/g	45.4 ± 4.2	38.1 ± 3.6	36.3 ± 3.4 ad
Kidney Cholesterol mg/g	8.87 ± 1.4	11.53 ± 3.1	10.2 ± 2.4 ad
Spleen Phospholipids mg/g	19.98 ± 2.4	14.53 ± 1.8	15.44 ± 1.7 ad
Spleen Cholesterol mg/g	2.59 ± 0.3	2.79 ± 0.64c	5.0 ± 1.2 ab

a = Value are expressed as mean ± SD; n = 10 rats

b = Significant difference between all groups p<0.05 (one way analysis of Variance ANOVA)

c = Significant difference between groups 1 and 2 p<0.05 (t-test)

d = No significant difference between all groups p<0.05 (one way analysis of Variance ANOVA)

e = No Significant difference between groups 1 and 2 p<0.05 (t-test)

* Unit of enzyme activity is that amount which catalyzes the transformation of micromole of the substrate per minute under standard conditions (expressed as unit/gm organ).

DISCUSSION

The main aim of this study is to evaluate the effect of feeding whole shea nut diet as well as the exfactory shea nut waste (defatted meal) as food on the biochemical characteristics necessary for metabolism in male albino rats and its adequacy as well as suitability as a supplementary for foods since the chemical composition is not adequate enough to evaluate its nutrient quality (Dei, *et al.*, 2007).

The evaluation of the food value of shea nut is seen as the responses of the body both in composition and in calorie status intake.

The general trend of weight loss in the shea nut diet compared to the control clearly shows that there was calorie inadequacy resulting in reduced food intake (Samaras, *et al.*, 2007). The reduction in weight loss also resulted in lowered organ weights like the liver, spleen and heart. The brain and heart was not considerably affected.

Serum total protein and albumin have always been indicators of the protein nutritional status (Banh, 2006). These indicators were found to be significantly reduced in rats fed the exfactory and shea nut diets. This is an indication of reduced protein intake. A reduction in protein intake has been observed to be rapidly followed by a decrease in the synthetic rate of albumin and since catabolic rate is not directly affected dietary protein intake or synthesis (Munro, 1964), thus the fall in serum protein and albumin indicates that the reduced nitrogen intake is responsible for the fall in urinary nitrogen output of the rats on shea nut diets compared to the control. This fall could be brought about by a decrease in the proportion of the amino acid flux that is metabolised to urea (Munro, 1964). The serum urea concentration and urinary urea output were both markedly reduced in rats fed shea nut diets (both raw and exfactory). Urea is used to measure the level of total nitrogen in tissues. It is well known that as protein intake decreases, it will result in low urinary total nitrogen (Eggum, 1973).

A close association between body weight and daily creatinine excretion has long been recognised and is used to predict muscle mass (Forbes and Bruining, 1976). This was found to be decreased in rats fed exfactory and raw shea nut diets, indicating a decreased metabolism in the muscle.

Serum liver Glycogen was greatly reduced in rats fed the shea nut meals when compared to the control. This was further reflected in the liver glycogen store which was depleted by approximately 65% and 15% in the raw and exfactory shea nut diets respectively. The fall in glycogen store was more significant in the raw shea nut. This could be due to the very low level of carbohydrate in the diet (about 10.8%).

Serum cholesterol was raised while the phospholipids remained normal with the experimental diets. Similar observation has been observed in malnourished children (Schelp, *et al.*, 1976).

Sodium, potassium and calcium are some of the important inorganic elements occurring in measurable large amount determined, which have biochemical and physiological inter relation to the evaluation of food value (Forbes and Bruining, 1976). Serum levels of these minerals were not significantly influenced.

The increased transaminase both in the serum and liver is assumed to be an indication of increased gluconeogenesis to furnish energy for maintenance of the rats (Heard, *et al.*, 1971). It is also an indication of the quality of protein in the foodstuff. As protein quality of foodstuffs decreases, transaminases activities in the serum and liver increases (Weber, *et al.*, 1969).

Total nitrogen intake was greatly reduced in rats fed the exfactory shea nut and raw shea nut diets. This is due mainly to the great reduction in food intake (Sonaka, *et al.*, 1993). The effect of the reduced nitrogen intake was reflected on the faecal nitrogen loss. Also, the percentage of absorbed nitrogen (% digestibility) was also low being 68.3% and 65% for exfactory and raw shea nut diets respectively as compared to the control of 8.2%. Due to the low nitrogen intake, the urinary nitrogen of the rats on the exfactory and raw shea nut diets was markedly reduced when compared to the rats on the control diet. However, the percentage urinary excretion of nitrogen intake was significantly higher at 87% and 87.5% for the exfactory and raw shea nut diets respectively than for the control (60.2%). This thus implies a strain on the nitrogen status of the animals on the exfactory and raw shea nuts diets.

The above observation along with excessive loss of hair would account for a negative nitrogen balance in the experimental rats, thus explaining the weight loss.

CONCLUSION

Results from this study indicate a situation of severe nutrient restriction resulting in malnutrition for the animals. The biochemical indices depict a poor response of the rats to both the exfactory and raw shea nut diets as food.

It is therefore recommended that for the purpose of utilization as a livestock feed, the shea nut meals must be compounded with other cereals or food to compliment its efficiency.

REFERENCES

- AOAC, 1997. Official methods of analysis of the Association of Analytical Chemists, 16th ed. Washington D.C.
- Banh, L., 2006. Serum Proteins as Markers of Nutrition: What Are We Treating? *P. Gastr.*, 43: 46-64.
- Dei, H.K., S.P. Rose, and A.M. Mackenzie, 2007. Shea nut (*Vitellaria paradoxa*) meal as a feed ingredient for poultry. *W. Poul. Sci. J.* 2007; 63:611-624.
- Eggum, B.O., 1970. Blood urea measurement as a technique for assessing protein quality. *Brit. J. Nutr.*, 24: 983-1012.
- Eggum, B.O., 1973. A study of certain factors influencing utilization in rats and pigs. *National institute of Animal Science, Copenhagen.*, 406: 121-135.
- Forbes, F.G. and M.D. Bruining, 1976. Urinary creatinine excretion and lean body mass. *Am. J. Clin. Nutr.*, 29: 1359-1366.
- Harris, E.K., and D.C. Demets, 1972. Method for the estimation of alkaline phosphatase activities in cow kidney extract. *Clin. Chem.*, 18: 602-605.
- Heard, C.R.C., S.M. Frangi, P.M. Wright and P.R. McCartney, 1971. Biochemical characteristics of the different forms of protein energy malnutrition on experimental model using young rats. *Brit. J. Nutr.*, 37: 1-21.
- Munro, H.N., 1964. General aspects of the regulation of protein metabolism by diet and by hormones. In: Munro HN and Allison JB (Eds). *Mammalian Protein*. Academic Press, New York.
- National Research Council, 2007. *Lost Crops of Africa: Vol. II: Vegetables*. National Academies Press.
- Reitman, S., and S. Frankel, 1957. A colorimetric method for the determination of serum Glutamic Oxaloacetic and Glutamic pyruvic transaminases. *Am. J. Clin. Path.*, 28: 56-83
- Samaras, T., A. Bartke and C.D. Rollo, 2007. *Human Body Size and the Laws of Scaling*. Nova Science Publishers. pp: 173-174.
- Schelp, F.P., P. Migasena, S. Saovakontha, P. Pongpaew and V. Supawan, 1976. Serum protein fractions from children of differing nutritional status analysed by polyacrylamide gel electrophoresis and electro immunoassay. *J. Nutr.* 1976; 35: 211-222.
- Sonaka, I., Y. Futami, T. Kobayashi, T. Umezawa and T. Maki, 1993. Effects of dietary protein restriction on nitrogen balance and cardiovascular functions in aged rats. *J. Gerontol.*, 48(4):B145-B150.
- Weber, C.W., W.P. Bermis, J.W. Berry, A.J. Deutschman, and B.L. Reid, 1969. Protein evaluation of two species of *Cucurbita* seeds. *Proc. Soc. Exp. Biol. Med.*, 130: 761.
- World Health Organization, Food and Agriculture Organization of the United Nations, and United Nations University, 2007. *Protein and amino acid requirements in human nutrition*. WHO Press.
- Zlatkis, A., B. Zak and A.J. Boyle, 1953. A new method for the determination of serum cholesterol. *J. Lab. Clin. Med.*, 41: 386.