INTRODUCTION

Food is the most basic necessity of life. Its purpose is to nourish the body, mind and soul (Turner, 2006). Processing food involves subjecting it to controlled conditions which helps to improve its safety, shelf life and to transform certain properties; however, it can produce desirable and undesirable effects (Ezeokonkwo, 2007). Heating is known to significantly reduce the effects of antinutrients and other factors which are not necessarily antinutrients in the food substances (Enujiugha, 2003). Heating also destroys microbes and inactivates enzymes and toxins (Henry and Hepell, 2002).

Tetracarpidium conophorum (conophor nut) commonly called the African walnut is a perennial climbing shrub found in the forest zones of sub-sahara Africa (Oluwole and Okusanya, 1993). It is a species in the tropical genus Tetracarpidium of the family Euphorbiaceae. The plants are cultivated principally for the nuts which are cooked or roasted and consumed as snacks. The nut is known as ukpa in the Igbo speaking tribes and awuse or asala in the Yoruba tribes in Nigeria. In Cameroon, it is known as kaso or ngak (Ajaiyeoba and Fadare, 2006). It takes about 15 days to germinate. The freshly harvested mature nuts are green in colour and turns dark brown when left to age. The nuts are encased in pods which contain four (4), three (3), or two (2) nuts per pod. The seed is made up of two cotyledons enclosed in a hard brown shell- like case within the pods.

In the western part of Nigeria, the nuts are harvested, boiled or roasted and served to visitors as snacks. The leaves and young shoots are known to be edible (FAO, 2006). The nut extracts has been reported to have antimicrobial and antifungal activities (Ajaiyeoba and Fadare, 2006).

Considering the effects of food processing on the nutrient quality of food substances, this work was undertaken to elucidate the impact of dry heated (roasted) T. conophorum based diet on some haematological parameters, liver and renal function biomarkers in male wister rats.

MATERIALS AND METHODS

All chemicals and biochemicals used were purchased from Sigma chemicals, St Louis USA, as analytical grade. The ingredients for diet formulation were bought from Harlan Teklad Inc, Madison, Wisconsin USA. Kits for evaluation of liver and renal functions were purchased from RANDOX laboratory Ltd, Ardmore, Diamond road, Crumin Co, Antrim UK. Kits for electrolyte estimation were purchased from TECO Diagnostics 1268 N.Lakeview Ave, Anaheim.CA 92807, USA.

Sample Collection

Fresh and mature conophor nuts were purchased from Ibeagwa market, a small market near the University of Nigeria, Nsukka. The fresh dehulled nuts were roasted in hot sand (100°C) for 40 minutes with constant stirring. The nuts were shelled, macerated and dried at 60°C after which they were ground and stored with freezer bags in a refrigerator at 4°C until used.

Proximate Analysis

The proximate composition analysis of roasted T.
conophorum nut was carried out using the standard procedures of AOAC by micro-Kjeldhal method and crude protein content calculated as N × 6.25.

Nutritional Evaluation

The diets were formulated using AIN 93G method for growth phase in laboratory rats (Reeves et al., 1993). Two main diets were used- Caesin based diet (CAS) which served as the control and the roasted T. conophorum diet (RTC). The diets were composed to provide 10% protein.

Feeding Experiment

Twelve weanling male albino rats supplied by the Veterinary Department of the University of Nigeria, Nsukka were divided into two groups of six rats each. The groups included, casein based diet (CAS) group, and Roasted Tetracarpidium conophorum based diet (RTC) group. They were housed in individual metabolic cages equipped to separate faeces and urine. After a six day adjustment period, the rats were weighed prior to access to the test diets. 15g diet /rat /day were fed to the test group and to the casein group for 35 days. There was daily urinary collection throughout the last seven days of the feeding period. The urine was pooled together for each group, measured, and treated with few drops of 2.5N HCl and stored with air.

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Alanine Transaminase (ALT)

Kit reagents

Buffer (phosphate buffer, L – alanine, α - oxoglutarate), 2, 4-dinitrophenylhydrazine, sodium hydroxide solution.

Aspartate Transaminase (AST)

Kit reagents

Buffer (phosphate buffer, L-aspartate, α -oxoglutarate), 2,4-dinitrophenylhydrazine, sodium hydroxide solution.

Urea

Kit reagents

R₃ solution (Urease-1g/l, EDTA-116mmol, sodium nitroprusside -6mmol/l), R₂ (diluted phenol-120mmol/l), R₁ (diluted sodium hypochorite-27mmol/l and sodium hydroxide-0.14N), CAL (standard-13.3mmol/l).

Table 1 Diets based on caesin and BTC nut flours as source of protein. Diet composition (g/kg)

<table>
<thead>
<tr>
<th>DIET INGREDIENT</th>
<th>DIET GROUPS</th>
<th>CAS</th>
<th>RTC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>114.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>RTC</td>
<td>-</td>
<td>415.22</td>
<td>-</td>
</tr>
<tr>
<td>Cornstarch</td>
<td>466.469</td>
<td>225.90</td>
<td>56.48</td>
</tr>
<tr>
<td>Sucrose</td>
<td>116.617</td>
<td>56.48</td>
<td>-</td>
</tr>
<tr>
<td>Fibre</td>
<td>50.0</td>
<td>50.0</td>
<td>-</td>
</tr>
<tr>
<td>Soy Bean Oil</td>
<td>70.0</td>
<td>70.0</td>
<td>-</td>
</tr>
<tr>
<td>Mineral mix</td>
<td>35.0</td>
<td>35.0</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin Mix</td>
<td>10.0</td>
<td>10.0</td>
<td>-</td>
</tr>
<tr>
<td>L-cystein</td>
<td>3.0</td>
<td>3.0</td>
<td>-</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2.5</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>BHT</td>
<td>0.014</td>
<td>0.014</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1. Diets based on cascin and BTC nut flours as source of protein. Diet composition (g/kg).

CAS- Caesin, RTC- Roasted T. conophorum, BHT- Butylated Hydroxytoluene. AIN-93G diet recommendations (Reeves et al., 1993).

Calculation

Urea concentration \(= \frac{A_{\text{sample}}}{A_{\text{stand}} \times 279.3} \)
Urea concentration $= \frac{A_{\text{Sample}}}{A_{S\ \text{tan}\ \text{dard}}} \times 279.3$ mmol/l

Urea concentration $= \frac{A_{\text{Sample}}}{A_{S\ \text{tan}\ \text{dard}}} \times 16.8$ (g/l)

(*) RANNOX conversion factor

Creatinine

Kit reagents

CAL (Standard), R1a (picric acid), R1b (Sodium hydroxide), trichloroacetic acid.

Method

Three clean test tubes were labeled blank, standard and sample respectively. Distilled water (0.5ml) was added to the blank test tube, 0.5ml CAL was added to the standard test tube, 0.5ml TCA was added into all the tubes. Urine (1ml) was diluted in 49ml of distilled water and 0.5ml was removed and added into the sample test tube. Equal volume of R1a and R1b was mixed together and 1ml of the mixture was pipetted into all the tubes. The solutions were mixed and allowed to stand for 20 minutes at 25°C. The absorbance of the sample (A sample) and (A standard) was read against the blank at 546nm.

$$\frac{A_{\text{Sample}}}{A_{S\ \text{tan}\ \text{dard}}} \times 8.85 = \text{mmol/L}$$

$$\frac{A_{\text{Sample}}}{A_{S\ \text{tan}\ \text{dard}}} \times 100 = \text{mmol/dl}$$

(*) RANNOX conversion factors.

Packed Cell Volume

The fresh blood samples were collected in capillary tubes and placed in haematocrit machine. The samples were centrifuged for fifteen minutes and the packed red blood cells were read off from a standard table.

Random Blood Sugar

Glucometer was used to take the readings of the blood samples of each rat in the different groups.

Electrolytes

Sodium

Kit reagents

Filtrate reagent (uranyl acetate, magnesium acetate, ethyl alcohol), acid reagent (diluted acetic acid), sodium colour reagent (potassium ferrocyanide, non-reactive stabilizers and fillers), sodium standard (sodium chloride solution).

Method

Four clean test tubes were labeled blank, standard, control and sample. Filtrate reagent (1ml) was pipetted into all tubes; 50µl of sample was pipetted into all tubes; sodium standard to the standard test tube and distilled water to the blank. The tubes were mixed vigorously and continuously for 3 minutes. The tubes were centrifuged at high speed of 1,500g for 10minutes. Four other test tubes were labelled as above, 1ml of acid reagent was pipetted into all tubes, 50µl of supernatant from the spun tubes were pipetted to their corresponding tubes and mixed. 50µl of colour reagent was added to all tubes and mixed. The spectrophotometer was zeroed with distilled water at 550nm and the absorbance of all tubes were read.

Calculation

$$\frac{\text{Absorbance of Blank} - \text{Absorbance of Sample}}{\text{Absorbance of Blank} - \text{Absorbance of Standard}} \times \text{Conc. of Standard} = \text{Conc. of Sample}$$

Potassium

Kit reagents

Potassium reagent (sodium tetraphenylboron 2.1mM, preservatives and thickening agents), potassium standard.

Method

Three test tubes were labeled standard, control and sample. Potassium reagent (1ml) was pipetted into all tubes, 0.01ml of sample was added to sample test tube and 0.01ml distilled water was added to the control. The solutions were mixed and left to stand at room temperature for 3 minutes. After 3 minutes, the spectrophotometer was set at 500nm and zeroed with the control. The absorbances of all tubes were recorded.

Calculation

$$\frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \times \text{Conc. of Standard (MEq/l)} = \text{Potassium concentration (MEq/l)}$$

Chloride

Kit reagents

Chloride reagent (Mercuric nitrate, mercuric thiocyanate, mercuric chloride, ferric nitrate, non-reactive ingredients, stabilizers in dilute acid and methanol), chloride calibrator (sodium chloride).

Method

Three test tubes were labeled blank, calibrator and sample respectively. Chloride reagent (1.5ml) was pipetted into each tube.
Chloride calibrator (0.01ml), was pipetted into the calibrator tube and distilled water (0.01ml) into the blank tube and mixed. They were all incubated at room temperature for 5 minutes. The spectrophotometer was set at 480nm and zeroed with blank. The absorbance of all tubes were recorded.

Calculation

\[
\text{Concentration of chloride (mEq/l)} = \frac{\text{Absorbance of Sample} \times \text{Conc. of Standard (MEq/l)}}{\text{Absorbance of Standard}}
\]

Statistical analysis

The data collected were analyzed using SPSS (version 12.0) analytical package. One way analysis of variance (ANOVA) and Fisher’s least significant difference (F-LSD) were used to separate the means. Results are presented as mean ± standard deviation of all parameters determined.

RESULTS

Proximate composition

The results of the proximate composition of *T. conophorum* (Fig. 1) which was subjected to roasting in comparison with the unprocessed nut (Fig. 1) showed that the crude protein content of the roasted nut (24.09%) was significantly different (p < 0.05) from that of the unprocessed nut (21.02%). The values for the crude fat, crude fibre and ash content of the RTC were higher and differed significantly (p <0.05) from those of the unprocessed (UTC) sample. The carbohydrate level (UTC-38.8%, RTC- 26.41%) as well as the ash content of the UTC were significantly higher (p <0.05) than those of the RTC samples.

Biochemical Assay

The rats fed CAS (control) diet had higher ALT and AST activities than those fed test diets (Fig. 2). The values differed significantly (p<0.05).

The renal function assays revealed that the serum levels of creatinine (Fig. 3) and urea (Fig. 4) for rats fed CAS diet were higher than those of rats fed test diets, however, the differences were significant (p<0.05) only in the urea levels.

The PCV levels of rats fed RTC diets were higher than those of the control (CAS) group while RBS values of rats fed CAS diet were higher but not significantly different (p> 0.05).

The differential white blood cell count showed comparable values; *Neutrophils*: for rats fed CAS diet (19.33 ± 3.88) and those of rats fed RTC diet had (19.17± 6.47). *Monocytes* of rats fed CAS diet was (1.50 ± 0.22) and those fed RTC diet had (1.50 ± 0.74). The values of *Lymphocytes* showed that rats fed CAS were higher than those fed RTC diet. None of the values differed from the other significantly (p>0.05). In the total white blood cell count, the rats fed RTC diet had a lower value than those of rats fed CAS diet.

DISCUSSION

The proximate composition of food crop is a major index of the nutritional potential of that crop. The proximate composition of unprocessed *T. Conophorum* nut (UTC) differed from those reported by other workers. Enujiugha (2003) reported *T conophorum* nut to have 29.09% (protein), 48.90% (oil), 12.58% (carbohydrate), 6.34% (fibre). Odoemelam (33002) reported 26.3% (protein), and 46.5% (fat). The FAO (2000) reported that the raw seed contained 22.7% crude protein, 56.0% fat (ether extract), 3.70% (fibre) and 9.10% carbohydrate. The results of the present work -20.7% (protein), 34.95% (fat, 36.81% (carbohydrate) and 1.62% (crude fibre) – were much lower than those of the RTC samples.

The proximate composition of *T. Conophorum*.
was comparable with that of groundnut (20.2%), and cotton seed (20.4%) but lower than those of cashew (21.4%) and tropical almond (25.81%) (FAO, 2000; Ezeokonkwo and Dodson, 2004). The higher fat content (34.95%) of *T. conophorum* than those of cotton (20.0%), soybean (10.0%), (FAO, 2000) suggests that it could be a potential source of vegetable oil. Its high fat as well as carbohydrate (36.81%) suggests that it could be a high energy plant. The crude fibre content of UTC (1.62%) is lower than that reported by FAO (3.7%) and Enujiugha (6.34%), the lower value could be attributed geographical location, varietal difference and growth conditions. This is a commonly observed phenomenon. The increases in the proximate composition of *T. conophorum* appear to be due to heat treatment. It is known that heat treatment hydrolyses some organic bonds to release more free nutrients such as protein, fat, minerals and sugars. The increases in protein could be due to reduction or destruction of certain protease inhibitors and other antinutrients such as tannins and phytic acid which form insoluble complexes with protein to make it unavailable. Enujiugha (2003), Obizoba and Atti (1994) had earlier observed this phenomenon. It is known that during heat treatment, plant food materials suffer considerable loss of low molecular weight carbohydrates (mono and disaccharides) due to leaching into processing water (FAO, 1998). The decrease in carbohydrate in the present work agrees with the report of Obizoba and Atti (1994) on pearl millet seeds.

**Biochemical studies - Liver function assessment.**

The lower levels of ALT of rats fed the test diet as compared with those of the rats fed the control diet (CAS) suggests that the test diet did not affect liver functioning adversely. It is generally known that an elevation of ALT is more specific for liver injury than that of AST (Kjeldsberg, 2000). More so, ALT is found primarily in liver and kidney while AST is found in high concentration in the heart muscles and low concentrations in liver (Jaeger and Hedegaard, 2002).

**Renal function assessment**

The nitrogen component of urea (blood urea nitrogen – BUN) is the end product of protein metabolism. Its concentration is known to influence the rate of BUN excretion. It is elevated in kidney damage, excessive protein intake and low fluid (Jaeger and Hedegaard, 2003). The negligible increase in levels of urea of rats fed RTC diets indicates that these diets had no adverse effect on protein metabolism. The normal creatinine levels of rats fed both control and test diets suggest that these diets did not precipitate kidney damage and protein starvation (Jaeger and Hedegaard, 2002). Urea and creatinine levels are basically used to determine functions of kidney. The normal levels of urea and creatinine excretion of rats fed processed *conophor* nut-based diets indicates that the diets had no adverse effects on kidney functions.

**Electrolytes**

Electrolytes are essential minerals critical to good health. They maintain fluid homeostasis by generating and conducting electrical impulses across cell membranes, nerve transmission, muscle function and cognition. The high Na, Cl, K levels for two groups of rats fed both control and test diets imply that these electrolyte balance was properly maintained in these rats fed these diets. The high K levels for rats fed control diet and similar values for rats fed RTC diet implies that processing had no adverse effect on K (Girvent, *et al.*, 2005).

**Haematological Parameters**

Hematocrit measures the percentage of red blood cells of total body blood. It is known that decreased PCV is indicative of
anaemia. The PCV levels of rats fed test diet that were within the normal range indicated that the rats fed test diets had adequate Fe and other nutrients needed to maintain homeostasis of haemoglobin.

The comparable blood glucose levels of rats fed CAS and RTC diets indicates that the test diets had no adverse effects on blood glucose. It is known that elevation of blood glucose level is indicative of low levels of insulin or damage to pancreas (Houten and Landaur, 2007). The total white blood count and the differential count helps to determine the state of the immune system of an organism. Disruption in the normal values predisposes one to pathogenic invasion. The comparable results noticed between the test diet and the control in the differential count indicates that the RTC diet supported
the biochemical processes leading to production and maintenance of the leucocytes. This is further buttressed by the results of the total white blood cell count, which showed that there were only numerical differences between the groups; however, they had no statistical significance (p > 0.05).

**Conclusion**

The absence of pathogenic tissues in rats fed processed diets (roasted) and the normal levels bioassay parameters seen suggests that *T. conophorum* contained no food toxicant. As seen by the results, *T. conophorum* nut has high food potentials that could be harnessed as source of dietary protein and needs wider use as a major contributor to our diets.

**REFERENCES**


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