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Biological Evaluation of *Thaumatococcus danielli* Waste Protein

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Abstract: Potential application of *T. danielli* waste as a raw material in livestock feed formulation had been suggested in earlier reports. In this study, the nutritional quality of protein in *Thaumatococcus danielli* seed and pericarp was evaluated using 16 male waster rats (20-25 g). The animals were randomly assigned to 4 groups and were maintained on four isocaloric diets, namely: casein, seed, pericarp and nitrogen-free diets, for 10 days. Daily weight gain and feed intake over the last 7 days were measured. Feacal, carcass, and feed nitrogen contents were also determined at the end of the feeding trial. Protein Efficiency Ratio (PER), Net Protein Retention (NPR), Biological Value (BV), Net Protein Utilization (NPU) and True Digestibility (TD), were thereafter calculated. Mean weight gain was significantly lower ($p>0.05$) in the rats fed the *T. danielli* pericarp feed (-2.60±0.80 g) than those placed on the seed (5.46±0.71 g). PER (-2.11±0.09); TD (59.37±1.72%); BV (16.96±1.61%); NPU (76.13±1.01%); and NPR (2.53±0.22) values were also significantly lower ($p<0.01$) in the pericarp than in the seed (2.19±0.23, 82.02±1.25%, 58.04±11.54%, 90.86±1.25% and 4.54±0.43 respectively). The quality of *T. danielli* seed protein compared favourably with that of the casein based diet. There was no significant difference ($p>0.05$) in the BV, PER, and NPU values between the casein based and the *T. danielli* based diets. The NPR value of the seed was significantly higher ($p<0.05$) compared to the casein diet.

Key words: *Thaumatococcus danielli*, protein quality, under-utilized crops, waste utilization

INTRODUCTION

Cereal grains are important index of food adequacy. This is because, worldwide, they account for about half of all food energy. While much grain is consumed directly, an increasing proportion (about one-third) is consumed via its rather inefficient conversion to meat, eggs and dairy products (Mc Michael, 2005). Meat is widely viewed as a desirable, high quality component of diets, hence, an increase in meat consumption is a central feature of the "nutrition transition" (Popkin, 2003). However, producing sufficient meat for a future global urban population of 5-6 million poses a major challenge to environmental sustainability. Domestication of animals as a food source generates among other things, the problem of substantial energy loss due to diversion of cereal grains from humans to animals (Mc Michael, 2005). The statistics for food-energy losses and water requirements for production of feed-lot and factory-farmed livestock are very high (Popkin, 2003; Flannery, 1994). To produce 1 kg of feed-lot beef requires about 9 kg of cereal grain, for pork the ratio is 4:1 and for chicken 2:1. These losses are mostly borne by poorer populations in lower-income countries that strive to generate foreign exchange by exporting feed grains rather than growing feed-grains for local consumption. Hence, suitable replacement for cereal grains must be developed if this increasing demand for meat protein must be sustained. The exploitation and development of such potential non-conventional crops as protein source for human beings/animals may offer a good scope to meet the increasing protein requirements at large, particularly in the developing countries. However, before such indigenous foodstuffs can be recommended, their nutritional properties and biological value should be thoroughly investigated (Pugalenthi *et al*., 2007).

One of the many underutilized plants in Nigeria is *Thaumatococcus danielli*, a non sacchariferous sweet plant which is common throughout the West African rain forest zone. In Nigeria, the plant grows predominantly in the cocoa-growing areas of the South-West, where it is called "Ewe-Eran" or "Adundunmitan" (Elemo *et al*., 1999). The arils of the seed contain an intensely sweet protein, thaumatin which is about 2000-3000 times the sweetness of an 8-10% sucrose solution on a weight basis (Higginbotham, 1979). Extraction of thaumatin from the arils of *T. danielli* leads to the generation of substantial waste, largely made up of the seed and pulp of the fruit, and which constitute over 99% of the entire fruit weight (Elemo *et al*., 1999a). Potential application of *T. danielli* waste as a raw material in livestock feed formulation was suggested in earlier reports (Elemo *et al*., 1999a; 2001). The seed is relatively high in protein, starch and minerals, especially calcium and magnesium (Elemo *et al*., 1999a; 1999b). Preliminary toxicological studies have indicated the presence of some antinutrients, particularly, tannins and trypsin inhibitors which could however be inactivated by simple processing methods (Elemo *et al*., 1998).

This study was therefore intended to evaluate the nutritional quality of *T. danielli* waste protein using an animal model.

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MATERIALS AND METHODS

Sample preparation: *Thaumatococcus danielli* (Benth) fruits were collected from Sagamu, Ogun state and Ogotun, Ekiti state. The fruits were washed, thoroughly drained and manually cut open with a scarpel to separate the pericarp and the seed. The aril was excised as well as the gelatinous membrane on the seed. The seed and pericarp were dried separately in a Gallenkamp 300 Plus oven at 60-80°C for 72 h. Thereafter, they were blended into powder with a National (MX-89) blender to pass through a 200-300 µm pore sized sieve. The seed was taken in a seed to water ratio of 1:10 (w/v) in a metal container and autoclaved at 15 lb pressure (121°C) for 30 min and stored in an air tight plastic container at -2°C.

Formulation of diets: Four different diets were prepared according to the method of Campbell (1963), as shown in (Table 1).

Animals: Sixteen male weanling rats of Wistar strain (aged 20-23 days) were obtained from the animal centre of the College of Medicine, University of Lagos, Idiaraba. The animals weighing 25-35 g were randomly assigned to four groups and kept in galvanized, wired screen metabolic cages. The animals were weaned on standard laboratory diet (Ladoke Feeds, Ibadan) for 1 wk so that at the beginning of the experiment, the animals were 28-30 days old. At the end of 1 wk, the animals were weighed and started on the different experimental diets for 28 days. Group 1 was fed the basal diet; Group 2 was fed the casein based diets and Groups 3 and 4 were assigned the seed and pericarp based diets respectively. Feeding for the first 3 days was regarded as the acclimatization period so no record of food consumption or collection of faeces or urine was taken. After the third day, daily records of weight changes, food consumption, as well as 24 h faecal and urine output were taken throughout the period of the experiment. The collected faeces and uneaten food were dried at 105°C for 48 h and ground into fine powder for nitrogen determination.

At the end of the 28 days, the animals were weighed, killed with chloroform and incisions were made on the skull, thoracic and body cavities. The carcasses, including the intestinal content were weighed before and after drying in an oven (Gallenkamp 300 plus) for 48 h at 105°C. The dried carcasses were then run 3 times through a kitchen mincer and kept at -4°C for nitrogen determination.

Analysis: Body water was determined by difference between the fresh and oven-dried weight of the carcass. Carcass, feed and faecal nitrogen was determined by the micro kjeldhal method (AOAC, 1990).

Protein quality determination: Protein Efficiency Ratio (PER), Biological Value (BV), Net Protein Utilization (NPU), Net Protein Retention (NPR) and True Digestibility (TD) were calculated as described by Campbell (1963).

Statistical analysis: Values were expressed as the mean and standard deviation of the four animals in each experimental group. Comparison of means was done using one way analysis of variance (ANOVA) and the Fisher least significant difference was used for *posteriori* test of significance (SPSS version 11).

RESULTS

Figure 1 showed the daily feed intake of rats fed *T. danielli* pericarp and seed diets during the first 7 days. Daily body weight changes in rats fed *T. danielli* pericarp and seed diets are shown in Fig. 2. Animals placed on casein- and *T. danielli* seed- based diets had weight gains ($p<0.01$) compared to the pericarp and basal diets which recorded weight losses. Table 2 showed the growth performance of rats placed on the different diets. There was no significant difference ($p>0.05$) in Protein Efficiency Ratio (PER) between the seed (2.19±0.46) and casein (2.55±0.26) diets. The pericarp diet was however highly significantly lower ($p<0.001$) with a PER of (-2.11±0.18). The seed based diet had the highest Net Protein Retention (NPR) (4.54±0.86) compared to the casein (3.66±0.38) and pericarp (2.53±0.43) diets. There was no significant difference in the Net Protein Utilization (NPU) ($p>0.05$) between the casein (93.10±0.65%) and the seed (90.86±2.50%). Both were however higher than

Table 1: Composition of diet (g)

Ingredient	Basal diet	Casein diet	Seed diet	Pericarp diet
Corn starch	650	550	-	-
Casein	-	-	-	-
Cellulose	50	50	50	50
Cotton seed oil	100	100	100	100
Mineral mix	40	40	40	40
Vitamin mix	10	10	10	10
T. danielli seed	-	-	105.3	-
T. danielli pericarp	-	-	-	222.2
Glucose	50	50	50	50
Sucrose	100	100	100	100

Table 2: Food intake, gain in body weight, Net Protein Retention (NPR), True Digestibility (TD), Biological Value (BV) and Net Protein Utilization (NPU) of casein and *T.danielli* seed and pericarp fed to albino rats (n = 4, mean±SD)

			T. danielli	
Assay	Basal	Casein	Seed	Pericarp
Weight gain for 10 d (g)	5.75±0.10[a]	13.10±0.76[b]	7.46±1.43[b]	-2.76±0.16[c]
BV (%)		70.17±7.73[a]	58.04±23.07[a]	16.96±3.22[b]
TD (%)		89.09±1.04[a]	82.32±4.84[b]	59.37±3.44[c]
NPU (%)		93.10±0.65[a]	90.86±2.50[a]	76.13±2.02[b]
NPR		3.66±0.38[a]	4.54±0.86[b]	2.53±0.43[c]
PER		2.55±0.26[a]	2.19±0.46[a]	-2.11±0.18[b]
Total feed intake for 10 d (g)	195.26±4.03[a]	421.13±4.83[b]	272.60±5.29[a]	214.90±5.40[a]

[abc]means in the same row for each parameter with different superscripts are significantly different (p<0.05)

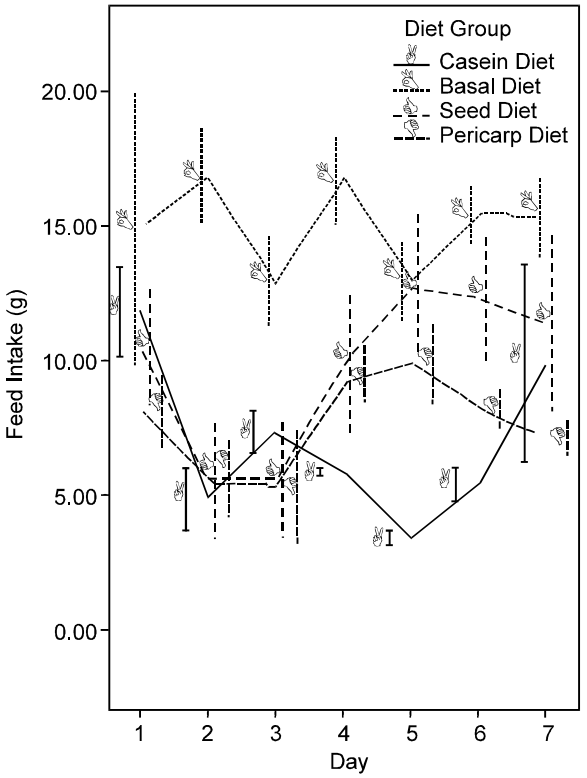

Fig. 1: Daily feed intake of rats fed *T. danielli* pericarp and seed diets

the pericarp (76.13±1.01%). The Biological Value (BV) and True Digestibility (TD) of the seed based diet (58.04±23.07% and 82.32±4.84% respectively) were also comparable to those of the casein diet (70.17±7.73 and 89.09±1.04% respectively).

DISCUSSION

The nutritional quality of any protein relates to its amino acid composition, digestibility and ability to supply the essential amino acids in the amounts required by the species consuming the protein (Satterlee *et al*., 1979). But while protein quality is dependent on the amino acid composition, efficiency of utilization is dependent not only on the quality and quantity of the protein in the diet, but also on the adequacy of the total diet, environmental conditions, age and physiological state of the recipient (FAO/WHO/UNU, 1985). This efficiency also has direct implications for the commercial value of the protein product (Boutrif, 1991).

The mean nitrogen intake of the different diets was a reflection of the feed intake by the animals in the different groups. Daily feed intake (Fig. 1) was relatively constant in the casein diet group over the first 7-day period of the experiment. However, in the other groups there was an initial drop in feed intake over the first 3 days, for the seed and pericarp groups and 6 days for the basal group. Animals fed the casein-based diet had significantly higher mean feed intake (15.04±4.38 g) compared to those on other diets. There was however no difference in the mean feed intake among the basal, seed and pericarp diets (6.74±4.03 g, 9.74±5.29 g and 7.68±3.32 g respectively). The feed intake might have been influenced by the palatability of the diet.

The body weight change of the animals over the 28-day period (Fig. 2) was highest in those fed casein diet (13.10±0.05 g), followed by the *T. danielli* seed diet (5.46±0.71 g). The pericarp and the basal diet-fed animals on the other hand had weight losses (-2.60±0.8 g and -5.75±0.38 g respectively).

The PER of *T. danielli* seed compared favourably with that of the casein diet but that of the pericarp was significantly lower. Protein Efficiency Ratio (PER) measures the ability of a protein to support the growth of a weanling rat; it represents the ratio of weight gain to the amount of protein consumed. However, one of the drawbacks of this method of protein quality assessment is that the PER measures growth but not maintenance (Sarwar and McDonough, 1990), therefore it may be of limited use in determining the protein needs of adult animals.

The Net Protein Retention (NPR) which is an improvement over PER (Pellet and Young, 1980) and therefore a better predictor of protein quality was significantly higher in *T. danielli seed* compared to the casein and pericarp diets.

Biological Value (BV) measures the amount of nitrogen retained in comparison to the amount of nitrogen

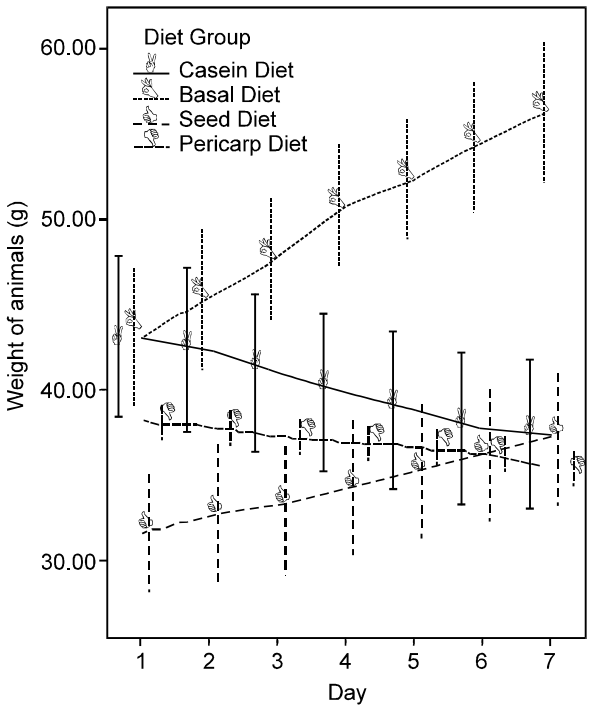

Fig. 2: Daily body weight changes in rats fed *T. danielli* pericarp and seed diets

absorbed. The BV of *T. danielli* seed was also comparable to that of casein, but that of the pericarp was significantly low.

Nitrogen Protein Utilization (NPU) of the *T. danielli* seed was very high and also compared favourably with the casein diet. The NPU of the *T. danielli* pericarp on the other hand was very low (16.86±1.61). NPU reflects the ratio of the nitrogen used for tissue formation versus the amount of nitrogen digested. The BV and the NPU methods reflect both availability and digestibility and they give an accurate appraisal of maintenance needs of the animal (Pellet and Young, 1980; Schaafsma, 1997).

True Digestibility (TD) value of *T. danielli* waste also indicated that the seed was better digested than the pericarp. The TD of the seed was not different from that of the casein diet. Protein quality determination based on nitrogen retention; include BV, NPU and TD methods. Conventionally, protein is fed at a level of 100 g/kg (10 per cent) of the diet and the result is designated NPU10. This conventional level permits comparisons among different proteins, although it is accepted that utilization is higher at lower levels of feeding and decreases as the dietary level is increased. On the other hand, this procedure provides a measure of TD that may be independent of the dietary protein level (Eggum, 1973).

On the whole, *T. danielli* seed protein had far better qualities than the pericarp and the seed protein was comparable to that of casein. Previous studies (Elemo *et al*., 1999) showed that *T. danielli* seed and pericarp contained 9.5% and 4.2% crude protein respectively and carbohydrate content of 69.4% and 6.3% respectively. Judging by the inclusion rate of both seed and pericarp in the experimental feed, there is every indication that the seed was able to meet both energy and protein needs of the animals. A possible explanation for the poor showing of the pericarp may be due to the fact that the inclusion of such a large quantity of pericarp needed to provide 10% protein in the diet might have also introduced excessive fiber into the diet. High fiber content interferes with digestion and bioavailability of proteins (Bhagya *et al*., 2006; Pugalenthi *et al*., 2007). The process of autoclaving might have contributed greatly to the high digestibility and bioavailability of the seed protein (Pugalenthi *et al*., 2007, Vijayakumari *et al*., 2007).

Conclusion:

- *Thaumatococcus danielli* is an under-utilized plant with great economic potential.
- The seed and pericarp of *T. danielli* represent over 99% of its weight and these constitute waste generated from processing of the fruit.
- An evaluation of the quality of the seed and pulp indicated that the seed of *T. danielli* had better quality protein compared to the pericarp.
- The quality of the seed protein compared favourably with that of casein.
- With adequate processing (autoclaving), the seed could serve as a good source of protein and energy for livestock.

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