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Nutritional value of rubber seed flour for poultry feed-stock, using adult insect *Tribolium castaneum* as a model

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ABSTRACT

The effect of raw flour of rubber (Hevae brasilensis) seeds on the growth and development of Tribolium castaneum was investigated, using some biochemical and physiological end point parameters. The rubber seed, obtained from Nigeria was ground to its flour and used as feed, unsupplemeted, for the insects. Cassava and wheat flour were also used for another set of the insects as the control. Analysis of total protein, carbohydrates and lipids in the homogenates of the insects after 15 weeks of rearing, show that is no significant differences (p>0.05) in the levels (mg/ml) of those nutrient in the insects when comparing the type of food in which those insects were reared. Also there was no significant difference in weight gains for these insects reared in rubber seed cake, wheat flour or cassava flour. We therefore suggest that rubber seed flour can be used as a cheap supplement in the formulation of animal or poultry feeds.

Keywords: Rubber seed, nutritional toxicity, *Tribolum casteneum*, poultry feed-stock.

INTRODUCTION

Insect models have been used in several food toxicity tests. For example the Housefly (*Musca domestica*) and cabbage looper moth (*Trichoplusia ni*) have been used to assess heavy metal toxicity in food and animal feed [1]. The sensitivity of flour beetles to shortages of nutrient has been put to advantage in assaying the values of various diets. Some agronomic crops contain anti-metabolites, which reduce their nutritional value [2]. These “inhibitors’ bind tightly to the proteolytic enzymes, thus causing indigestion and poor utilization of the nutrients.

The insect, *Tribolium castaneum* enjoys a wide range of food habits and conditions such as temperature and moisture. It has, however a high ability to survive starvation. The insect is generally of small size and therefore could not easily be detected until a sizeable population is built up. tribolium species are among the most numerous of stored-products insect pests, the two most common being *T. castaneum* (the flour beetle) and *T. confusm* (confused flour beetle). According to some reports[3,4], *T castaneum* (red flour beetle) is associated with a vast range of stored-food products in Nigeria and they include cereals, yam flour, cassava flour cowpea, groundnuts, maize, sorghum, rice, soybean.

The possibility of using *T. castaneum* as test organism for evaluating the nutritive value of different samples of maize has been tested. The data obtained agreed well with relative biological values of the samples as determined with rats. The general nutritional requirements of beetles are in many ways similar to those of mammals, and as such they have been used for biological assessment of nutritional values of certain foodstuff for man and livestock [5]. The flour beetles are especially suitable for this purpose due to their wide distribution and the ease of rearing on synthetic diet [6], a task which is accomplished at a great cost with rodents.

This study is aimed at evaluating the *in vivo* toxicity and nutritional value of rubber seed flour for the possibility of using it as food supplement to the poultry and animal feeds.

MATERIALS AND METHODS

Rubber Seed

Rubber (*Havea brasiliensis*) seeds were obtained from the faculty of Agriculture Rubber Plantation of the University of Nigeria, Nsukka. They were dried in an oven at 45^0C for 72 hrs and shelled. The kernels were milled using attrition mill, and the cake prepared by adding a little quantity of distilled water to a specified weight.

Collection and rearing of insects.

Adult insects, *Tribolum castaneum* were collected from infected rubber seeds. They were picked at random and placed inside plastic dimension vials maintained in our laboratory at temperature of 27 + 2^oC, with a relative humidity of 75%. The containers were covered with net gauze to prevent escape of the insects. They were starved for 24 hours prior to their feeding on the test diets.

Experimental procedure

Several batches of the starved insects, each numbering 500 were randomly picked into different plastic containers in which were placed rubber seed cake, wheat flour cake or cassava flour cake. At a weekly interval, 50 insects were at randomly picked from these containers and separately homogenized with pestle and mortar in 5 ml of 0.05M phosphate buffer (pH 7.4). Sample for lipid assay were homogenized in chloroform-ethanol mixture (1.1) for 5 min.

Quantitative analysis of protein

The homogenate (in the phosphate buffer) was centrifuged at 850g for 15 min and the sediment consisting of chunks of cuticle and cellular debris were washed in the buffer and the washing added to the supernatant the nucleic acid. After 15 mins. of standing, the mixture was centrifuged at 15,000g for 20 mins and the pellets discarded. Equal volume (5 ml) of trichloroacetic acid (TCA) was added and centrifuged again at the same speed and time. The supernatant was discarded and the sediment (pellets) suspended in the buffer after adjusting the pH to 6.3. Thereafter, 0.2 ml of 0.1M NaOH was added. The tubes were heated in a water bath at 85^oC for 3 hours. The tubes were cooled to room temperature, centrifuged and the supernatant assayed for protein using a modified Lowry method as reported elsewhere [7]. bovine serum albumin (BSA) standard curve was prepared for quantification.

Quantitative analysis for carbohydrate

To a portion (2 ml) of the freshly homogenized insects in phosphate buffer was added an equal volume (2 ml) of 4M HCI. The mixtures (in the various test tubes) were placed in a boiling water bath with the mouth of the tubes covered with cork. At intervals of 15 mins, 0.5 ml aliquots were withdrawn from the mixtures and introduce into test tubes containing 2.5 ml of 1 M phosphate buffer. The above was diluted to 10ml with distilled water before assaying for reducing equivalent by the Somgyi method [8] quantified against a glucose standard. The assay was continued to a constant value of reducing sugar equivalent when hydrolysis of the carbohydrate was deemed complete.

Analysis of total lipids

The homogenate of the insects in chloroform-ethanol mixture was poured into a conical flask and corked. After 10 mins of standing, it was filtered into a separating funnel using sintered glass wool. The debris was re-extracted with the mixture and filtered. The filtrates were pooled and to this was added 5 ml of 0.1% (v/v) NaCl and then thoroughly shaken after about 5 mins. standing, the lower organic phase was collected and the lipids in this extract was estimated by the method of Tietz [9]. Standard lipids were also assayed as above for quantification.

RESULTS AND DISCUSSION

Biological methods of estimating nutritional values of food items include weight gain or nitrogen retention in the test animals, when fed with protein containing diet [10]. Rats are usually the test animals, but insects such as the flour beetles have been found adequate due to their sensitivity to shortages of basic nutrients [1] and toxic compounds [11]. The parameters assayed include weight gain (or loss), levels of carbohydrate, protein and lipids in the tissue of the insects. Fig. I shows changes in mean weights of the insects reared on wheat, cassava or rubber seed flour cakes. Growth may mean an increase in height over a period of time.

Table I. Duncan multiple range test (from one-way ANOVA) for comparisons of the variable: mean total protein, carbohydrates, and lipids in the insect homogenate, and mean weight after 5 weeks of rearing on various diets

Combination variables	Total protein (mg/m1)		Total lipids (mg/m1)		Total carbohydrate (mg/m1)		Mean wt/(mg)	
	Differences	Least significant ranges	Difference	least significant ranges	Difference	least significant ranges	Difference	least significant ranges
Rubber seed cake/Wheat flour cake	0.0172	0.0828^{ns}	0.1275	0.5945^{ns}	0.0055	0.0713^{ns}	0.107	0.112^{ns}
Rubber seed cake/Cassava flour cake	0.0343	0.0828^{ns}	0	0.5945^{ns}	0.0103	0.0744^{ns}	0.02	0.107^{ns}
Wheat flour cake/Cassava flour cake	0.0515	$0.-865^{ns}$	0.1275	0.6205^{ns}	0.0048	0.0744^{ns}	0.087	0.107^{ns}

Ns: not significant (confidence coefficient (1-α)=0.95)

In this investigation, growth was assayed as increase in weight, with or without an increase in height. Results in Fig. 1 show that the mean weights of the insects fed with rubber seed cake declined on the second week relative to the observed weight in week 1, and was least at the 5th

week. However, there were weight gains in insects fed on wheat and cassava flour from the first week to the second week, with some decline in week three. By means of one-way ANOVA, it was found out that mean weights recorded against the different feeding requirements differed significantly ($p>0.05$). However, the multiple range tests for comparisons of these mean weight variations shows that the difference were not significant, with respect to their respective least significant range (Table I). In spite of the above, it is axiomatic that these insect consumed more than 200 fold their body weight of rubber seed. They have been observed to destroy rubber seed in storage (50 kg load) in one year. In this case, they consumed the rubber seed, reproduce rapidly and die, thus resulting in destruction of the seed.

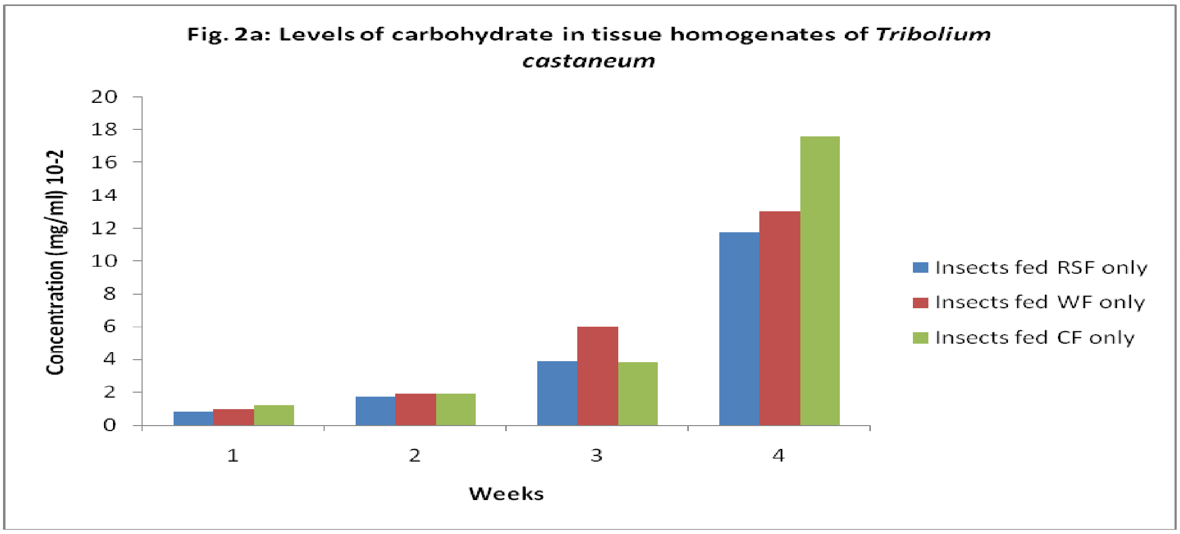
Table II: Mean daily consumption of Rubber seed cake, wheat flour cake and cassava flour cake by each group of 500 *T. castaneum* within the period of study

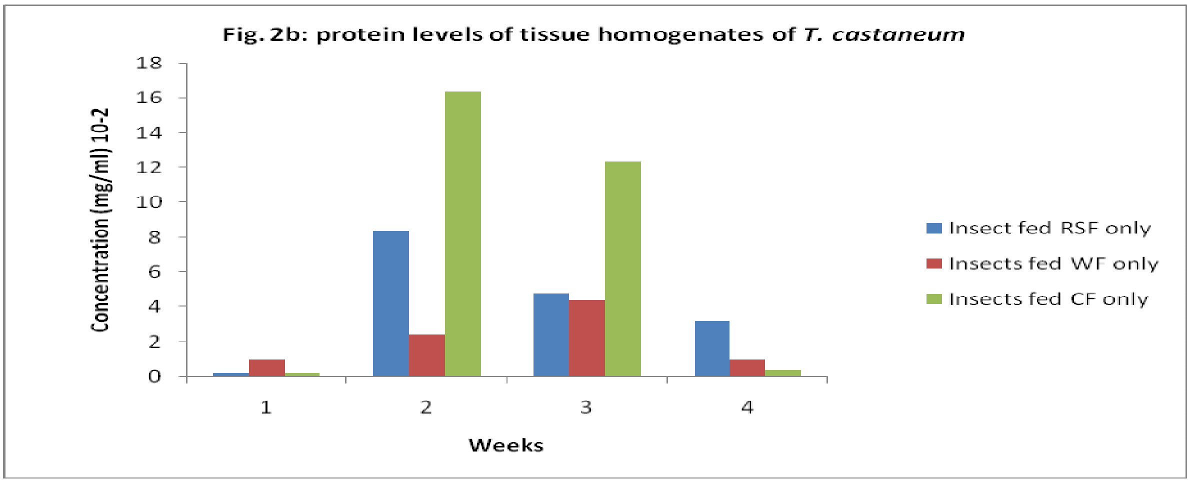
Substrates	Mean quantity consumed (mg/day)
Rubber seed cakes	164.3±54.5
Wheat flour cake	2.60±3.3
Cassava flour cake	58.4±30.6

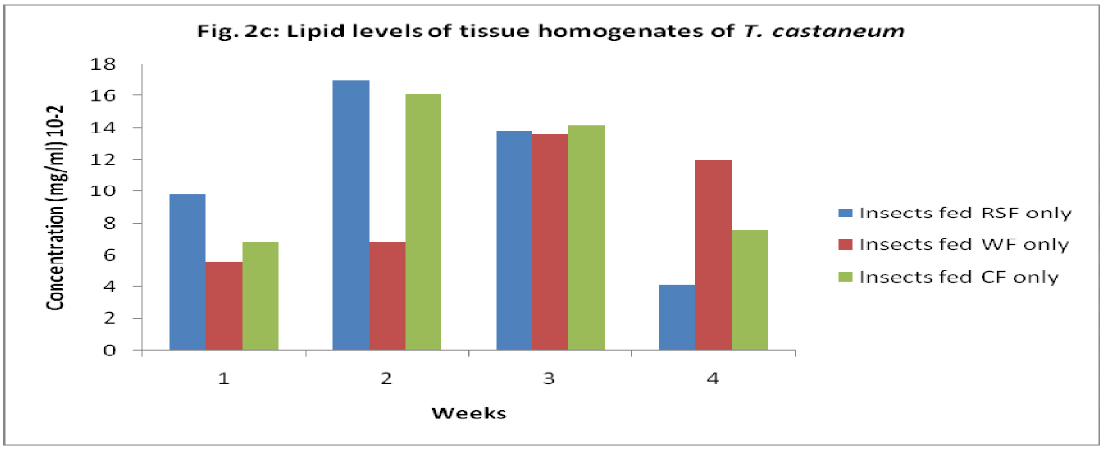
Table III: Composition of rubber seed cake (Njoku, 1994)

Compositions	Percent
Carbohydrates	29.0
Protein	22.3
Lipid (Ether extract)	42.51
Ash	2.6
Water	3.89

Assay of the levels of carbohydrate, protein and lipids in the homogenate of the insects fed on rubber seed cake, wheat flour cake and cassava flour cake, was carried out for 5 weeks and the results are presented in Fig 2 (a, b and c). The data shows that metabolism of wheat flour by the insects within weeks two and three yielded high amount of carbohydrates. In week one and five also contained high value of carbohydrate. However, insects reared in rubber seed cake contained carbohydrate level that was below those reared on wheat flour cake in all the weeks. Also for protein level, the values obtained in insects reared on rubber seed cake were lower throughout the studies. Conversely, the lipid levels in insects reared on rubber seed cake were high, especially in weeks three and five. It may be observed (Table II) that the average daily consumption of rubber seed cake by each group of 500 insects was highest (1644.3$\pm$ 54.5mg/d), followed by cassava flour cake (58.4 $\pm$30.6mg/d). This tends to suggest that the beetles had a greater preference for the fact that the insect were previously adapted to the rubber seeds (from where they were picked). The preference might also be due to the attractive aromatic odour of the rubber seed cake. In spite of the preference and high consumption by the insects, the level of carbohydrate in the homogenate was low. This seemingly low level observed, (though the rubber seed had a very high concentration of carbohydrate (29.0%), may be attributed to its roles in the metabolism of nucleotides (such as AMP, ADP, UDP, Xanthine etc) under optimal conditions [12]. The F-cal for carbohydrate, protein and lipid levels in the insects (homogenate), after 5 weeks of rearing on rubber seed, wheat flour or cassava cakes respectively 0.047,0.925 and 0.156 and these values are not significantly difference with the table of 1.9 (F095=2,9). This then suggests that a measure of nutritional value could be derived from the rubber seed, which is in abundance in Nigeria.







Proximate analysis carried out by [13] on this rubber seeds (Table III) shows the relative amounts of these dietary components that could be derived when it is used in formulating animal or poultry feed. Chemical and toxicological properties of the rubber seed meals and oil have also been documented [14] based on these studies, the rubber seed could be harnessed as a supplement for formulating animal or poultry feed. It is important to note that high amount of protein was found in the body tissue of the insects reared on cassava flour cake. In spite of this, the observed increase in growth (weight) did not justify this high level. This may be due to non-utilization of the protein or amino acid present in the cassava, especially during egg production and larvae development. This is because; it was observed that the larvae of the insects reared on cassava flour cake were abnormally small, and few in number. The larvae in the group also took a relatively longer time to develop than those reared on rubber seed cake and wheat flour cake. The basic function of protein in nutrition is to supply adequate amount of the needed amino acid. Proteins differ in their nutritive value due to the varying composition of essential amino acids and digestibility. Thus the protein requirements of the animals or insects depend on the type, composition and availability of the amino acids in the test diet. Hence the seemingly low level of protein in the insect reared on rubber seed cake, relative to those reared on what flour cake, might be due to their utilization during metabolism of the aforementioned nucleotides and rapid utilization of the absorbed protein, which result into the production of may available eggs and larvae. It was also noted that the insects had quite a good increase of lipids in their body fluids (homogenate) within two weeks, but these were rapidly used up within the next weeks. The main lipids found in the body fluids of these insects are lecithin [15]. These phospholipids play important roles in fat metabolism and transmethylation reception in insects. The lipid level (42.51%) in the rubber seed cade3 was considered adequate to meet the nutritional requirement of *T. castaneum.*

It is well know that the nutrition characteristic and toxicity index of a diet alters the time course of development, reproductive ability and survival of organisms [10]. This study shows that rubber seed cake could be used as a cheap supplement in the formation of animal or poultry feeds. The availability and utilization of the nutrients – carbohydrate, protein and lipids – could be extrapolated to livestock and poultry feeds. The suitability of the rubber seed cake to the beetles' physiology implies that these basic nutrients are biologically available for growth and development.

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