Research Journal of Biological Sciences 5 (2): 140-145, 2010
ISSN: 1815-8846

Dietary Incorporation of Boiled Fluted Pumpkin (*Telfairia occidentalis* Hook F.) Seeds 1: Growth and Toxicity in Rats

[1, 2]Chukwuonso E.C.C. Ejike, [1]Paschal C. Ugboaja and [2]Lawrence U.S. Ezeanyika
[1]Departments of Biochemistry, Michael Okpara University of Agriculture,
Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria
[2]University of Nigeria, Nsukka, Enugu State, Nigeria

Abstract: The seeds of fluted pumpkin (*Telfairia occidentalis* Hook F.) are known to have some nutritional properties that suggest they are good food sources but their effects on growth and toxicity is not known. Twenty four adult male albino rats were divided into 4 groups: A, B, C and D. Group A served as the control group while groups B, C and D received 5, 15 and 45% boiled *T. occidentalis* supplemented diets for 21 days. Growth indicators and toxicity markers were assessed in all rats at the end of the study using standard protocol. The results show similar feed consumption index at the start and end of the study. Rats in the control group had the highest final weight (212.23±39.35 g) while those in groups C and D had slightly lower (though statistically similar) weights (192.03±23.64 and 192.01±27.98 g, respectively). Rats in group B weighed, however significantly ($p<0.05$) less (181.25±18.66 g) than the control group at 21 days. The percentage relative weight gain of the different groups was nevertheless proportional to the *T. occidentalis* content of the diets. Test rats also had better feed utilization index at week 3 relative to the control. The relative weights of the liver and kidneys of rats in the 4 groups and the levels of alanine and aspartate transaminases in the sera of the rats were similar ($p>0.05$). Dietary incorporation of the seeds of *T. occidentalis* resulted in good growth and did not have any detectable toxicity after 21 days. Other aspects of health that may be affected by the consumption of oil-seeds need to be investigated.

Key words: Fluted pumpkin, growth, *Telfairia* occidentalis, toxicity, weight gain, Nigeria

INTRODUCTION

The population of many developing countries is growing rapidly. However, the production of food to feed the teeming population is insufficient making food security a burning issue in the developing world (FAO, 1985). In such places, the supply of animal protein no longer suffices for the protein needs of the population. In fact by the year 2000, there was an estimated 20-25 metric tons deficit in meat production (Bender, 1992). According to the Food and Agricultural Organization (FAO, 1990) the low level of meat supplies in the developing world is traceable to the low purchasing power of the vast majority of the population. In response to this scenario, research efforts have been geared towards finding food crops that are locally available but hitherto underutilized or utterly neglected which can provide the energy and proteins required to augment food supply (Giami and Wachuku, 1997; Enujiugha and Ayodele-Oni, 2003). One of such food crops with considerable value of energy and protein in Nigeria is fluted pumpkin (*T. occidendalis* Hook F.). Fluted pumpkin is a tropical vine with large lobed leaves and long twisting tendrils (Okoli and Mgbeogu, 1983). Its common names include fluted guard, costillada (Spanish), krobonko (Ghana), gonugbe (Sierra Leone) and ugu (South-East Nigeria). The plant belongs to the curcubitaceae family and is grown mainly for its nutritional value (Axtell, 1992). The seeds of fluted pumpkin are valuable both as an oilseed (54%) and also as a protein source (27%) with a fairly well balanced amino acid composition (Akwaowo *et al*., 2000, Hamed *et al*., 2008). Unfortunately, 78-91% of the fruits are wasted annually (Fagbemi *et al*., 2005).

A lot has been documented on the nutritional properties of the seeds of fluted pumpkin (Asiegbu, 1987; Giami and Bekebian, 1992; Giami and Isichei, 1999; Akwaowo *et al*., 2000; Hamed *et al*., 2008). However, little is known about growth and toxicity in animals fed diets supplemented with the seeds of *T. occidentalis*. The objective of this study is to provide information in this direction.

Corresponding Author: Chukwuonso E.C.C. Ejike, Departments of Biochemistry, Michael Okpara University of Agriculture, Umudike, P.M.B. 7267 Umuahia, Abia State, Nigeria

MATERIALS AND METHODS

Preparation of fluted pumpkin seeds: Fluted pumpkin fruits were bought from a local market in Umudike, Nigeria. The fruits were sliced open and the pulp and seeds removed. The seeds were cleaned and freed from unwanted materials before they were shelled manually. Bad seeds were promptly removed. The good seeds were washed thoroughly and boiled for 1 h. Thereafter, the seeds were dried in an oven at 40°C until a constant weight was achieved. The boiled and oven-dried seeds were then milled in a laboratory miller and used for the study.

Experimental design: Twenty four adult male wistar rats were obtained from the Animal Breeding Unit of the Faculty of Biological Sciences, University of Nigeria, Nsukka. They were housed in four cages and acclimatized to the animal house for 1 week. After 1 week, the rats, weighing approximately 154 g each were randomly (while controlling for weight differences) assigned to four groups: A, B, C and D. Rats in group A served as the control and received semi-purified rat chow (Bendel Feed and Flour Mills Ltd., Nigeria). Those in group B, C and D received the semi-purified rat chow supplemented with 5, 15 and 45% boiled *T. occidentalis* seeds, respectively. The supplemented diets were thoroughly homogenized, manually palletized and oven dried at 40°C to a constant weight and stored in air-tight containers from where they were dispensed to the rats daily.

The study lasted for 21 days. During this period, each animal received 25 g of feed daily and had unrestricted access to water. The animal house was airy, under tropical conditions and had 12 h light and dark cycles. After 21 days, the rats were fasted overnight and sacrificed humanely. Each rat was dazed by a cervical blow and blood collected by cardiac puncture. The blood was transferred into clean sample containers allowed to stand at ambient temperature until clotting took place and then centrifuged at 2000 g for 5 min. The serum was carefully removed and placed in clean and appropriately labeled sample containers. The sera were used for analysis immediately.

The carcass was immediately dissected and the liver and kidneys excised carefully. The organs were then washed, separately in normal saline. Excess liquid was removed by blotting with filter papers before the organs were weighed.

Assays/measurements

Feed consumption: Left over feed was collected per group daily. From that the quantity of feed consumed was calculated by difference and reported as group weekly averages.

Weights of rats: The weights of rats in each group were measured at the start of the study and weekly thereafter, to ascertain if there was gain/loss in weight in the rats. Values were reported as group weekly averages.

Percentage weekly/cumulative relative weight gain of rats: At the end of every week during the study, the difference between the average weekly weight per group and its weight in the preceding week was represented as a percentage of the average weight of that group in the preceding week. That gave the weekly relative weight gain for that group.

The percentage cumulative relative weight gain was calculated by representing the difference between the average weight of each group and its average weight at the beginning of the study as a percentage of the group's average weight at the beginning of the study.

Feed utilization index: At the end of each week, the weekly weight gain per group was represented as a percentage of the quantity of feed consumed by that group in that week. The cumulative feed utilization index was calculated by representing the cumulative weight gain per group as a percentage of the quantity of feed consumed by that group in the course of the study.

Organ/relative organ weights: The excised liver and kidneys from each rat were weighed using a sensitive digital balance. For the kidneys, the average weights of two kidneys were recorded for each rat. From the weights of the excised organs, their relative weights were calculated by dividing the weight of the respective organ with the weight of the animal to which it belonged just before sacrifice.

Alanine and aspartate amino transferase assays: Both liver enzymes were assayed in serum by the enzymatic colorimetric method of Reitman and Frankel (1957) using commercially available test kits purchased from Randox Laboratories Ltd., Crumlin, UK.

Data analysis: Means were calculated and differences between means separated using the one way ANOVA test with the least significant difference fixed at 0.05. All data analyses were done using the statistical software SPSS for windows version 11.0 (SPSS Inc. Chicago IL).

RESULTS AND DISCUSSION

At the end of week 1, rats in group C consumed the largest quantity of feed while those in group D consumed the least quantity (Table 1). There was, however no significant ($p>0.05$) difference between the quantity of

feed consumed in any of the three test groups compared to the control group. This suggests that the supplemented diets were palatable and acceptable to the test rats. Rats in group D consumed similar quantity of feed as those in the control group at the end of week 2. During the same period, rats in group B, however consumed significantly ($p<0.05$) lower quantity of feed while those in group C consumed significantly ($p<0.05$) higher quantity of feed, compared to the control group. It is difficult to explain the drop in feed consumption in group B, as well as the surge in feed consumption in group C. However, the fact that the rats that received 45% *T. occidentalis* supplemented diet (group D) consumed similar quantity of feed as those in the control group suggests that the reason for the noticed fluctuations in feed consumption may not be due to unacceptability or high demand for the supplemented diets. Rats generally eat in relation to their energy requirements (Kleiber, 1975; NRC, 1978) so the rats may have taken the quantity of feed they required. At the end of week 3, all the groups consumed similar quantities of feed. Rats in the test groups had overcome the perturbations of week 2 and consumed as much feed as rats in the control group. There was no significant difference ($p>0.05$) in the weights of the rats in group B, C and D compared each to the control group at the beginning of the study. At the end of week 1, rats in all groups but group B gained appreciable weight. The average weights of rats in group B was significantly ($p<0.05$) lower than that of the control group, while the others had similar average weights to the control group. The negligible weight gain in rats in group B was surprising especially as they consumed similar quantity of feed as the others that gained appreciable weight. At the end of week 2, rats in group A and B gained each approximately 18 g. However, rats in group C gained only about 8 g while those in group D lost about 10 g. It is possible that rats in group C and D were not utilizing their diet appropriately as their weight gain dropped from that of the previous week even though their feed consumption was higher or similar to those in group C or D and the control group, respectively. It may also be that the loss in weight that apparently started earlier found clear expression at week 2. However, as seen in Table 2 at the end of the study, only rats in group B had an average weight that was significantly ($p<0.05$) lower than that of the control. This improvement in weight in all the test groups may be in response to their re-established comparable feeding (quantity-wise) to the control group. The animals may have become better suited to exploit the supplemented diets maximally for growth and tissue maintenance.

Usually weight gain is related to feed intake. Inadequate dietary protein content or an increase in dietary energy content usually depresses feed intake (Peterson and Baumgardt, 1971; Menaker and Navia, 1973). The similarity in both feed intake and weight gain of the four groups at week 3 may suggest that the test diets contained good measures of protein and energy. The percentage relative weight gain of the rats show that those in the control group plummeted from 22.0% at week 1 to 4.7% at week 3. Only rats in group B had a percentage relative weight gain at week 3 that was higher than their value for week 1. Apart from rats in group B whose percentage relative weight gain was significantly ($p<0.05$) less than that of the control, the others had values that were similar ($p>0.05$) to the control at each week. At the end of the study, percentage relative weight gain virtually became proportional to the quantity of *T. occidentalis* in the diet (Fig. 1). On the other hand, the percentage cumulative weight gain shows that all the groups (except group D) had an ascending pattern. Though, this index was highest in group A that of rats in group B followed

Table 1: Quantity of feed (g) consumed by rats in the test and control groups

Factors	Group A (g)	Group B (g)	Group C (g)	Group D (g)
Day 7	144.66±22.37	150.20±22.74	152.36±30.29	115.03±44.28
p-value		0.743	0.649	0.088
Day 14	144.27±14.07	125.50±12.65	163.47±7.57	139.46±14.36
p-value		0.010	0.008	0.477
Day 21	145.69±36.74	142.46±21.02	144.63±20.84	141.99±18.66
p-value		0.814	0.939	0.787

Table 2: Weight (g) of rats in the test and control groups

Factors	Group A (g)	Group B (g)	Group C (g)	Group D (g)
Day 0	156.39±41.40	146.65±18.40	155.76±30.10	156.56±28.00
p-value		0.529	0.968	0.991
Day 7	188.01±39.50	149.03±18.30	169.16±24.20	180.00±27.40
p-value		0.011	0.196	0.578
Day 14	206.59±44.30	167.38±19.70	177.49±21.70	169.59±23.80
p-value		0.012	0.055	0.017
Day 21	212.23±39.35	181.25±18.66	192.03±23.64	192.01±27.98
p-value		0.030	0.088	0.113

by group C were the steepest (Fig. 2). These suggest that rats in the test groups were slower in exploiting the nutrients in their diets but the diets were more steadfast in maintaining weight gain in the animals. The percentage cumulative weight gain of the rats in the control group was higher than the others at the end of the study because of the buffering effect of the surge in weight gain in that group at week 1 when the test animals were still acclimatizing with their new diet. This makes the percentage relative weight gain considerably more informative.

Figure 3 shows the feed utilization index of the different groups. At the end of the first week, rats in the control group and group D had the highest feed utilization index (21.9 and 20.4%, respectively). However, by week 3, rats in the control group had the least feed utilization index while those in group D had the highest. Cumulatively, the feed utilization index for the different groups became narrow (range 7.9-12.8%). Since the feed utilization index estimates the quantity of feed converted to body mass, the test diets, especially 5% *T. occidentalis* supplemented diet appeared better than the control diet. Rats in the control group had the largest average liver weight (8.21±1.84 g) while those in group B had the least (6.64±0.75 g). Only the difference between the average liver weights of rats in group B, compared to the control was significant ($p<0.05$). The average kidney weights of rats in all the groups were similar. Similarly, the relative liver weights and relative kidney weights of the test groups were each similar to that of the control group (Table 3). The liver is the organ responsible for the detoxification and biotransformation of both xenobiotics and toxins produced by normal cellular metabolism. The kidneys are major excretory organs (Parkinson, 1996). Both organs play significant roles in carbohydrate and protein metabolism. An unusual increase in their weights may be suggestive of a derangement in the system and is often linked to the accumulation of toxic substances in the system. The significantly low average liver weight of rats in group B may be a reflection of the low weight of animals in the said group compared to the control and not of disease. This is made clearer by the similar average relative weights of the liver of rats in all the groups. This may in turn suggest an absence of toxicity induced by the consumption of the supplemented diets. Both ALT and

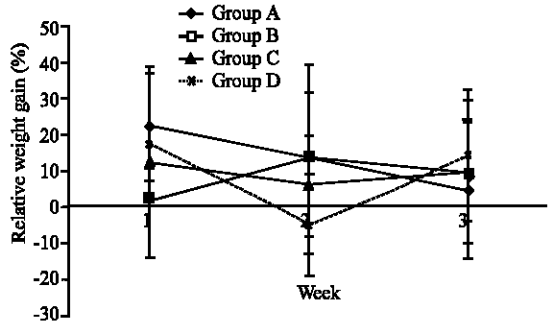

Fig. 1: Relative weight gain of rats in the test and control groups expressed as percentages

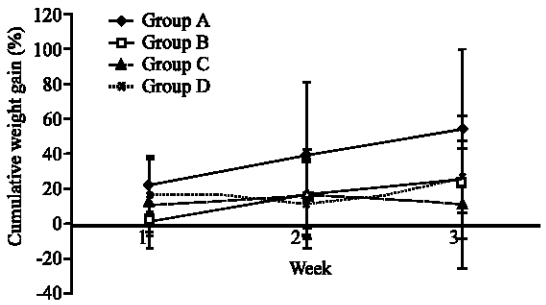

Fig. 2: Cumulative weight gain of rats in the test and control groups expressed as percentages

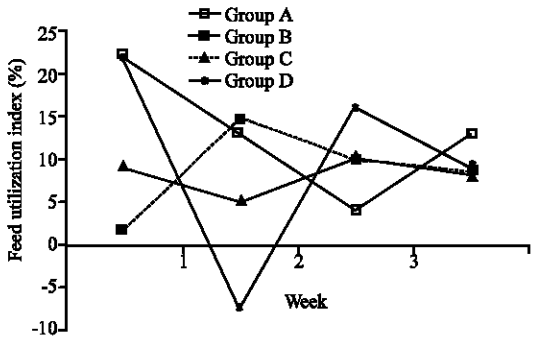

Fig. 3: Feed utilization index of rats in the test and control groups, expressed as percentages

Table 3: Organ weights and relative organ weights (g) of selected organs of rats in the test and control groups

Facrors	Group A (g)	Group B (g)	Group C (g)	Group D (g)
Liver weight	8.21±1.84	6.64±0.75	7.15±1.36	6.96±1.25
p-value		0.028	0.128	0.076
Kidney weight	1.45±0.24	1.35±0.23	1.50±0.23	1.51±0.30
p-value		0.433	0.694	0.623
Relative liver Wt ×1000	39.00±2.45	37.38±1.92	39.25±3.81	37.38±4.69
p-value		0.347	0.884	0.347
Relative kidney Wt ×1000	7.04±1.30	7.68±1.22	8.14±0.98	8.09±1.13
p-value		0.282	0.069	0.081

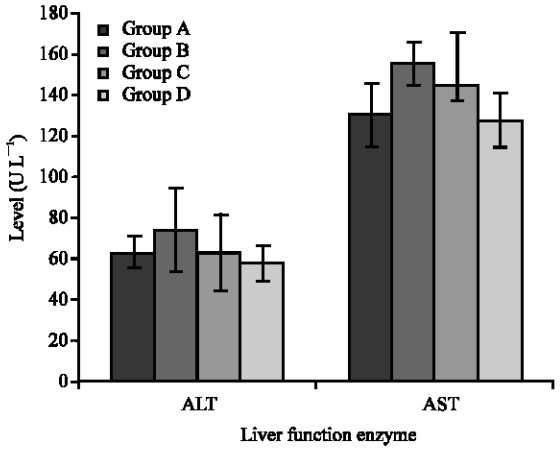

Fig. 4: Levels of ALT and AST in the serum of test and control rats

AST were highest in group B and lowest in group D (Fig. 4). The differences in the means of both enzymes in the test groups, compared to their respective controls, showed no significant differences ($p<0.05$). AST level was however marginally ($p = 0.05$) higher when group B is compared to the control. The destruction of the hepatic architecture is the principal culprit for the presence of elevated liver enzymes in serum (Ejike *et al*., 2008). This destruction often occurs in the presence of high amounts of toxins and xenobiotics which become challenges to the liver (ASCP, 2003). The lack of clearly significant elevations of these enzymes in the test rats compared to the control rats may then suggest that the diets were well tolerated. Though, fluted pumpkin seeds are known to contain anti-nutrients like tannins and phytic acid (Giami and Isichei, 1999; Akwaowo *et al*., 2000) which may be toxic and can reduce mineral availability and inhibit protein digestability (Lopez *et al*., 2002) processing methods like boiling are known to reduce them significantly in seeds (Bradbury *et al*., 1984; Hassan *et al*., 2005; Hamed *et al*., 2008). The processing (boiling and oven-drying) which we subjected the fluted pumpkin seeds to may be responsible for the absence of toxicity in the test groups as seen in this study.

CONCLUSION

Results from this study show that dietary incorporation of *T. occidentlis* seeds gave good results from the point of growth and toxicity. The seeds may therefore be used to augment energy and protein requirements in resource-poor countries. Further research is required to investigate other aspects of health that may be affected by the consumption of oil-seeds.

REFERENCES

ASCP, 2003. Educational commentary: Liver function enzymes. American Proficiency Institute 2003 1st Test Event.

Akwaowo, E., B.A. Ndon and E.U. Etuk, 2000. Minerals and antinutrients in fluted pumpkin (*Telfairia occidentalis* Hook). Food Chem., 70: 235-240.

Asiegbu, I.E., 1987. Some biochemical evaluation of fluted pumpkin seed. J. Sci. Food Agric., 40: 151-155.

Axtell, B.L., 1992. Minor oil crops. FAO Agricultural Services Bulletin No. 94. FAO of The United Nations.

Bender, A., 1992. Meat and Meat Products in Human Nutrition in Developing Countries. FAO., Rome, Italy.

Bradbury, J.H., J.G. Collins and N.A. Pyliotis, 1984. Digestability of protein of the physiological components of cooked and raw rice. Br. J. Nutr., 52: 507-513.

Ejike, C.E.C.C., E.O. Alumanah, L.U.S. Ezeanyika, A.A. Ngene and E.E. Ojefua, 2008. Antibiotics administration and its possible liver damage. Bio Res., 6: 351-354.

Enujiugha, V.N. and O. Ayodele-Oni, 2003. Evaluation of nutrients and some anti-nutrients in lesser known underutilized oil-seeds. Int. J. Food Sci. Tech., 38: 525-528.

FAO, 1985. The Fifth World Food Survey. Food and Agricultural Organization, Rome.

FAO, 1990. World meat situation and outlook. Commodities and Trade Division. ME 90/1. Rome.

Fagbemi, T.N., O.O. Oshodi and K.O. Ipinmoroti, 2005. Processing effects on some antinutritional factors and *in vitro* mutienzyme protein digestibility (IVPD) of three tropical seeds: Breadnut (*Artocarpus altillis*), cashewnut (*Anacardium occidentale*) and fluted pumpkin (Telfairia occidentalis). Pak. J. Nutr., 4: 250-256.

Giami, S.Y. and D.A. Bekebian, 1992. Proximate composition and functional properties of raw and processed full fat fluted pumpkin (*Telfairia occidentalis*) seed flour. J. Sci. Food Agric., 59: 321-325.

Giami, S.Y. and I. Isichei, 1999. Preparation and properties of flours and protein concentrates from raw, fermented and germinated fluted pumpkin (*Telfairia occidentalis* Hook) seeds. Plant Foods Hum. Nutr., 54: 67-77.

Giami, S.Y. and O.C. Wachuku, 1997. Composition and functional properties of unprocessed and locally processed seeds from three underutilized food sources in Nigeria. Plant Foods Human Nutr., 50: 27-36.

Hamed, S.Y., N.M. El-Hassan, A.B. Hassan, M.M. Eltayeb and E.E. Babiker, 2008. Nutritional evaluation and physicochemical properties of processed pumpkin (*Telfairia occidentalis* hook) seed flour. Pak. J. Nutr., 7: 330-334.

Hassan, A.B., G.A. Osman and E.E. Babiker, 2005. Effect of domestic processing on antinutrients and availability of protein and mineral of lupin seeds. J. Food Technol., 3: 255-262.

Kleiber, M., 1975. The Fire of Life: An Introduction to Animal Energetics. RF Kreiger Publ. Co., New York.

Lopez, H.W., F. Leenhardt, C. Coudray and C. Remesy, 2002. Minerals and phytic acid interactions: Is it a real problem for human nutrition? Int. J. Food Sci. Technol., 37: 727-739.

Menaker, L. and J.M. Navia, 1973. Appetite regulation in the rat under various physiological conditions: the role of dietary protein and calories. J. Nutr., 103: 347-352.

NRC, 1978. Nutrient Requirement of Domestic Animals: Nutrient Requirements of Laboratory Animals. National Academy of Science, Washington, DC.

Okoli, B.E. and C.M. Mgbeogu, 1983. Fluted Pumpkin, *Telfairia occidentalis*: West African vegetable crop. Econ. Bot., 37: 145-149.

Parkinson, A., 1996. Biotransformation of Xenobiotics. In: Casarett and Doull's Toxicology, The Basic Science of Poisons, Klaassen, C.D. (Ed.). McGraw-Hill, New York, pp: 113-186.

Peterson, A.D. and B.R. Baumgardt, 1971. Food and energy intake of rats fed diets varying in energy concentration and density. J. Nutr., 101: 1057-1067.

Reitman, S. and S. Frankel, 1957. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminase. Am. J. Clin. Pathol., 28: 56-63.