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Composition and nutritional properties of seeds from *Pachira aquatica* Aubl, *Sterculia striata* St Hil et Naud and *Terminalia catappa* Linn

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Abstract

The seeds of three wild plants (*Pachira aquatica*, *Sterculia striata* and *Terminalia catappa*) were analyzed to establish their chemical compositions and nutritional properties in order to investigate the possibility of using them for human and/or animal consumption. Proximate analyses showed that they have high amounts of protein and oil. However, they are deficient in various essential amino acids but *P. aquatica* seeds have tryptophan, threonine and phenylalanine + tyrosine contents higher than those reported for human milk, chicken egg and cow's milk. Haemagglutinating and trypsin inhibitor activities were found to be present in the seeds of *P. aquatica* and *T. catappa* but absent in *S. striata*. Coincidentally the rats fed on *S. striata* diet gained slightly in weight and presented alterations in the key internal organs which were less drastic throughout the 10-day test period. On the other hand, the rats fed on *T. catappa* diet maintained their body weight but suffered from stomah, small intestine and pancreas hypertrophy as well as spleen atrophy. Five out of six rats fed on *P. aquatica* diet died within 6–8 days. The remaining rat experienced enlargement of the stomach, liver, pancreas, kidneys, heart and lungs and had spleen atrophy when compared with the same organs of rats fed on egg-white diet. Hypertrophy of the pancreas and kidneys was very marked and these organs nearly doubled in dry weight in comparison with those of the egg-white control group, demonstrating that the raw seed of *P. aquatica* are highly toxic when fed to rats even at a meal protein concentration half that of *S. striata* or *T. catappa*, which were better tolerated by the experimental animals.

Keywords: *Sterculia striata*; *Terminalia catappa*; *Pachira aquatica*; Protein quality; Antinutritional factors; Toxicity

1. Introduction

Near to 60% of the world's food supply comes from rice, wheat and corn (Wilson, 1988) although approximately 250,000 plant species have been described worldwide (Pimentel et al., 1997; Raven & Johnson, 1992). There are hundreds of species of trees which provide food for people in both the humid and semi-arid tropics but they have received much less attention from the scientific community than the annual crops. Some species have been exploited for centuries and are widely distributed around the world. Others have been cultivated only in limited areas, while most are still essentially wild (Cannell, 1989). Certain wild plants contain proteins and other nutritionally important components that could be used as alternatives for human and animal diets. The search for alternative feed ingredients, especially for developing countries, is of utmost importance, particularly because of the high cost of animal protein sources. Preliminary compositional studies carried out on seeds of *Pachira aquatica*, *Sterculia striata* and *Terminalia catappa* showed that they deserve to be investigated as promising sources of low cost protein, fat and carbohydrate for possible use as food/feed to meet the gap of protein and energy deficiency.

P. aquatica Aubl (common name, Malabar Chestnut) is a tree belonging to the Bombacaceae family, native to an area from Southern Mexico to Guyana and Northeastern Brazil. Its seeds are eventually consumed raw (taste like peanuts) or as roasted beans with a flavour of chestnuts, being considered a delicacy. Furthermore, its young leaves and flowers are cooked and used as a vegetable (Lorenzi, 1992).

S. striata St Hil et Naud (common name, Chicha), from the Sterculiaceae family, is native to almost all-Brazilian regions. Sometimes its raw or cooked seeds

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are consumed by man and also by several species of the Brazilian fauna. Studies conducted with other species of the genus *Sterculia* have shown the possible medicinal and industrial use of different parts of these trees (Lorenzi, 1992).

T. catappa Linn, that belongs to the Combretaceae family, is native to Malasia and was introduced to Brazil as an ornamental tree. Often, in Brazil, it is common to witness children eating its fruit tissue, which contains 95.9 ± 5.8 mg ascorbic acid 100 g^{-1} tissue (Keshinro, 1985). Several species of the genus *Terminalia* have long been used in the traditional medicine in both East and West African countries to treat infectious diseases (Fabry, Okemo, Mwatha, Chhabra & Ansorg, 1996).

In view of these properties and uses, the objective of this study was to assess the nutritional potential of *P. aquatica*, *S. striata* and *T. catappa* seeds by analyzing the protein, amino acid and oil contents and the level of some antinutritional constituents such as trypsin inhibitor, lectin and urease. Feeding trials were also carried out to evaluate these seeds as sources of dietary proteins fed to growing rats since it is well known that the nutritive value of several plant materials is limited by low digestibility, low level of sulfur-containing amino acids and also by the presence of antinutritional and/or toxic substances which produce adverse effects upon animal health (Liener, 1986; Oliveira et al., 1994; Vasconcelos et al., 1997).

2. Materials and methods

2.1. Seeds

Mature seed samples of *P. aquatica*, *S. striata* and *T. catappa* were obtained from plants that grow widely in the state of Ceara in Northeastern Brazil.

2.2. Proximate analysis

Seed ash and oil contents were determined as described by Triebold (1946). Seeds, diets, rat carcasses and ground fecal samples were analyzed for moisture and nitrogen contents according to Triebold (1946) and to Baethgen and Alley (1989), respectively. Crude protein contents were calculated as $N \times 6.25$. Seed carbohydrate was estimated by difference.

2.3. Amino acid composition

Amino acid analysis was performed after hydrolysis of seed flours with 6 M HCl containing 1% phenol at 110°C for 22 h, in sealed glass tubes under N_2 atmosphere. HCl plus phenol were removed by evaporation and the amino acid compositions established after chromatography on Biochrom 20 system (Pharmacia). Tryptophan content was measured colorimetrically (Pintér-Szakács & Molnár-Perl, 1990).

2.4. Aqueous extracts

The dehulled seeds were ground in a coffee grinder to a fine powder and stored at −20°C until used. The seed meal was suspended in the extracting buffer (0.5 M borate buffer, pH 6.0, containing 0.15 M NaCl) in a proportion of 1.0 g of meal to 10.0 ml of buffer. The suspension was maintained under continuous stirring, overnight, at 4°C, and then filtered. The filtrate was centrifuged at $10\,000 \times g$, for 30 min, at 4°C and the clear supernatant dialyzed (cut-off MW 12 000) against the extracting buffer.

2.5. Protein determination

The protein content in the extracts was evaluated by the method described by Bradford (1976) using bovine serum albumin as standard.

2.6. Agglutination assay

Haemagglutinating activity was determined by serial two-fold dilution (1:2) of the extracts (Vasconcelos, Cavada, Moreira & Oliveira, 1991). The extracts were diluted with 0.15 M NaCl, containing 5×10^{-3} M Ca^{++} and 5×10^{-3} M Mn^{++} in glass tubes or microtiter plates and mixed with rabbit red cell (2% suspension prepared in 0.15 M NaCl). The degree of agglutination was monitored visually after the tubes or plates had been left to stand at 37°C for 30 min and at room temperature for an additional 30 min. The results are reported as haemagglutination titer (HU) which is the reciprocal of the highest dilution giving a visible agglutination.

2.7. Trypsin inhibitor activity

Trypsin inhibitor assay was carried out by a slight modification of the method originally described by Kakade (Hamerstrand, Black & Glover, 1981). The meal was suspended in 1 ml of 0.01 M NaOH. The suspension was stirred magnetically for 3 h. After this period, the mixture was left for 30 min without stirring and then 0.5 ml of the supernatant was mixed with 0.5 ml 0.01 M NaOH in an Eppendorf centrifuge tube. This solution was centrifuged 5 min at $14\,000 \times g$. After centrifugation, 0.1 ml of the alkaline extract was mixed with 1.6 ml 0.05 M Tris–HCl, pH 8.2 containing 0.02 M $CaCl_2$, with 0.1 ml of trypsin (Sigma, type I) solution (from a stock solution of 0.4 mg in 10 ml 0.001 M HCl) plus 0.1 ml of Nα-benzoyl-L-arginine *p*-nitroanilide solution (10 mg/ml in dimethyl sulfoxide plus H_2O). The mixture was incubated for 45 min at 37°C and then 0.2 ml 30% acetic acid solution was added. The absorbance

at 410 nm was measured. Activity was expressed as the amount of trypsin inhibited, calculated from a calibration curve using soybean trypsin inhibitor (Sigma, type I-S).

2.8. Urease assay

Urease assay was carried out by minor modifications of the procedure described by Kaplan (1969). 0.1 ml of urea solution (0.5 M) plus 0.7 ml 2% EDTA buffered with 0.2 M potassium phosphate, pH 6.5, were mixed with 100, 200, or 300 μl of the seed crude extract (this material was prepared in 0.01 M sodium phosphate buffer, pH 7.0, containing 0.15 M NaCl, using the same protocol as previously described). The mixture was incubated for 15 min at 37°C and then 1.0 ml phenol plus sodium nitroprusside solution (62.0 g of phenol with 0.25 g of sodium nitroprusside l^{-1}) and 1.0 ml sodium hypochlorite plus alkali solution (43.0 ml of 5.25% hypochlorite with 20.0 g of alkali l^{-1}) were added. The mixture was incubated for a further 5 min at 37°C. After that, 7 ml of distilled water was added. The tubes were covered with parafilm and shaken vigorously to mix. The absorbance at 625 nm was measured. The enzyme activity was calculated from a calibration curve using urease (Sigma 41H7008—870 000 units g^{-1}).

2.9. Diets

A protein-free basal diet was composed of 500 g maize starch, 100 g potato starch, 150 g glucose, 150 g corn oil, 50 g mineral mixture and 50 g vitamin mixture kg^{-1}. Vitamin and mineral mixture were formulated as before (Oliveira et al., 1994). Casein (Merck), egg white (Sigma Chemical Co.) or *T. catappa* or *S. striata* seed meal alone or a 1 + 1 mixture of egg white and *P. aquatica* seed meal were incorporated to the basal diet to give an equivalent of 100 g protein kg^{-1} by substitution with the maize starch. *P. aquatica* seed flour comprised only half of the total dietary protein because, in a previous experiment carried out using the seed meal alone, the rats died within 2–3 days.

2.10. Feeding trials

Male Wistar rats were weaned at 21 days of age and given a commercial stock diet until their weights reached around 50 g. They were then fed the casein diet ad libitum for 3 days as a period of adaptation to pulverized diets and selected according to food consumption and body weight. Groups of six rats were placed in screen-bottomed cages, each containing three rats, and fed for 10 days with the control (egg-white, non-protein containing) or experimental (containing the seed flours) diets. Feed and water were supplied ad libitum. Rat weights, diet spillage and refused diet were recorded daily. Feces were collected during the last 5 days of the experimental period, bulked for three rats, freeze-dried, weighed and ground in a coffee grinder. At the end of the trials, the rats were killed by ether overdose and the internal organs dissected. These were then freeze-dried while the carcasses were dried in an oven at 105°C for 24 h. Dry weights were recorded before incorporating the organs with their original carcasses which were ground and kept in a desiccator for appropriate analyses. True protein digestibility and net protein utilization (NPU) were calculated following, essentially, the method described by Miller and Bender (1955). Student's *t*-test was used to evaluate statistical differences.

3. Results and discussion

3.1. Proximate composition

Moisture, ash, protein, oil and carbohydrate contents of *P. aquatica*, *S. striata* and *T. catappa* are shown in Table 1. Moisture content and total ash values varied between 60.0 and 114.5 g kg^{-1} dry matter and 24.0 and 35.0 g kg^{-1} dry matter, respectively. The crude protein content for *P. aquatica* was 129 g kg^{-1} dry matter, a value within the range found for cereal seeds (84.0 to 148 g kg^{-1} dry matter) such as corn, triticale and wheat (Heger & Eggum, 1991). The data for *S. striata* (225 g kg^{-1} dry matter) and *T. catappa* (294.0 g kg^{-1} dry matter) are higher than the protein contents in seeds of important grain legumes (180–250 g kg^{-1} dry matter) (Singh & Singh, 1992). However, these values are comparable to the seed protein contents of underutilized legumes such as *Canavalia ensiformis*, 260 g kg^{-1} dry matter (Ajah & Madubuike, 1997), *Bauhinia purpurea*, 271.7 g kg^{-1} dry matter (Vijayakumari, Siddhuraju & JanardLanan, 1997) and some species of the genus *Crotalaria*, 200.0–396.0 g kg^{-1} dry matter (Pandey & Srivastava, 1990). In addition, the seed oil contents are found to be higher than those reported for various soybean cultivars, 183.0–215.3 g kg^{-1} dry matter (Vasconcelos et al.,

Table 1
Proximate composition[a] (g kg^{-1} dry matter)[b] of seeds from *Pachira aquatica*, *Sterculia striata* and *Terminalia catappa*

Component	Species		
	P. aquatica	*S. striata*	*T. Catappa*
Moisture	60.0 ± 1.2a	114.5 ± 2.6b	77.0 ± 1.9c
Protein (N×6.25)	129 ± 4.5a	225 ± 6.8b	294 ± 11.2c
Oil	539.0 ± 28.6a	286.4 ± 15.1b	583.0 ± 29.1a
Carbohydrate[c]	297.0	458.2	99.0
Ash	35.0 ± 0.9a	30.4 ± 0.8b	24.0 ± 0.6c

[a] Values in a horizontal row with different following letters differ significantly ($P < 0.05$).
[b] Means of triplicate analyses.
[c] Calculated by difference.

1997). The values for *P. aquatica* and *T. catappa* are comparable to those of other oilseeds such as peanut (Grosso, Zygadlo, Lamarque, Maestri & Gusman, 1997), castor bean (Polit & Sgarbieri, 1976), rapeseed (Zhou, He, Yu & Mukherjee, 1990) and sunflower (Saeed & Cheryan, 1988) which have contents varying from around 437.0 to 547.0 g kg^{-1} dry matter. However, the oil of another species of *Pachira*, *P. insigenes*, was considered unfit for human consumption due to the presence (21%) of potential toxic cyclopropenoid fatty acid (CPFA) which cause numerous physiological disorders in experimental animals (Berry, 1980). Of nutritional importance is the fact that there are several reports showing that the high level of CPFA is common among the representatives of the *Sterculia* genus (Miralles, Bassene & Gaydou, 1993) and that its proportion in the oil did not decrease upon cooking the nuts (Berry, 1982). Concerning *T. catappa*, Nag and De (1995) found a similar oil content for the seeds of *T. bellirica* and stated that this oil may become the olive of the East and it appears very promising for edible purposes.

3.2. Amino acid patterns

Table 2 shows the amino acid composition of each seed meal, the minimal requirements established for 2–5 and 10–12 year old children (FAO/WHO/UNU, 1985) and the requirements for rats (Coates, O'Odonoghue, Paiyne & Ward, 1969). Comparison of the essential amino acid level with the amino acid requirement for children shows that *S. striata* seeds have histidine as the first limiting amino acid (chemical score 83% for both groups). Phenylalanine + tyrosine are the second ones (chemical score 87%) but only for 2–5 year old children. All other essential amino acids meet the children needs including methionine + cysteine and tryptophan that are limiting amino acids in most legumes. The seeds of *T. catappa* are severely deficient in lysine for 2–5 and 10–12 year old children (chemical scores 40% and 53%, respectively), as are the majority of the cereal seeds and they have histidine as the second limiting amino acid (chemical score 88%). Otherwise *P. aquatica* seeds have methionine + cysteine as the first limiting amino acids with chemical scores of 61 and 69% when compared to the requirements for 2–5 and 10–12 year old children, respectively. Further they are deficient in lysine for children aged 2–5 years and in histidine for both groups (chemical score 84%). It is worthwhile to mention that their contents of the essential amino acids tryptophan, threonine and phenylalanine + tyrosine are higher than those reported for human milk, chicken egg and cow's milk (FAO/WHO/UNU, 1985). It is concluded that these seeds could be useful as alternative sources of some essential amino acids and that further investigations are needed to ascertain what proteins could provide these amino acids.

Comparing the amino acid data with the standards for growing rats (Coates et al., 1969), the studied seeds are well below the minimal requirements for rats. The seeds of *T. catappa* showed to be deficient in all essential amino acids, except tryptophan. Similarly, *S. striata* and *P. aquatica* seeds are deficient in eight and seven of them, respectively; the former only has sufficient amounts of valine, lysine and tryptophan and the second one of threonine, valine, leucine and tryptophan. Phenylalanine + tyrosine and methionine + cysteine are the first limiting amino acids for *S. striata* (chemical score 61 and 62%, respectively), lysine for *T. catappa* (chemical score 39%) and methionine + cysteine for *P. aquatica* seeds (chemical score 34%).

3.3. Antinutritional compounds

The presence of lectins and trypsin inhibitors is depicted in Table 3. Haemagglutinating activity, measured against rabbit erythrocytes, was found to be present in the seeds of *P. aquatica* and *T. catappa* but absent in the seeds of *S. striata*. Using other types of red cells Grant et al. (1995) found low levels of lectin activity

Table 2
Amino acid composition (g kg^{-1} protein) of seeds from *Pachira aquatica*, *Sterculia striata* and *Terminalia catappa* compared to FAO/WHO/UNU scoring pattern for children and the amino acid target requirements for growing rats

Amino acid	Species			Child[a]		Rat,[b]
	P. aquatica	*S. Striata*	*T. Catappa*	2–5 years	10–12 years	young
Essential						
Thr	54.8	36.6	31.4	34	28	40
Val	63.8	57.6	47.6	35	25	55
Ile	41.3	36.1	29.7	28	28	50
Leu	79.9	72.2	79.2	66	44	80
Lys	48.5	59.8	23.2	58	44	60
Phe	39.0	37.5	42.8	63[c]	22[c]	50
Tyr	29.8	17.0	22.8			40
Met	8.2	18.4	12.5	25[d]	22[d]	45[d]
Cys	7.0	9.6	13.2			
Trp[e]	25.3	14.7	15.2	11	9	15
His	16.0	15.8	16.8	19	19	25
Non-essential						
Asx	136	119	88.4			
Glx	123	179	189			
Ser	73.6	56.1	55.1			
Gly	87.7	76.5	113			
Ala	66.1	70.6	59.5			
Arg	65.7	96.7	130			50
Pro	34.2	27.3	30.2			

[a] Patterns of amino acid requirements for different age groups (FAO/WHO/UNU, 1985).
[b] Amino acid target requirements for rats (Coates et al., 1969).
[c] Tyr + Phe.
[d] Met + Cys.
[e] Trp was determined according to the method described by Pintér-Szakács and Molnár-Perl (1990).

in *T. catappa* seeds and Sachdeva and Bhalla (1997) detected haemagglutinating activity in the seed extracts of *T. bellirica* and *T. citrina*, two other species of the Combretaceae family. It is well documented that several seed lectins are resistant to proteolysis by gut enzymes and are detrimental to rat health when orally fed, leading to impaired growth and alterations of key organs, particularly hypertrophy of the small intestine (Oliveira et al., 1988; Pusztai et al., 1996; Rios et al., 1996). The trypsin inhibitor content of *P. aquatica* was about two-times higher than the content found for *T. catappa*. Indeed it was already reported that *T. catappa* seeds contain only moderate amounts of trypsin inhibitors and negligible quantities of α-amylase inhibitors (Grant et al., 1995). On the other hand, the *S. striata* seeds seem to be free of trypsin inhibitor activity, at least under the conditions of assay employed. Dietary trypsin inhibitors are thought to be responsible for the poor digestibility of dietary protein, by interference with the proper functioning of trypsin leading to growth inhibition and hypertrophy of the pancreas (Liener, 1994). In spite of their antinutritional effects, lectins and trypsin inhibitors are often inactivated by proper heat treatment. However, excessive heating could lead to damage of proteins and consequent unavailability of amino acids. If this is the case, it may pose a problem for the seeds studied here, since they are deficient in several essential amino acids. In our study, none of the three seeds presented urease activity.

3.4. Feeding trials

Table 3 shows that the rats fed on the seed protein-based diets ate much less food than those on the egg-white control. The loss of appetite was particularly pronounced in rats fed on *P. aquatica* seeds since they ate about 6 times less food than the control group fed on egg-white diet. As a consequence, they experienced a drastic body weight reduction. Furthermore, five out of six rats fed on the *P. aquatica* diet, in which the seed flour comprises only half of the total dietary protein (100 g kg^{-1}), died within 6–8 days. The rat that survived suffered from steady loss of weight and developed bald skin. To allow macroscopical examination of key organs, it had to be killed at day 8, before the end of the experimental period (10 days). On the other hand, rats fed on the *S. striata* diet gained slightly in weight and those fed on *T. catappa* maintained their body weight. Nevertheless the net protein utilization (NPU) values were very low for rats fed on the seed diets, varying from 52.0 to 58.2%, compared to the NPU calculated for the egg-white fed rats (80.8%). The NPU value (58.2%) found for *T. catappa* was very close to the data previously reported (Grant et al., 1995). Rukmini and Udayasekhara-Rao (1986) reported that, whereas rats were not adversely affected by intake of *T. bellirica* oil, a diet containing 100 g kg^{-1} *T. bellirica* kernel protein as a raw diet as well as a cooked diet fed to rats, mice and chicks caused low food intake and death in all animals. They suggested that these effects were probably due to heat-stable antinutritional factors in the kernel. Although the true protein- and dry matter-digestibility for *T. catappa* (88.3 ± 6.9% and 86.4 ± 2.4%, respectively) and for *S. striata* (91.3 ± 3.3% and 96.5 ± 0.5%, respectively) are, in general, below the value found for the egg white diet (97.2 ± 0.5% and 96.1 ± 0.5%, respectively) they are not sufficiently low to explain the poor nutritional performance of the rats. As the *S.*

Table 3
Comparison[a] of the nutritional parameters of rats fed on a protein-free basal diet, diets containing 100 g protein kg^{-1} from egg white, *Sterculia striata* or *Terminalia catappa* meals and a diet containing a mixture of 50 g egg white + 50 g protein from *Pachira aquatica* meal

Parameters	Diets				
	Non-protein ($n=6$)	Egg white ($n=6$)	*P. aquatica*[b] ($n=1$)	*S. striata* ($n=6$)	*T. catappa* ($n=6$)
Initial body weight[c] (g)	50.6 ± 3.1a	50.9 ± 3.9a	49.9 ± 3.3	50.4 ± 3.2a	51.7 ± 3.7a
Final body weight[c] (g)	40.6 ± 2.0b	88.1 ± 6.8a	30.8	54.2 ± 5.4c	51.0 ± 5.2c
Daily food intake[d] (g)	75 ± 1.5b	25.2 ± 3.3a	3.9 ± 0.6d	13.2 ± 1.8c	14.4 ± 2.2c
NPU[d] (%)	–	80.8 ± 3.3a	–	52.0 ± 3.4b	58.2 ± 5.2c
True Protein digestibility[d] (%)	–	97.2 ± 0.5a	–	91.3 ± 3.3b	88.3 ± 6.9b
Dry matter digestibilty[d] (%)	94.6 ± 0.8b	96.1 ± 0.5a	–	96.5 ± 0.5a	86.4 ± 2.4c
Body nitrogen[c] (g kg^{-1})	106 ± 6.0b	84.0 ± 3.0a	131	85.0 ± 4.0a	89.0 ± 4.0a,c
Lectin[e] ($HU.10^{-3}$ kg^{-1} flour)	–	–	113	ND[f]	32
Trypsin inhibited (g kg^{-1} flour)	–	–	2.60 ± 0.20a	ND	1.36 ± 0.12b
Chemical score[g] (%)	–	–	34 (met + cys)	61 (phe + tyr)	39 (lys)

[a] Values in a horizontal row with different following letters differ significantly ($P<0.05$).
[b] Initial body weight and daily food intake were calculated until day 6.
[c] Per rat.
[d] Per three rats.
[e] Haemagglutinating units $\times 10^{-3}$ per kg seed flour.
[f] Not detected.
[g] Chemical scores of the respective first limiting amino acid calculated using the minimal requirements for rats (Coates et al., 1969).

Table 4
Relative dry weights (g/kg body dry matter)[a] of organs and tissues of rats fed on a protein-free basal diet, diets containing 100 g protein kg^{-1} from egg white, *Sterculia striata* or *Terminalia catappa* meals and a diet containing a mixture of 50 g egg white + 50 g protein from *Pachira aquatica* meal

Organ	Diets				
	Non-protein ($n=6$)	Egg white ($n=6$)	*P. aquatica* ($n=1$)[b]	*S. striata* ($n=6$)	*T. catappa* ($n=6$)
Stomach	7.9 ± 0.7b	5.8 ± 0.6a	7.0	6.8 ± 0.8c	7.4 ± 0.6b,c
Intestine	33.1 ± 3.6b	25.1 ± 1.9a	22.3	29.2 ± 3.8b	31.1 ± 5.4b
Caecum + colon	7.7 ± 0.9a	7.9 ± 0.8a	7.9	8.4 ± 0.8a	8.6 ± 0.9a
Liver	39.8 ± 1.9a	39.7 ± 3.1a	47.4	45.2 ± 3.3b	43.7 ± 2.2b
Pancreas	3.8 ± 0.6a	3.6 ± 0.3a	6.6	3.4 ± 0.6a	4.5 ± 0.4b
Thymus	1.3 ± 0.2b	2.3 ± 0.3a	ND[c]	1.8 ± 0.4c	1.7 ± 0.3c
Spleen	1.6 ± 0.3b	2.3 ± 0.2a	1.3	1.3 ± 0.2b	1.1 ± 0.6b
Kidneys	10.8 ± 1.0b	7.7 ± 0.6a	14.5	8.7 ± 0.6c	8.3 ± 0.7a,c
Heart	3.9 ± 0.4b	3.0 ± 0.3a	4.0	3.2 ± 0.4a	3.0 ± 0.5a
Lungs	6.9 ± 0.6b	4.9 ± 0.6a	6.6	4.5 ± 0.7a	4.7 ± 0.8a

[a] Values in a horizontal row with different following letters differ significantly ($P<0.05$).
[b] Organ dry weight taken from the rat killed two days before the end of the experimental period.
[c] Not determined.

striata seeds are free of lectin and trypsin inhibitors it is likely that the observed deficiency in some essential amino acids led to the poor performance of the rats. *P. aquatica* and *T. catappa* seeds, in addition to amino acid imbalance, also presented lectin and trypsin inhibitor activities and, coincidentally, both induced hypertrophy and/or atrophy of several key internal organs of rats more markedly than those fed on *S. striata* throughout the 10-day test period (Table 4). Actually, the diet containing the *P. aquatica* seed meal was very toxic. Thus, the remaining rat fed on *P. aquatica* diet experienced enlargement of the stomach, liver, pancreas, kidneys, heart and lungs and had spleen atrophy when compared with the same organs of rats fed on egg-white diet. Hypertrophy of the pancreas and kidneys was very marked and they nearly doubled in dry weight in comparison with egg-white control group. This effect must have led the rats to die. The rats fed on *S. striata* and *T. catappa* also suffered from stomach, small intestine, and liver hypertrophy as well as spleen and thymus atrophy but these seeds were less detrimental than *P. aquatica* seeds since the rats survived the entire experimental period. Enlargement of the small intestine and pancreas had been observed previously in rats fed with diets containing purified lectins from *Canavalia brasiliensis* (Oliveira et al., 1994), *Phaseolus vulgaris* (Oliveira et al., 1988) and *Glycine max* (Gelencser et al., 1994).

In conclusion the raw seeds of *P. aquatica* were highly toxic when fed to rats even at a meal protein concentration half that of *S. striata* or *T. catappa*, which were better tolerated by the experimental animals. As the trypsin inhibitor and lectin contents are higher in *P. aquatica* than in the other two seeds it is tempting to suggest that these compounds, together with other yet-unknown deleterious substances, may have led to the overall toxicity of this particular seed to growing rats. For instance, the oil of another species of *Pachira, P. insigenes*, was considered unfit for human consumption since it caused numerous physiological disorders in experimental animals (Berry, 1980).

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