Amino acid composition and short-term toxicological evaluation of *Artocarpus heterophyllus* seed cake in rat diet

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Abstract: The amino acid composition and short-term toxicological evaluation of *Artocarpus heterophyllus* seed cake (AHSC) in rat diet was carried out in order to determine its suitability for animal feed/human consumption. The mean values obtained for the proximate composition of the seed cake were moisture: 12.34 ± 0.78 %, ash: 3.79 ± 0.34 %, crude protein: 15.10 ± 0.79 %, crude fibre: 6.09 ± 0.04 %, crude fat: 1.20 ± 0.02 % and carbohydrate: 62.68 ± 0. 25 % while compounded feed gave moisture: 9.26 ± 0.57 %, ash: 9.73 ± 0.01 %, crude protein: 22.11 ± 0.31 %, crude fibre: 6.33 ± 0.11 % and carbohydrates: 45.30 ± 0.25 %. The amino acid content of the seed cake was as high as 74.526 g/100g for both essential and minor amino acids. The phytochemical screening of *Artocarpus heterophyllus* seed cake indicated the presence of some secondary metabolites such as alkaloids, cardiac glycosides and carbohydrates. Albino rats were fed with feed that was compounded with 7.08 % of AHSC as total replacement for corn bran in the control group for six weeks. The rats were looking normal throughout the duration of the experiment and no mortality was recorded. There was no significant difference between the heamatological result of the test and control rats. Histopathological analysis revealed no lesion in the heart, liver and kidney of the experimental rats. *Artocarpus heterophyllus* seed cake seemed to be suitable for animal feed/human consumption.
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1. Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) belonging to the family Moraceae, is a fairly large sized tree and bears the largest fruit among the edible fruits. Jackfruit tree is native to India and is popular in several tropical and sub-tropical countries. The fruit is known as the 'poor man's fruit' in eastern and southern parts of India because it is a major part of their diet as a vegetable and nutritious food during the season (Chowdhury *et al*., 1997). In Nigeria, its cultivation has not been encouraged, though it is found in the south-coastal parts of the country where it grows wild or semi-conserved. Jackfruit tree is medium to large in size; it is grown mainly for its fruits, timbers, and foliage. Being a good source of vitamin A, vitamin C and pectin, jackfruit helps in alleviating the pancreatic ailments and also aids in blood purification (Bobbio *et al*., 1978).

Some functional properties of jackfruit seed flour and its protein digestibility have been reported (Ocloo *et al*., 2010; Odoemelan, 2005; Rajarajeshwari *et al*., 1999; Singh *et al.*, 1991). The proximate and physicochemical composition of parts of jackfruit has also been reported (Ajayi 2011; Bobbio *et al.*, 1978; Hossain *et al.*, 1990; Selvaraj *et al.*, 1989 and Tulyathan *et al*., 2002). Jain (2011) gave the anti-inflammatory activity of the bark. A study was conducted by Kumar *et al.* (1988) and Burkill (1977) on the proximate composition of jackfruit seed flour. The variations of carbohydrates and the distribution of free sugar and fatty acid composition of different parts of ripe jackfruit varieties have been reported (Chowdhury *et al.*, 1997 and Rahman *et al.*, 1999). The incorporation of seed flour to deep fat fried products has been found to reduce the fat absorption to a remarkable extent (Rajarajeshwari *et al.*, 1999). There is no report in literature on the use of jackfruit seed cake in rat feeding. This study reports on the amino acid composition and toxicological evaluation of *Artocarpus heterophyllus* seed cake in rat diet. This is in continuation of previous works on seeds, seed oil and seed cake and their nutritional /industrial applications (Ajayi *et al*., 2007; 2013).

2. Materials and methods

Fruits and seeds of *A. heterophyllus* were collected from the Botanical Garden, University of Ibadan, Ibadan, Nigeria in the month of February, 2011. The seeds were manually decorticated to expose the seeds of *A. heterophyllus* which were ground using a domestic grinder to give the grits. The seed oil was extracted with a soxhlet extractor using normal hexane (40–60 ^{o}C). The oil obtained, after distilling off the hexane, was stored in a labelled flask. The seed cake was desolventized and air dried at room temperature for 48 h. They were then packed in transparent polyethylene bag prior to further analysis. The moisture, oil, protein, ash and crude fibre contents of the seeds were analyzed according to AOAC (1990).

Carbohydrate content was determined by difference [100 - (protein+ crude fat + ash + crude fibre)].

Fourteen albino rats of both sexes were obtained from the Central Animal House, University of Ibadan, Ibadan, Nigeria. The animals were divided randomly into two groups: control group (n=7) and experimental group (n=7). They were allowed to acclimatize for one week prior to the experiment. The animals were housed in plastic cages, in a well ventilated room at room temperature and were given feed and water *ad-libitum* for an experimental period of 6 weeks.

A basal diet was formulated to meet the entire nutrient requirement for young albino rat of 8 weeks (*Rattus norvegius*). *A. heterophyllus* seed cake (AHSC) was used to totally replaced corn bran in the experimental diet formulated because they have similar nutrient composition and amino acid profile. The diets were prepared according to the procedure described by Souza *et al*. (2007) with slight modification. The basic ingredients used were 2800.0 g of maize, 1274.7 g of soy bean, 231.0 g of calcium, 55.3 g of salt, 994.0 g of groundnut cake, 495.6 g of palm kernel cake, 495.6 g of wheat, 495.6 g of corn bran and 158.2 g of oyster shell for the control diet with 495.6 g of AHSC replacing corn bran in the experimental diet (Table 1). Ingredients of the diets were mixed thoroughly with the mixing machine to obtain a homogenous mixture which was pelletized and packed into two different transparent sterile plastic containers.

The weight of the Wistar rats at the beginning of the experiment was about 114 to 124 g. The rats were fed with the compounded diets for 6 weeks. The rats in the control group were fed the basal diet only while the ones in the experimental group were fed a diet in which 7.08 % of AHSC totally replaced corn bran. All the rats were fed *ad libitum* and had unrestricted access to drinking water. The feed intake and body weight gain was noted every week (Leontowicz *et al*., 2007).

Three milliliters of blood was collected from each rat by cardiac puncture into heparinized vials and stored at 10 ^{o}C for haematological analysis the same day that they were sacrificed. The haematological analysis was carried out the same day. The abdominal wall of each rat was dissected through the linear alba and peritoneum using the scalpel blade. The liver, heart, kidney and spleen were carefully examined for gross lesions and weighed after removal of blood by blotting on a filter paper.

The packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) white blood cell (WBC), differential WBC counts, mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were determined and calculated respectively using the standard technique described by Jain, 1986. This entails spinning of capillary tube containing blood samples at 25,000 rpm for 5 minutes after which value was read on the microhaematocrit graphic readers and recorded in percentages.

The determination of haemoglobin concentration was carried out by measuring 5ml of Drabkins solution into a tube, and then into the spectrophotometers cuvettes; this was used to zero and standardize the spectrophotometer (after been stabilized for 30 minutes before putting the tube). The reading was taken using absorbance at wavelength of 540mm. 20ml of each blood sample was dispensed into 5ml Drabkins haemoglobin solution, mixed thoroughly and allowed to stand for 15 minutes. These were then transferred into the spectrophotometer cuvettes. The value of haemoglobin concentration was recorded using a standard prepared chart.

In determining the White Blood Cells, the graduated pipette was used to take 0.5ml of WBC fluid into 5ml bijour bottle and 25µl of the blood sample was also dispensed using 25µl automatic micropipette and then mixed thoroughly. The cover slip was fixed on the counting chamber properly. A little amount of the dilute blood was dispensed under the cover slip and left for 3 minutes after which it was mounted on the stage. Counting was done at X40 objectives. The leucocytes in the four corner primary squares were counted and the value obtained was multiplied by a factor of 50 to obtain the number of cells in 1µl of blood.

The Red Blood Cells (Erythrocytes) Count was done by withdrawing 1ml of (RBC) fluid with pipette into 5ml bijour bottle. Micropipette was used to take 5µl of blood sample and dispensed into 1µl bijour bottle and mixed effectively. Little amount of the dilute blood was dispensed into counting chamber beneath the cover slip. This was allowed to settle down for about 3 minutes and counting was done at objective. The number of cells in five secondary squares (80 tertiary squares) was determined and multiplied by 10,000. This was accomplished by adding four zeros to the total number of cells counted. This represents the number of erythrocytes per ml of blood.

Histological analyses of the heart, liver, kidney, lungs intestine, spleen and brain samples were carried out. Small portions of these tissues already harvested and stored in formalin were fixed and put through series of dehydration in graded concentration of xylene. They were embedded in wax, sectioned at 5 µ and transferred to clean glass slides. The thin sections were stained with haemotoxylin and eosin (H and E) dyes for examination under the light microscope for histological changes (Ajayi *et al*.,

2007; Jain, 1986). These tissues were observed for gross lesions.

Differences between groups were tested for by two-way ANOVA and Duncan test. The p values of <0.05 were considered significant.

3. Results

The results of the study carried out are given below:

Table 1: Feed and proximate compositions of the different diets

Feed	Amount (%)	Control diet (g)	Experimental diet (g)
Maize	40.0	2800.0	2800.0
Soy bean	18.21	1274.7	1274.7
Bone	3.3	231.0	231.0
Salt	0.79	55.3	55.3
Groundnut cake	14.2	994.0	994.0
Palm kernel cake	7.08	495.6	495.6
Wheat	7.08	495.6	495.6
Corn bran	7.08	495.6	-
AHSC[b]	7.08	-	495.6
Oyster shell	2.26	158.2	158.2
Proximate (%)[a]	AHSC	Control diet	Experimental diet
Moisture content	12.34 ± 0.78	9.09 ± 0.78	9.26 ± 0.57
Ash content	3.79 ± 0.32	9.77 ± 0.02	9.73 ± 0.01
Crude protein	15.10 ± 0.79	20.36 ± 0.31	22.11 ± 0.31
Crude fibre	6.09 ± 0.04	6.25 ± 0.03	6.33 ± 0.11
Crude fat	1.20 ± 0.02	7.00 ± 0.01	7.22 ± 0.05
Carbohydrate	61.36 ± 2.0	47.53 ±0.27	45.30 ±0.43

[a] Values are expressed as mean±SD; [b] *Artocarpus heterophyllus* seed cake

Table 2: Amino acid content of *Artocarpus heterophyllus* seed cake

Amino acid	Content (g/100g)
Glycine	2.300
Alanine	2.857
Serine	3.857
Proline	2.332
Valine	3.688
Threonine	2.834
Isoleucine	3.666
Leucine	5.571
Asparte	8.424
Lysine	4.664
Glutamate	11.629
Methionine	1.531
Phenylalanine	3.764
Histidine	1.749
Arginine	5.814
Tyrosine	2.749
Cystine	7.097

The composition of both control and experimental diets in combination with the results of the proximate composition of AHSC and the diets are given on Table 1. The percentage composition of the feed is maize: 40%; soy bean: 18.21%; groundnut cake: 14.02%; palm kernel cake, wheat 7.08%; corn bran7.08%; bone: 3.3%; oyster shell: 2.6%; salt: 0.779% with 7.08% of AHSC replacing corn bran in the experimental diets. AHSC has moisture content of 12.43±0.78; ash content: 3.79±0.32; crude protein: 15±0.79; crude fat: 1.2±0.02; crude fibre: 6.09±0.04 and carbohydrate: 61.36±2.0. There was no significant difference in the results of the proximate analysis obtained for both control and experimental diets.

Table 3: Result of phytochemical analysis of *Artocarpus heterophyllus* seed cake

Test	Result
Saponins	-
Tannins	-
Flavonoids	-
Alkaloids	+
Cardiac glycosides	+
Steroids	-
Carbohydrates	+
Terpenoids	-
Phenol	-
Phlobatanins	-

- Not present/detected; + present/detected

The result of the amino acid analysis of AHSC showed significant variation among the amino acids (Table 2) with the highest concentration level being that of glutamate: 11.629 followed by asparte: 8.424; cystine: 7.097; leucine : 5.571 and lysine:

4.664. The concentration of isoleucine: 3.688 and serine: 3.857 was greater than that of the other amino acids and the amount of methionine, histidine, threonine, alanine and glycine in the cake was minimum from 1.531to 2.857.

AHSC tested positive to alkaloids, cardiac glycosides and carbohydrates whereas tannins, reducing sugar, flavonoids, phlotabatanins, steroids, terpenoids, saponins and phenol were absent (Table 3).

Table 4: Weight increase and tissue weight of the rats in the control and experimental groups

Week increase[a]	Control group (g)	Experimental group (g)
0	114.29 ± 9.76	124.29 ± 7.87
1	120.71 ± 9.32	132.14 ± 12.20
2	124.17 ± 3.76	128.57 ± 10.69
3	129.17 ± 12.42	140.00 ± 10.41
4	143.33 ± 9.83	147.86 ± 5.67
5	146.67 ± 5.16	141.43 ± 9.00
6	148.33 ± 7.53	157.14 ± 11.13
Tissue weight[ab]		
Kidney	0.75 ± 0.27[c]	0.71 ± 0.27[c]
Liver	4.25 ± 0.69[c]	4.64 ± 0.99[c]
Lungs	1.00 ± 0.00[c]	0.93 ± 0.35[c]
Heart	0.50 ± 0.00[c]	0.50 ± 0.00[c]
Spleen	0.50 ± 0.00[c]	0.57 ± 0.19[c]
Intestine	1.33 ± 0.26[c]	1.36 ± 0.48[c]
Brain	1.42 ± 0.20[c]	1.43 ± 0.19[c]

[a]Values are expressed as mean ± SD
[b]Values in the same row with the same superscripts are not significantly different at (P< 0.05)

There was no significant difference between the organs of the control and experimental rats (Table 4).

Table 5: Result of the haematological analysis of the rats in the control and experimental groups

Parameter	Control group	Experimental group
PCV (%)	42.17 ± 2.64[c]	38.86 ± 5.24[c]
RBC (10^6/µl)	6.99 ± 0.43[c]	6.48 ± 1.07[c]
Hb (mg/dl)	13.88 ± 0.89[c]	12.56 ± 2.01[c]
MCV (fl)	19.86 ± 0.65[c]	19.42 ± 0.86[c]
MCHC (%)	32.93 ± 0.68[c]	33.22 ± 1.20[c]
WBC (10^3/µl)	5791.67 ± 2218.43[c]	5635.71 ± 936.18[c]
Lymphocyte (%)	69.33 ± 5.54[c]	57.71 ± 12.72[c]
Neutrophyl (%)	28.17 ± 4.96[c]	39.00 ± 12.41[c]
Eosinophyl (%)	0.00 ± 0.00[c]	0.71 ± 0.76[d]
Monocyte (%)	2.17 ± 2.14[c]	2.43 ± 0.54[c]
Absolute Lymphocyte	3994.58 ± 1913.11[c]	3296.79 ± 1058.59[c]
Absolute Neutrophyl	1575.50 ± 446.25[c]	2146.93 ± 556.07[c]
Absolute Eosinophyl	0.00 ± 0.00[c]	41.57 ± 44.99[d]
Absolute Monocyte	127.58 ± 125.01[c]	139.43 ± 49.97[c]
Platelets	154666.67 ± 26590.73[c]	132285.71 ± 16819.77[d]

[a]Values are expressed as mean ± SD for (n=6) for group A and (n=7) for group B
[b]Values in the same row with different superscripts are significantly different at (P< 0.05)

The results of haematological studies of both the control and test rats are presented in Table 5. There were significant increases ($p<0.05$) in the values of eosinophyl and platelets counts of the control rats in comparison with the experimental rats but there was no significant difference in the values of Hb, MCHC, PCV, RBC, monocytes, neutrophyl and WBC of the rats in the two groups.

Table 6: Histological result of the rats in the control and experimental groups

Tissue	Control group	Experimental group
Heart	No visible lesion	No visible lesion
Brain	The endothelial cells are swollen and prominent	No visible lesion
Lung	There is moderate thickening of the inter alveolar septae by proliferation of macrophages pneumonocytes.	There is moderate thickening of alveolar interstitium
Liver	No visible lesion	No visible lesion
Kidney	The renal blood vessels are moderately congested.	No visible lesion
Intestine	There are a few locally extensive foci of necrotic surface epithelial cells.	There are a few swollen and degenerate surface columnar epithelial cells. There are however foci of hyperplasia (piling of cells on each other giving the appearance of pseudo stratified epithelium)
Spleen	No visible lesion	There is expansion of the per arteriolar lymphoid shealth (PALS)

4. Discussion

Moisture provides a measure of the water content of the seed cake and its total solid content. It is also an index of storage stability of the flour. The moisture content of the seed cake was 12.34 ± 0.78 % (Table 1). The value was higher than 6.09 % reported by Ocloo *et al*. (2010) for jackfruit seed flour. The crude fat content of the jackfruit seed cake was 1.2 ± 0.2 %. This value is relatively low when compared with 3.10 % of wheat flour reported by Akubor *et al*. (2004). Values of 32.13 % and 35.43 % were obtained for two varieties of tigernut (*Cyperus esculentus*) flour by Oladele *et al*. (2000). The observed value was however comparable to 1.3 % reported by Bobbio *et al*. (2010) and 2.1-2.5 % by Kumar *et al*. (1988) for jackfruit seeds. The crude ash content of the seed cake was 3.79 ± 0.32 %. The ash content is the organic residue remaining after the organic matter has been burnt away. It is not necessarily of exact composition as the mineral matter present in the original flour as there may be losses due to volatization or some interactions between constituents. Oladele *et al*. (2000) reported ash content values of 3.97 % and 4.25 % for tiger nut flours. The crude protein content of the seed flour was 15.10 ± 0.79 %. This value was lower than 17.18-18.3 % obtained by Kumar *et al*. (1988) and Singh *et al*. (1991) and 31.9 % by Bobbio *et al*. (1978) for jackfruit seeds. The differences observed may be due to varietal differences, maturation of the seeds and environmental conditions. Values of 6.34-8.57 % have also been reported for jackfruit seed flour (Mukprasirt *et al*., 2004). The crude fibre content of the seed cake was 6.09 ± 0.04 %; this value was higher than 2.36 % and 3.06 % reported by Singh *et al*. (1991) and Tulyathan *et al*. (2002) respectively. The major component of the seed cake was carbohydrate. The value obtained for this study was 61.48 %. This value was lower than 74 % reported by Singh *et al*. (1991), 76.1 % by Kumar *et al*. (1988), 79.34 % by Ocloo *et al*. (2010) and 81.64 % by Tulyathan *et al*. (2002) for jackfruit seed flour. However, it was similar to 66.2 % reported by Bobbio *et al*. (1978) for jackfruit seeds.

The proximate composition of compounded feed for both the control and experimental rats is also shown on Table 1. The moisture content for the test diet was 9.26 ± 0.75 % while that of the control diet was 9.09 ± 0.78 %. This indicated that the moisture content was reduced after feed compounding as seen in Table 1 (12.34 ± 0.78 %). The values of ash content obtained for both the control and test diets were 9.73 ± 0.01 % and 9.77 ± 0.00 % respectively. The crude protein values for control (22.11 ± 0.31 %) and experimental feeds (20.38 ± 0.31 %) were higher than that of the seed cake (15.10 ± 0.79 %). These crude protein values were comparable to 22.0 % reported by Souza *et al*. (2007). The crude fat value (7. 22 ± 0.05 %) of the control diet was higher than that of the test one (7.00 ± 0.01 %).

AHSC contains eight out of the nine essential amino acids. The amino acid profile of *A. heterophyllus* seed cake showed that it was highest in glutamate (11.629g/100g) followed by aspartate (8.424g/100g) and lowest in methionine (1.531g/100g) (Table 2). The indispensable amino acids are leucine, isoleucine, valine, lysine, threonine, tryptophan, methionine, phenylalanine and histidine. Histidine is considered to be an indispensable amino acid because of the detrimental effects on haemoglobin concentrations that have been observed

when individuals are fed histidine-free diets FAO/WHO/UNU (1973). The composition ratio of essential amino acids of AHSC compared favourably with those of soy bean (Sarwar *et al*., 1983), egg and milk proteins (FAO/WHO/UNU, 2002). This is very significant from a nutritional point of view and based on the value obtained for the total amino content of the cake, consumption of about 100g of diet formulated by the seed cake would provide more than half of the recommended daily requirement of protein as recommended by (FAO/WHO, 2002) for children aged between 5 and 19 years. This fact suggests that AHSC may be useful as food supplement for the alleviation or prevention of protein malnutrition in developing countries.

Table 3 presents the qualitative phytochemical screening of *A. heterophyllus* seed cake. Alkaloids, cardiac glycosides and carbohydrates were detected. Alkaloids are known to show medicinal activity as well as physiological activity (Sofowora, 1979). The presence of these compounds in the seed cake suggests its usefulness in pharmaceuticals since they can serve as potent starting materials in the synthesis of sex hormones (Okwu, 2001).

The physical appearance of both the control and test rats showed that the rats were looking normal throughout the duration of the experiment and no mortality was recorded.

At the beginning of the feeding period, the experimental rats had the average body weight of 124.29 ± 7.87 g while that of the control rats was 114.29 ± 9.76 g (Table 4) but at the end, the test rats had higher mean body weight of 157.14 ± 11.13 g than those of the control (148.33 ± 7.53 g). This is a good indication that AHSC could probably serve as a total replacement for corn bran in rat feed.

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PVC (%) in the blood varied from 38.86 ± 5.24 to 42.17 ± 2.64 for all the rats in both groups. Haemoglobin concentration (mg/dl) of the rats was between 12.56 ± 2.01 to 13.88 ± 0.89 for both the test and control groups (Table 5). There seemed to be no difference in most parameters studied in the blood of rats from both groups with the exception of eosinophyl, absolute eosinophyl value and blood platelets. RBC (10^6/ml) of the control group (6.99 ± 0.43) was a bit greater than that of the test group (6.48 ± 1.07). MCV (fl) value 19.42 ± 0.86 of the test group was similar to 19.86 ± 0.65 value obtained for the control group. An analysis of variance showed that there was no significant difference between the two groups. The percentage MCHC, 33.22 ± 1.20, of the rats in the test group was greater than 32.93 ± 0.68 of those in the control group. White blood cell count (10^3/µl) of the control and test groups was 5791.67 ± 2218.43 and 5635.71 ± 936.18 while the platelets value were 154666.67 ± 26590.73 and 132285.71 ± 16819.77 respectively. The percentage neutrophilis 39.00 ± 12.41 of the test group rats was greater than 28.17 ± 4.96 of those in the control group. There was significant difference between the percentage eosinophyl and absolute eosinophyl of the control and the test group rats. The value 139.43 ± 49.97 for absolute monocyte of the test group rats were greater than that of 127.58 ± 125.01 obtained for the control group.

No lesion was observed in the heart, liver and spleen of the rats in the control group but there were mild lesion in their brain, lungs, kidney and intestine (Table 6). The heart, brain, liver and kidney of the experimental rats did not show any lesion. However, there was some lesion observed in their lung, intestine and spleen. The seed cake might not be harmful to most organs and tissues of rats fed with 7.08 % of AHSC inclusion level in the feed indicating that it could probably be used to replace corn bran or any other carbohydrate component in the diet of livestock and even man.

5. Conclusion

Artocarpus heterophyllus seed cake has a potential of being utilized successfully as a source of dietary energy and protein for livestock because of its

high carbohydrate and protein values. The essential amino acid content is nutritional significant. The presence of alkaloids in the seed cake suggests its usefulness in drug manufacture. The test rats showed appreciable weight gain during the experimental period and the feed intake also showed that the rats consumed reasonable quantity of the diet. Haematological analysis showed no significant adverse effect on the rats; AHSC might therefore be safe for human consumption.

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