

## Evaluation of *Cucumeropsis mannii* Seed Cake

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**Abstract:** The chemical composition of *Cucumeropsis mannii* seed cake was analyzed for in order to evaluate its suitability and application in albino rat feed. The proximate analysis of *Cucumeropsis mannii* seed cake showed that it had high protein ( $43.63 \pm 0.13\%$ ) but low carbohydrate ( $5.28 \pm 0.14\%$ ) and crude fibre value ( $4.39 \pm 0.03\%$ ) contents. The predominant mineral was K ( $181.90 \text{ g/l}$ ) followed by Mg ( $83.90 \text{ g/l}$ ) and Na ( $39.93 \text{ g/l}$ ). Other mineral elements such as Ca, Fe, Zn, Cu and Mn were 3.75, 2.58, 1.86, 0.695 and  $0.417 \text{ g/l}$  respectively. The amino acid content ( $\text{g}/100\text{g}$ ) of *C. mannii* seed cake was appreciably high especially for arginine (9.19), alanine (5.74) and glutamine (16.82), asparagines (16.25). The rat experiment lasted for eight weeks; rats were divided into two groups A and B and fed with diets that contained groundnut cake (control group) and 14.45% *C. mannii* seed cake as a total replacement for groundnut cake (test group) and in the compounded feed. Both control and test feed had high values of carbohydrate ( $50.03 \pm 0.11\%$  and  $44.4 \pm 0.27\%$ ) and crude protein ( $22.40 \pm 0.13\%$  and  $23.71 \pm 0.13\%$ ) than *C. mannii* seed cake respectively. The albino rats appeared to suffer no toxicological effect and weekly monitoring showed good physical appearance. Haematological and histopathological examination of sections of the heart, liver, kidney, spleen, lung, intestine and brain results obtained showed no significant difference between the test and control groups.

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### 1. Introduction

*Cucumeropsis mannii*, belonging to the *Cucurbitaceae* family, is a climber that grows in wet humid climate, particularly in the South western Nigeria. This white seed melon is grown mostly as an oil rich seed crop and it is also a source of dietary proteins Badiru *et al.* (1991) and Fokou *et al.* (2004). The seeds are obtained either in shelled or unshelled forms in West African markets and are used greatly in West African cookery. The shelled seeds can be ground or milled before and after roasting and are used in soups and as soup condiments. Melon seeds may be eaten as snacks, either as whole toasted seeds or as fried cake prepared from milled seeds Ogbonna *et al.* (2000). Various ethnic groups have different local names as follows: EFIK òkòkò, IGBO àhù, àhù elu or aki (Onitsha), ògìlì (Owerri) àhù, YORUBA ègúsí-itòò or itòò, (Ilorin) itoro and HAUSA àgùshìi (Burkill, 1985).

Developing nations do not produce enough food and of the right nutritional quality to meet daily needs of their ever growing population, therefore, there is a need to search for nutritious and locally available underutilized food products in order to ensure that all the potential sources of foods are effectively exploited, Oshodi *et al.* (1999). The effective utilization of any plant protein sources in food supplementation or in new food product formulation is based on the knowledge of their

nutritional composition and functional properties. *Cucumeropsis mannii* is a good source of dietary oil and protein Fokou *et al.* (2004). Its industrial application, however, depends on the knowledge of their protein quality and functional properties. The aim of this work therefore is to determine the amino acid content, the chemical composition, the nutritional evaluation as well as to evaluate the toxicological effect of using defatted *C. mannii* seeds as feed supplement in albino rat. This is in continuation of previous works on seeds and seed cake and their nutritional /industrial applications (Ajayi *et al.*, 2004; 2008; 2012).

### 2. Material and methods

*Cucumeropsis mannii* fruits used for this work were collected from a farm at Bode-saadu in Ilorin, Kwara state, Nigeria. After collection, fruits were cracked, packed in a heap and left for 14–20 days to allow the fruit pulp to rot. Then the seeds were removed and thoroughly washed to remove thick mucilage covering them. They were covered with sand to prevent sticking, which would make hulling difficult and were dried at room temperature before packing. The seeds obtained were shelled manually and left to air dried for few days at room temperature and ground into a paste using a previously cleaned and dried mortar and pestle. The paste was then stored in an air tight container for analysis.

*C. mannii* seed oil was extracted using cold extraction method. The residue remaining after oil extraction was air dried, pulverized and passed through a 200 mesh size to obtain the defatted powder known as *C. mannii* seed cake which was used as experimental material. Percent moisture, crude fat, ash and crude fibre contents *C. mannii* seed cake was determined using the methods of Association of Official Analytical Chemists (AOAC, 1990). Evaluation of percent crude protein content of the seed cake was carried out using the micro-Kjeldahl method and crude protein was calculated ( $N \times 6.25$ ). Moisture, oil, protein, ash and crude fiber contents of the seeds were also analyzed for. Carbohydrate contents were determined by difference [ $100 - (\text{protein} + \text{crude fat} + \text{ash} + \text{crude fiber})$ ].

0.5 g of *C. mannii* seed cake was digested with 20 ml mixture of concentrated  $\text{HNO}_3$  and perchloric acid (2:1 v/v) until the solution became a clean one. It was thereafter, transferred to a 100 ml volumetric flask, and diluted. It was made up to the mark with deionized water and stored in a clean polyethylene bottle. The mineral element content was determined using an atomic absorption spectrophotometer (Perkin–Elmer model 703, USA) as described by Onyeike and Acheru (2002) with the exception of sodium and potassium which were determined by flame photometric method.

The seed cake was hydrolysed in 6 M HCl at 105 °C for 22 hours in nitrogen flush. The hydrolysate was further analysed for amino acids using the sequential multi-sample amino acid analyzer as described by Spackman *et al.* (1958). The chromatogram of the sample was compared using norleucine as a standard. The amino acid content of the sample was recovered by extracting with 30 ml of dichloromethane three times before concentrating to 1.0 ml for gas chromatography analysis (AOAC, 2006).

Fourteen albino rats (aged 6 weeks, weighing between 80-86 g) were obtained from the Physiology Department, University of Ibadan, Nigeria. The animals were divided into 2 groups (A and B) of seven rats per group. At the beginning of experiment, the control group (A) was fed with control diet (Feed A) while the test group (B) was fed with the compounded feed (Feed B) made from *C. mannii* seed cake.

A basal diet feed was formulated to meet the entire nutrient requirement for young rats. The diet was prepared according to the formula and procedure used by Souza *et al.* (2007) with little modification. The basic ingredients used were 4000.0 g of maize, 1820.0 g of soy beans, 330.0g of dicalciumphosphate, 79.0 g of salt, 1445.0 g of groundnut cake, 700.0 g of palm kernel cake, 700.0 g of wheat, 700.0 g of corn

bran and 226.0 g of oyster shell. For the experimental feed, 1445.0 g of *C. mannii* seed cake was used to replace 1445.0 g of groundnut cake which was used in the control feed (Table 1). The ingredients were mixed thoroughly with the mixing machine, pelletized, air-dried and packed into two different transparent containers.

At the end of the feeding period of eight weeks, the rats were fasted over night. Blood sample was immediately collected from the eye into test tubes containing EDTA to prevent blood coagulation, and then, they were harvested. The tissues collected were kidney, heart, spleen, lungs, small intestine, brain and liver. These organs were weighed immediately after collection and preserved in formalin for pathology studies.

The packed cell volume (PCV), haemoglobin (Hb) concentration, red blood cell (RBC) and white blood cell (WBC) counts were determined using the standard techniques described by Dacie and Lewis (1991) and Jain (1986). The differential WBC counts mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated (Jain, 1986).

The internal organs were exposed by dissection and the liver, spleen, kidney and lungs were observed for gross lesions. Small portions of each organ already stored in formalin were fixed and put through timed series of dehydration in graded concentrations of xylene. They were embedded in wax, sectioned at 5 $\mu$  and transferred on to clean glass slide. The thin sections were stained with haematoxylin and eosin (H and E) dye for examination under the light microscope for histological changes.

### 3. Results

The results of the experiment are shown on Tables 1-7 and Figures 1-5 below.

Table 1. Composition of control and test feeds

| Feed components (g)                        | Amount (%) | Control Feed        | Test Feed           |
|--|------------|---------------------|---------------------|
| Maize                                      | 40.0       | 4000.0              | 4000.0              |
| Soy bean                                   | 18.2       | 1820.0              | 1820.0              |
| Bone ( $\text{Ca}_2\text{PO}_4$ )          | 3.3        | 330.0               | 330.0               |
| Salt                                       | 0.79       | 79.0                | 79.0                |
| <i>C. mannii</i> seed cake/ Groundnut cake | 14.45      | 1445.0 <sup>a</sup> | 1445.0 <sup>b</sup> |
| Palm kernel cake                           | 7.0        | 700.0               | 700.0               |
| Wheat                                      | 7.0        | 700.0               | 700.0               |
| Corn bran                                  | 7.0        | 700.0               | 700.0               |
| Oyster shell                               | 2.26       | 226.0               | 226.0               |

Table 2. Result of proximate composition of *C. mannii* seed cake and diets A and B

| Parameters (%)   | <i>C. mannii</i> | Control feed | Test feed    |
|------------------|------------------|--------------|--------------|
| Moisture content | 12.74 ± 0.02     | 14.11 ± 0.11 | 15.33 ± 0.08 |
| Ash content      | 5.15 ± 0.01      | 12.40 ± 0.13 | 12.79 ± 0.20 |
| Crude protein    | 43.63 ± 0.13     | 22.40 ± 0.13 | 23.71 ± 0.13 |
| Crude fibre      | 4.39 ± 0.03      | 7.50 ± 0.02  | 7.60 ± 0.02  |
| Crude fat        | 33.21 ± 0.04     | 0.49 ± 0.01  | 3.77 ± 0.19  |
| Carbohydrate     | 5.28 ± 0.14      | 50.03 ± 0.11 | 44.42 ± 0.27 |

Values are expressed as mean ± SD for n= 3 except for carbohydrate

Table 3. Mineral element content of *C. mannii* seed cake

| Metals    | Concentration (mg/l) |
|-----------|----------------------|
| Magnesium | 83.903               |
| Calcium   | 3.753                |
| Sodium    | 39.930               |
| Manganese | 0.417                |
| Copper    | 0.695                |
| Iron      | 2.583                |
| Zinc      | 1.857                |
| Potassium | 181.897              |

Table 4. Amino acid content of *C. mannii* seed cake

| Amino acid    | Content (g/100g)     |
|---------------|----------------------|
| Glycine       | 2.346                |
| Alanine       | 5.739                |
| Serine        | 2.258                |
| Proline       | 3.356                |
| Valine        | 1.373                |
| Threonine     | 3.419                |
| Leucine       | 4.293                |
| Isoleucine    | 4.857                |
| Lysine        | 4.122e <sup>-1</sup> |
| Aspartate     | 16.252               |
| Glutamate     | 16.817               |
| Phenylalanine | 3.481                |
| Methionine    | 3.267e <sup>-1</sup> |
| Arginine      | 9.193                |
| Histidine     | 2.117                |
| Cystine       | 1.168                |
| Tyrosine      | 2.369                |

Table 5. Weight of rat tissues (g)

| Tissue    | Control group            | Test group               |
|-----------|--------------------------|--------------------------|
| Kidney    | 1.34 ± 0.21 <sup>a</sup> | 1.24 ± 0.18 <sup>a</sup> |
| Liver     | 6.29 ± 0.76 <sup>a</sup> | 5.48 ± 0.58 <sup>b</sup> |
| Lungs     | 1.60 ± 0.27 <sup>a</sup> | 1.34 ± 0.18 <sup>b</sup> |
| Heart     | 0.73 ± 0.14 <sup>a</sup> | 0.90 ± 0.35 <sup>a</sup> |
| Spleen    | 0.69 ± 0.09 <sup>a</sup> | 0.80 ± 0.16 <sup>a</sup> |
| Intestine | 1.37 ± 0.16 <sup>b</sup> | 1.78 ± 0.22 <sup>a</sup> |
| Brain     | 1.49 ± 0.12 <sup>a</sup> | 1.54 ± 0.11 <sup>a</sup> |

Values are expressed as mean ± SD for (n=7) for both control and test groups

Values in the same row with different superscripts are significantly different at P<0.05

Table 6. Result of haematological and biochemical analyses

| Parameter                 | Control group                     | Test group                       |
|---------------------------|-----------------------------------|----------------------------------|
| PVC (%)                   | 49.14 ± 4.38 <sup>a</sup>         | 43.40 ± 6.19 <sup>b</sup>        |
| RBC (10 <sup>6</sup> /μl) | 7.98 ± 0.76 <sup>a</sup>          | 7.21 ± 1.23 <sup>b</sup>         |
| Hb (mg/dl)                | 15.60 ± 1.60 <sup>a</sup>         | 13.70 ± 1.77 <sup>b</sup>        |
| WBC (10 <sup>3</sup> /μl) | 4692.86 ± 1276.53 <sup>b</sup>    | 4970 ± 1449.40 <sup>a</sup>      |
| MCHC (%)                  | 31.70 ± 0.98 <sup>a</sup>         | 31.64 ± 1.26 <sup>a</sup>        |
| MCV (%)                   | 61.64 ± 1.68 <sup>a</sup>         | 60.43 ± 2.27 <sup>a</sup>        |
| MCH (%)                   | 19.54 ± 0.82 <sup>a</sup>         | 19.13 ± 1.22 <sup>a</sup>        |
| Lymphocyte (%)            | 74.29 ± 5.41 <sup>a</sup>         | 67.80 ± 12.81 <sup>b</sup>       |
| Neutrophyl (%)            | 22.86 ± 6.18 <sup>b</sup>         | 30.20 ± 14.02 <sup>a</sup>       |
| Eosinophyl (%)            | 1.57 ± 0.53 <sup>a</sup>          | 1.00 ± 1.41 <sup>b</sup>         |
| Monocyte (%)              | 2.00 ± 1.00 <sup>a</sup>          | 1.20 ± 0.84 <sup>b</sup>         |
| Absolute Lymphocyte       | 4393.36 ± 1017.07 <sup>a</sup>    | 3392.10 ± 1373.97 <sup>b</sup>   |
| Absolute Neutrophyl       | 1058.64 ± 380.11 <sup>b</sup>     | 1478.00 ± 811.17 <sup>a</sup>    |
| Absolute Eosinophyl       | 76.50 ± 38.98 <sup>a</sup>        | 52.60 ± 72.48 <sup>b</sup>       |
| Absolute Monocyte         | 90.50 ± 43.60 <sup>a</sup>        | 58.20 ± 35.98 <sup>b</sup>       |
| Platelets                 | 88428.57 ± 19,346.47 <sup>b</sup> | 91000.00 ± 24728.53 <sup>a</sup> |
| Total protein             | 8.00 ± 0.30 <sup>a</sup>          | 7.66 ± 0.37 <sup>a</sup>         |
| Albumin                   | 4.91 ± 0.29 <sup>a</sup>          | 4.58 ± 0.28 <sup>a</sup>         |
| Globulin                  | 3.04 ± 0.18 <sup>b</sup>          | 3.28 ± 0.40 <sup>a</sup>         |
| Albumin-Globulin ratio    | 1.60 ± 0.13 <sup>a</sup>          | 1.36 ± 0.13 <sup>b</sup>         |

Values are expressed as mean ± SD for (n=6) for control group and (n=7) for test group

Values in the same row with different superscripts are significantly different at P<0.05

Table 7: Pathology examination of rat tissues

| Tissue    | Control group   | Test group  |
|-----------|---|---|
| Heart     | No visible lesion   | No visible lesion   |
| Lung      | No visible lesion   | There is slight desquamation of the bronchiolar epithelium  |
| Liver     | No visible lesion   | No visible lesion   |
| Kidney    | There is moderate sloughing off of the tubular epithelium in the renal medulla.                                   | There is moderate multifocal sloughing off of the tubular epithelium in the renal medulla.  |
| Brain     | There is marked vacuolation of the neurons and neutrophils  | There are a few foci of moderate to severe vacuolation of the neurons and neutrophils   |
| Intestine | There is marked sloughing off of the villi; there are numerous amounts of cellular debris in the intestinal lumen | The villi are shortened, moderately depleted in numbers; at the tip of the villi, there are inflammatory cellular aggregates (mostly neutrophils and lymphocytes) |

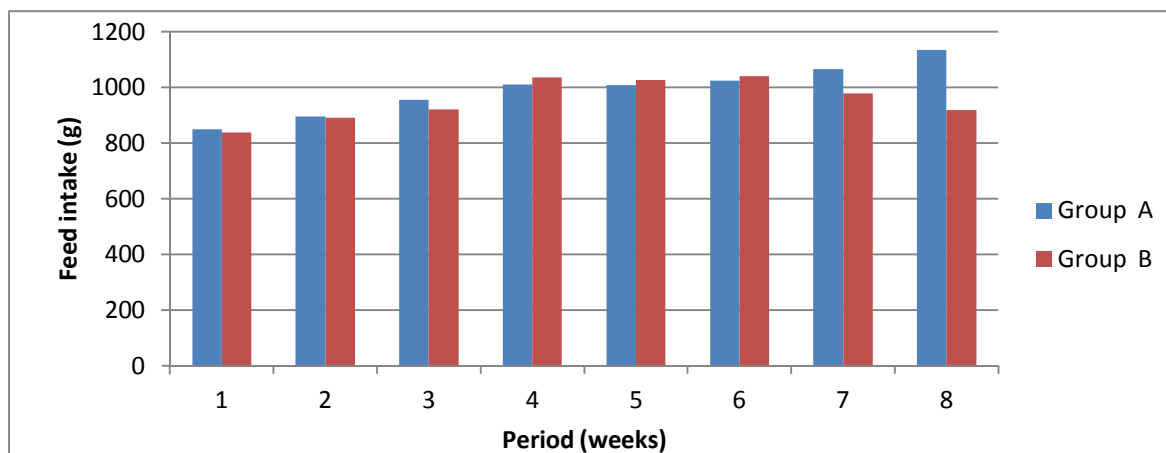


Figure 1. Graph of feed intake of control (A) and test (B) rats

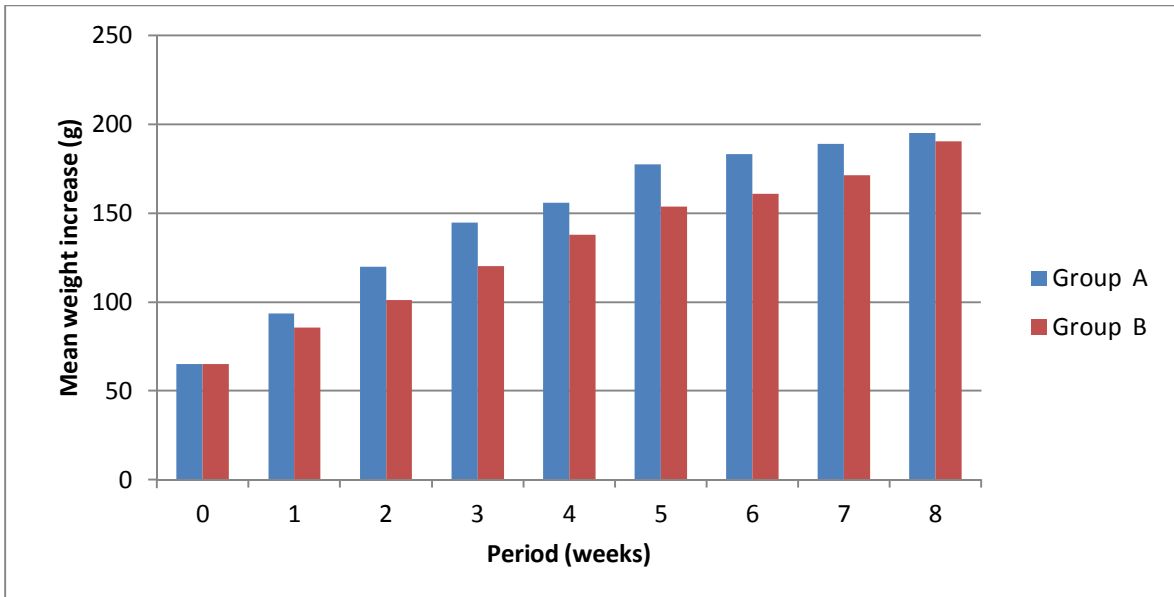


Figure 2. Graph of weight increase of control (A) and test (B) rats

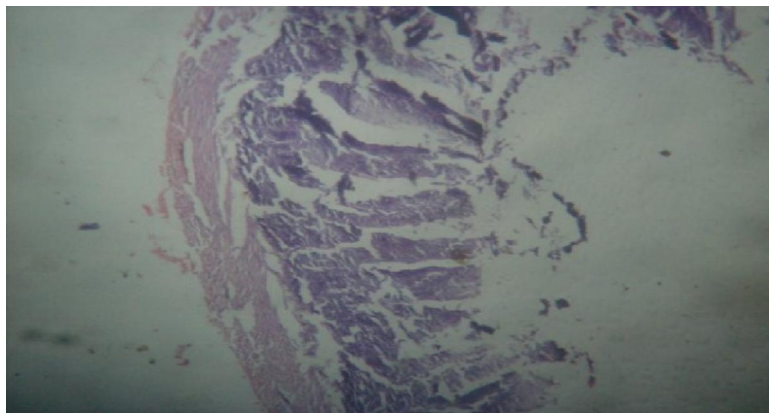


Figure 3. Photomicrograph of the intestine of one of the test rats showing that the villi are shortened, moderately depleted in numbers and have inflammatory cellular aggregates. H&E= X 100

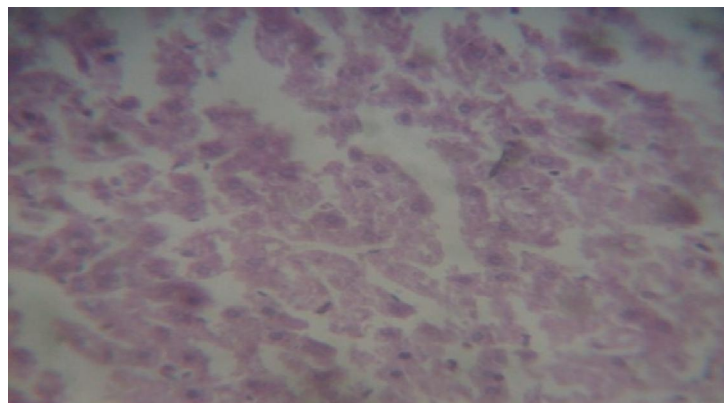


Figure 4. Photomicrograph of the lung of one of the test rats showing a slight desquamation of bronchiolar epithelium. H&E= X 100



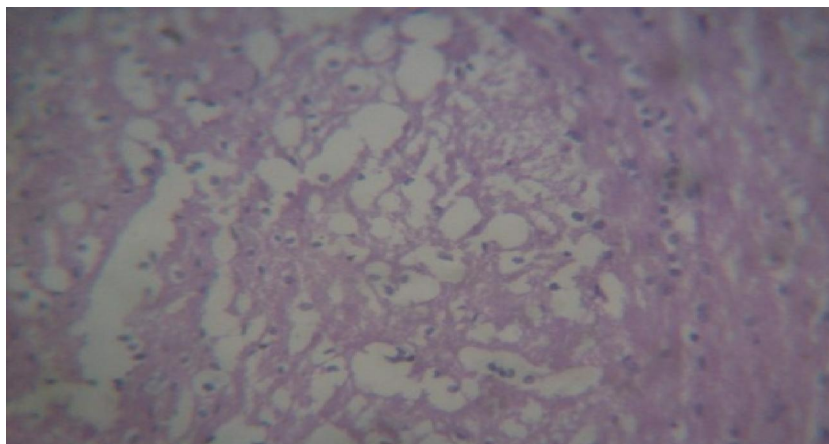


Figure 5. Photomicrograph of the brain of one of the test rats showing few foci of moderate to severe vacuolation of the neurons and neurophilis. H&E=100

### Discussions

The result of proximate analysis of *C. mannii* seed cake is shown on Table 2. The result obtained showed that defatted cucumeropsis seed contained  $12.74 \pm 0.02$  moisture,  $43.63 \pm 0.13$  crude protein,  $4.39 \pm 0.03$  crude fibre,  $33.21 \pm 0.04$  fat, and  $5.15 \pm 0.01$  ash content. The carbohydrate content is very low,  $5.28 \pm 0.14\%$ . *C. mannii* seed cake had high fat content ( $33.21 \pm 0.04$  g/100g) when compared with 23.5g/100g reported for soybean by Njoku et al. (2010). However, the crude protein content in the seed cake is higher than the one that is given in literature for legumes and oil seed flours such as fluted pumpkin (30.42), pigeon pea (21.64) *C. citrullus* (28.44) and compared well with (33.8) in lupin seed flours as reported respectively by Fagbemi et al. (2007); Aremu et al. (2006) and Lqari et al. (2002). It was found to be  $43.63 \pm 0.13\%$  which was very high when compared with *A. heterophyllus* (20.19%), *T. africana* (27.44%) and groundnut 26.5% (Onyeike and Acheru 2002). Both the high protein and crude fiber values are in accordance with the values obtained for *C. mannii* seed cake by Eunice et al. (2012); *C. mannii* could be an alternative source of dietary protein. The value obtained for crude fibre was  $4.39 \pm 0.03\%$ . The ash content shown ( $5.15 \pm 0.01\%$ ) was slightly lower when compared with 6.72% and 5.50% for *A. heterophyllus* and *T. africana* (Ajayi, 2008) but higher than the values of 1.5% and 2.27% for palm kernel and groundnut (Onyeike and Acheru, 2002).

The result of the mineral elements indicates that *C. mannii* seed cake contained significant amount of important minerals (Table 3). The K concentration (181.897 mg/L) was the highest, followed in descending order by Mg (83.903 mg/L), Na (39.930mg/L), Ca (3.753 mg/L), Fe (2.583 mg/l) Zn (1.857mg/L), Cu (0.695mg/l), and Mn (0.417 mg/L). These minerals in the diet are generally required for

normal growth, activities of muscles and skeletal development, cellular activity and oxygen transport, chemical reaction in the body and intestinal absorption, fluid balance and nerve transmission, as well as the regulation of acid-base balance (Ogbe and Affiku, 2011).

Among the 17 amino acids determined in *C. mannii* seed cake as shown on Table 4, glutamate had the highest concentration (16.817 g/100g) followed by aspartate 16.252 g/100g, arginine 9.193 g/100g, alanine 5.739 g/100g, isoleucine 4.857 g/100g, leucine 4.293 g/100g, phenylalanine 3.481 g/100g. Ojieh et al. (2008) reported the following values for these essential amino acids: arginine (9.0), isoleucine (4.8), leucine (4.2), and phenylalanine (3.2) as well as glutamic acid (16.9) and aspartic acid (16.3) for *Citrullus lanatus* (egusi, melon) flour. Lysine had a very low value of  $4.122e^{-1}$  which was the same with the one reported by Oyenuga (1978) for melon species.

Generally, the rats maintained fine and smooth hairs all through. There was no significant smell except that of their urine. Both the control and experimental groups had the normal rats smell. It was important to note that cases of natural mortality were recorded in the experimental group with symptoms weight drop and weakness of the body in the seventh and eighth weeks respectively.

Figs. 1 and 2 show the feed intake and body weight changes per week of the rats in both the test and control groups. There were positive weight changes in each group within the period of this study. At the beginning of the experiment, the feed consumed by both control and experimental groups increased from the first week to the eighth week. The average body weight of rats for both the test and control groups increased from the beginning till the end of the experiment as shown on Fig. 2. This is a

good indication that *C. mannii* seed cake might actually be good as total replacement for groundnut cake in rat feed due to rapid weight gained and the good physical appearance of the rats.

The organs whose weights were noted were liver, kidney, heart, lungs, intestine, brain and spleen (Table 5). The liver weight obtained for rats in the control and test groups ( $5.48 \pm 0.58\text{g}$  and  $6.29 \pm 0.76\text{g}$ ) respectively is higher than the report given by Vishnu *et al.* (2010) where the control group was  $4.1 \pm 0.26$  and the test group ranged from  $3.94 \pm 0.94\text{g}$  to  $4.01 \pm 0.31\text{g}$ . Vishnu *et al.* (2010) also found no significant difference between the organs of the rats in the control group and the various doses of *G. mangostana* pericarp applied on rats. The values of heart, brain,  $0.73 \pm 0.1\text{g}$ ,  $1.49 \pm 0.12\text{g}$  obtained for rats in the control group are similar to those for test group rats. Average lungs weight of rats in control group ( $1.60 \pm 0.27\text{g}$ ) was slightly higher than that of test group ( $1.34 \pm 0.18\text{g}$ ). The kidney weight for control group ( $1.34 \pm 0.21\text{g}$ ) was higher than those of the test group ( $1.24 \pm 0.18\text{g}$ ). The difference in the kidney weight of the rats may emanate from the initial body differences in the rats. The spleen weight of the control group ( $0.69 \pm 0.09\text{g}$ ) was lower than that of the test group ( $1.78 \pm 0.22\text{g}$ ). The values obtained for the brain weight of both groups are almost the same ( $1.49 \pm 0.12\text{g}$  and  $1.54 \pm 0.11\text{g}$  for the control and test group respectively).

Table 6 shows the result of packed cell volume (PVC) and total white blood cell (WBC) count of the control and test rats. The mean packed cell volume (PVC) and total white blood cell count (WBC) of rats in the test group are  $43.40 \pm 6.19\%$  and  $4970 \pm 14.49$ , respectively. These values did not differ significantly from  $49.14 \pm 4.38\%$  and  $4692.86 \pm 1276$  respectively in the control rats. The result indicate that rats in the two groups were not anaemic, as their haematological values were similar to those reported for healthy rats and related murine species (Ogunsanmi *et al.*, 2002). The similarity of WBC, both in the test and control rats suggests that the rats had no infection. This is similar to previous report on *T. occidentalis* by Ajayi *et al.* (2004).

No lesions were observed in the heart, lung and liver of rats from the control group fed with normal rat feed. In the rats fed with *C. mannii* seed cake, the pathology result showed no major complications and no significant differences in the tissues (heart, lung, liver and kidney) of rats in both groups as shown on table 6. The intestine and the brain of rats fed with normal feed showed a marked sloughing off of the villi, numerous amounts of cellular debris in the intestinal lumen (Fig. 3) and a marked vacuolation of the neurons and neutrophil. Rats fed with *C. mannii* seed cake showed that the villi

were shortened, moderately depleted in numbers; at the tip of the villi, there are inflammatory cellular aggregates mostly neutrophils and lymphocytes and there were a few foci of moderate to severe vacuolation of the neurons and neutrophil in the brain (Fig. 5). No lesion were observed in the liver and heart of rats fed with *C. mannii* seed cake. These findings indicate that *C. mannii* seed cake is not harmful to most organs and tissues of rats. Hence it can be used to replace similar conventional seed cake in the diet of livestock.

#### 4. Conclusion

*Cucumeropsis mannii* seed cake had a high value of crude protein but low crude fibre and carbohydrate; it can be supplemented for with high carbohydrate feed components such as maize and corn bran. The high values of some amino acids found in the seed cake indicate the good protein nature of defatted. *C. mannii* seed cake contains mineral element that are all useful in making the body strong. The rats in the test group displayed fairly similar body weight gain when compared to those from the control group. There was no significant change in the haematological parameters as well as in the tissue pathology studies of the rat in both groups; *C. mannii* seed cake might be a good component of rat feed.

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