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PROCESSING OF FLUTED PUMPKIN SEEDS, *TELFAIRIA OCCIDENTALIS* (HOOK F) AS IT AFFECTS GROWTH PERFORMANCE AND NUTRIENT METABOLISM IN RATS

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ABSTRACT

This study determined the nutrient and some anti-nutrient components in *Telfairia occidentalis* seeds. The work also evaluated the effects of processing on some of the anti-nutritional factors in the seeds as well as growth and animal metabolism. Fresh seeds of *T. occidentalis* were divided into three groups based on heat treatment: group 1, the unprocessed (raw) seeds; group 2, the under-processed seeds (heat-treated at 70^{o}C for 30 min); and group 3, the processed seeds (cooked at 100^{o}C for 1 hr). Seeds from each group were de-hulled, sun-dried and pulverized. Portions from each group were subjected to proximate composition analysis; trypsin inhibitor and lectin content were also measured in the seeds. The dried seed samples were incorporated into the diets of experimental animals. Twenty albino rats were randomly divided into four groups and fed with the control or experimental diets for a period of 21 days. During this period, body weights of the animals and feed intake were recorded daily and feces and urine were collected. At the end of experimental period, blood samples were collected from the animals for heamatological analysis, then the animals were sacrificed and some key organs were excised for histopathological analysis. Results showed that the seeds contained essential nutrients and that processing significantly affected the lectin and trypsin inhibitor (anti-nutrients) in the seeds. In comparison with control animals, the parameters measured which included body weight gain, nutrient digestibility, nitrogen balance, nitrogen retention and hematological parameters were markedly different among the three groups of animals fed diets incorporated with the seeds. Histopathological analysis indicated that the spleen and small intestines were adversely affected in the experimental animals. In conclusion, *T. occidentalis* seeds have high nutritive value, but could have deleterious effects in animals if ingested without adequate processing. It could, however serve as a high quality and low cost plant protein source for animal feed formulations provided adequate seed processing is ensured.

Key words: seeds, anti-nutrient, processing, metabolism, growth

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INTRODUCTION

Plant seeds form an important part of human diets and are usually regarded as good foods [1]. The significance of plant seeds especially in the diet of the population in developing countries is increasing for several reasons. First, the seeds have nutritive and calorific values, which make them necessary in diets as good sources of proteins, edible oils and fats. The seeds are also potential raw materials for local industries, especially, in the oleo chemical and animal feeds industries [2].

Fluted pumpkin (*Telfairia occidentalis* Hook F.) is a perennial plant with great economic importance in Nigeria. It belongs to the family, Cucurbitaceae and originated from tropical West Africa [3, 4]. Fluted pumpkin is dioecious with male and female flowers borne on different plants [5]. Previous studies on fluted pumpkin reported an XY system of sex chromosomes with homogametic XX female and heterogametic XY male [6]. Cross pollination in fluted pumpkin is undertaken by insects after fertilization; the seeds produced are enclosed by young drupe-like pods which usually contain male and female seeds. The harvesting of fluted pumpkin usually takes place 120-150 days after sowing. The popularity of the plant stems from the high nutritional value of its leaf and seed which are eaten as food [7, 8]. The seeds are eaten roasted or boiled and are also sometimes used as soup thickeners [9]. The seeds contain 13% oil and are used for cooking, marmalade manufacturing and cookie formulations [10, 11]. The oil of *T. occidentalis* seeds have a high iodine and a high content of unsaturated fatty acids when compared to palm oil. The seed oil is also suitable for manufacturing of soaps, paints and vanishings [12]. The fermented seeds of fluted pumpkin are used in the production of “Ogiri ugu”, alocally made custard [2]. Seed residue after oil extraction is also used as animal feeds [13]. Fluted pumpkin seeds have been reported to be rich in proteins [14]. There is, however, a major limitation in the utilization of many plant seeds, including *T. occidentalis* seeds because they contain anti-nutritional factors such as enzyme inhibitors, allergens, lectins, and other naturally occurring substances that may influence diet intake, digestibility, absorption and metabolic processes in animals and humans. There have been reports that the existence of these anti-nutritional factors in food causes growth inhibition as well as digestive and histological perturbations in laboratory models [15]. Many lectins either directly or indirectly cause profound morphological and physiological modifications in the small intestine. Such alterations characteristically lead to increased shedding of brush border membranes, accelerated cell-loss and shortened, sparse and irregular enterocyte microvilli, thus disrupting digestion and absorption. In animals, the effects of lectins depend on their origin, dose and duration of feeding [16, 17].

Removal of undesirable components is essential to improving the nutritional quality of foods and effectively utilizes their full potential as food ingredient. There have been reports that anti-nutritional factors in food may be partially inactivated by processing methods such as boiling, soaking, fermenting or sprouting [18]. There is, however, little data to show that any of these methods remove the anti-nutritional factors completely from the food. Consequently, ingestion of diets based on raw seeds

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containing these factors has been found to result in weight loss and eventual death of experimental animals [19].

As a good source of protein, fluted pumpkin seeds play an important role in the world of food production both for humans and animals. However, the seeds contain many kinds of anti-nutritional factors such as lectin, trypsin inhibitors, phytates, and tannins. Some of these anti-nutritional factors such as saponins, oxalates and phytates are removed by washing, soaking and parboiling but others such as lectins and trypsin inhibitors are more resistant and will require higher temperature and prolonged cooking in order to be inactivated. This study, therefore, was aimed at investigating the effects of some anti-nutritional factors in the seeds of *Telfairia occidentalis* (processed and unprocessed) on growth performance and nutrient metabolism in rats

MATERIALS AND METHODS

Seed collection and preparation

Fresh *T. occidentalis* seeds (Figure 1A and B) were purchased from a local market in Ile-Ife, Nigeria and authenticated in the IFE Herbarium, Department of Botany, Obafemi Awolowo University, Ile-Ife, Nigeria. The seeds were divided into three groups. Group A was the raw uncooked seed (unprocessed), group B was heat-treated at 70^{o}C for 30 minutes (under- processed), and group C was cooked for 1 hr at 100^{o}C (processed). Seeds from each group were de-hulled, sliced and sun-dried and were then pulverized using warring blender. The dried samples were kept at -4°C until use.

Figure 1A: Pod containing fluted pumpkin seeds

Figure 1B: Fluted pumpkin seeds

Proximate composition analysis of seed samples

Moisture, protein, crude fat, crude fibre and ash contents of the seed powder were determined by the standard official methods of analysis of the AOAC [20] while carbohydrate content was calculated by difference. Analyses were done in triplicates.

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Analysis of anti-nutrients

The content of trypsin inhibitor was determined by the procedure of Smith *et al.* [21]. One gram of the dried seed sample was extracted by shaking with 50 ml of 10 mM NaCl and left overnight at 4ºC. The resulting slurry was adjusted to pH 9.4 - 9.6 with 1 M HCl or 1 M NaOH, and left overnight. The extract was diluted with water so that 1 ml produced trypsin inhibition of between 40 and 60%. Bovine trypsin (20 µg/ml) was used as standard.

The assay was carried out as follows:
reagent blank: 2.0 ml distilled water + 2.0 ml of standard trypsin
sample blank: 2.0 ml diluted sample extract + 2.0 ml distilled water
sample(s): 1.0 ml diluted sample extract + 1.0 ml distilled water + 2.0 ml standard trypsin

After mixing and pre-heating to 37ºC for 10 min, 5.0 ml benzoyl-D-L-arginine-P-nitroanilide (BAPNA) solution (previously warmed to 37ºC) was added to each tube, mixed and incubated for 10 min at 37ºC. One ml acetic acid (30% v/v) was then added to stop the reaction. The solution in each tube was filtered and the *p*-nitrophenol released was measured spectrophotometrically at 410nm (CINTRAL 101/ version 2.2).

Trypsin inhibitor activity (TIA) was expressed as: $\frac{2.632 \times D \times \Delta A}{S}$ mg trypsin inhibited g^{-1} sample

Where: D = dilution factor
S = sample weight
ΔA = change in absorbance = $A_{sample} - A_{blank}$

The lectin content in the saline extract of the seed was determined by hemagglutination assay according to the method of Kuku and Eretan [22]. The concentration of protein in the extract was measured by the method of Lowry *et al.* [23]. The hemagglutination unit of activity (HU) was taken as the reciprocal of the highest dilution (titre) of the extract showing visible agglutination of erythrocytes (rabbit). Hemagglutinating activity was expressed as HU/mg protein.
All determinations were carried out in triplicates.

Animal experiment

The dry seed sample powders (unprocessed, under-processed and processed) were incorporated into the diets of the experimental animals. The diets were formulated using the American Institute of Nutrition (AIN) method as described by Reeves et al. [24]. The diet originally contained 50% maize starch, 10% potato starch, 15% corn oil, 5% mineral mixture and 5 % vitamin mixture. The test and control diets were formulated by substitution of maize starch with the amount of a particular protein (casein) to give 10% protein requirement. Silicic acid was added to mimic animal food. The composition of the diets is shown in Table 2.

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Albino rats weighing 80 ± 5 g (6 weeks old) bred from the same colony were obtained from the Animal House, Faculty of Pharmacy of the Obafemi Awolowo University. The rats were maintained on a standard stock pellet diet, and then fed with the casein control diet for a period of one week and allowed to acclimatize under a controlled atmosphere (temperature, relative humidity and a fixed 12- hr light/dark cycle). Only those rats which had a regular food intake and matched on the basis of body weight during the adaptation period were subsequently used in the experiment.

For this experiment, 20 rats were randomly divided into four groups of five animals each and were individually housed in Techniplast metabolism cages (Biotech, Clackmannanshire) fitted with a feeding tunnel to prevent food spillage and ensure minimal contamination of the feces and urine samples with food. The rats were fed on different diets as follows: group 1 was fed on a test diet incorporated with raw (unprocessed) seed powder; group 2 was fed on diet containing under-processed powder and group 3 on diet incorporated with the processed seed powder. A control group was fed on the reference caseinated diet formulation (200 g casein/kg).

The diets were isoenergetic and were given in powdered form for a period of 21 days. Water was available *ad libitum* during this period, meanwhile, the care and use of laboratory animals followed the institutional guidelines of Obafemi Awolowo University, Ile-Ife, Nigeria.

Food consumption was recorded daily. Body weight was recorded daily for rats from each group before feeding in the morning. To determine the nutritional efficiency of diets, urine and feces were collected daily. All samples were stored at -4°C. At the end of the experimental period, blood samples were collected from the rats for hematological analysis. The animals were sacrificed by cervical dislocation. The gastrointestinal tract was thoroughly rinsed out with a large amount of distilled water to remove food and feces. Spleen, heart, kidney and liver were excised and fixed immediately in 10% formyl saline for histological analysis.

Growth performance assay

Body weight and feed consumption were recorded in rats before feeding in the morning. Body weight gain and feed conversion ratio (Fcr) were calculated. The feed conversion ratio was expressed as:

$$\frac{\text{Weight gain (g)}}{\text{Feed intake (g)}}$$

Nutrient digestibility determination

The samples of experimental diets and feces were homogenized using a motor and pestle and analyzed by standard methods [20]. The nutrient digestibility was measured by the method of Noreen and Salim [25]. The dry matter was determined by oven-drying at 105^{o}C for 16 hr; crude fat by petroleum ether extraction, crude fiber by digestion with H_2SO_4 and NaOH and the crude protein by Kjeldahl method [20]. Nitrogen Free Extract (carbohydrates) was calculated by taking the sum of values for

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crude protein, crude fat, ash, crude fiber and subtracting from 100. Analyses were done in triplicates.

Nitrogen balance and nitrogen retention assay

Intake nitrogen, fecal nitrogen and urinary nitrogen were analysed for total nitrogen by Kjedahl method, and nitrogen balance and nitrogen retention were calculated according to the following formulas:

Nitrogen balance = intake nitrogen - urinary nitrogen - fecal nitrogen
Nitrogen retention = (intake nitrogen - urinary nitrogen - fecal nitrogen)/intake nitrogen

Hematological and histopathological analysis

The packed cell volume (PCV), Hemoglobin concentration and white blood cell (WBC) count of the blood samples collected from both control and experimental animals were determined using standard hematological methods [26].

Organs collected from the animals including the gastrointestinal tract, spleen, heart, lungs, kidney and liver were fixed in 10%, formyl saline for 24 hr, dehydrated in ascending concentration of ethanol (50%, 70%, 90% then twice in 100%) for interval of 1 hr to enable the tissue to be embedded in paraffin [27]. The tissues were sectioned to 6- micron thin films using a rotary microtome and stained with Hematoxylin and Eosin and then examined with a Zeiss EM light microscope.

Statistical analysis

Data were expressed as mean ± SEM using Graph Pad Prism Graphical –Statistical package version 5. Statistical analysis was performed using ANOVA, followed by significant difference test for comparisons between individual groups. The non-parametric Dunnett Comparison Test was applied to discriminate differences in variables with 5% level of significance ($p<0.05$).

RESULTS

Proximate composition of T. occidentalis seeds

The proximate composition (% dry weight) of *T. occidentalis* seeds is presented in Table 1. The results showed that the seeds are rich in essential nutrients. The crude protein content of the processed seed (28.09%) was higher than that of the unprocessed seed (26.93%). There was, however, no significant difference in the values of the crude fat, crude fiber, and ash content.

Content of Trypsin inhibitor and Lectin in T. occidentalis seeds

There were significant differences ($p< 0.05$) in the contents of trypsin inhibitor and lectin among the three groups of fluted pumpkin seeds. The trypsin inhibitor content in unprocessed seeds (23.18 TIU/mg) was higher than in the under-processed seeds (2.13 TIU/mg). No trypsin inhibitor activity was detected in the processed seeds. The lectin content expressed in HU/mg protein of unprocessed seeds (2048) was higher

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than that of under-processed (32), and was extremely low for processed seeds (2) (Table 3).

The effects of T. occidentalis seeds on growth performance

Results of body weight gain and feed conversion ratio are presented in Figure 2A & B. The body weight and feed conversion efficiency significantly ($p<0.05$) decreased in animals fed diet containing unprocessed seeds when compared to other groups fed with diet incorporated with under-processed and processed seeds where body weight gain was observed.

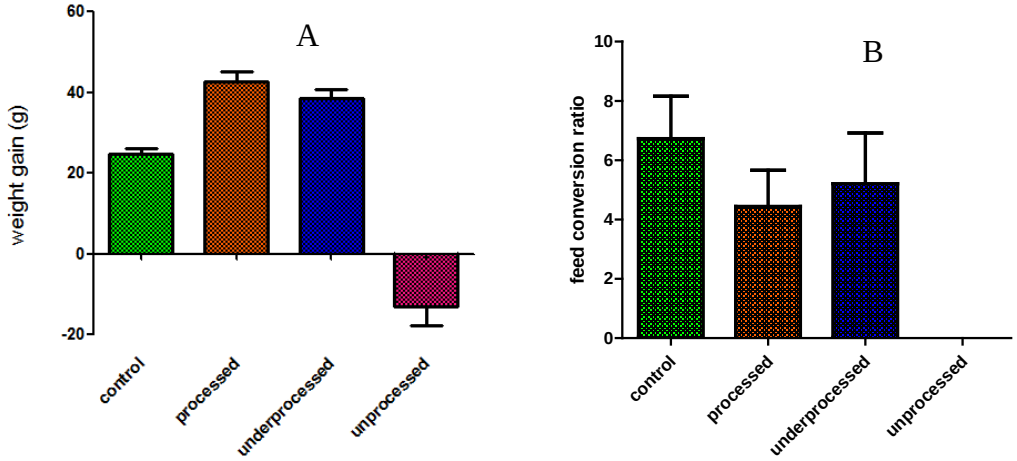

Figure 2: (A) Body weight gain and (B) Feed conversion ratio (Fcr) in rats fed diet incorporated with *T. occidentalis* seeds

The effect of T. occidentalis seeds on nitrogen metabolism

Nitrogen balance and nitrogen retention indicate the state of the body in regard to ingestion and excretion of nitrogen. Nitrogen balance and retention values were significantly lower in treatment group when compared to the control group as shown in Figure 3A and B. Nitrogen balance and nitrogen retention among treatment groups were highest in the processed group and lowest in the unprocessed group, and were significantly different from the control and the under- processed groups ($p < 0.05$). The striking difference in these parameters in the three treatment groups could be attributed to the different levels of anti-nutritional factors.

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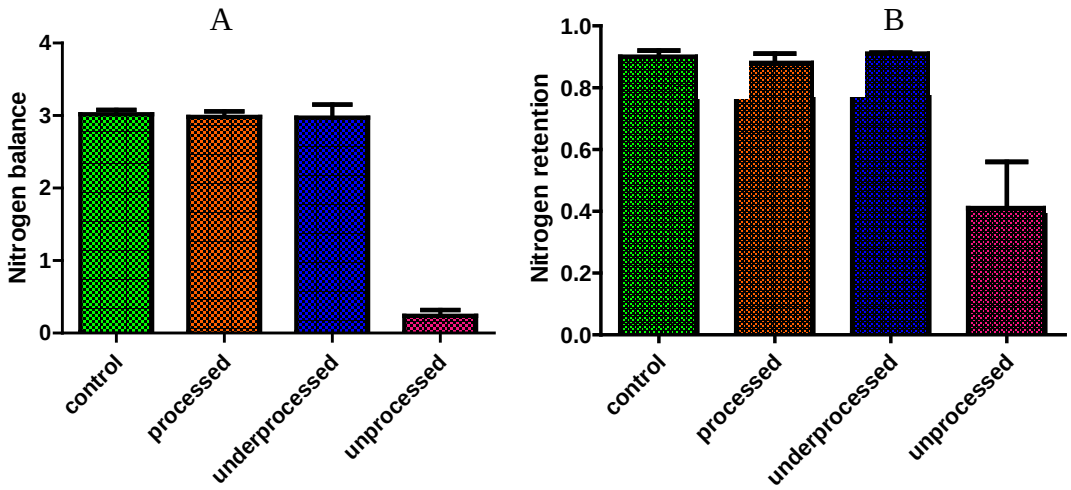

Figure 3: (A) Nitrogen balance and (B) Nitrogen retention in rats fed diet incorporated with *T. occidentalis* seeds

The effect of T. occidentalis seeds on nutrient digestibility

Nutrient digestibility is a measure of how efficient a particular nutrient is utilized by the body and was lower in the experimental diets when compared to the control group (Figure 4). The digestibility of dry matter, protein and carbohydrate were lower in unprocessed group and higher in processed group with significant differences between the values ($p < 0.05$). There was, however, a significant increase in fat digestibility, which was highest in unprocessed group and lowest in the processed group ($p< 0.05$).

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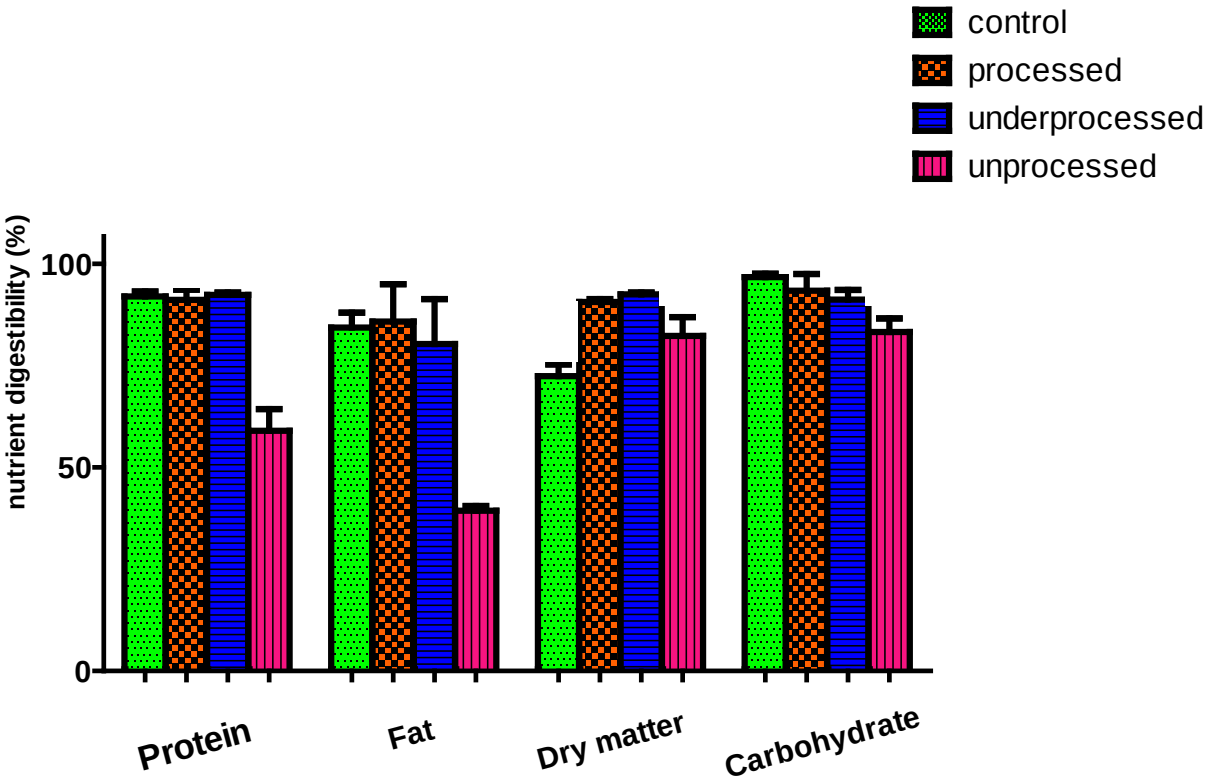

Figure 4: Nutrient digestibility in rats fed diet incorporated with *T. occidentalis* seeds

The effect of T. occidentalis seeds on blood parameters

Results of the haemoglobin, PCV and WBC count are presented in Table 4. A numerical decrease was observed in the haemoglobin level in treated groups compared with the control. The PCV was significantly lower in animals fed a diet incorporated with unprocessed fluted pumpkin seeds when compared to the control and other treatment groups. The white blood cell count was significantly lower in animals fed with unprocessed and under-processed fluted pumpkin seeds. This effect was more pronounced in animals in the unprocessed group.

Histological analysis

Histopathological analysis revealed that the spleen and the small intestine of the animals in the treatment groups were affected (Figures 5 and 6) while the other organs were essentially normal.

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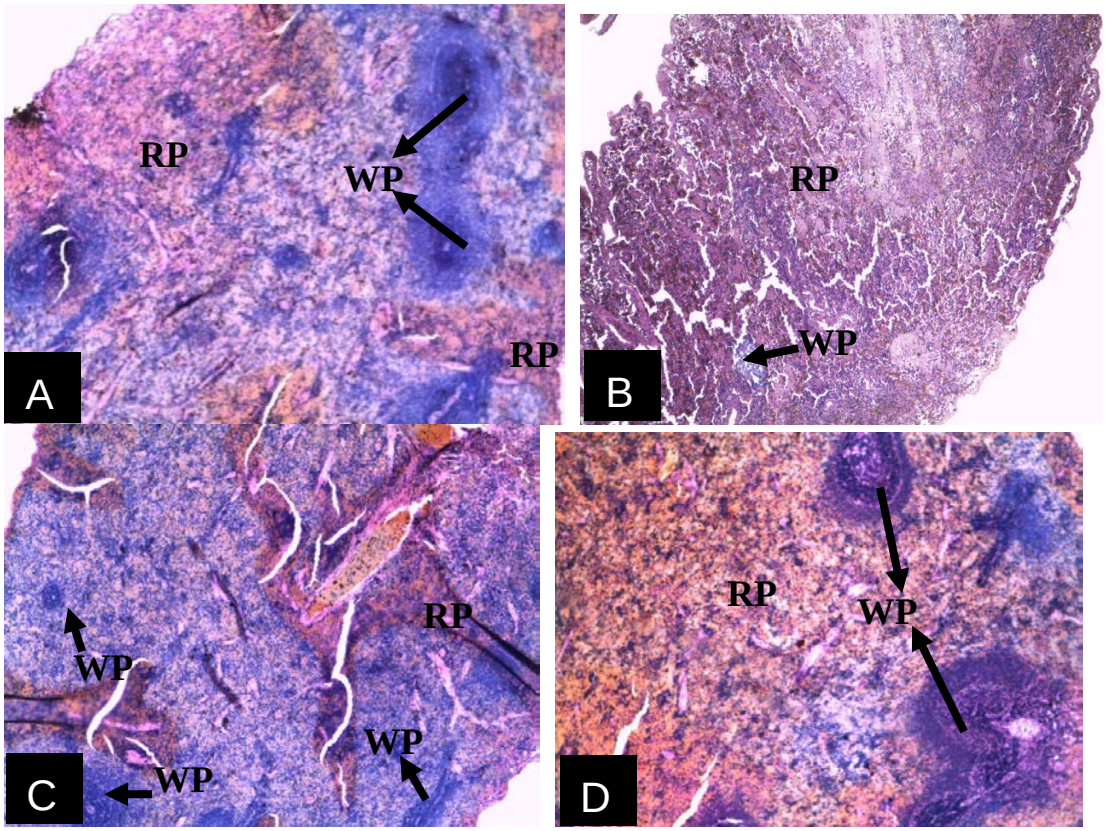

Figure 5: Photomicrographs of sections of the spleen of the control and experimental animals. Magnification, x100

A **(control)**. Photomicrograph showing the transverse section of the normal control spleen showing its general architecture. Note the white pulp (WP) which are discrete white nodules embedded in a highly vascularized red matrix called red pulp (RP).

B **(unprocessed)** – Photomicrograph showing the transverse section of the spleen of rats fed the unprocessed fluted pumpkin seeds. The section showed highly reactive splenic follicles marked by reduction in the number of lymphoid aggregation of white pulp and several lymphocytes are scattered in the red pulp.

C **(under-processed)** - Photomicrograph showing the transverse section of the spleen of rats fed the under processed fluted pumpkin seeds. There are few number of small sized white pulp (WP) embedded in the vascularized red pulp (RP). A few lymphocytes are also seen scattered in the red matrix.

D **(processed)**. Photomicrograph showing the transverse section of the spleen of rats fed the processed fluted pumpkin seeds. Note the white pulp (WP) consisting of lymphoid aggregations embedded in the vascularized red pulp (RP).

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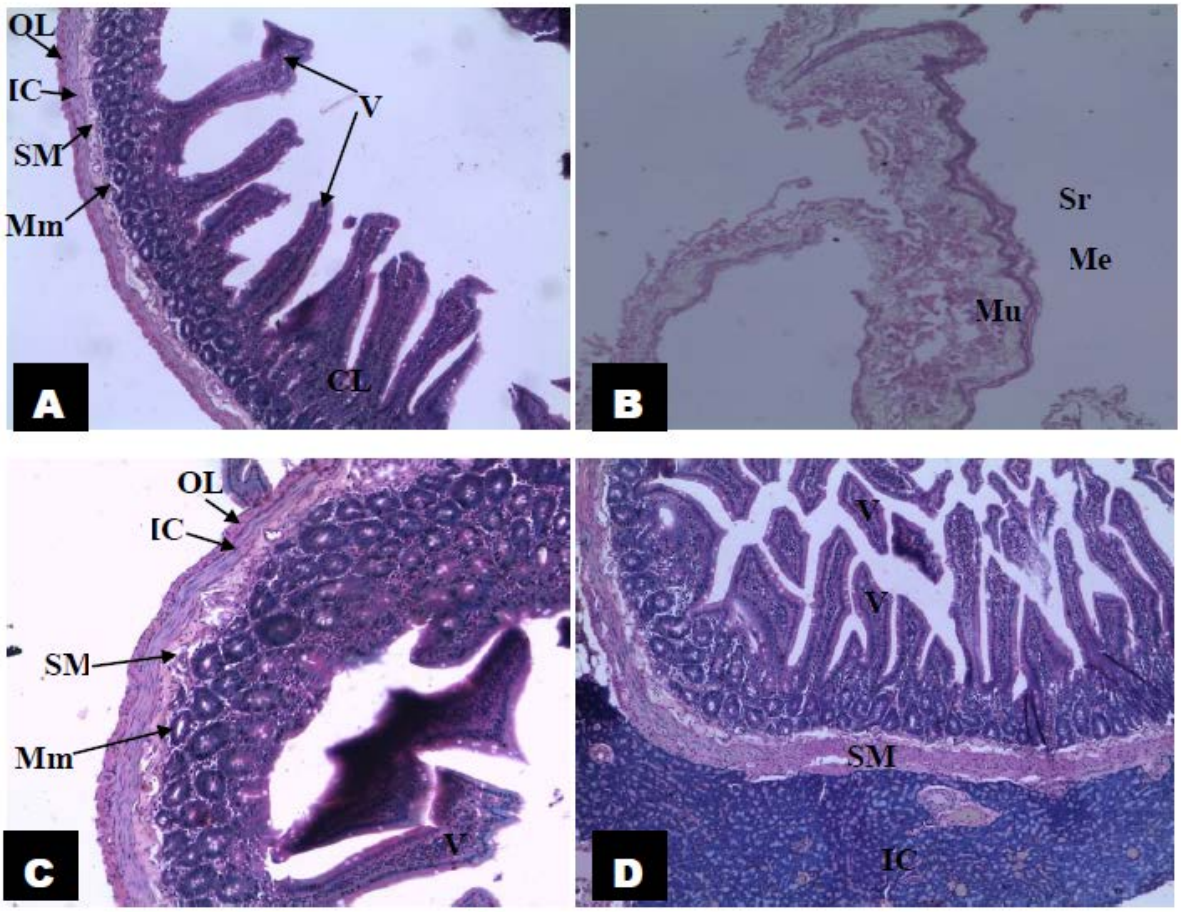

Figure 6: Photomicrographs of sections of small intestine of the control and experimental animals

A (Control): The transverse section of the small intestine (jejunum) of normal control rat showing the general architecture of a normal jejunum. The intestinal villi (V) are lined by a simple columnar epithelium which is continuous with that of the Crypts of Lieberkuhn (CL). The muscularis mucosa (Mm) lies immediately beneath the base of the crypts. Also note the inner circular (IC) and outer longitudinal (OL) layers of the muscularis externa. The peritoneal aspect of the muscularis is invested with serosa (Sr). Magnification, x100.

B (Unprocessed): The transverse section of the jejunum of rats fed with unprocessed fluted pumpkin seeds. The wall of the jejunum is very thin as degenerative changes appear in its sub mucosa (SM) and the muscular wall. Most of the intestinal villi are eroded together with the crypts of lieberkuhn. Magnification, x40.

C (Under-processed): The transverse section of the jejunum of rats fed with under processed fluted pumpkin seeds. The intervilli distance is increased in this section

indicating erosion/degeneration of some villi. The sub mucosa, crypts and muscular layer appear normal. Magnification, x100.

D (Processed): The transverse section of the jejunum of rats fed with processed pumpkin seeds. The section appears normal. Magnification, x100.

DISCUSSION

The seeds of *T. occidentalis* were found to contain sufficient amounts of the essential nutrients required by man such as protein and fat. These nutrients could supplement other dietary sources if the seed is served in adequate amount in diet [2]. The crude protein content (28.09 %) of the unprocessed seeds obtained in this study is comparable to the 24.40% value reported by Akwaowo *et al*. [8]. Christian [2] had also reported that *T. occidentalis* seeds have 31.38% crude fat, 2.02% ash, 50.08% carbohydrate and fibre content of 2.12% while Akwaowo *et al.*[8] reported 56.24% crude fat, 1.80% crude fibre, 1.60% ash and 16.10% carbohydrate. The fat (18.4 %), carbohydrate (2.41%), ash (1.53%) and crude fibre (0.88%) contents of the seeds as determined in the present study are lower than the values reported by these earlier workers. These differences may be attributed to genetic variations, as well as climate, environmental and geographical factors.

The study showed that trypsin inhibitors in the seeds were inactivated by heat treatment; trypsin inhibitors are known to be heat-labile and can be partially or completely denatured when exposed to elevated temperature. Lectin content was very high in the saline extract of the unprocessed *T. occidentalis* seeds and was in agreement with a previous research report [28] while the lectins were also almost completely inactivated by heat treatment at 100^{o}C for 1 hr.

Animals fed diets incorporated with the processed and under-processed seeds showed body weight gain that was not significantly different from the control group. This showed that the level of anti-nutritional factors in these diets was insufficient to affect growth. However, incorporation of the unprocessed fluted pumpkin seeds in animal diet impaired feed conversion efficiency and significantly lowered the body weight gain. This could be attributed to the effect of some anti-nutrients such as trypsin inhibitors and lectins in the seeds. It has been reported that trypsin inhibitors interfere with the physiological process of digestion through disruption of the normal functioning of pancreatic proteolytic enzymes in non-ruminants leading to severe growth depression [29]. Reports have also shown that lectins bind to complementary carbohydrates present on the surface of enterocytes, for example in glycolipids and glycoproteins of the brush border membrane and as a result of enterocyte atrophy, the nutrient absorption surface area becomes reduced and the transport of nutrients through the epithelium gets impeded, which leads to inhibited growth of animals. [15,30]. It was also reported that dietary lectins and trypsin inhibitors did interfere with body metabolism and nutrient availability [31]. Likewise, 50% of growth inhibition in rats fed raw soybean diet was attributed to lectin, 40% to trypsin inhibitor, and 10% to other anti-nutrients [32].

Many other reports have also related growth performance of animals with the level of lectin and trypsin inhibitor in diets [33, 34]. In this study, body weight gain and feed conversion efficiency of rats fed diets incorporated with unprocessed fluted pumpkin seeds (which contained higher values of trypsin inhibitor and lectin) were the lowest, while those fed diets incorporated with under-processed and processed seeds were the highest. Therefore, the results showed that the higher the anti-nutritional factor content, the lower the body weight gain and feed conversion efficiency.

Nutrient digestibility and deposit are the two parameters used to measure digestion and absorption of nutrients. Earlier studies have shown that nutrient digestibility decreased in animals fed with diets containing high levels of trypsin inhibitor [35]. In the present study, unprocessed fluted pumpkin seeds that contained the highest level of trypsin inhibitor led to a significant decrease in protein, fat, dry matter and nitrogen free extract digestibility. Other studies have also confirmed that protein digestibility was decreased by 20-40% in animals fed diets containing raw soybean or high levels of trypsin inhibitor when compared to those fed diets containing heated soybean which had lower trypsin inhibitor content [36, 37]. Protein digestibility in the present study was decreased by 35.83% in animals fed diets incorporated with unprocessed fluted pumpkin seeds containing the highest level of trypsin inhibitor when compared to the control group. It is possible that the anti-nutrient effect of trypsin inhibitors is due to their direct interaction with pancreatic proteolytic enzymes and a corresponding reduction in the digestibility of the proteins in the diet [38]. It was also reported that lectins combine with a specific receptor (polyose) on the epithelial cell surface in the small intestine, interfere with the function of many enzymes in the brush border mucosa and cause a decrease in protein utilization efficiency [39]. The results of this study also showed that protein digestibility was lower in animals fed diet that contained high level of lectins.

Nitrogen analysis of faeces of pigs fed soybean seeds showed that the inclusion of lectin in diets increased the loss of endogenous nitrogen, which resulted in decreased nitrogen balance and nitrogen retention [40]. With increase in lectin content of diets, the nitrogen loss showed a linear increase, but the nitrogen balance and nitrogen retention showed a linear decrease [41]. It was reported that the level of trypsin inhibitor affected these two parameters [35]. These results indicate that these anti-nutritional factors could lower nitrogen deposit, thereby affecting digestion and absorption of the nutrient. The results also showed that diets containing high levels of these anti-nutritional factors led to a significant decrease in nitrogen balance and nitrogen retention in treatment groups when compared to the control group, which is in agreement with previous reports [40, 41].

A progressive decrease in blood parameters was observed for the different treatment groups. Rats fed diets containing unprocessed seeds showed the lowest PCV, HB and WBC count values. White blood cell count plays a most important role in phagocytosis and immunity and, therefore, in defenses against infection such that an alteration in the normal values predisposes individuals to pathogenic invasion. The

reduction in WBC count of the treatment groups may be due to the damage to the spleen, which can have negative effect on the immune system. This was corroborated by the splenic morphology of the rats showing that animals fed diets containing unprocessed and under-processed fluted pumpkin seeds had reactive splenic follicles with increased lymphopoiesis. The apparent depopulation of these follicles may be related to the mobilization of the lymphocytes into the blood stream in response to the presence of these anti-nutritional factors. Previous reports [42, 43] have shown that epithelial cell microvilli in particular are affected by lectin exposure, which initiates disruption and shedding of these membrane rich surface projections. The small intestine morphology of rats fed with unprocessed fluted pumpkin seeds in this study showed degenerative changes in the mucosa and muscular wall with most of the microvilli eroded. These villi and microvilli provide the small intestine a large surface area for absorption of nutrients; thus the bioavailability of nutrients in animals fed with the unprocessed seeds was negatively affected. A decrease in intestinal villi was also observed in rats fed with underprocessed fluted pumpkin seeds. This could result in improper digestion and malabsorption of nutrients which could eventually lead to vomiting and diarrhea.

The biological utilization of most plant seeds and legumes in animal feed production is on the increase because of their availability and as cheaper source of protein. However, due to processing costs, some of these plant foods are used raw or not properly processed during feed formulation. For example, it has been shown that most farmers would prefer to use raw *Lablab purpureus* (lablab beans) to feed chicken in order to eliminate the cost of transporting the raw beans to the processor and back to the farm as well as trying to avoid the processing and other handling costs involved [44]. Thus, improper processing of plant foods may expose animals to high concentrations of these anti-nutritional factors. This study has shown that improper processing of fluted pumpkin seeds when being utilized for animal feed production could lead to deleterious effects that may be fatal to health.

CONCLUSION

This study showed that *T. occidentalis* seeds have high nutritive values, but their utilization could be affected by the presence of anti-nutritional factors such as lectins and trypsin inhibitor that are present in the seeds. The seeds when incorporated into animal diets impaired body weight gain and affected nutrients metabolism; bioavailability of nutrients was impaired due to damage to the small intestine. In addition, ingestion of the seeds caused damage to the spleen and affected the immune system of the animals. All these effects could, however, be reduced or removed if the seeds are adequately processed by heating.

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RECOMMENDATION

The essential nutrients in fluted pumpkin seeds need to be exploited in the formulation of animal feeds. Adequate processing of the seeds however needs to be ensured to ameliorate the deleterious effects of the anti-nutritional factors.

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Table 1: Proximate Composition (% dry wt) of *Telfairia occidentalis* Seeds

Seed	Moisture	Protein	Fat	Ash	Crude Fiber	Carbohydrate	Dry Matter
Processed	50.70 ±0.21	26.93 ±0.07	15.15 ±0.24	1.54 ±0.11	0.81 ±0.16	4.93±0.27	49.40 ±0.13
Under - processed	49.50 ±0.09	27.12 ±0.25	16.82 ±0.07	1.53 ±0.16	0.83 ±0.22	4.20±0.21	50.50 ±0.14
Un processed	48.67 ±0.10	28.09 ±0.08	18.43 ±0.26	1.53 ±0.32	0.88 ±0.17	2.41±0.11	41.33 ±0.24

Values are the means of triplicate determinations expressed on a dry weight basis

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Table 2: Animal diet composition (g/kg)

Ingredients	**Control diet**	**Diet containing unprocessed seed**	**Diet containing under-processed seed**	**Diet containing processed seed**
Casein	125	-	-	-
Pumpkin seed	-	356	360	371
Corn starch	375	191	189	186.3
Glucose	150	150	150	150
Corn oil	150	103	103.2	92.4
Potato starch	100	100	100	100
Vitamin/mineral premix	100	100	100	100
Silicic acid	0.4	0.4	0.4	0.4

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Table 3: Effect of Processing on Some Anti-nutrients in *Telfairia occidentalis* Seeds

	Unprocessed	Under-processed	Processed
Trypsin inhibitor (TIU/mg)	23.18±0.07 [a]	2.13±0.12[b]	0.00[c]
Haemaglutinating activity (HU/mg)	2048	32	2

Values are the means of triplicate determinations. Mean values bearing different superscripts are significantly different ($p < 0.05$)
TIU – Trypsin Inhibitor Unit of activity
HU – Heamagglutinating Unit of activity

Table 4: Haemoglobin (Hb), Packed Cell Volume (PCV) and White Blood Cell (WBC)

	Count of Control and Test Animals		
Group	**Hb (g/dL)**	**PCV (%)**	**WBC (10^9/L)**
Control	15.32± 3.36[a]	46.40 ± 3.58[a]	9760 ± 1307[a]
Unprocessed	10.70± 0.56[a]	29.60± 3.21[b]	2300 ± 444.4[b]
Under- processed	12.36± 3.23[a]	41.80± 3.70[a]	7400 ± 2672[c]
Processed	11.34± 0.75[a]	42.20± 3.96[a]	9160 ± 1381[a]

Values are expressed as Mean ± SD (n=5). Mean values bearing different superscripts on the same column are significantly different ($p < 0.05$)

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