

Full Length Research Paper

Effect of processing on the lectin and trypsin inhibitor content of *Plukenetia conophora* seeds as it affects growth performance and nutrients metabolism in rat

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The study evaluated the effect of processing on lectin and trypsin inhibitor contents of *Plukenetia conophora* seeds and assessed such effect on growth and nutrient metabolism in rats. Mature conophora seeds were divided into three groups based on processing. The seeds were sun-dried and pulverized, and portions from each group were subjected to proximate composition analysis and measurement for the contents of trypsin inhibitor and lectin. The dried samples were incorporated into diets of experimental rats. 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, feces and urine were collected, and body weights of the animals were measured daily. At the end of the experimental period, blood samples were collected from the rats for hematological analysis, then the animals were sacrificed and some key organs were excised for histopathological analysis. The results showed that trypsin inhibitor and lectin contents of the raw seeds were higher than the under processed and processed seeds. All measured parameters including gain in body weight, feed utilization efficiency, nutrient digestibility, nitrogen balance, nitrogen retention and hematological parameters were markedly different among the three groups of animals fed on diets incorporated with the conophora seeds, in comparison with the control animals. Histopathological analysis indicated that the lung and the spleen were adversely affected in animals treated with the under processed and the unprocessed seeds with the severity of toxicity more pronounced in the unprocessed group. These findings showed that *P. conophora* seeds have high nutritive value but ingestion of the unprocessed seeds impaired growth, affected nutrients utilization and caused damage to the lung and the spleen in experimental animals.

Key words: Conophor seeds, trypsin inhibitor, lectin, processing, metabolism, growth.

INTRODUCTION

Legume seeds have high acceptability and utilization as an important source of energy and protein for both animal and human consumption in the developing countries. *Plukenetia conophora* Müll.-Arg, formerly known as *Tetracarpidium conophorum* Hutch. and Dalz. is a woody perennial climber that belongs to the family Euphorbiaceae.

It has a long history as food plant and is grown by peasant farmers across West African rain forest and in India (Petrova, 1980). Its common name is African walnut and it is cultivated principally for the nuts which are cooked and consumed as snacks. There have been reports on the high nutrient potentials of conophora

nuts/seeds (Ogunsua and Adebona, 1983) and also on the impact of traditional processing on the nutrient and sensory qualities of the nut (Adesioye, 1991). However, a bitter taste is usually observed upon drinking water immediately after eating conophora nut. This has been attributed to the presence of alkaloids and some other antinutritional factors. Many legume seeds contain lectins which are toxic when being fed to humans or laboratory animals.

The existence of these antinutritional factors affects the nutritional value and digestibility, and has been a major limitation in the utilization of many of these unconventional protein-rich and high caloric seeds. Animal diets containing legume seeds with lectin constituents, for example, have been shown to impair the absorption of various nutrients such as amino acids, carbohydrates etc. and elicit a variety of biochemical and histological perturbations in laboratory animals (Pusztai et al., 1981). This has been attributed primarily to the binding of the lectins to the surface of the intestinal epithelial cells causing among other alterations, a non-specific interference with the final digestion and absorption of nutrients (Thompson, 1993; Thompson et al., 1986). There has been some information that lectins and other antinutritional factors in legume seeds may be inactivated by processing methods such as soaking, sprouting, cooking or fermenting although not completely (Pusztai et al., 1991). Many legume lectins are relatively resistant to both heating and digestion. Some of these lectins have a high thermal stability (70°C) and do not completely degrade with cooking while some are relatively resistant to stomach acid and proteolytic enzymes (Kilpatrick et al., 1985). Consequently, they are able to pass through the gut and about 1 to 5% are absorbed into the bloodstream in humans, a significant amount to cause an immune response (Pusztai et al., 1990). Therefore, lectins are a danger when consumed in their raw state (unprocessed), or by persons deficient in stomach acid or proteolytic enzymes.

Reports from studies over the years have also looked at trypsin inhibitors in some food items such as grains, nuts, seeds, fruits and vegetables (Daniel, 2005). Proponents of plant-based diets generally believe these diets provide plenty of protein, but failed to take into account the fact that protein ingested is not the same as protein digested when protease inhibitors are in the picture. Without high quality and proper bioavailability of protein, growth, repair, immunity, hormone formation and all metabolic processes suffer. These protease inhibitors found in foods are resistant to inactivation by most processing methods, only the old-fashioned fermentation techniques come close to deactivating them while with all other processing methods, they remain active. Even small quantities of these protease inhibitors in diet can adversely affect people whose digestive capacities are already compromised by low acid levels, pancreatic insufficiency, bowel diseases, gluten intolerance and other health challenges (Rackis and Gumbman, 1981).

Protease inhibitors keep the pancreas from producing enough proteases, but the body compensates by increasing the number of pancreatic cells (hyperplasia) and their size (hypertrophy). Over time, the pancreas rises to the challenge and then recovers; however, when the pancreas is stressed, pancreatitis and even cancer become distinct possibilities (Liener, 1995). There were also reports on the chemical and functional characteristics of conophora nuts and the effect of processing on some of the antinutritional factors (Enujiugha, 2003).

In traditional medicine, the seeds are considered as herbs and are used to tonify kidneys, strengthen the back and knees, moisten the intestines as well as to motivate bowel movement by the use of concoctions made from the raw seeds (Ayoola et al., 2011). It is also possible that the conophora seed may be utilized for animal feeds because of its ready availability and cost. By incorporating the seeds (processed and unprocessed) into animal diets, it is of interest to assess the effect of ingestion of the seeds on the growth performance and nutrient metabolism in rat as well as to determine if the effects could be ameliorated by processing such as cooking.

MATERIALS AND METHODS

Seed collection and processing

Mature unshelled conophora seeds (Figure 1) were purchased from Better Life Market in Ile-Ife in the month of June. The seeds were authenticated at the IFE Herbarium, Department of Botany of Obafemi Awolowo University. The seeds were divided into three groups. Group A was the raw uncooked seed (unprocessed), group B was heat-treated at 60°C for 45 min (under processed) and group C was cooked for 2 h at 100°C (processed). Seeds from each group were carefully cracked, sliced separately to about 1 to 2 cm thickness using a kitchen knife with steel blade, sun-dried and milled into powder using Warring blender. The dried sample powders were stored in screw-capped bottles at 4 to 6°C until use.

Proximate composition analysis of seed samples

The proximate composition of the seed powder was determined according to Official Methods of Analysis (AOAC, 1995). The dry matter was determined by oven-drying at 105°C for 16 h; crude fat by petroleum ether extraction, crude fiber by digestion with H₂SO₄ and NaOH and the crude protein by Kjeldahl method. Nitrogen free extract (CHO) was calculated by taking the sum of values for crude protein, crude fat, ash, crude fiber and subtracting from 100. Analyses were done in triplicates.

Analysis of antinutrients

The content of trypsin inhibitor was determined by the procedure of Smith et al. (1980). One gram of the dried seed sample was extracted by shaking with 50 ml of 10 mM NaCl and left overnight at 4°C. The pH of the resulting slurry was adjusted from 9.4 to 9.6 with 1M HCl or 1M 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:



Figure 1. *Plukenetia conophora* seeds released from the pod.

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 37°C for 10 min, 5.0 ml benzoyl-DL-arginine-P-nitroanilide (BAPNA) solution (previously warmed to 37°C) was added to each tube, mixed and incubated for 10 min at 37°C. 1 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 410 nm.

Trypsin inhibitor activity (TIA) was expressed as:

$$\frac{2632 \times D \times \Delta A}{S} \text{ mg trypsin inhibited g}^{-1} \text{ sample}$$

Where: D = dilution factor

S = sample (gram)

ΔA = change in absorbance = $A_{\text{sample}} - A_{\text{blank}}$

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The lectin content in the saline extract of the seed was determined by hemagglutination assay according to the method of Kuku and Eretan (2004). The concentration of protein in the extract was measured by the method of Lowry et al. (1951). 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.

Bioassay

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. (1993). 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 1. Albino rats weighing 80 ± 5 g (six 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- h 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, twenty 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

Table 1. Composition of diets (g/kg).

Ingredient	Control diet	Diet incorporated with processed seed	Diet incorporated with under processed seed	Diet incorporated with unprocessed seed
Casein	125	-	-	-
Conophor seed	-	350	391	450
Corn starch	375	240	130	100
Glucose	150	150	150	150
Corn oil	150	60	130	175
Potato starch	100	100	100	100
Vitamin/mineral premix	100	100	100	100
Silicic acid	0.4	0.4	0.4	0.4

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.

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. Other organs including 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.

Nutrient digestibility determination

The samples of experimental diets or feces were homogenized using a mortar and pestle and analyzed by standard methods of the AOAC (1995). The nutrient digestibility was measured by the method of Noreen and Salim (2008). The dry matter was determined by oven-drying at 105°C for 16 h; crude fat by petroleum ether extraction, crude fiber by digestion with H₂SO₄ and NaOH and the crude protein by Kjeldahl method. Nitrogen free extract (CHO) was calculated by taking the sum of values for 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 analyzed as total nitrogen by Kjeldahl method. 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

Haematological 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 (Coles, 1986). The organs collected from the animals including spleen, heart, lungs, kidney, liver and small intestine were fixed in 10% formyl saline for 24 h, dehydrated in ascending concentration of ethanol (50%, 70%, 90%, then twice in 100%) for interval of 1 h to enable the tissue to be embedded in paraffin (Drury and Wallington, 1980). The tissues were sectioned to 6- micron thin films using a rotary microtome and stained with Hematoxylin and Eosin, which were 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 (30 days demo version). 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 conophora seeds

The proximate chemical composition (% dry weight) of freshly harvested conophor nuts is presented in Table 2. The crude protein content of the processed seed (28.62%) was higher than that of the unprocessed seed (22.24%). The values for the crude fat, crude fibre and ash content of the processed seeds were higher than those of the unprocessed seeds. However, the carbohydrate (CHO) level and the ash content of the unprocessed seeds were higher than those of the processed seeds.

Table 2. Proximate composition (% dry weight) of conophor seeds.

Seed	Protein	Moisture	Fat	Ash	Crude fiber	CHO	Dry matter
Un processed	22.24± 1.75	15.85± 0.87	38.90±1.92	2.55± 1.45	2.18±0.17	20.29± 0.82	84.15±0.13
Under processed	25.56± 1.00	21.70± 0.50	30.02± 1.75	2.20 ±0.17	1.90± 0.42	14.00±0.25	70.68±0.17
Processed	28.62 ± 0.05	30.04 ± 0.75	27.46± 0.62	2.81± 0.32	1.59± 0.01	11.57 ±1.25	69.96± 0.90

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

Table 3. Effect of processing on trypsin inhibitor and lectin content of conophor seeds.

Conophor seed	Trypsin inhibitor (mg/g)	Heamagglutinating activity (HU/mg)
Unprocessed	7.32 ± 2.20 ^c	2048
Under processed	4.72± 0.10 ^b	64
Processed	2.61 ± 0.07 ^a	2

Values are the means of triplicate determinations. Means bearing different superscripts are significantly different ($p < 0.05$).

Content of trypsin inhibitor and lectin in conophora seeds

Table 3 shows significant differences in the content of trypsin inhibitor and lectin among the processed and unprocessed seeds. The content of trypsin inhibitor in unprocessed seed (7.32 mg/g) was highest compared with that of under processed and processed seed. Processing brought about a reduction in the trypsin inhibitor content of conophora seeds. The lectin content expressed in HU/mg protein of unprocessed seeds (2048) was higher than that of under processed (64), and was extremely low for processed seeds (2).

The effects of conophora seeds on growth performance

The body weight and feed conversion ratios (Fcr) were significantly reduced ($p < 0.05$) in the animals fed with diets incorporated with under processed and unprocessed seeds as shown in Figures 2A and B. There was no body weight gain in the group fed on diet with unprocessed conophora seed rather there was weight loss, while that of the group fed on diet with processed seed was higher than those of the under processed group. Feedstuff by gain weight ratio in processed group was the highest and was significantly different from other treatment groups ($p < 0.05$).

The effect of conophora seeds on nutrient digestibility

The nutrient digestibility was lower in the experimental diets compared to the control group (Figure 3A). 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 ($p < 0.05$).

The effect of conophora seeds on nitrogen metabolism

The nitrogen balance and retention were significantly lower in treatment groups compared with the control group as shown in Figure 3B and C. The striking difference in these parameters in the three treatment groups could be attributed to the different levels of antinutritional factors. Nitrogen balance and nitrogen retention among treatment groups were highest in the processed group and the lowest in the unprocessed group, and were significantly different from the control and the under processed groups ($p < 0.05$).

The effect of conophora seeds on blood parameters

Table 4 shows the results of the packed cell volume (PCV), haemoglobin concentration (Hb) and white blood cell count (WBC) in rats in both control and treatment groups. There was a significant difference between the control and treatment groups ($p < 0.05$). The values were highest in processed group and lowest in group fed diet incorporated with unprocessed seed.

Histopathological analysis

Histopathological analysis revealed that only the lung, spleen and the small intestine of the animals in the treatment groups were affected while the liver, the heart and the kidney were essentially normal.

Figures 4, 5 and 6 show the photomicrographs of sections of the lung, the spleen and the small intestine of the rats in the control and treatment groups. In rats fed on

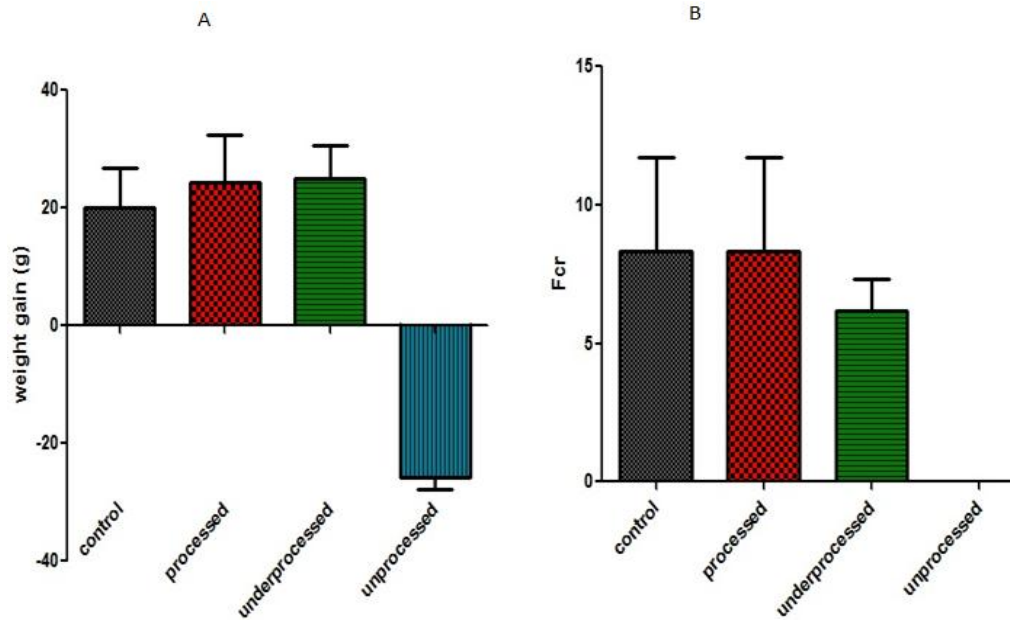


Figure 2. (A) Weight gain and (B) feed conversion ratio (Fcr) in rats fed diet incorporated with *P. conophora* seeds.

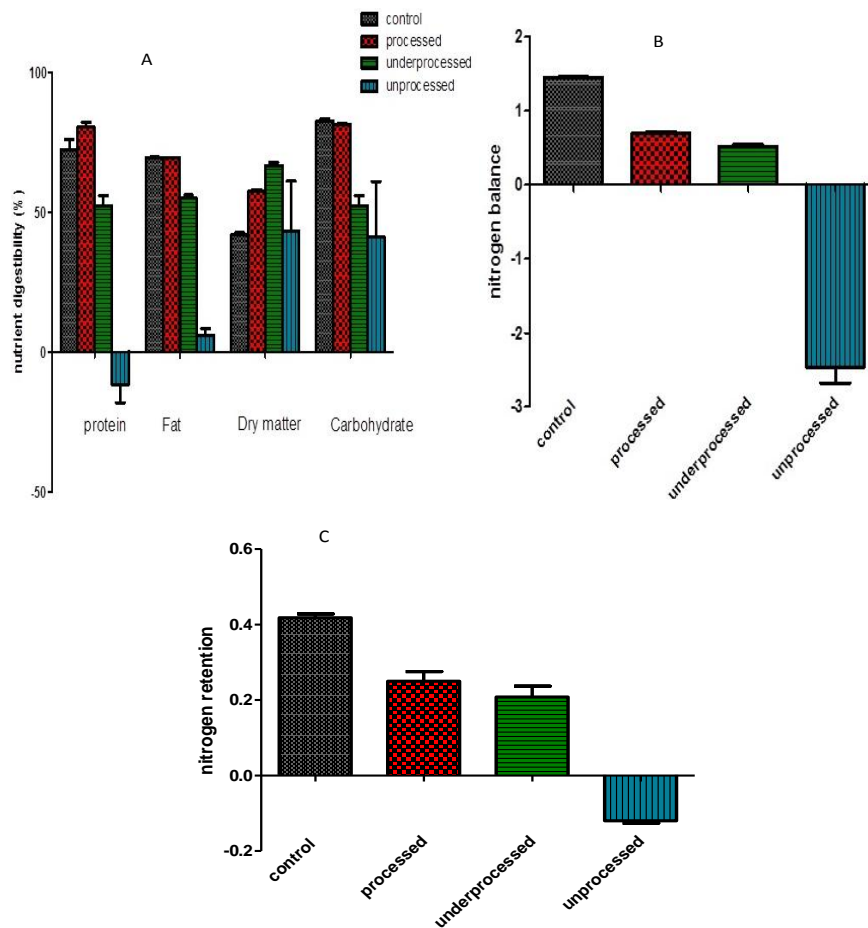


Figure 3. Nutrient digestibility (A), Nitrogen balance (B) and Nitrogen retention (C) in rats fed diet incorporated with *P. conophora* seeds.

Table 4. Hemoglobin, packed cell volume and white blood cell count of control and test animals.

Group	Hb (g/dL)	PCV (%)	WBC (10 ⁹ /L)
Control	12.76 ± 0.53 ^a	38.40 ± 1.60 ^a	10280 ± 1860 ^a
Unprocessed (raw)	4.3 ± 1.76 ^b	11.00 ± 4.59 ^c	660 ± 293 ^d
Underprocessed	11.08 ± 0.85 ^a	33.20 ± 2.56 ^b	4690 ± 517 ^c
Processed	11.52 ± 1.29 ^a	34.60 ± 3.89 ^a	7520 ± 2032 ^b

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

diet incorporated with unprocessed seeds, there was a reduction in the respiratory portion of the lung section due to the thickening or inflammation of the inter alveolar septum as compared with the lung of animals fed on normal diet in which respiratory bronchioles were well defined with numerous alveoli and alveolar sacs appearing on the pulmonary parenchyma giving the lung a spongy appearance. The spleen of rats fed on diet with the unprocessed seed 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 while in the control animals, the white pulp are discrete white nodules embedded in a highly vascularized red matrix (red pulp).

The transverse section of the jejunum of rats fed with unprocessed seeds showed degenerative changes in the mucosa and the muscular walls with most of the intestinal villi eroded together with the crypts of lieberkuhn as against the architecture of a normal jejunum observed for the control animals.

DISCUSSION

The proximate composition of food crop is a major index of the nutritional potential of that crop. The proximate composition of *Plukenetia conophora* seeds obtained in this study differed from those reported by other workers. Enujiugha and Ayodele-Oni (2003) reported *P. conophora* seeds to have 29.09% protein, 48.90% oil, 12.58% carbohydrate and 6.34% fibre. Odoemelam (2003) reported 26.3% protein and 46.5% fat. The FAO (2000) reported that the raw seeds contained 22.7% crude protein, 56.0% fat (ether extract), 3.70% fibre and 9.10% carbohydrate. The results showed that conophor nut is an oil seed with high oil content and adequate protein to satisfy the calorie and protein needs of the consuming populations. The results of this study showed 22.24% protein, 38.90% fat, 20.29% carbohydrate and 2.18% crude fibre. These values were lower than those of earlier studies. These differences may be attributed to distinct genetic varieties, or accompanied with climatic, environmental and geographical factors (Wang et al., 2000). The study also showed that processing by cooking had a marked effect on the content of antinutritional factors like trypsin inhibitor and lectin. The content of antinutritional factors in unpro-

cessed (raw) seed was significantly higher (p < 0.05) than in the under processed and was lowest in the processed seed. Many studies have confirmed that daily gain in body weight and feed utilization efficiency are lower in animals fed with diets containing trypsin inhibitor and lectin (Liener, 1996).

In the present study, such two characteristic parameters of rats fed on diet incorporated with raw unprocessed seed were significantly lower than those fed on the under processed seed, while those animals fed on the processed seed had the highest values. One of the possible reasons for this result is that trypsin inhibitor leads to the loss of endogenous nitrogen, and the other is that lectin could combine with small intestine epithelium and induce constitutional and functional changes in the small intestine (Pusztai et al., 1991). Lienier (1996) reported that 50% of growth inhibition in rats fed with raw soya bean was attributed to lectin, 40% to trypsin inhibitor and 10% to other antinutritional factors. Schulze et al. (1993) discovered that by adding 2.4 g/kg units of Trypsin Inhibitor (TI) to a control diet, reduction in the growth of animals by 13% was observed and it was about 3-fold lower (32%) with the addition of 7.2 g/kg TI. This indicated that the effect of trypsin inhibitor on animal growth was related to its level in the diet. Grant et al. (1995) also reported that the effect of lectin on growth performance of animals changed with its dosage. Li (2003) observed no obvious change of growth performance in rats fed on diet containing lectin in the range of 0 to 1.2 mg/g within 20 days, but the growth performance was lower to a decrease by 23% compared with control rats from the effect of diet having lectin above 2.0 mg/g. In the present study, the results on body weight gain together with the feed utilization efficiency of animals interpreted that the higher the antinutritional factor content was, the lower the body weight gain and feed utilization efficiency were. It further demonstrated that heat treatment of *P. conophora* seed had such a remarkable effect on the trypsin inhibitor and lectin that it led to significant differences in body weight gain and feed conversion ratio in the treatment groups.

Digestion and absorption of nutrients could be measured by two parameters, including nutrient digestibility and deposit. In the present study, feeding on raw unprocessed seed diet caused a significant decrease in dry matter, protein and carbohydrate digestibility. Similar results

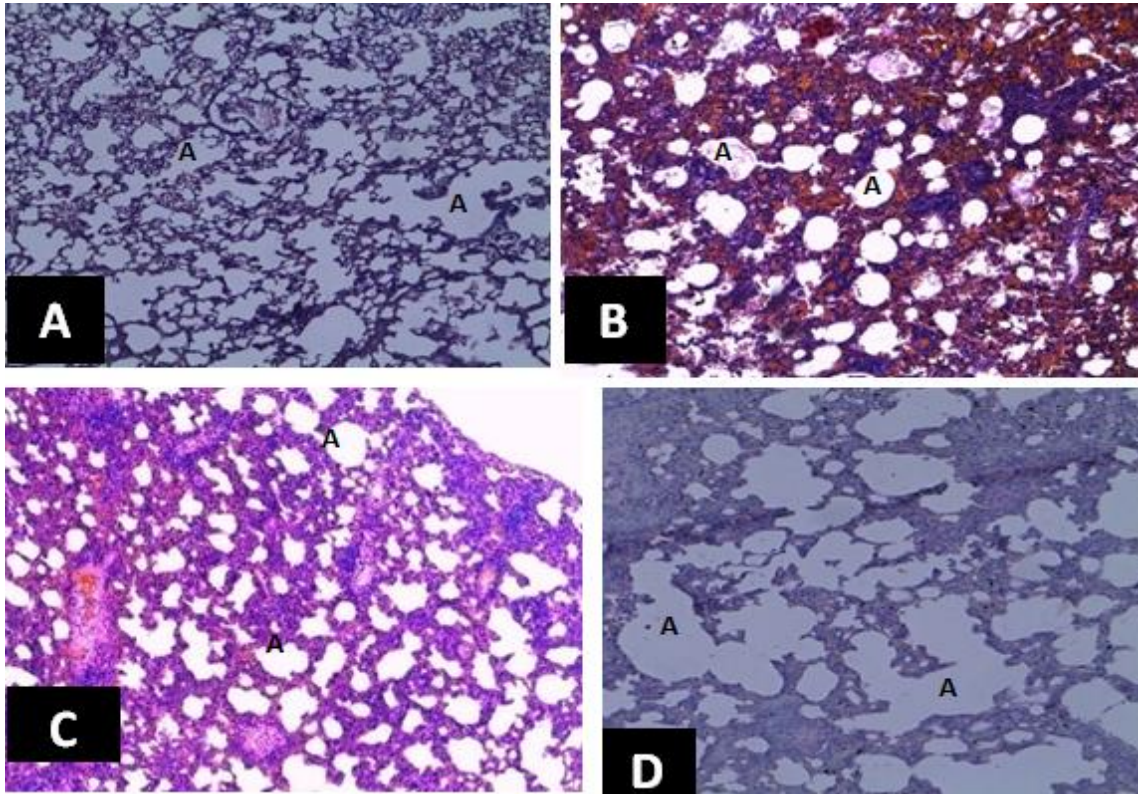


Figure 4. Photomicrographs showing transverse sections of the lung of the control and experimental animals; A (Control): The transverse section of the lung of a rat fed with the control diet. The respiratory bronchioles are well defined. Numerous alveoli (A) and alveolar sacs appear on the pulmonary parenchyma giving the lung a spongy appearance (x 100); B (Unprocessed): The transverse section of the lung of rat fed diet incorporated with the unprocessed conophora seeds. There is a reduction in the respiratory portion of the lung section due to the thickening or inflammation of the inter alveolar septum. (x 100); C (Under processed): The transverse section of the lung of rat fed diet incorporated with the under processed conophora seeds. The lung shows morphological similarities to the lung of the rat fed with the unprocessed seed (x 100); D (Processed): The section through the lung of rat fed with diet incorporated with the processed conophora seeds. The lung shows morphological similarities to the lung of control rat (x 100).

were previously reported (Qin et al., 1996). Nutrient digestibility was decreased in animals such as pig, chicken, rat, mouse and so on, fed with trypsin inhibitor, and the decrease of protein digestibility was more pronounced (Chunmei et al., 2010; Gilani et al., 2012). Li et al. (1996) reported that protein digestibility was decreased by 20 to 40% in animals fed with diets containing high levels of trypsin inhibitor (raw soybean) compared with those fed with diets containing such a factor at low levels (heated soybean or soybean meal). This study found that protein digestibility was significantly decreased in the animals fed diet containing the under processed and unprocessed (raw) seed compared with the control group. This could be explained by the findings of Qin (2003) that lectin could combine with a specific receptor (polyose) of the epithelial cell surface in the small intestine wall, destroying the brush border mucosa structure of the small intestine, interfering with the function of many enzymes in the brush border mucosa, so as to decrease protein

utilization efficiency. Previous study had also indicated the effect of lectin on nitrogen metabolism (Li et al., 1998).

The effect was found to be obvious, and lectin mainly increased the effluence of endogenous nitrogen in the way that it lowered nitrogen balance and nitrogen retention. The increase of lectin content in diet resulted in a continuous increase in nitrogen loss, and a continuous decrease in the nitrogen balance and nitrogen retention. The level of trypsin inhibitor also affects these two parameters. Hagenleister and Barth (1993) reported that with an increase of trypsin inhibitor content in the diet, endogenous nitrogen in chymus was obviously increased whereas nitrogen balance and nitrogen retention were obviously decreased. These results indicated that trypsin inhibitor and lectin could hamper nitrogen deposit, thereby affecting digestion and absorption of the nutrient. In the present study, similar results were obtained. Trypsin inhibitor and lectin led to a significant decrease of

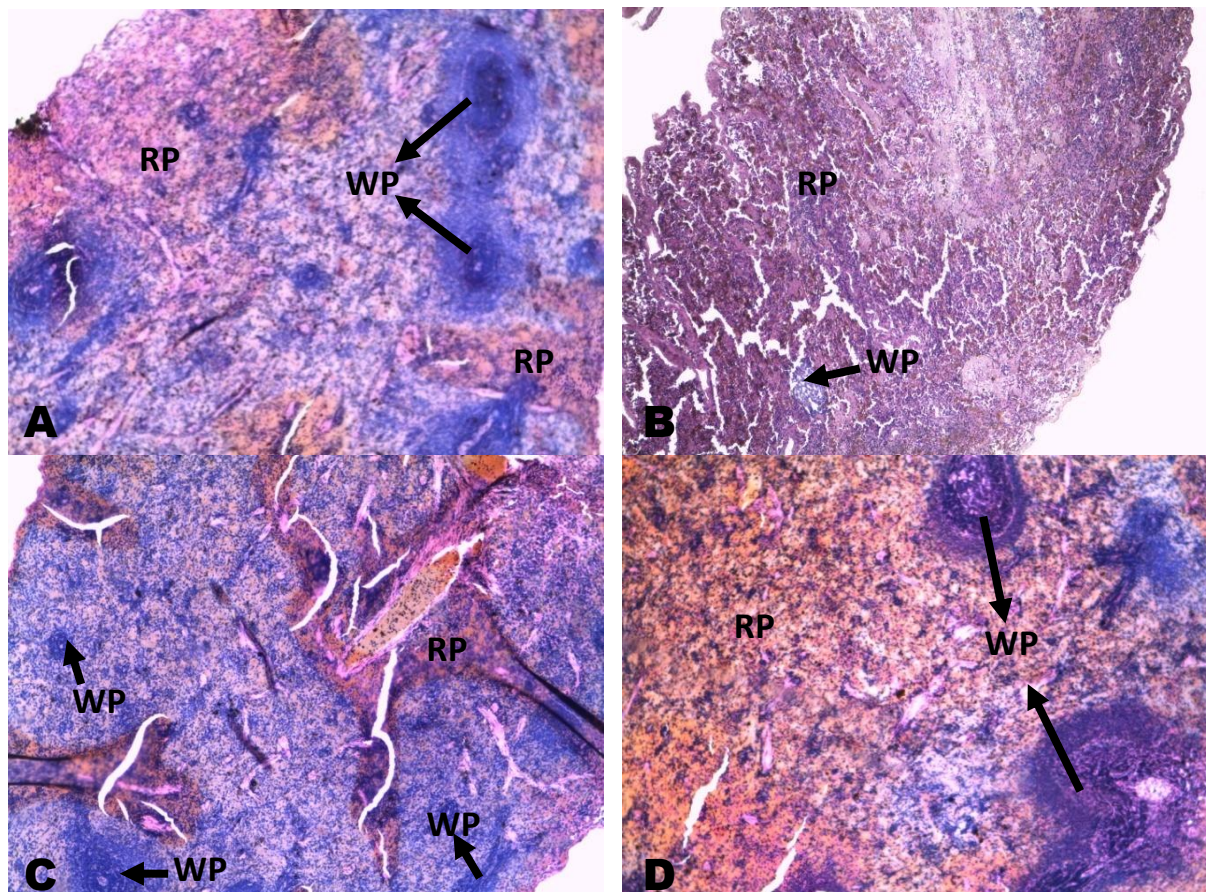


Figure 5. Photomicrographs showing sections of spleen of the control and experimental animals; A, A section of the spleen of the control rat. Note the white pulp (WP) which are discrete white nodules embedded in a highly vascularized red matrix called red pulp (RP) (x100); B, A section of the spleen of the rat treated with unprocessed conophora seed. 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 (x100); C, A section of the spleen of the rat treated with under processed conophora seed. 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 (x 100); D, A section of the spleen of the rat treated with processed conophora seed. Note the white pulp (WP) consisting of lymphoid aggregations embedded in the vascularized red pulp (RP) (x 100).

nitrogen balance and nitrogen retention in animals in the treatment groups compared to those in the control group. The extent of the decrease in the raw unprocessed group was the highest, while that of the processed group was the lowest. These data showed that the higher the level of trypsin inhibitor and lectin content in the diet, the lower the nutrient digestibility, nitrogen balance and nitrogen retention.

A progressive decrease in blood parameters was observed for the different treated groups. Rats fed on diets incorporated with unprocessed seeds had the lowest values in the PCV, Hb concentration and WBC count. Hematocrit in terms of PCV measures the percentage of red blood cells of total body blood and a decrease in PCV is consequently an indicator of anaemia (Kjeldsberg, 2000). The PCV levels of rats treated with processed seed diet were within the normal range as those of nor-

mal control diet. This suggested that the rats had adequate Fe and other nutrients needed to maintain homeostasis of hemoglobin. However, there was significant decrease in the PCV of animals fed with diet comprising of under processed and unprocessed seeds. The total white blood cell count and the differential count helps to determine the state of the immune system of an organism. Disruption in the normal values predisposes one to pathogenic invasion. The reduction in WBC count in the rats fed with unprocessed seeds may be due to the damage to the spleen, thereby affecting the immune system (Walker et al., 1990). The abnormal spleen morphology of the animals corroborated the consequence of markedly low WBC count. The histopathological analysis showed inflammation of the lungs of rats fed with under processed and processed conophora seeds. The alveolar wall thickening may be caused by emphysema, edema,

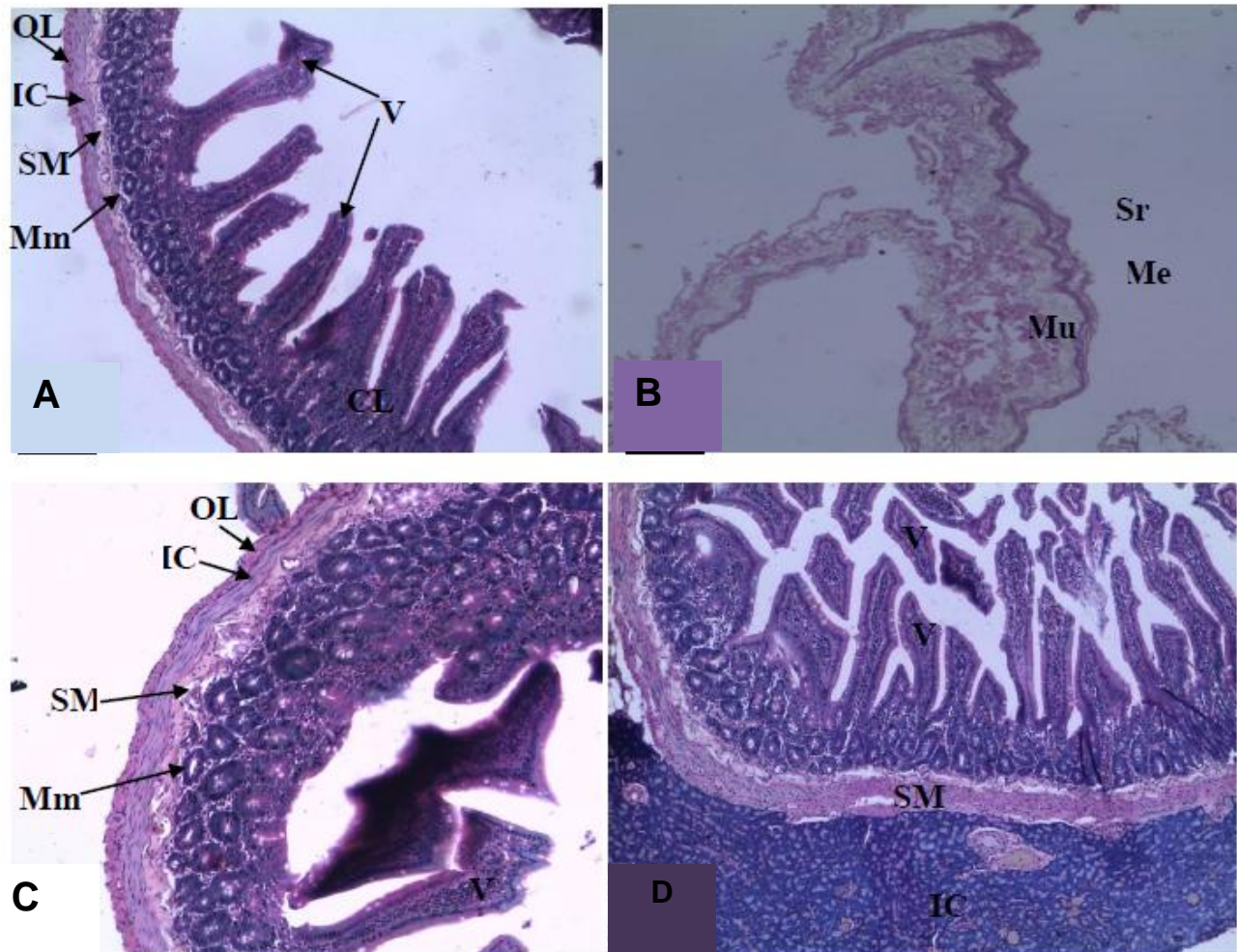


Figure 6. Photomicrographs showing sections of small intestine of the control and experimental animals; A (Control): The transverse section of the small intestine (jejunum) of normal control rat showing the general architecture of a normal jejunum. The intestinal villi (V) are lined by a simple columnar 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) (x 100); B (Unprocessed): The transverse section of the jejunum of rats fed with unprocessed conophora seed. 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 (x 40); C (Under processed): The transverse section of the jejunum of rats fed with under processed conophora seed. The intervilli distance is increased in this section indicating erosion/degeneration of some villi. The sub mucosa, crypts and muscular layer appear normal (x100); D (Processed): The transverse section of the jejunum of rats fed with processed conophora seed. The section appears normal (x 100).

interstitial infiltration with neutrophils and macrophages, as well as air-space cellularity (Vadiviel et al., 2010). This could be as a result of malnutrition due to the effect of lectin in hindering nutrient uptake or the trypsin inhibitor in reducing protein utilization.

The apparent depopulation of splenic follicles in the treated rats may also be related to mobilization of the lymphocytes into the blood stream in response to the presence of lectin in the diet fed to rats in the under processed and unprocessed groups (Mebius and Kraal, 2005). Hart et al. (1988) showed that epithelial cell microvilli particularly are affected by lectin exposure, which initiates

disruption and shedding of these membrane rich surface projections. In this study, the small intestine morphology of rats fed with unprocessed and underprocessed seeds showed degenerative changes in the mucosa and muscular wall with most of the microvilli eroded which could be attributed to the lectin in the diet. These villi and microvilli provide the small intestine a large surface area for absorption of nutrients. Reduction in the number of the villi could result in improper digestion and malabsorption of nutrients.

No visible pathological lesions were observed in the kidney, liver and heart of the experimental animals.

Conclusion

This study concluded that *P. conophora* seeds have high nutritive values, but their utilization could be affected by the two antinutritional factors, including lectins and trypsin inhibitor present in the seeds. The seeds when incorporated into animal diets impaired growth and affected nutrient metabolism. In addition, ingestion of the seeds caused damage to the lung, the small intestine and the spleen. The latter effect impaired the cellular immune system of the animals. All of these effects could, however, be reduced or removed if the seeds are well cooked by heating.

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