RESEARCH ARTICLE

Biochemical and haematological indices of rats fed with different processed fluted pumpkin (*Telfairia occidentalis*) seeds

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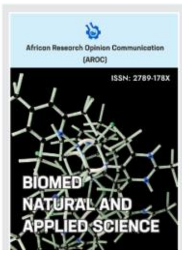

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ABSTRACT

Fluted pumpkin (*Telfairia occidentalis)* seed is a perennial plant that belongs to the family of *Cucurbitaceae.* Several nutritional values of the seeds have been reported*.* In this present study, thirty rats (average weight= 133 g ± 1.25 g) were randomly allocated to 5 groups of 6 rats each and placed on a conventional diet supplemented with groundnut meal (control), unprocessed fluted pumpkin seed flour (UF_Diet), boiled fluted pumpkin seed flour (BF_Diet), germinated fluted pumpkin seed flour (GF_Diet), and soaked fluted pumpkin feed flour (SF_Diet) for 28 days after which animals were sacrificed and blood samples collected for biochemical and haematological analysis. Results revealed that processing improved the protein content of the seed flours. The aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) range from 210.70±2.14 to 517.5±5.39 U/L, 60.6±0.30 to 386.3±3.53 U/L, and 221.7±1.21 to 328.4±3.31 U/L respectively. Processing significantly decreased ($p< 0.05$) the creatinine content of rats fed supplemented fluted pumpkin seed flours when compare with the control and unprocessed groups. The hematological parameters were also significantly ($p<0.05$) improved by the dietary treatments. The result of this study revealed that germination, soaking, and boiling were effective processing methods for improving the nutritive values of fluted pumpkin seed meals.

Keywords: Fluted pumpkin seed*;* Biochemical; haematological; Processing methods

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1.0 Introduction

Nutrition is an important factor that affects animal physiology and the nutritional status of an individual is dependent on dietary intake and effectiveness of metabolic processes [1,2]. Feed is an important aspect of livestock production for human consumption. The importance of feed supplementation in animal production has increased in the last few years [3]. An increase in meat production can be achieved through proper nutrition, inclusion of feed ingredients at normal or required levels [4]. Food insecurity is a global problem and more than 1 billion people around the world are estimated to lack adequate dietary energy availability, and more than 2 billion people around the world suffer micronutrient deficiencies [5]. Exploitation and utilization of available food sources and resources are the only options for achieving nutrition security.

Fluted pumpkin (*Telfairia occidentalis*) is a perennial plant with great economic importance in Nigeria [6]. it belongs to the family of *Cucurbitaceae*, a creeping vegetative that spreads low across the ground with large lobed leaves, and long twisting tendrils [7]. Processing of food includes cooking, germination, soaking, freezing and so on and it could improve their digestibility, taste, odour and palatability [8].

Fluted pumpkinis widely consumed in Nigeria, the seed part reportedly has high oil content, tannins, oleic acid, linoleic acid which makes it capable of treating infertility and improving fertility and nutritive values [9]. However, despite the high protein content of its seeds, it is annually wasted only to be replanted [10]. The seeds are still among the lesser-known crops and are under-utilized despite their usefulness and nutritional value [11].

In spite of the nutritional and medicinal benefits of these seeds, their effect on biochemical and haematological indices are underreported. Therefore, there is a need to investigate its safety for consumption since biochemical and haematological parameters are important diagnostic tools for health status and have been an indispensable tool in the diagnosis, treatment and prognosis of many diseases. Hence the present study was aimed to investigate the haematological and biochemical implications of feeding rats with processed fluted pumpkin seed to ascertain its safety for consumption.

2.0 Materials and methods

2.1 Sample collections

The seeds of fluted pumpkin were purchased from a market in Madalla, Niger State, Nigeria. The seeds were cleaned of stones, sand, and other particles, the seeds were authenticated at the Plant Biology Department, Federal University of Technology, Minna, Nigeria by Mr Audu. Each of the seeds was divided into four portions and each portion was processed differently.

2.2 Experimental Animals

Thirty Albino rats weighing 133 g ± 1.25 g were used in this study. The animals were acclimatized for the period of one week prior to the start of the experiment. They were housed and fed normal rat chows and water ad libitum. The animal experiment was conducted in strict compliances with the principles for humane handling and use of laboratory animals as contained in the Animal Care Guidelines and Protocol Review of National Institutes of Health Guide for the Care and Use of Laboratory Animals

2.3 Sample Processing

Boiling: The methods described by Ahamefule et al. [12]was used to prepare the boiled samples, 1 kg of the seed sample was subjected to boiling at 100 °C for 15 minutes at the rate 1 kg of the seed to 5 L of water after which water was drained off by means of 10 mm sieve and the boiled seeds was spread on hessian sacks for 48 to 72 hours under sunlight until dry. After drying, the seeds were milled and kept in plastic containers at room temperature for further analysis.

Soaking: The methods described by Sotolu et al. [13] were used to prepare the soaked samples, 1 kg of each seed sample was weighed into 5 L plastic containers, which were then filled with 5 L cold water for 24 to 48 hours at room temperature. Thereafter, water was drained by means of a 10 mm sieve, and the soaked seed was spread on hessian sacks for 48 to 72 hours under sunlight until dry. After drying, the seeds were milled and kept in plastic containers at room temperature for further analysis.

Germination: Germination was achieved as described by Kayembe et al. [14], by soaking the seeds for 24 hours. Afterward, they were spread indoors on hessian sacks on the floor, covered with aluminum foil to exclude light, and were allowed to germinate for three days. Water was applied once daily to provide moisture during sprouting. Thereafter, the germinating seed was dried for 48 to 72 hours under sunlight, ground, and then kept at room temperature pending further analysis.

2.4 Feed Formulation

The experimental diet was formulated based on the American institute of nutrition (AIN) method [15]. The animal diet composition with *Telfaira Occidentalis* (Fluted pumpkin) Seed flour is shown in table 1**.** The premix composition (1.25 kg) included; Vitamin D3 (1,7000,000 IU), Vitamin A (8,000,000 IU), Vitamin E (5,000 mg), Vitamin K3 (1,500 mg), Folic acid (200 mg) Niacin (15,000 mg), Vitamin B2 (3,000 mg), Vitamin B12 (5 mg), Vitamin B1 (1000 mg), Vitamin B6 (1000 mg), Biotin, Antioxidant (125,000 mg) Calpan (5,000 mg). Cobalt (100 mg), Selenium (100 mg), Iodine (1,000 mg), Iron 25,000 mg, Manganese (45,000 mg), Copper (3,000 mg), Zinc (35,000 mg), Choline Chloride (100,000 mg)

Table 1 Animal diet composition with *Telfaira Occidentalis* (Fluted pumpkin) Seed Flours (per kg diet)

Ingredient	Control Diet	UF_Diet (g)	BF_Diet (g)	SF_Diet (g)	GF_Diet (g)
Seed Sample	-	680	490	610	620
G.nut cake	130	-	-	-	-
Cornflour	670	120	310	190	180
G.nut oil	5	5	5	5	5
Maize bran	100	100	100	100	100
Bone meal	25	25	25	25	25
Glucose	43	43	43	43	43
Premix	5	5	5	5	5
Salt	17	17	17	17	17
Methionine	3	3	3	3	3
Lysine	2	2	2	2	2

SF; Soaked fluted pumpkin, GF; Germinated fluted pumpkin, BF; Boiled fluted pumpkin, UF; Unprocessed fluted pumpkin

2.5 Experimental Design

Thirty rats were randomly allocated to 5 groups of 6 rats each and were place on different experimental diet as shown below. The experimental diet and water were offered ad libitum to the rats for 28 days. The rats were weighed to obtain their initial weights so that their growth rate was determined at the end of experimental feeding.

Group 1: Control (groundnut meal)
Group 2: Conventional diet with unprocessed fluted pumpkin seed flour (UF_Diet)
Group 3: Conventional diet with boiled fluted pumpkin seed flour (BF_Diet)
Group 4: Conventional diet with germinated fluted pumpkin seed flour (GF_Diet)
Group 5: Conventional diet with soaked fluted pumpkin Seed Flour (SF_Diet)

2.6 Collection of Blood Samples

At the end of the 28 days feeding experiment, the rats were weigh to obtain their final weights. Each rat was anaesthetized with diethyl ether in a desiccator and sacrificed. Blood samples was collected in EDTA sample bottles for haematological analysis and plain bottles for biochemical analysis.

2.7 Biochemical Analysis

Blood collected for biochemical parameters was spinned using centrifuge at 3000 rpm for 10 minutes to separate the serum from the plasma. All biochemical analyses were conducted using Randox Diagonistic kit (Randox Laboratories Ltd, Crumlin, UK). Standard methods of analysis were used to analysed the serum biochemical parameters including alanine transaminase (ALT) [16], aspartate transaminase (AST) [17], alkaline Phosphatase (ALP) [18], gamma-glutamyl transferase, creatinine [19], albumin [20], total protein [21], total cholesterol, LDL cholesterol, HDL cholesterol and triglyceride concentrations

2.8 Hematological Analysis

Blood was collected into sample bottles containing a speck of tetraacetic ethylene diamine acid (EDTA) powder. The following haematological parameters; packed cell volume (PCV), white blood cell count (WBC), red blood cell count (RBC), haemoglobin concentration (Hb) and mean corpuscular haemoglobin concentration (MCHC) were estimated using automated hematologic analyzer (Sysmex, KX-21,Japan) as described by Dacie and Lewis [22].

2.9 Statistical Analysis

All values were expressed as mean ± SEM, Statistical analyses was performed using analysis of variance (ANOVA) followed by Duncan's multiple range tests using SPSS program 20.0. p values $p \leq 0.05$ was considered to be significant.

3.0 Result

3.1 Proximate composition of unprocessed and processed *Telfaira occidentalis* seeds

There was a significant increase ($p< 0.05$) in the crude protein content of germinated (25.70 %) and boiled (22.20 %) seed flours of fluted pumpkin when compared to its unprocessed (21.07 %) seed. A significant increased ($p< 0.05$) was also seen in fat and carbohydrate content of boiled and soaked seed flours of fluted pumpkins when compared to their unprocessed seed flour (Table 2).

Table 2: Proximate Composition of Unprocessed and Processed *T. occidentalis* (Fluted pumpkin) Seed Flour

Sample(%)	SF	GF	BF	UF
Moisture	0.62±0.01^{b}	1.32±0.03^{c}	0.45±0.03^{a}	1.44±0.03^{d}
Ash	2.57±0.15^{a}	4.21±0.05^{b}	4.30±0.17^{b}	4.87±0.09^{c}
Carbohydrate	17.88±1.41^{c}	14.81±0.70^{a}	16.16±0.70^{b}	19.27±3.59^{a}
Protein	19.90±1.55^{a}	25.70±0.60^{c}	22.20±0.60^{b}	21.07±3.63^{a}
Fibre	2.51±0.09^{c}	2.30±0.03^{b}	2.16±0.05^{b}	0.15±0.05^{a}
Fat	56.52±0.01^{d}	51.73±0.05^{a}	54.73±0.03^{c}	53.20±0.01^{b}
Energy(Kcal/100g)	669.82±0.67^{d}	636.77±0.07^{a}	654.63±0.72^{c}	640.76±0.51^{b}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p< 0.05$). SF; Soaked fluted pumpkin, GF; Germinated fluted pumpkin, BF; Boiled fluted pumpkin, UF; Unprocessed fluted pumpkin

3.2 Effect of different processing methods of *T. occidentalis* seeds on biochemical parameters of rats

The ALT activity of rats fed with germinated fluted pumpkin seed flour diet was statistically higher ($p<0.05$) than those fed with other fluted supplemented seed diets. There was a significant difference ($p < 0.05$) in the serum bilirubin and total protein levels of rats on fluted pumpkin diets. There was no significant difference ($p>0.05$) in the serum albumin levels of animals fed with different supplemented diets. The potassium level of rars fed with germinated T. occidentalis seed flour supplemented diet was significantly higher ($p<0.05$) when compared to the levels in other groups. There was no significant difference ($p>0.05$) in the serum urea level of animals fed on boiled and unprocessed seed flour supplemented diets of T. occidentalis when compared to the control diet (Table 3).

Table 3: Biochemical parameters of animals fed with supplemented diets of unprocessed and processed *Telfaira occidentalis* (Fluted pumpkin) seed flours

Parameters	Control	SF_Diet (g)	GF_Diet (g)	BF_Diet (g)	UF_Diet (g)
AST (U/L)	210.70±2.14^{a}	517.5±5.39^{e}	392.3± 3.94^{c}	436.7±4.36^{d}	244.5±2.15^{b}
ALT (U/L)	60.6±0.30^{a}	114.4±2.80^{b}	386.3± 3.53^{e}	267.3±2.42^{d}	118.3±2.32^{c}
ALP (U/L)	221.7±1.21^{a}	308.9±3.17^{d}	298.7±2.20^{c}	276.7±2.53^{b}	328.4± 3.31^{e}
Total Protein(g/dL)	6.4±0.15^{b}	7.4±0.16^{e}	7.10±0.23^{d}	6.80 ±0.55^{c}	6.0±0.06^{a}
Albumin (g/dL)	3.0±0.01^{b}	3 .0±0.02^{b}	3.0±0.01^{b}	3.1±0.01^{c}	2.6±0.01^{a}
T B (mg/dL)	17.7±1.15^{a}	42.6±2.43^{d}	48.5±1.98^{e}	21.7±2.11^{b}	22.6±1.04^{c}
CB (mg/dL)	15.7±0.50^{a}	44.4± 2.22^{e}	39.0±1.04^{d}	18.8±1.43^{c}	18.7±2.13^{b}
Urea (mg/dl)	8.3±0.80^{a}	10.7±1.01^{d}	11.5±1.09^{e}	8.7±1.02^{b}	9.4.±1.05^{c}
Sodium(mmol/L)	130.7±4.2^{e}	87.6±2.38^{b}	46.6± 2.75^{a}	112.7± 3.15^{c}	118.7±2.31^{d}
Potassium(mmol/L)	6.4±0.24^{a}	10.1±0.15^{c}	16.8±1.09^{e}	9.0±0.35^{b}	12.9±1.04^{d}
Chloride(mmol/L)	98.0±1.12^{b}	101.4± 1.60^{c}	97.2±2.10^{b}	106.4±3.05^{d}	93.9±2.12^{a}
Creatinine(mg/dl)	0.9±0.01^{d}	0.40±0.02^{a}	0.40±0.01^{a}	0.50±0.03^{b}	0.6±0.02^{c}

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p< 0.05$). SF; Soaked fluted pumpkin, GF; Germinated fluted pumpkin, BF; Boiled fluted pumpkin, UF; Unprocessed fluted pumpkin

3.3 Effect of different processing methods of *Telfaira occidentalis* seeds on of heamatogical parameters of rats

The RBC was significantly higher ($p< 0.05$) in rats fed soaked and germinated fluted pumpkin seed flour supplemented diets when compared to rats fed unprocessed fluted pumpkin seed flour supplemented diet. The hemoglobin level was significantly higher in rats fed soaked and boiled fluted pumpkin seed flour supplemented diet when compared to animal fed unprocessed fluted pumpkin seed flour supplemented diet. RBC, platelet, and lymphocyte levels were higher in rats fed soaked fluted pumpkin seed flour supplemented diet while eosinophil, neutrophil, basophil, and monocyte counts were significantly different ($p< 0.05$) in rats fed processed fluted pumpkin seed flour supplemented diets when compared to rats fed on the unprocessed diet (Table 4).

Table 4: Haematological parameters of rats fed unprocessed and processed *Telfaira occidentalis* supplemented diets.

Parameter	Control	SF_Diet (g)	GF_Diet (g)	BF_Diet (g)	UF_Diet (g)
HB (g/dl)	16.2 ± 0.15^d	12.1 ± 0.15^c	10.5 ± 0.07^a	12.0 ± 0.13^c	11.7 ± 0.09^b
PCV (%)	48.0 ± 1.2^e	36 ± 1.25^c	34 ± 1.03^a	36 ± 1.58^d	35.0 ± 1.15^b
MCV (Fl)	87 ± 0.04^e	74 ± 0.06^d	73 ± 0.04^c	71 ± 0.02^b	70.0 ± 0.01^a
MCH (Pg)	22 ± 0.23^a	25 ± 0.31^d	24 ± 0.24^c	24 ± 0.22^b	24.0 ± 0.23^c
MCHC (g/dl)	33 ± 0.56^b	32 ± 1.08^a	33 ± 1.12^c	33 ± 1.09^c	31.0 ± 1.11^a
RBC($\times10^9$/l)	4.9 ± 0.03^a	6.0 ± 0.09^d	6.4 ± 0.13^e	5.1 ± 0.11^c	4.9 ± 0.07^b
WBC ($\times10^9$/l)	5.2 ± 0.31^d	3.7 ± 0.11^a	4.7 ± 0.13^c	6.6 ± 0.17^e	4.2 ± 0.15^b
PLC($\times10^9$/l)	278 ± 45.21^e	265 ± 35.57^d	184 ± 24.72^a	233 ± 30.21^c	201 ± 28.16^b
L	58 ± 5.25^c	78 ± 5.24^e	47 ± 4.58^b	$28.0\pm .21^a$	63.0 ± 6.02^d
NEU	42 ± 6.13^d	19 ± 2.21^a	35 ± 5.24^c	52.0 ± 7.54^e	31.0 ± 4.92^b
BASO	01 ± 0.17^c	00 ± 0.00^a	02 ± 0.04^d	01 ± 0.02^b	01 ± 0.01^b
ECO	03 ± 0.07^c	01 ± 0.02^a	03 ± 0.05^c	04 ± 0.04^d	02 ± 0.02^b
MONO	06 ± 0.09^c	02 ± 0.01^a	13 ± 1.21^d	15 ± 1.54^e	03 ± 0.03^b

Values are expressed as means of triplicate determinations ± SEM. Values along rows with different superscript are significantly different ($p< 0.05$). SF; Soaked fluted pumpkin, GF; Germinated fluted pumpkin, BF; Boiled fluted pumpkin, UF; Unprocessed fluted pumpkin. Haemoglobin concentration (Hb), Packed cell volume (PCV), mean cell volume (MCV), Mean cell haemoglobin (MCH) Mean corpuscular Haemoglobin concentration (MCHC), Red blood cell count (RBC), white blood cell count (WBC), Platelet count (PLC), Lymphocytes (L), Neutrophils (NEU), Basophils (BASO), Eosinophils (ECO) and Monocytes (MONO).

4.0 Discussion

The increased protein content seen in germinated *Telfaira occidentalis may be* attributed to an extensive breakdown of seed storage compound and synthesis of structural protein and other cell components occurring during germination. High amino acids biosynthetic activities in seedlings could result in increased content of free amino acids to support protein synthesis [23], this report is similar to that of Rumiyati et al. [23] who reported that germination increase the protein content of Australian sweet lupin *(Lupinus angustifolius L)* and is also similar to the findings of other studies which reported that protein level of germinated and fermented seeds increases when compared with unprocessed products [24,25] .

The processed *Telfaira occidentalis* seed flours revealed a significant increase in liver enzymes when compared to the unprocessed. Previous studies reported that changes in serum protein, albumin, bilirubin, ALT, AST, and ALP concentrations indicate changes in the normal liver functions [26]. The increase seen in protein and liver enzymes levels suggests impairment in normal liver functioning. The level of creatinine in all the groups significantly decrease when compared with the control. This might be due to the seeds antioxidant and phenol content that have a scavenging effect on the free radicals and it may Indicate that the functioning of the kidney is normal, this is in line with a previous study which stated that seeds restored the normal renal function and histology of kidney with no pathological changes [27].

Results obtained in table 4, showed that the value of HB, PCV, MCHC, RBC, increases significantly ($p<0.05$) when compared to that of the unprocessed seed flour for *Telfaira occidentalis.* This increment is an indication that the processing methods significantly improved the quality of the *Telfaira occidentalis* seed flour, improvement may be due to among other factors inactivation of the antinutrient factor present in the seed flour and transformation of some of the component nutrients to non-toxic, more readily digestible and absorbable forms [28].

5.0 Conclusion

Based on the results obtained from this study, it is concluded that soaking, boiling, and germination are more effective in improving the nutritive values of *Telfaira Occidentalis* seed compared to unprocessed seed flour. It could be another cheap and available source of protein that can be incorporated into the diet when subjected to different processing methods without any negative effect on the hematological parameters.

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Ethical Approval: Not applicable

Authors contributions: The work was conducted in collaboration of all authors. All authors read and approved the final version of the manuscript.

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