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Research Article

Effects of Nigerian processed cassava flake (Garri) on fasting blood glucose and some physiological markers in male Wistar rats

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Abstract

Garri, made from processed cassava (*Manihot esculenta Crantz*), is a widely consumed dish in West Africa, especially in Nigeria. Although it is an important dietary source of carbohydrate, there are reports of its toxic effects on the body. This study was designed to evaluate the effect of garri on fasting blood glucose levels and some physiological markers in male Wistar rats. A total of twenty male Wistar rats were used in this study, and divided into four groups of five animals each. Group I was fed with grower's feed, Group II was fed with 25% garri, Group III was fed with 50% garri and Group IV was fed with 100% garri. The animals in all groups were allowed access to vitamix in their drinking water, and were fed for a period of four weeks. The results obtained showed that 50% and 100% garri, significantly ($p < 0.05$) lowered the fasting blood glucose level, body weight and body mass index of the male Wistar rats. There was a significant decrease in high density lipoprotein and triglyceride levels, but a significant increase in low density lipoprotein levels in the animals fed with 100% garri. The gastric mucus content and aspartate aminotransferase (AST) levels were significantly elevated. The differential neutrophil cell count was significantly elevated by garri. Histological sections of the pancreas, lung and stomach of animals fed with garri, showed mild histoarchitectural alterations and inflammation. It can be concluded that garri, especially at 100% concentration may have altered some physiological parameters in male Wistar rats.

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Garri, cassava, liver, pancreas, lungs, stomach, blood glucose, body weight, physiological parameters.

1. Introduction

One of the most popular and traditional ways of consuming cassava is through the preparation of cassava flakes, commonly known as "garri" [1]. Garri plays an essential role in the diets of numerous communities in West Africa, particularly in Nigeria, where it holds cultural significance and is a dietary mainstay [2, 3]. Cassava (*Manihot esculenta Crantz*) is a crucial staple crop in many tropical and subtropical regions worldwide, serving as a primary source of carbohydrates for millions of people [4]. The optional fermentation and addition of palm oil affect the classification of garri which is typically classified as "white garri" and "yellow garri". Cassava mash is fermented before roasting to create white garri, which yellow garri is handled without fermentation but with red palm oil added first [5]. In contrast to its high

carbohydrate content, garri has poor dietary fibers, proteins, vitamins and minerals, which are lost when processing cassava into garri [6]. One aspect of garri's composition responsible for some of its toxicity, it is cyanogenic glycoside content [7, 8]. Although, simple preparation methods like boiling, frying or roasting are used to prepare cassava plant roots with low cyanogenic glycoside concentrations for consumption, however, some traces of cyanogenic glycoside, particularly linamarin is consumed with garri, which has health implications [9-11]. Aflatoxin, a toxic substance produced by fungi that contaminates garri, also poses a potential health challenge, even small amount is continuously consumed over a long period of time [12].

The high glycemic index of garri has raised concerns about its impact on individuals with diabetes [13]. However, cassava consumption has been associated with a lower incidence of diabetes in some populations [14]. Despite being an immediate source of energy [15], consumption of garri, particularly the inadequately processed, is toxic to the liver and impairs its detoxification capabilities [16]. There have been reports of vacuolation of liver cells suggesting degenerative changes [17]. Tewe et al. [18] have demonstrated that cyanide, either in synthetic inorganic or organic forms, can cause marked changes in weight gain, nutrient utilisation, liver enzymes and thiocyanate concentration in the serum.

Studies conducted by Adewoye and Akintayo [19] suggested that certain anti-nutritional factors present in cassava may have adversely affect the gastric health, potentially leading to gastric ulcers and other gastrointestinal disorders. Another study by Joseph et al. [20] revealed that a significant decrease occurred in the body weight after prolonged consumption of cassava flakes, which may indicate nutritional imbalance with adverse health implications. Although, the direct effects of garri consumption on the lungs, pancreas, stomach and lipid profile have not been reported, given the popularity of garri and its place in many people's diets, especially in Nigeria, a thorough investigation of its effects on the body's systems is necessary. Hence, the justification for evaluating the effect of garri consumption on fasting blood glucose levels and some physiological parameters in male Wistar rats.

2. Materials and methods

2.1. Materials

2.1.1. Equipment and reagents

A digital weighing machine (TG Medical Sdn. Bhd. Selanger D. E., Malaysia), digital glucometer (fine test Auto-coding™ infopia limited, Korea), glucose strips (fine test) EDTA and plain sample bottles, normal saline, 10% buffered formalin, ketamine hydrochloride and diazepam, dissecting kits.

2.1.2. Experimental animals

Twenty (20) male Wistar rats weighing between 100-120 g were purchased from the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. The rats were housed in the animal house of the Department of Human Physiology, Ahmadu Bello University, Zaria. They were kept in well ventilated laboratory cages and allowed to acclimatize for two weeks before the commencement of the experiment.

2.1.3. Other materials

Garri (cassava flakes) was sourced from Dumes camp, Ajaokuta, Kogi State, Nigeria. Grower's feed (Crown Flour Mill Limited, Lagos State, Nigeria), thread, wing infusion set and Vitamix (Vetindia Pharmaceuticals Limited, India).

2.2. Methodology

2.2.1. Experimental design

2.2.1.1. Formulation of garri and vitamix

Garri 25%: 25g of garri mixed with 75g of grower's feed.

Garri 50%: 50g of garri mixed with 50g of grower's feed.

Vitamix 1% (w/v): 0.05g of vitamix dissolved in 100 mL of distilled water, and administered as drinking water.

2.2.1.2. Proximate analysis of garri and growers' feed

Samples of garri and grower's feed were taken to the Department of Animal Production and Health, Ahmadu Bello University, for quantification of their nutritional compositions according to the Association of Official Analytical Chemists International [21].

2.2.1.3. Animal grouping

The twenty (20) male Wistar rats were randomly divided into four groups of five (5) rats each (n=5).

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Group 1: Male Wistar rats fed with 100% grower's feed (control group)
Group 2: Male Wistar rats fed with 25% garri
Group 3: Male Wistar rats fed with 50% garri
Group 4: Male Wistar rats fed with 100% garri

All groups were fed with the various formulated diets and administered Vitamix 1% (w/v) *ad libitum* for four weeks.

2.2.2. Determination of body weight, body mass index and fasting blood glucose level

The body weights (g) of the rats were taken every week using a digital weighing scale untill the end of the experiment. A ruler (cm) was used to measure the length from the nose to the base of the tail (snout-anus length). Each rat was handled gently and stretched to ensure that its body was straight. The body mass index (BMI) formula for rats is as follows.

BMI= weight (g)/ [length (cm)]^2 [22].

The fasting blood glucose levels of the rats were also determined weekly after 12-hours overnight fasting, using fine test Auto-coding™'glucometer [23].

2.2.3. Collection of blood sample and bronchoalveolar lavage fluid (BALF)

At the end of the four weeks feeding period, the animals were anaesthetized using 75 mg/kg ketamine hydrochloride and 25 mg/kg diazepam [24]. Blood samples were collected via cardiac puncture and centrifuged at 5000 x g for 5 min. The sera was collected and stored in plain sample bottles, which were then used for biochemical analyses. The thoracic cavity was dissected to expose the lungs and the trachea. Bronchoalveolar lavage was performed by cannulating the trachea using a thread. A 2 mL syringe connected to the wing infusion set and 2.0 mL of normal saline (0.9% NaCl) was instilled into the lungs according to the method described by Chaudhari et al. [25], with slight modification. The lavage fluid was collected and stored in EDTA bottles and used for total and differential white blood cell counts.

2.2.4. Gastric tissue sample collection and analysis of mucus content

A circular incision was made in the upper abdominal region using surgical scissors. The stomach was carefully slit open and a small portion was cut, rinsed with saline to remove any food materials and weighed. The collected glandular portion of the gastric tissues was transferred to 0.1% alcian blue solution prepared in 0.25 M sucrose and 0.5 M sodium acetate (pH 5.8) and stained for 2 h at room temperature. Afterwards, the segments were rinsed twice with 0.25 M sucrose solution for 15 and 45 min and the dye complexed with the gastric mucus was extracted with 0.5 M magnesium chloride solution for 2 h (during this period the soaked stomach was shaken every min for 30 min). The extract was then mixed with an equal volume of diethyl ether and centrifuged at 3000 rpm for 15 min. The absorbance was determined at 580 nm. Mucus amount was calculated using standard curves of alcian blue.

$$\text{Mucus content} = \frac{\text{Alcian blue (}\mu\text{g/mL)}}{\text{glandular tissue (g)}}$$

2.2.5. Determination of serum lipid profile

Serum levels of total cholesterol, triglycerides, high-density lipoprotein and low-density lipoprotein were also determined spectrophotometrically [26-29].

2.2.6. Estimation of serum liver enzymes

The serum levels of alanine transaminase (ALT), aspartate transaminase (AST) [30], and alkaline phosphatase (ALP) were determined spectrophoto-metrically [31].

2.2.7. Hematological analysis

Total and differential white blood cell counts in bronchoalveolar lavage fluid was done according to the method of Cheesebrough [32].

2.2.8. Histological analysis of tissues

Samples of stomach, lung and pancreas were collected for histology using H & E techniques as described by Bancroft and Stevens [33].

2.2.9. Data analysis

Weekly data collected (body weight and fasting blood glucose levels) were analyzed using mixed design analysis of variance (ANOVA), while other collected data were analyzed using one-way ANOVA followed by Tukeys' *post hoc* test for multiple comparisons. All results were expressed as mean ± standard error of mean (SEM). SPSS version 25.0 was used for the analyses. Values of $p < 0.05$ were considered statistically significant.

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Table 1. Proximate analysis of the Grower's Feed and Garri.

Description	D.M (%)	C.P. (%)	C.F. (%)	Oil (%)	Ash (%)	NFE (%)
Garri	88.52	5.35	1.56	0.46	1.43	91.20
Grower's feed	90.23	30.75	3.93	4.74	6.34	54.24

D.M. = dry matter, C.P. = crude protein, C.F. = crude fiber, NFE = nitrogen-free extract.

3. Results

3.1. Proximate analysis of Garri and Grower's feed

Table 1 showed the percentage composition of the proximate analysis of garri and grower feed in this study. The results obtained for garri were dry matter (88.52%), crude protein (5.35%), crude fibre (1.56%), oil (0.46%), ash (1.43%) and nitrogen-free extract (91.20%). The results obtained for the grower's feed were dry matter (90.23%), crude protein (30.75%), crude fiber (3.93%), oil (4.74%), ash (6.34%) and nitrogen-free extract (54.24%).

3.2. Effect of varying doses of garri on body weight, BMI and fasting blood glucose level in Wistar rats

The result in Fig. 1 shows the effect of garri on the fasting blood glucose levels of male Wistar rats.

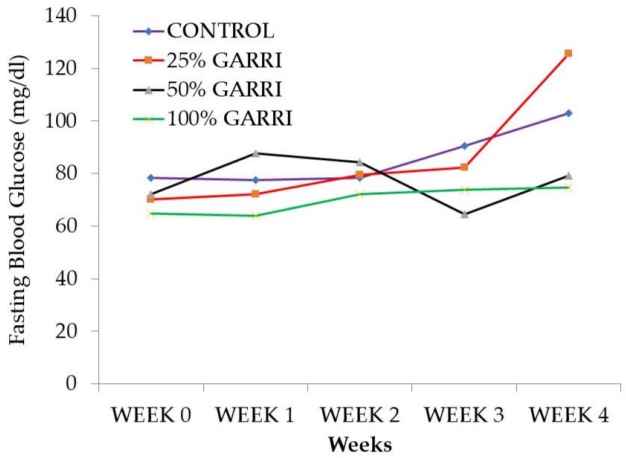

Figure 1. Effects of varying doses of garri on fasting blood glucose level of male Wistar rats.

There was a significant decrease ($p < 0.05$) in fasting blood glucose levels in the group fed with 100% garri compared to the group fed with 50% garri in week 1. In week 4, there was a significant ($p < 0.05$) decrease in the groups fed with 50% and 100% garri compared to the group fed with 25% garri and the control. There was no significant difference ($p > 0.05$) across the weeks in the groups fed with 50% and 100% garri.

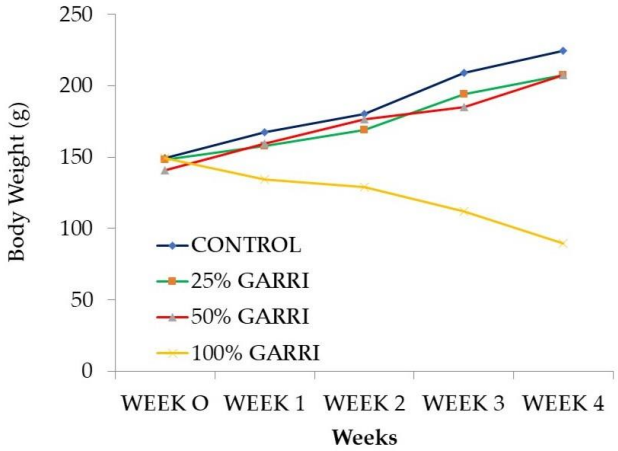

Figure 2. Effects of varying doses of garri on body weight of male Wistar rats.

The results of the effect of varying doses of garri on body weights are presented in Fig. 2. The results showed the effect of cassava flakes (garri) on the body weight of male Wistar rats. There was a significant decrease ($p < 0.05$) in body weight in the group fed with 100% garri compared to the group fed with 50% garri and the control in week 1. However, in week 4, there was also a significant decrease in body weight in the group fed with 100% garri compared to the group fed with 25% garri, 50% garri and the control. In the group fed with 100% garri, there was a significant decrease in body weight in weeks 3 and 4 compared to weeks 0, 1 and 2. The results of the effect of garri on the BMI of Wistar rats are presented on Fig. 3. There was a significant decrease in BMI in the group fed with 100% garri compared to the control group, those fed with 25% and 50% garri.

3.3. Effect of varying doses of Garri on total and differential WBC count in BALF

Table 2 shows the effect of garri on the total and differential white blood cell counts in the BALF of male Wistar rats. There was a significant increase ($p < 0.05$) in WBC count in the groups fed with 25% garri and 100% garri, compared to the control group.

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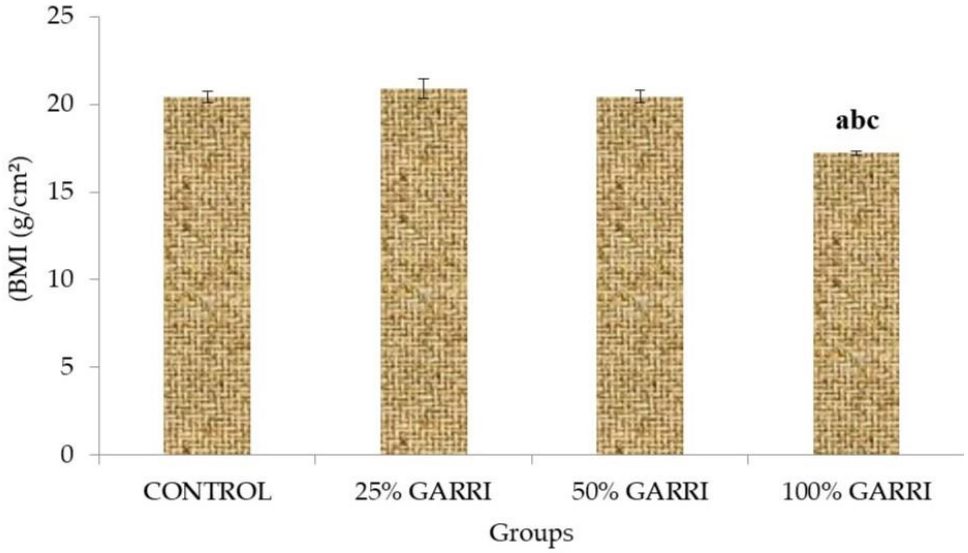

Figure 3. Effects of varying doses of garri on body mass index of male Wistar rats.
(Superscripts a b, c = statistically significant difference ($p < 0.05$) compared to the control, 25% Garri and 50% Garri, respectively).

Table 2. Effect of varying doses of Garri on total and differential WBC count in BALF.

GROUPS	Total WBC (x10⁹ cells/µl)	NEUTRO (%)	LYMPHO (%)	EOSIN (%)	MACRO (%)
CONTROL	3.38±0.54	13.60±1.57	78.20±5.83	3.60±0.75	2.51±1.46
25% GARRI	5.72±0.24^{ac}	11.63±0.50^{a}	80.00±2.95	4.60±0.24	2.00±0.84
50% GARRI	3.70±0.61	14.20±0.97	82.80±2.42	3.40±0.20	2.20±0.66
100% GARRI	6.06±0.54^{ac}	17.00±1.30^{b}	82.40±2.80	3.60±0.51	2.00±0.45

a, b, c = statistically significant difference ($p < 0.05$) compared to the control, 25% Garri and 50% Garri respectively. NEUTRO = Neutrophils; LYMPHO = Lymphocytes, EOSIN = Eosinophils, MACRO = Macrophages, WBC = white blood cells.

However, there was no significant difference ($p > 0.05$) between the group fed with 50% garri, compared to the control group. There was a significant increase in the differential count of neutrophils in the group fed with 100% garri compared to the control group and the group fed with 25% garri. However, there was no significant difference ($p > 0.05$) in the differential counts of macrophages, lymphocytes and eosinophils compared to the control.

3.4. Effect of varying doses of Garri on serum liver enzymes in male Wistar rats

Fig. 4 shows the effect of garri on serum liver enzymes. There was no significant difference ($p > 0.05$) in the serum level of alanine aminotransferase (ALT) of male Wistar rats when all the groups were compared with each other. The result on the serum level of aspartate aminotransferase (AST) in male Wistar rats showed a significant increase in AST in the group fed with 50% garri compared to the groups fed with 25% and

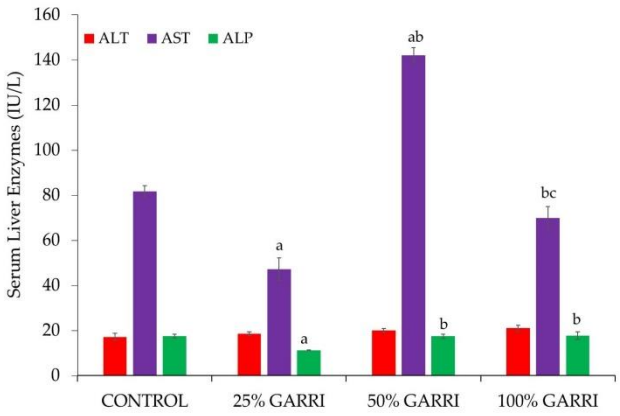

Figure 4. Effects of varying doses of garri on serum liver enzymes of male Wistar rats.
Superscripts a b, c = statistically significant difference ($p < 0.05$) compared to the control, 25% Garri and 50% Garri respectively. **ALT=**Alanine Aminotransferase**, AST =**Aspartate Aminotransferase**, ALP=**Alkaline Phosphatase.

100% garri as well as the control group. In addition, the group fed with 100% garri showed a significant

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Table 3. Effects of varying doses of garri on serum lipid profile in male Wistar rats.

Groups	CHOL (mg/dl)	TRIG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
CONTROL	83.60 ± 10.17	153.5 ± 15.26	20.00 ± 3.54	43.48 ± 5.75
25% GARRI	87.40 ± 8.52	200.9 ± 18.90	11.80 ± 0.92[a]	47.22 ± 11.12
50% GARRI	49.00 ± 6.78[b]	107.2 ± 6.017[b]	8.40 ± 0.68[a]	20.80 ± 2.59
100% GARRI	58.40 ± 12.21	78.44 ± 6.63[ab]	8.20 ± 0.92[a]	69.50 ± 4.56[c]

a, b, c = statistically significant difference ($p < 0.05$) compared to the control, 25% Garri and 50% CHOL= total cholesterol; TRIG= triglycerides; HDL= high-density lipoprotein; LDL= low-density lipoprotein

increase in AST compared to the group fed with 25% garri. The results on the serum level of alkaline phosphatase (ALP) in male Wistar rats showed a significant decrease in the group fed with 25% garri compared to the control group, the group fed with 50% garri and the group fed with 100% garri.

3.5. Effects of varying doses of garri on serum lipid profiles of male Wistar rats

The result in Table 3 showed the effect of garri on the serum lipid profile of male Wistar rats. There was a significant decrease in total cholesterol in the group fed with 50% garri, compared to the group fed with 25% garri. There was no significant difference ($p > 0.05$) in the groups fed with 100% garri compared to the other groups fed with garri as well as the control group. There was a significant decrease in serum triglyceride levels in the groups fed with 50% and 100% garri compared to the group fed with 25% garri. In addition, the group fed with 100% garri was significantly lower compared to the control group. However, there was no significant difference between the groups fed with 25% garri and the control group. The result on the serum level of high-density lipoprotein (HDL) of male Wistar rats showed a significant decrease ($p < 0.05$) in the groups fed with 25%, 50% and 100% garri when compared to the control group. However, there was no significant difference when the other groups were compared with each other. There was a significant increase in LDL level in the group fed with 100% garri compared to the group fed with 50% garri. However, there was no significant difference in the group fed with 25%, 50% garri compared to the control group.

3.6. Effects of varying doses of garri on gastric mucus content of male Wistar rats

Fig. 5 shows a significant increase ($p < 0.05$) in gastric mucus content in groups fed with 25%, 50% and 100% garri compared to the control group.

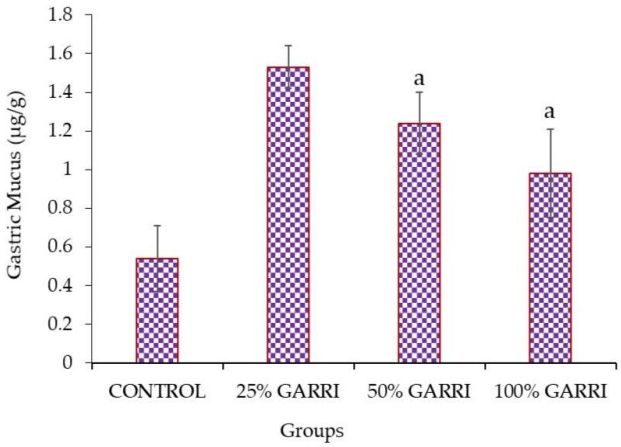

Figure 5. Effects of varying doses of garri on gastric mucosa of male Wistar rats.
Superscripts 'a' = statistically significant difference ($p < 0.05$) compared to the control.

3.7. Effects of varying doses of garri on histology of some tissues

Fig. 6 shows the photomicrograph of the pancreas of Wistar rats fed with 100% grower's feed showing normal islets of Langerhans, acinar cells and ducts. However, the rats fed with 50% and 100% garri showed an increased number of islet cells. Fig. 7 shows the photomicrograph of the lungs of male Wistar rats that feed with 25% and 50% garri, had normal alveolar spaces. However, the group fed with 100% garri showed slight alveolar necrosis with slight cellular infiltration. The photomicrograph of the gastric tissue as presented in Fig. 8 shows normal epithelial cells and muscularis mucosae in the mucosa in all the groups, except the group fed with 100% garri which showed proliferation of the epithelial cells of the gastric mucosa.

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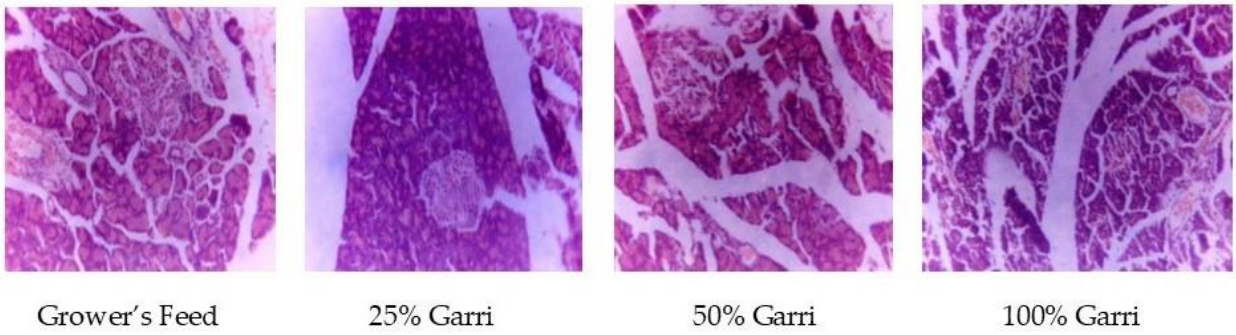

Figure 6. Photomicrographs of pancreas of Wistar rats fed with varying doses of Garri (H & E, Magnification X100).

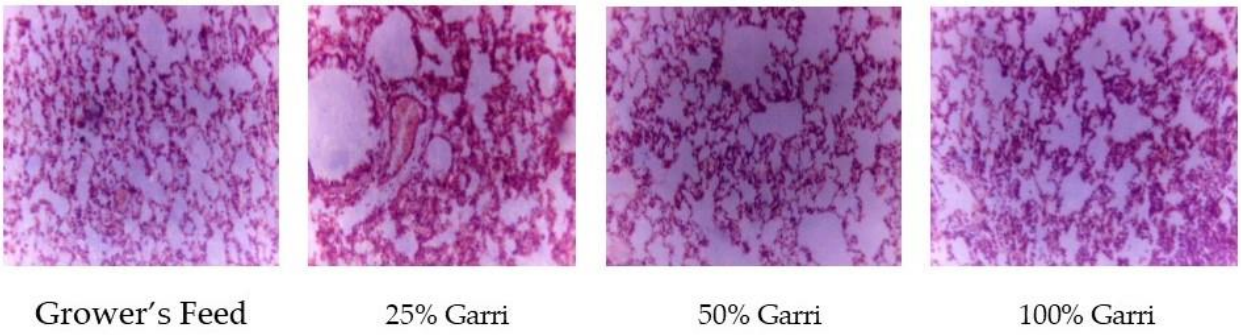

Figure 7. Photomicrographs of Lungs of Wistar Rats fed with Varying Doses of Garri (H & E, Magnification X100).

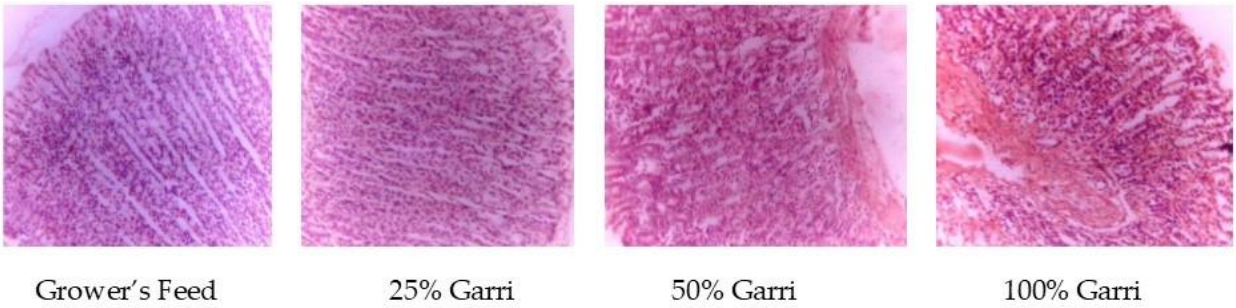

Figure 8. Photomicrographs of the gastric tissue of Wistar rats fed with varying doses of Garri (H & E, Magnification X100).

4. Discussion

Garri is a popular staple food in Nigeria made from cassava tubers. It is known for its high carbohydrate content and is a good source of energy [34]. The preliminary proximate analysis of garri and grower's feed showed a high nitrogen-free extract (NFE) content in garri compared to grower's feed. The NFE content is considered the cheapest dietary energy source in livestock and aqua feeds, and it is more available than proteins [35]. Cyanide and phytate are components that contribute to the nitrogen content of feed. The higher NFE content in garri observed in this study might be due to the decrease in cyanide during the fermentation process [36, 37]. Garri also had a higher dry matter content and low crude protein in comparison with the sample of growers' feed. This result is in agreement with the findings of a preliminary study on a sample of garri conducted by Mohammad et al. [38], and Nwabueze et al. [39], who also reported high dry matter, low crude protein and high NFE contents.

In this study, the consumption of garri (100%) decreased the fasting blood glucose levels in Wistar rats. Considering that garri is known for its high

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carbohydrate content, this result may be paradoxical. However, the dietary fiber found in garri has been reported to slow down the digestion and absorption of carbohydrates, resulting in a more gradual release of glucose into the bloodstream, thus, preventing spikes in blood sugar levels [40]. A study conducted by Myke-Mbata et al. [41], found that cassava is digested and absorbed more slowly, resulting in a slower and more gradual increase in blood glucose levels.

The body weight of the animals fed with only garri, was significantly reduced, which was attributed to the composition of garri itself, as reported by Levin et al. [42]. Dietary fiber has also been shown to have a satiating effect, leading to reduced food intake and subsequent weight loss [43]. Additionally, garri is low in fat and protein, which are known to contribute to weight gain [44]. Therefore, the lower calorie and nutrient content of garri compared to normal feeds could contribute to the decrease in body weight observed in the garri-fed animals. The result of body weight in this study align with the body mass index of the animals fed only garri. Although high NFE is an indication of reduced cyanide content in garri, even in trace amounts, cyanide may be responsible for the decrease in BMI observed in this study [45].

The histological analysis of pancreatic tissue in the Wistar rats fed only with garri, showed an increase in the number of cells in the islets of Langerhans. Islets of Langerhans are clusters of cells within the pancreas that secrete hormones like insulin and glucagon, which are involved in glucose homeostasis [46]. Considering that the fasting blood glucose level in this study was significantly reduced after feeding with garri, implying that the beta- cells of the islets of Langerhans possibly increased in number and activity, resulting in increased insulin secretion, leading to reduced blood glucose levels.

The results of the total and differential white blood cell counts in BALF showed that garri increased the total white blood cell and differential neutrophil counts in BALF, which can be observed in lung inflammations [47, 48]. Cassava, was reported to contain proteins and compounds that can potentially trigger allergic reactions that may elevate white blood cells [49]. Histological examination of lung tissues showed cellular infiltration in rats fed only with garri, which is also a result of lung inflammation.

Cellular infiltration refers to the migration or accumulation of various types of cells into tissues or organs where they are not usually found. This can occur in response to various pathological conditions such as infection or inflammation [50].

In this study, consumption of garri by male Wistar rats caused a significant increase in aspartate aminotransferase (AST) levels. The increase in AST level aligns with the findings of Jemai et al. [51] who reported that increases in AST and other liver enzymes are crucial indicators of liver function. Changes in enzyme can reflect various liver conditions, including inflammation, injury, or dysfunction [52]. Although we did not observe a change in the other liver enzymes, an increase in AST level is an indication of a possible effect of garri's glycosides, which are implicated in periportal vacuolation of the liver accompanied by elevated liver enzymes [53].

A decrease in total cholesterol levels was also observed in animals fed garri. This is in accordance with the study of Airaodion and Ogbuagu [54], who reported that cassava dietary intake decreases total cholesterol level, which was attributed to the presence of high dietary fiber in cassava. The ability of dietary fiber to lower lipid levels is achieved through the action of soluble fiber, which binds to bile acids, leading to increased removal of these acids in feces and disrupting the continuous entero-hepatic circulation of bile salts. Another study by Anderson et al. [55], reported that the increased dietary fiber often results in a reduction in the availability of cholesterol for incorporation into lipoproteins. A high dietary fiber intake has been reported to increase the excretion of cholesterol, thereby lowering blood cholesterol levels. A decrease in triglyceride levels was also observed in the groups fed 50% and 100% garri. This is in accordance with Lin et al. [56] who reported that the starch intake was associated with a significant decrease in triglyceride levels in animal models.

The results on the serum level of high density lipoprotein (HDL) of male Wistar rats showed a decrease in HDL level in all the groups fed with garri,

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compared to the control. This is in accordance with the study reported by Oladunjoye et al. [57], in which a low HDL cholesterol level was observed in cassava peel meal. Cassava peel contains high hydrocyanic acid and the presence of hydrocyanic acid in cassava can also exert hypocholesteronic influence as glucosides have ability to interfere with the intestinal absorption of dietary cholesterol and lipid. An increase in LDL level in the group fed with 100% garri was observed in this study. The increased level of LDL is comparable with the research conducted by Ravnskov [58], who concluded that approximately 60 to 80 percent of the cholesterol in the blood is carried by LDL, commonly referred to as "bad" cholesterol. Therefore, the increase in low-density lipoprotein required a higher concentration of cassava flakes, as it was observed only in the group feed with 100% garri.

The results on gastric mucus content showed a significant increase in the group fed with garri (100%) when compared to the control group. This is in contrast with the study of Satchithanandam et al. [59], who reported a significant decrease in both luminal and tissue mucus levels, this contrast may be due to the duration of this study. The photomicrograph of the gastric mucosa of male Wistar rats in the control group showed damaged and dilated gastric glands, which is in contrast to the study by Oghobase et al. [60] who reported an intact morphological pattern of the gastric mucosa characterized by the normal morphology of the gastric glands, which includes different types of cells and regions within the glands.

5. Conclusions

The administration of varying doses of garri for four weeks reduced fasting blood glucose levels and body weight, altered the lipid profile, and liver enzymes, elevated the mucus content of the stomach and infiltration of inflammatory cells in the lungs as well as caused some histological changes in the pancreas, lungs and stomach.

Ethical statement

The experimental protocol was reviewed and certified by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number (ABUCAUC/2023/009). Strict adherence to the Ethical Committee's directives was observed.

Disclaimer (artificial intelligence)

Author(s) hereby state that no generative AI tools such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators were utilized in the preparation or editing of this manuscript.

Authors' contributions

Conceptualization, B.D.A.; Methodology, R.A.A., Z.A.M. and H.G.B.; Validation, Z.A.M. and D.M.I.; Software, M.H.A. and R.U.A; Formal Analysis, B.D.A. and D.M.I; Investigation, B.D.A. and H.G.B; Resources, R.A.A; Data Curation, D.M.I and R.U.A; writing—Original Draft Preparation, B.D.A; and M.H.I.; Visualization, H.G.B.; Writing- Review & Editing, B.D.A., Supervision, B.D.A. and R.A.A.; Project administration, B.D.A. and H.G.B; Funding acquisition, Z.A.M., R.U.A and B.F.M.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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