Original Research Article

Biochemical Changes Associated with Consumption of Garri Processed by Traditional and Instant Mechanical Methods in Wistar Rats

ABSTRACT

Aim: The aim of this study is to investigate the biochemical changes associated with the consumption of garri processed by traditional and instant mechanical methods in Wistar rats.

Methods: Cassava samples were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan and were processed using traditional and instant mechanical methods. Fifteen adult male Wistar rats were purchased from the Animal Holding Unit of the Department of Physiology, University of Ibadan, Nigeria with body weight between 100 and 120 g. They were acclimatized for seven (7) days during which they were fed *ad* libitum with standard feed and drinking water. They were randomly divided into three groups of five rats each. The rats in group A were then fed with pure standard feed, those in group B were fed with garri processed by instant mechanical method while those in group C were fed with garri processed by traditional method. After twenty-eight days of feeding, the animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture. Hepatic and renal indices were determined using standard methods.

Results: Perturbations were observed in the liver and renal indices when animals fed with garri processed by traditional method were compared with those of animals fed with garri processed by instant mechanical method and control group respectively.

Conclusion: In this study, both garri samples did not cause hepatic nor renal damage but perturbation of biochemical parameters were observed. These perturbations were more severe in animals fed with garri processed by instant mechanical method. This could be attributed to the high cyanide content in it. Processors of garri should be enlightened on the dangers of garri high in cyanide to human health and should be encouraged to avoid short-cut practice in the production of garri. Garri should be allowed to ferment for at least 72 hours before roasting.

Keywords: *Hepatic indices, renal indices, garri processed by traditional method, garri processed by instant mechanical method.*

1. INTRODUCTION

Cassava (*Manihot esculenta* Cranz) is a woody shrub native to South America of the spurge family, Euphorbiaceae [1]. It is extensively cultivated as an annual crop in tropical and subtropical regions for its edible starchy tuberous root, a major source of carbohydrates [2]. Cassava is the third largest source of food carbohydrates in the tropics, after rice and maize. It is a major staple food in the developing world, providing a basic diet for over half a billion

people [3]. It is one of the most drought-tolerant crops, capable of growing on marginal soils. In 2014, global production of cassava root was 268 million tonnes, with Nigeria as the world's largest producer of nearly 55 million tonnes or 21% of the world total [4].

Cassava is classified as either sweet (*Manihot palmata*) or bitter (*Manihot esculenta* or *Manihot utilissima*). Like other roots and tubers, both bitter and sweet varieties of cassava contain anti-nutritional factors and toxins, with the bitter varieties containing much larger amounts [5]. It must be properly prepared before consumption, as improper preparation of cassava can leave enough residual cyanide to cause acute cyanide intoxication, goiters, and even ataxia, partial paralysis, or death [6]. The more toxic varieties of cassava are a fall-back resource (a "food security crop") in times of famine or food insecurity in some places. Farmers often prefer the bitter varieties because they deter pests, animals, and thieves [7].

In Nigeria, as in most African countries, cassava is one of the most important carbohydrate sources. About 95 percent of cassava is consumed as food and less than 5 percent of it is used for industrial purposes [8]. It is usually consumed in processed forms. In recent times, several processing options have emerged from cassava such as garri, fufu, starch, flour, tapioca and chips. Irrespective of these options, garri (roasted granules) and edible starch (which is a by-product from drying the grated tubers) have maintained an important position in the food timetable of many households in Nigeria and other countries of the world, although starch consumption is most notable in the south-south region of Nigeria [9].

Toxicity of cyanide in cassava products has been reported. There are as well few reported cases of death linked to consumption of cassava meals [10]. The incidents of cassava toxicity parallel severe hunger condition associated with drought or wartime when processors adopt “shortcut” (shortened process time) in order to meet market demand. Consumption of garri has always been a trend in Nigeria and some other parts of the world. The consumption of this product has been accompanied with some side effects like food poisoning and other related effects due to inefficiency in the course of production, which inevitably leads to improperly processed product, and when this product is consumed, it results to food poisoning and its effect can be fatal [10].

On 20th March 1994, one Mrs. Loveth Osueke was reported in the National dailies to have died after eating African Salad made from cassava which she bought from Ariaria market in Aba, Abia State, Nigeria. More recently, it was also reported in the national dailies on Tuesday 1st November, 2016 that six persons (including a mother, her three children and her two neighbours) died after consumption of cassava product (‘lafun’) in Ogaminana area of Okene in Kogi State, Nigeria [11].

Fermentation is an important processing technique for cassava, especially in Africa. Three major types of fermentation of cassava roots are recognized: the grated root fermentation, fermentation of roots under water and mould fermentation of roots in heaps [12]. The grated cassava roots are allowed to ferment in sacks for 3-7 days, which encourages lactic acid fermentation. The pH after 3 days decreases from 6 to 4 and the fermentation is dominated by lactic acid bacteria [5]. Grating is important for bringing linamarin into contact with linamarase allowing its hydrolysis to glucose and cyanohydrin and then to HCN [13]. The hydrolysis continues during the fermentation process. Lactic acid fermented products are reported to have significant concentrations of cyanohydrins because pH decreases during fermentation and cyanohydrin is stable at low pH. The processes of garri production reduce cyanogen contents by more than 95 % [12]. Fermentation of cassava roots under water, followed by sun drying, is reported to be the best for cyanogens removal [14]. This type of fermentation is used more in areas where there is a sufficient supply of water such as near a river or lake, and is common in countries such as Nigeria, Democratic Republic of Congo, Tanzania and Malawi [12]. Heap fermented cassava root products are produced in Tanzania [15], Uganda and Mozambique [16]. The process involves peeling of cassava roots, sun drying for 1 to 3 days, heaping and covering, fermentation, scraping off the molds, crushing into crumbs, sun drying, pounding and sieving into flour. During the fermentation of the roots, the temperature inside the heaps increases between 23 and 29 ℃ higher than the temperature outside the heaps (2 to 12 ℃). According to Sani and Farahni, [16], heap fermentation is dominated by the *Neurospora*

sitophila, Geotrichum candidum and *Rhizopus oryzae.* Heap fermentation of cassava roots followed by sun drying is capable of reducing the cyanogen levels by 95 % [16].

2. MATERIALS AND METHODS

2.1. Production of Garri

Cassava samples were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria and were processed using traditional and instant mechanical methods. For garri processed by instant mechanical method, cassava was grated and dewatered using hydraulic press and were roasted (fried) within 24 hours of harvest. For garri processed by traditional method, the grated cassava mash was allowed to stay for 24 hours in the sack before dewatering using sticks. The dewatering process took 3 days before roasting. The two methods of processing were as described by Olukosi *et al*., [17].

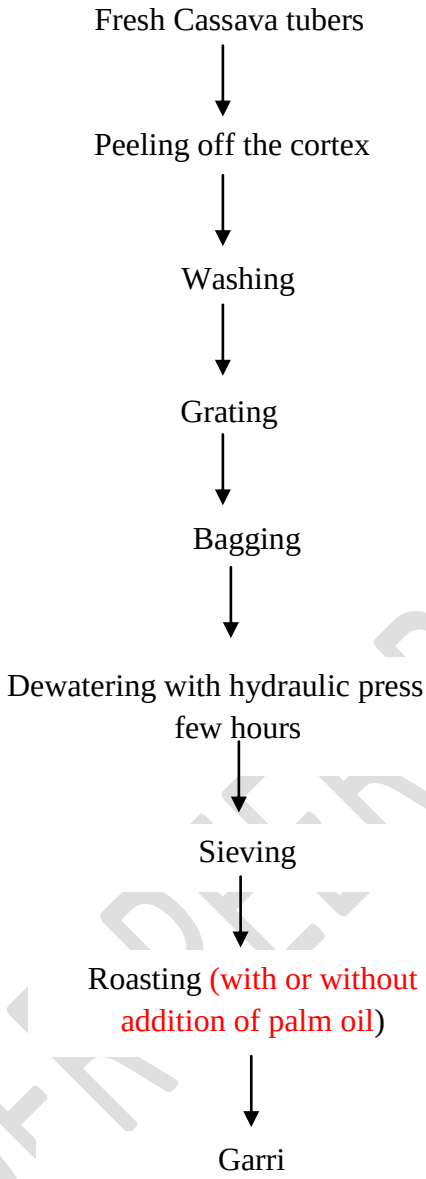

Figure 1: Stages of processing Garri by Instant Mechanical Method

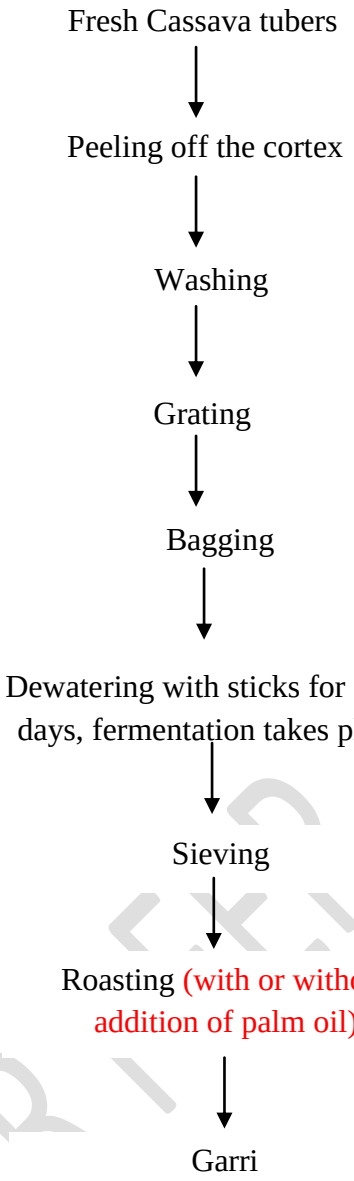

Figure 2: Stages of processing garri by traditional method

2.2 Experimental Design

15 adult male Wistar rats (*Rattus norvegicus*) were purchased from the Animal Holding Unit of the Department of Physiology, University of Ibadan, Nigeria with body weight between 100 and 120 g. They were housed in Imrat animal house, Ibadan. They were acclimatized for 7 days during which they were fed *ad* libitum with standard feed and drinking water. Throughout the experiment, the animals were housed in clean cages placed in well-ventilated housing conditions (under humid tropical conditions). The rats were randomly divided into three groups consisting of five rats in each group. Prior to the rats being fed with different garri samples, they were given known amount of standard feed and the remaining feed was weighed after 24 hours to ascertain the actual quantity of feed each group took. This was done prior to proper feeding and the average was computed. On the average, it was observed that each group sufficiently ate 150 g of mash feed meaning that each rat can take 30 g of meal per day. The rats in group A were then fed with 150 g of pure standard feed, rats in group B were fed with 150

g of Garri processed by instant mechanical method and the rats in group C were fed with 150 g of garri processed by traditional method. After 28 days of feeding, the animals were fasted overnight and anaesthetized using diethyl ether. Blood samples were collected by cardiac puncture.

2.3 Determination of Hepatic Indices

Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) activities were determined using Randox commercial Enzyme kits according to the method of Reitman and Frankel [28]. Alkaline Phosphatase (ALP) activity was determined by Phenolphthalein Monophosphate method described by Babson *et al.,* [19]. Total Protein concentration was carried out using Biuret method described by Henry *et al.* [20]. Estimation of albumin was done by bromocresol green (BCG) method described by Doumas *et al.* [21]. Globulin concentration was determined by subtracting albumin from total protein.

2.4 Determination of Renal Indices

Creatinine concentration was determined using Jaffe reaction described by Toora and Rejagopal [22]. Urea concentration was determined using a Randox Commercial Kit based on the methods of Fesus *et al.* [23]. Total bilirubin concentration was determined by diazo method described by Royden and Alfred [24]. Conjugated bilirubin concentration was determined by the method of Compernolle [25]. Unconjugated bilirubin was determined by subtracting conjugated bilirubin from total bilirubin.

2.5 Statistical Analysis

Data were subjected to analysis of variance using the Statistical Package for Social Sciences (SPSS), version 20.0. Results were presented as Mean ± Standard Error of the mean (SEM). 2–tailed t–test was used for comparison of the means. Differences between means was considered to be significant at $p<0.05$.

3. RESULTS

The result of the effect of garri processed by traditional and instant mechanical methods are presented in tables 1 and 2 below.

Table 1: Effect of Garri Samples on Liver Indices of Animals after 28 days of Feeding

Liver Indices	Control	Instant Mechanical Garri	Traditional Garri
AST (IU/L)	130.63 ± 7.65^{a}	95.15 ± 4.02^{b}	95.84 ± 5.78^{b}
ALT (IU/L)	41.72 ± 2.17^{a}	36.28 ± 3.72^{b}	37.49 ± 3.64^{b}
AST/ALT	3.13 ± 0.03^{a}	2.62 ± 0.02^{b}	2.56 ± 0.02^{b}
ALP (IU/L)	14.60 ± 0.57^{a}	22.59 ± 0.70^{b}	19.79 ± 1.02^{c}
TP (g/dL)	5.40 ± 0.13^{a}	4.54 ± 0.06^{b}	4.55 ± 0.10^{b}
Albumin (g/dL)	3.12 ± 0.24^{a}	2.61 ± 0.24^{b}	2.63 ± 0.14^{b}
Globulin (g/dL)	2.28 ± 0.01^{a}	1.93 ± 0.02^{a}	1.92 ± 0.01^{a}

Values are presented as Mean±S.E.M, n = 5. Values with different superscript along the same row are significantly different at $p<0.05$

Legend: AST = Aspartate Amino Transferase, ALT = Alanine amino Transferase, ALP = Alkaline Phosphatase, TP = Total Protein

Table 2: Effect of Garri Samples on Renal Indices of Animals after 28 days of Feeding

Renal Indices	Control	Instant Mechanical Garri	Traditional Garri
Creatinine (mg/dL)	0.82 ± 0.01^{a}	0.60 ± 0.05^{b}	0.63 ± 0.02^{b}
Urea (mg/dL)	37.27 ± 0.47^{a}	30.38 ± 0.36^{b}	23.64 ± 0.54^{c}
TB (mg/dL)	0.72 ± 0.11^{a}	0.44 ± 0.06^{b}	0.30 ± 0.10^{c}
CB (mg/dL)	0.33 ± 0.06^{a}	0.21 ± 0.02^{b}	0.12 ± 0.02^{c}

UB (mg/dL)	0.39 ± 0.04^{a}	0.23 ± 0.02^{b}	0.18 ± 0.01^{b}

Values are presented as Mean±S.E.M, n = 5. Values with different superscript along the same row are significantly different at p<0.05

Legend: TB = Total Bilirubin, CB = Conjugated Bilirubin, UB = Unconjugated Bilirubin

4. DISCUSSION

Studies on the tissue enzyme alterations might reflect the metabolic abnormalities and cellular injuries in some organs. The liver and kidney have extremely important function in detoxification and excretion of metabolic wastes and xenobiotics [26]. Exposure to toxic chemicals causes alterations in some tissue enzyme activities [27,28]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are distributed extensively in several different organs and have important roles in carbohydrate and amino acid metabolic pathways and their activities is established to change under several physiological and pathological circumstances [29].

There was reduction in the activities of AST and ALT in garri fed rats when compared with those of the control group. Airaodion et al. [30], has reported that garri processed by both traditional and instant mechanical methods contain some proportions of cyanide but this proportion was higher in garri processed by instant mechanical method. The result of AST and ALT observed in this study corresponds with the work of Kadiri and Asagba [31] who reported an increase in the activities of AST and ALT when animals were exposed to cyanide directly above 2 mg but a decrease in animals exposed to food contaminated with less than 2 mg cyanide. The suppression of enzymes by garri in this study is also similar to the reports of Chilaka *et al*. [32] and Eze *et al*. [33]. Feeding a diet high in simple carbohydrates to rats or mice results in increased transcription of at least 15 genes involved in glucose uptake, glycolysis and lipogenesis [34]. Glucose represses the induction of inducible operons by inhibiting the synthesis of cyclic Adenosine monophosphate (cAMP) a nucleotide that is required for the initiation of transcription of a large number of inducible enzyme systems including the Lac operon. Cyclic AMP (cAMP) is required to activate an allosteric protein called catabolite activator protein (CAP) which binds to the promoter CAP site and stimulates the binding of ribonucleic acid (RNA) polymerase to the promoter for the initiation of transcription, but cAMP must be available to bind to CAP which binds to deoxyribonucleic acid (DNA) to facilitate transcription. In the presence of glucose, adenylase cyclase (AC) activity is blocked. AC is required to synthesize cAMP from Adenosine Triphosphate (ATP) [35]. Therefore if cAMP levels are low, CAP is inactive and transcription does not occur. Thus the effect of glucose in suppressing these inducible enzymes is by lowering cyclic AMP level. The garri feeding might have lowered cAMP in garri-treated albino rats thus causing inhibition of these inducible enzymes. The decrease in ALT and AST level may be attributed to the fact that slowly digested carbohydrate diet gives a less rapid flow of glucose into the circulating system [36]. ALT is considered most reliable hepatocellular injury because it is solely confined to the liver, unlike AST which is also abundantly present in other body organs such as the kidneys, brain, and hearts [36]. The significant decrease observed in the activities of ALT and AST in garri-fed animals when compared to the control groups showed that garri samples caused no organ damage. However, no significant difference was observed when the activities of ALT and AST in animals fed with garri processed by traditional method were compared with those in animals fed with garri processed by instant mechanical method.

Alkaline phosphatase (ALP) is involved in the hydrolysis of a wide range of phosphomonoester substrates. Significant alterations of ALP activity associated with sublethal long term cyanide exposure have been documented in hepatic and renal tissues of rabbits [37,38]. Indeed, Okolie and Osagie [37], reported a significant decrease in ALP activity in the lungs of rabbits subsequent to chronic exposure to cyanide and suggested the existence of variabilities in tissue susceptibilities to the toxic effect of chronic cyanide exposure.

As noticed above, some variations exist in the literature concerning the effects of cyanide poisoning on the tissue enzyme profile that might be associated to diversities in toxicokinetic parameters of cyanide compounds in various species, utilized dose, route and timing of exposure, tissue susceptibilities, experimental situations and procedures or other unknown factors.

In this study, a significant increase in the activity of ALP was observed in animals fed with garri samples when compared to the control group at $p<0.05$. Similarly, a significant increase was observed when the activity of ALP in rats fed with garri processed by instant mechanical method was compared with those fed with garri processed by traditional method. This is in agreement with the work of Kadiri and Asagba [31], who reported a significant increase in the activity of ALP when animals were exposed to cyanide directly above 2mg and food contaminated with less than 2 mg cyanide respectively. ALP is a marker enzyme for the plasma membrane and endoplasmic reticulum of the tissues [39]. It is often employed to assess the integrity of the plasma membrane, since it is localized predominantly in the microvilli in the bile canaliculli, located in the plasma membrane. Since ALP hydrolyses phosphate monoesters, its significant increase in animals fed with garri processed by instant mechanical method could constitute a threat to the life of the cells that are dependent on a variety of phosphate esters for their vital process as it may lead to indiscriminate hydrolysis of phosphate ester metabolite of the liver [40]. Consequently this may adversely affect the facilitation of the transfer of metabolites across the cell membrane of animals fed with garri processed by instant mechanical method.

The increase in the activity of ALP in animals fed with garri processed by instant mechanical method when compared with those in animals fed with garri processed by traditional method could result from the high concentration of cyanogenic glycosides, phenolic compounds (Tannin) in garri processed by instant mechanical method reported by Airaodion et al. [30]. These substances cause lipid peroxidation and oxidative stress in most tissues. These anti-nutrients are characterized by their diverse toxic effects [41,42]. This study corresponds with work done by Eze *et al.* [33], in which there was a significant increase in the activity of ALP, when rats were fed with chloroform and methanol extracts of garri.

Yohei *et al.* [43], reported that the relationship between high renal restitive index (RI) and cardiovascular and renal outcomes is significant and persisted after multivariate Cox regression analysis, including traditional risk factors. The serum creatinine concentration is widely interpreted as a measure of the glomerular filtration rate (GFR) and it is used as an index of renal function in clinical practice [44]. Glomerular filtration of creatinine, however, is only one of the variables that determine its concentration in serum. Alterations in renal handling and metabolism of creatinine and methodological interferences in its measurement may have a profound impact on the serum concentration of creatinine metabolism and is constant among individuals and over time, with the creatinine production rate being equal to the renal excretion rate. In the theoretical situation where both criteria are satisfied, the serum creatinine is inversely proportional to the GFR, so that each halving of the GFR results in a doubling of the serum creatinine concentration [45]. Secretion of creatinine was observed even in early studies of the clearance of exogenously administered creatinine [45]. In 2003, Mandell *et al.* [44], reported that the exogenous creatinine clearance decreased as the concentration of creatinine in the blood was acutely increased10-fold by creatinine infusion. This decrease was thought to be due to saturation of the tubular secretory mechanism, because the inulin clearance was not affected by this exogenous increase of the creatinine concentration in the blood. Creatinine reabsorption during low rates of urine flow is thought to result from its passive back-diffusion from the lumen to the blood. Thus, when urine flow rate is very low, passive reabsorption of creatinine might result in a lower creatinine clearance and a higher concentration of serum creatinine than what one would expect solely on the basis of the GFR [44,46]. Dietary protein deficiency leads to negative nitrogen balance and loss of muscle mass, thereby decreasing creatinine production. Less severe alterations in the diet, however, also may have important effects on the size of the creatine pool and creatinine excretion, which are independent of nitrogen balance and muscle mass. In this study, a significant decrease observed in the serum creatinine concentration in experimental animals when compared with that of the control

animals might be as a result of poor protein content of garri samples.

Bilirubin is the breakdown product of heme moiety of hemeoglobin; other hemeoproteins include cytochromes, catalase, peroxidase, tryptophan pyrrolase and a small pool of free heme. Increase in concentration of direct reacting bilirubin in blood causes hyperbilirubinaemia, which is toxic under certain conditions inducing jaundice, hyperbilirubinemia-induced auditory dysfunction and neurotoxicity resulting in brain damage [47]. On the other hand, mild unconjugated hyperbilirubinaemia behaves as mild antioxidant and might offer protection against cardiovascular diseases and tumour development [48]. Recent research survey has reported that low concentration of direct reacting bilirubin induces stroke in body and sometimes causes cardiac problems too. Serum bilirubin levels are often enhanced under a variety of clinical conditions. In the circulation of blood, bilirubin is bound to serum albumin, which prevents its potential toxicity thought to be caused by free bilirubin [49]**.** Despite its high-affinity of binding to albumin, bilirubin is rapidly and selectively taken up by the liver, biotransformed upon conjugation with glucuronate, and secreted into bile [50]. Thus bilirubin is converted into bilirubin glucuronic acid in the liver and excreted along with bile.

Free bilirubin is the breakdown product of hemoglobin (Hb) of aged erythrocytes in the reticuloendothelial cells of the spleen. This free bilirubin is not bound to albumin and its toxic effect is believed to occur even at a concentration of 0.005 mg/dL. So far, no reliable method has been developed for measuring free bilirubin content in plasma (or alternatively for measuring the free binding capacity of albumin for free bilirubin). The free bilirubin bound to albumin is called unconjugated bilirubin. The splitting of heme ring at different positions (α, β, γ or δ) leads to the formation of its various isomers which cannot form hydrogen bonds, and are therefore more readily water-soluble and get excreted through the urine [51].

The free or unconjugated bilirubin bound by albumin is carried to liver, where it is conjugated with glucuronic acid by the enzyme glucuronyltransferase. The enzyme, glucuronyltransferase transforms the albumin-bound bilirubin to monoglucuronide or diglucuronide conjugated bilirubin urine [51].

Total bilirubin has been reported to be a potent physiologic antioxidant that may provide important protection against atherosclerosis, coronary artery, and inflammation [52], total serum bilirubin level concentrations is directly proportional to the protective factor high-density lipoprotein-cholesterol [53]. Decrease in total bilirubin and conjugated bilirubin level in the experimental animals when compared with that of the control group showed that there was no organ damage due to garri consumption.

The significant decrease in total protein concentrations in experimental groups treated albino rats is in agreement with the work of Sunmonu and Oloyede [54]. The significant reduction in total protein and serum albumin concentrations may be a consequence of poor diet or an indication of liver dysfunction amongst others. Thus, it is possible that the garri samples consumed by the rats which contain cyanide may affect the liver thereby preventing it from synthesizing enough total protein and albumins for release into the serum.

CONCLUSION AND RECOMMENDATION

In this study, both garri samples did not cause damage to the liver or kidney, but perturbations of biochemical parameters were observed. These perturbations were more severe in animals fed with garri processed by instant mechanical method. This could be attributed to the high cyanide content in it. Processors of garri should be enlightened on the dangers of garri high in cyanide to human health and should be encouraged to avoid short-cut practice in the production of garri. Garri should be allowed to ferment for at least 72 hours before roasting.

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