Effect of varied fermentation periods on the diabetogenic potential of toasted cassava granules

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Abstract. The effects of exclusive gari diets prepared using varied fermentation periods on the fasting blood glucose levels (FBGL) and glucose tolerance (GT) of rats were studied. Thirty growing male albino rats divided randomly into 5 groups of 6 rats each were used. The 5 groups were each fed gari diets fermented for 0, 24, 48, 72 hours; and a standard rat diet, respectively, for 8 weeks. Data on the total cyanogen content and % crude protein of the diets; FBGL, GT, body weight, water:feed consumption ratio and clinical observations of the rats were collected. Results showed that the total cyanogen content and % crude protein of the diets were depleted as fermentation periods increased. The FBGL of all the gari-fed rats were significantly elevated and their GT significantly impaired with a significant variation between the groups. The elevated FBGL and impaired GT which were found to increase with increase in fermentation period were strongly inversely correlated to the % crude protein content of the diets ($r = -0.92$ and -0.96 respectively) suggesting that the diabetogenic potential of gari diets strongly depended on its % crude protein content. Shortened fermentation periods leading to production of gari with high total cyanogen content did not induce higher diabetogenicity.

Key words: Cassava (gari), Diabetogenic potential, Fasting blood glucose levels, Glucose tolerance, Varied fermentation periods

Introduction

The tuberous roots of cassava (*Manihot esculenta* Crantz) are a very valuable source of food energy for hundreds of millions of people in the tropics especially in sub-saharan Africa where it ensures food security for large numbers of people living under unpredictable sociopolitical and ecological circumstances. These tubers can be traditionally processed into various forms, one of which is the toasted granules (gari).

The utilization of cassava and its processed products as food is constrained by its content of toxic principles mainly cyanogenic glucosides (linamarin and lotaustralin) [1, 2]; and its poor nutritional value – the protein content being poor in quality and low in quantity [3]. These limiting factors to the use of cassava as a food form the basis for associating cassava diets with

such diseases as endemic goiter, konzo, tropical ataxic neuropathy, tropical calcifying pancreatitis, pancreatic diabetes and malnutrition-related diabetes mellitus (MRDM) [4]. It is noteworthy that the outbreaks of these diseases have always occurred in populations which almost exclusively subsist on cassava diets and which, under conditions of socioeconomic deprivation such as is seen in food shortages, wars or poverty, resorted to short cuts in traditional processing methods [4].

Traditional processing of cassava to gari involves peeling of the washed tubers, grating them into a pulp, fermenting for at least 3 days, draining out water under physical pressure, and finally toasting. These processes reduce the content of toxic principles to levels safe for human consumption [5] and increase its shelf life. The increasing demand for gari and the recent widespread mechanization of some aspects of the processing such as grating, milling and draining [1] have led to drastic reductions in the fermentation period during processing such that the entire processing could be completed within 5 hours in contrast to the traditional minimum of about 72 hours. Considering the vital role of fermentation in cyanogen reduction during processing [6], it is speculated that these shortened fermentation periods may have a role to play in the increasing occurrence of diabetes mellitus which is fast reaching an epidemic proportion especially in developing countries [7].

Malnutrition-related diabetes mellitus (MRDM) or tropical diabetes has been associated with 'malnutrition alone or in combination with cassava consumption. . .' [8]. The pathogenesis had been hypothesized to be due to the toxic effects of cyanide on the pancreatic islets and the unavailability of amino acids required for detoxification which causes its accumulation and aggravation of toxicity [9]. Though the World Health Organization states that the frequency of MRDM in the general population is unknown [10] and that there is, at present, no reliable data on the prevalence of diabetes mellitus in Nigeria, the disease remains the most common endocrine disease seen in hospitals in tropical Africa [11] and hospital records show a yearly increase in the number of newly diagnosed cases [12].

This study was therefore designed to investigate the effects of feeding rats gari produced at varied fermentation periods on the diagnostic indices of diabetogenicity – fasting blood glucose levels (FBGL) and glucose tolerance (GT).

Materials and methods

Experimental design.   Thirty albino rats of the Sprague-Dawley strain were used for the study. They were divided into 5 groups each of which was fed a specified diet for the 8-week experimental period. The total cyanogen content

and % crude protein of these diets were determined. Reference values of the parameters assessed for in the rats were collected in duplicates during a two-week pre-experimental period and, thereafter, all through the experimental period. Parameters assessed included fasting blood glucose levels (FBGL), glucose tolerance (GT) (2-hour post prandial blood glucose levels), body weight, water:feed consumption ratio and observable clinical signs of disease.

Experimental animals. Thirty growing male Sprague-Dawley rats, 10–14 weeks of age, were bred at the Faculty of Veterinary Medicine Laboratory Animal House, University of Nigeria, Nsukka. These were randomly divided into 5 groups of 6 each and kept in neatly bedded separate cages.

Experimental diets

Group 1 diet – Gari toasted without fermentation

Group 2 diet – Gari toasted after 24 hours of fermentation

Group 3 diet – Gari toasted after 48 hours of fermentation

Group 4 diet – Gari toasted after 72 hours of fermentation

Group 5 diet – Standard growing rat feeding (supplied by Bendel Feeds and Flour Mills PLC, Nigeria)

To each of the gari diets was added 0.5% by weight of iodized table salt (Union Dicon Salt PLC, Lagos, Nigeria).

Analytical methods. The total cyanogen content of the gari diets was determined by the alkaline titration method specified by the Association of Official Analytical Chemists [13]; the results were converted to the standard mg HCN equivalent per kg dry weight of sample. The % crude protein was determined by the Kjeldahl method of the AOAC [13]. The FBGL of the rats was determined at two-week intervals using the modified 0-Toluidine method [14] after a 16 hour fast of the rats. The absorbance of each sample was compared to a standard using an SP-8 ultraviolet spectrophotometer (Pye Unicam) at 630 nm wavelength. The results were converted to the standard mmol per liter. The GT was assessed at four-weekly intervals as the 2-hour post prandial blood glucose level [10] after a 0.3 g/100 g body weight oral glucose challenge (administered using a graduated plastic pipette) following a 16 hour fast [14]. This was also converted to the standard mmol per liter. The body weights of the rats were measured at two-weekly intervals using a 5 kg capacity balance (Ohaus Scale Co, NJ). The water:feed consumption ratio was obtained from measurements of 24 hour water and feed consumption monitored at two weekly intervals. The rats were carefully observed daily all through the experimental period and obvious clinical signs were noted and recorded.

Table 1. Fermentation periods, total cyanogen content and percentage crude protein of the experimental diets

	Group 1 gari diet	Group 2 gari diet	Group 3 gari diet	Group 4 gari diet	Group 5 control diet[a]
Fermentation period (hours)	0	24	48	72	NA[b]
Total cyanogen content (mg HCN equiv./kg)	6.70	4.75	3.59	3.00	NA[b]
Percentage crude protein (%)	1.09	0.89	0.76	0.66	14.50

[a] Group 5 control diet was standard rat diet supplied by Bendel Feeds and Flour Mills PLC, Nigeria. It was not used in computing the correlation coefficient.
[b] Fermentation periods and total cyanogen determination do not apply to the standard control diet.

Statistical analyses. Data collected are presented as means with standard deviations. Results obtained at the 8th week were statistically analysed for significant variations using the one way analysis of variance for multiple samples [15]. A 'before and after' t-test [15] was used to compare data obtained during the pre-experimental period with that obtained at the 8th week for statistically significant differences. The degree of association between related variables was sought following the Pearson product moment correlation using the raw score formula [15]. The correlation coefficient (r) was accepted as significant at 5% probability.

Results and discussion

The total cyanogen determination of the gari diets showed a gradual loss of cyanogens with increase in fermentation period (Table 1). About 55% of the total cyanogens in the unfermented gari were lost after 3 days of fermentation. This finding confirms the relevance of fermentation as one of the most important steps in the detoxification of cassava during processing. A 3-day fermentation brings to completion the two-stage fermentation postulated by Collard and Levi [16] leading to hydrolysis of cyanogenic glucosides and subsequent liberation of HCN. Fermentation thus provides the conducive microbial environment which aids the spontaneous pH-dependent decomposition of cyanohydrins with liberation of HCN [17].

It was also found that increased fermentation periods led to a depletion of the meager protein content of the gari diets with loss of about 0.43% out of 1.09% contained in the unfermented gari after 3 days of fermentation
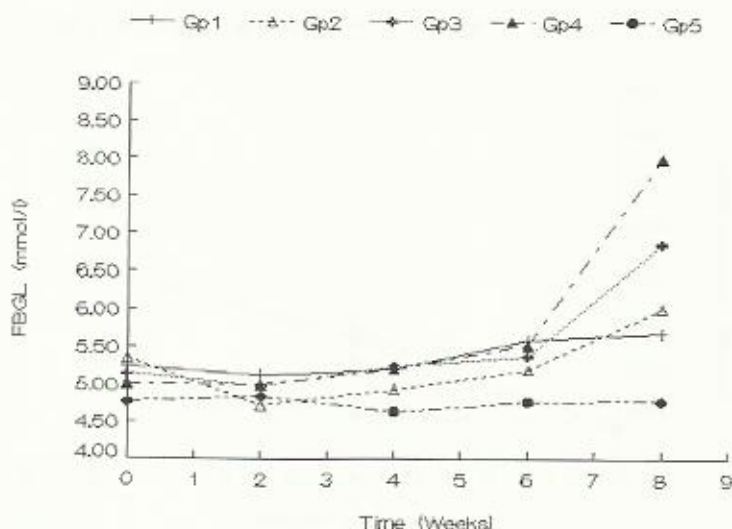
Figure 1. Mean fasting blood glucose levels (FBGL) of rats fed control and gari diet of varied fermentation periods. Gp1 – Unfermented gari diet; Gp2 – 24 hr fermented gari diet; Gp3 – 48 hr fermented gari diet; Gp4 – 72 hr fermented gari diet; Gp5 – Standard growing rat diet.

(Table 1). This confirms the speculations of Silano et al. [18] who observed that about 50% of the nitrogen content of cassava is in the form of free amino acids which they indicated may be lost during processing. Further processing was thus found to aggravate the already poor nutritional value of the gari diets.

The mean FBGL (mmol/l) of all the gari-fed rats showed a slight fall in week 2, then rose in week 4 and continued till the end of the study (Figure 1). From the 6th week, there were variations between the groups, but a 'one way analysis of variance' of the results at the 8th week did not find these variations statistically significant ($p < 0.05$). The mean GT measured as the 2-hour post prandial blood glucose levels (mmol/l) showed that there was impaired glucose tolerance from week 4 till the end of the study (Figure 2). A t-test of the difference between the GT of individual groups at week 0 and week 8 showed that the GT of all the gari-fed rats were significantly ($p < 0.01$) impaired. Also a 'one way analysis' of the variations observed between the groups at week 8 showed that these variations were statistically significant ($p < 0.01$). Thus the significant impairment of the GT of the gari-fed rats varied with the fermentation period of the gari diet; the longer the fermentation period, the more severe was the impairment.

The findings of elevated FBGL and signficantly impaired GT in the gari-fed rats confirmed that exclusive gari-feeding of rats is diabetogenic. This is believed to be due to hypoinsulinemia consequent upon exhaustion of the
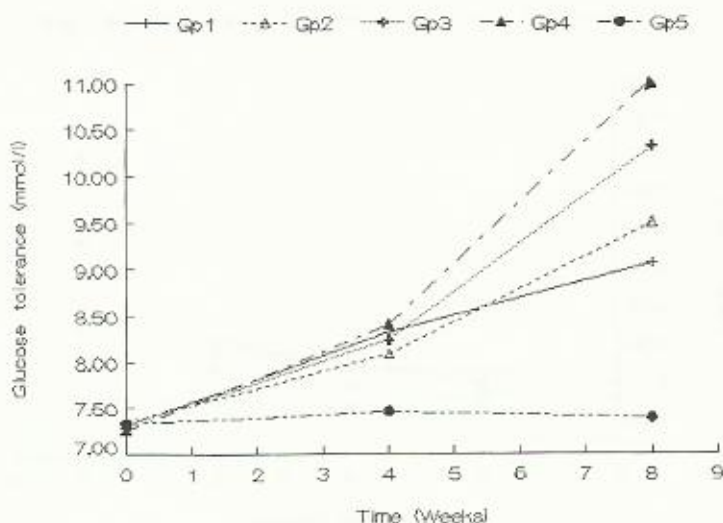
Figure 2. Mean glucose tolerance (2 hour post prandial blood glucose level) of rats fed control and gari diet of varied fermentation periods. Gp1 – Unfermented gari diet; Gp2 – 24 hr fermented gari diet; Gp3 – 48 hr fermented gari diet; Gp4 – 72 hr fermented gari diet; Gp5 – Standard growing rat diet.

beta cells of the pancreas due to prolonged elevation of blood glucose levels by excessive indulgence in carbohydrate diets [19]. It agrees with the findings of impaired GT in rats fed cassava based diets by Akanji [9], and 'increased disposition of rats fed 80% gari to the development of diabetes' by Echeta [20]. It further lends weight to the epidemiological findings of increased occurrence of pancreatic diabetes in indigenes of Kerala state of India who almost exclusively depend on their cassava staple-tapioca [21], and increased prevalence of diabetes in Kalene Hills of Zambia in contrast to adjoining areas because of their high level consumption of cassava products [22].

A strong inverse correlation was found between the % crude protein of the diets and the variations in the FBGL/GT between the groups at week 8 ($r = -0.92$ and -0.96, respectively). The relationship is presented graphically in Figures 3 and 4. Thus the depletion of the protein content of the diets associated with increased fermentation periods led to significant differences in the level of impairment of GT and elevation of FBGL. These differences in FBGL and GT between the groups were not found to be related to the total cyanogen content. This finding emphasizes the importance of proteins as 'molecules of the first rank' in all biological processes, and cassava diets are grossly deficient in proteins. The finding also strengthens the suggestion that the increased disposition of rats fed 80% gari diets to the development of tropical diabetes was a metabolic response to high carbohydrate:protein ratio
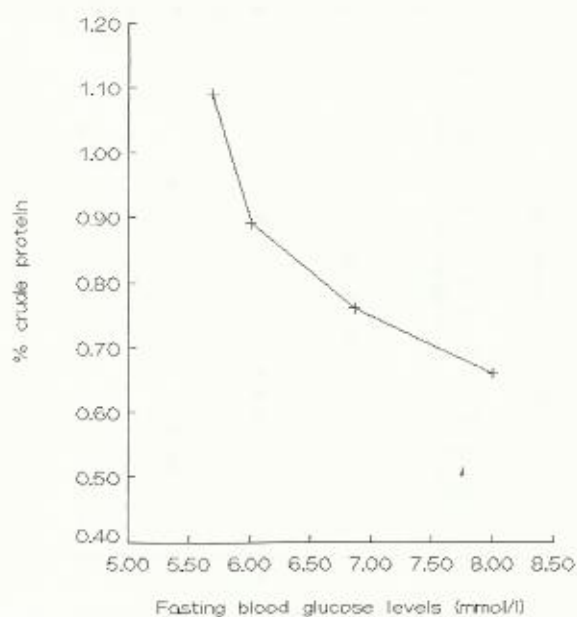
Figure 3. Relationship between the mean fasting blood glucose level of rats at the end of the study and the percentage crude protein content of their gari diets.
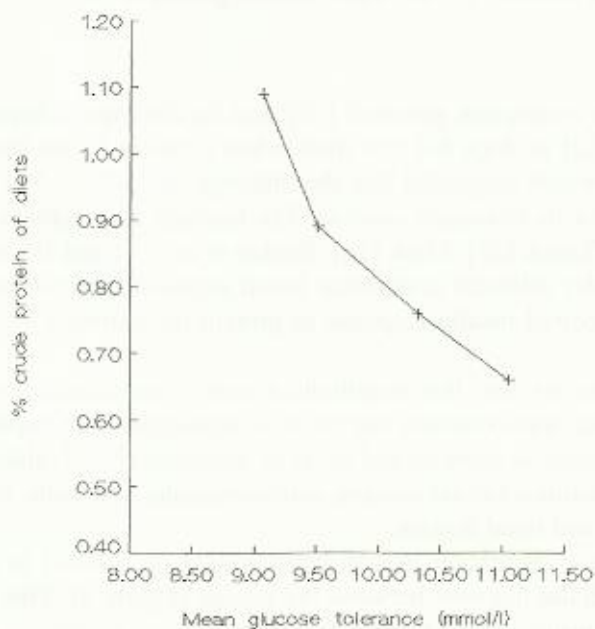

Figure 4. Relationship between the glucose tolerance (2 hr post prandial blood glucose level) of rats at the end of the study and the percentage crude protein of their gari diets.
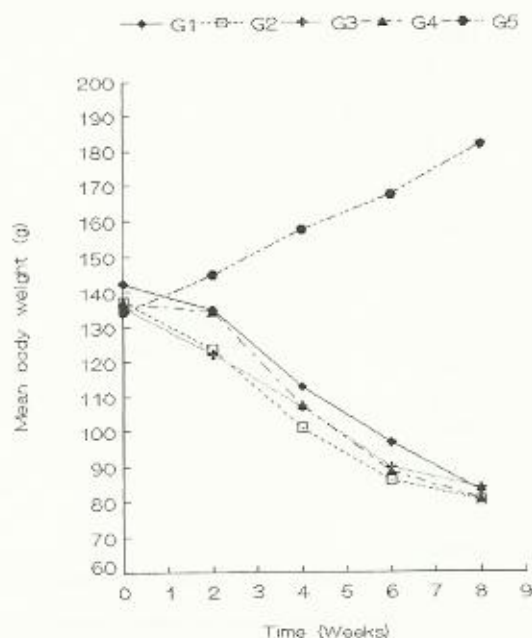
Figure 5. Mean body weight of rats fed control and gari diets of varied fermentation periods. Gp1 – Unfermented gari diet; Gp2 – 24 hr fermented gari diet; Gp3 – 48 hr fermented gari diet; Gp4 – 72 hr fermented gari diet; Gp5 – Standard growing rat diet.

rather than its cyanogenic potential [20]; and the findings of hypoinsulinemia by Kamalu [23] in dogs fed gari diets when compared with those fed rice-cyanide diet which suggested that the diabetogenic potential of gari diets is independent of its cyanogen content. The findings also agree with those of Kajubi [24], Yajnik [25], Cook [26], Becker et al. [27] and James and Coore [28] who under different conditions found impaired glucose tolerance and persistent impaired insulin response in protein malnutrition associated with kwashiokor.

It is worthy to note that longitudinal studies coordinated by the WHO [7] suggest that approximately one third of individuals with impaired glucose tolerance progress to diabetes and are at an increased risk of related complications such as cardiovascular disease, cerebrovascular accidents, retinopathies, neuropathies and renal disease.

There was a significant loss in body weight ($p < 0.01$) in all the gari-fed rats which did not vary between the groups (Figure 5). This is typical of protein malnutrition characterized by growth failure, marasmus and wasting of the body tissues due to catabolism of tissue proteins to supply the body's basal metabolic needs [29].

The water:feed consumption ratio at week 8 was found to be reasonably higher in the gari fed groups than in the control group being highest in the group 2 rats (4.00) and lowest in the group 4 rats (2.12) compared to the control group (1.82). This pattern was found to concur with the severity of the clinical signs of disease observed in the gari-fed rats. The signs observed included irritation, alopecia, loss of appetite, loss of body condition, huddling together, general weakness, hunching of the back and mortality. These were attributed partly to chronic cyanide toxicity and partly to protein malnutrition. On rating these signs based on onset and severity they were found to be most severe in group 2 rats fed the 24-hour fermented gari. This is understandable because fermentation studies [16] reveal that about the 24th hour of fermentation most of the cyanogens are in the most toxicologically active and readily available form (cyanohydrins and free HCN).

In conclusion, the study showed that shortened fermentation periods lead to production of gari with relatively high cyanogen content which did not translate to increased diabetogenicity in rats, rather the depletion of its protein content associated with increased fermentation periods led to significantly increased diabetogenicity. The diabetogenic potential of gari diets in albino rats was found to be dependent on the protein content of the diets. Considering the vital role that fermentation plays in cyanogen reduction and the relatively negligible low quality protein content of cassava, it is recommended that cassava mash be fermented for at least 3 days in the production of gari, and supplemented with proteins from other sources during consumption. Further work is proposed to determine the level of protein supplementation of gari diets that may overcome its diabetogenicity.

Acknowledgments

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