Nutritional Evaluation of Gari Diets from Varying Fermentation Time Using Animal Model

C.I. Owuamanam[1] , A.D. Hart[2] and I.S. Barimalaa[2], L.I. Barber [2] and S.C. Achinewhu[2]

1. Department of Food Science and Technology Federal University of Technology,   PMB 1526, Owerri, Nigeria.
2. Department of Food Science and Technology River State University of Science and Technology, Port Harcourt, Nigeria.
e-mail icliffowums@yahoo.com; achinewhu@yahoo.com

Abstract: The implications of consumption of dietary cyanide from gari were investigated by feeding trials on experimental rats (*Rattus norwagicus)* in this study. The biological assay technique was used to assess diets formulated from gari (fermented cassava product) processed for zero, 24, 48, 72h and control (corn starch) which contain varying amounts of residual cyanide (ranging from 111.6 – 18.6 mg HCN/kg). The diets were balanced in minerals, vitamins and casein. The only source of variability among the diets was the levels of residual dietary cyanide as affected by the duration of fermentation. Weaning male albino rats (21 day old) were grouped in tens (10 rats per group) and fed the diets. The feeding trials was carried out for twenty eight (28) days and thereafter repeated for another set of rats giving a total of 100 rats. The results show that average weight gain, feed intake, feed conversion ratio and protein efficiency ratio (PER) differed significantly ($P > 0.05$). The highest PER, 0.22 was obtained from rats on the control diet while the least was obtained for gari diet from 24h fermented product, 0.15. The red blood cell count (RBC), packed cell column (PCV) differed significantly, $P > 0.05$. The poor performance of rats on 24h diet might be associated with presence of high level of cyanogenic glucoside intermediates – cynohydrin, which readily interacts with β-glucosidase in the gut and triggers wide range biological effects. Hence fermenting for periods beyond 24h makes garri safe for consumption. [Researcher. 2010;2(8):1-10]. (ISSN: 1553-9865).

Key words: Dietary cyanide; gari; Feed conversion ratio; PER; Blood parameters.

1.0 Introduction

Gari is a gritty, starchy staple with high energy content which is derived from cassava (*Manihot esculenta Cranz*) (Ernesto et al., 2000). It is a convenient product because it is stored and marketed in a ready-to-eat form; and can be prepared with hot and cold water depending on the type of meal (Nweke *et al*., 2002; Adindu and Aprioku, 2006). Gari is the most common form in which cassava is sold in Nigeria and many other African countries (Ngoddy, 1977; Oluwole *et al*., 2004). However the traditional methods of processing cassava into gari have been found to be deficient in reducing the amount of residual cyanide in the product (Achinewhu et al., 1998; Achinewhu and Owuamanam, 2001). There have been many reported cases of acute and chronic conditions associated with consumption of cassava derived meals (Osuntokun, 1970; Osuntokun, 1973). There are as well few reported cases of death linked to consumption of cassava meals (Akintonwa, 1994). According to Rosling (1989), 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. Currently the populations of Africans in dare need of gari (the most stable cassava product) is critical and some processors (mainly rural women) nowadays adopt short cuts so as to take the advantage of market demand and make quick profit. The extent to which such harmful practices leave high levels of cyanide in diets and the attendant consequence of nutrients from gari diets in supporting growth in weaning rats is hereby investigated.

2.0 Materials and Methods

The test diets for the study were formulated in accordance with the AOAC (1990) procedure for determination of protein efficiency ratio (PER) as presented in Table 1. Fifty, 21-day-old weaning male albino rats were used for the study. Cassava roots were peeled washed, grated, sifted and roasted to produce gari for zero hour that is without fermentation. And thereafter, the cassava mash was fermented for 24h, 48h, and 72h, and roasted into gari. These samples and the control (corn starch) were used to formulate the five diets that were used for the feeding trials. The diets were each fed to a group of ten albino rats (*Rattus nowegicus*) which fitted into experimental arrangement of 10 albino rats x 5 diets (control, 0h, 24h, 48h and 72h) randomized design. The rats were fed *ad libitum* with constant

introduction of fresh diets and drinkable water every day, the rats were individually housed in galvanized steel cages and the study lasted for 28 days for a run. The feeding trial was replicated twice which involved a total of 100 rats.

Table 1 composition of basal diet for protein efficiency ratio (PER) study ingredients and amounts

INGREDIENT	Quantity
Protein	10
Vegetable Oil	5
Salt mixture [a]	5
Vitamin mix.	1
Non nutritive fibre (cellulose)	1
Corn starch or gari	(to make up 100)

a&b: prepared by F. Hoffman-La Roche and Company Ag. Switzerland based on formulation for Laboratory animals. a: Salt mixture composition (content/kg), calcium 6g; chloride 5g; copper 10 mg; iodine 0.2 mg; iron 100 mg; magnesium 2.0 g; manganese 75 mg; phosphorous 5g; potassium 5.0 g; sodium 5.0g; zinc 18 mg. b: Vitamin mixture contained international units, iu or mg or μg per kg of diet): vitamin A 7,000 (I.U); vitamin D300 (I.U); vitamin E 60 I.U; vitamin K 2.9 mg; Thiamin HCl 4.0 mg; Riboflavin 5 mg; pyridoxine HCl 6 mg; Niacin 10 mg; pantothenic acid 12.0 mg; cyanocobalamin (B12) 5.0 μg.

The rats were acclimatized for 5 days before taking the initial weight for the PER study. The digestibility study was commenced on the 14th day of the PER and lasted for 7 days. The average feed intake, average weight gain/loss, feed efficiency ratio and protein efficiency ratio (PER) were determined for 28 days study.

2.1 Proximate Composition the Diets
The proximate composition of the diet (crude protein, fat, crude fiber, ash and carbohydrate) was determined following the method of AOAC (1990).

2.2 Dietary Cyanide Content
The method of Esser et al. (1993) for determination of cyanide was followed.

(i)Preparation of KCN standard
A stock solution was prepared by dissolving 50 mg of KCN in 0.2 M NaOH. The stock solution was diluted 1:50 with 0.2 M NaOH. The automatic pipette was used to pipette into marked tubes: 0.025, 0.050, 0.075 and 0.100 ml of the diluted KCN stock, the volume was made up to 0.100 ml corresponding to 5, 10, 15 and 20 μg/ml with 0.2 M NaOH. 0.5 ml of 0.1 m phosphate buffer pH 6.0 was added followed by addition of 0.6 ml, chloramines –T and 0.8 ml of the colour reagent. The absorbance reading was obtained using (visible) spectrophotometer against blank at 605 nm wavelength.

(ii) Determination of cyanide in gari
Thirty (5.0 g) grammes of gari was milled and homogenized with 50 ml of 0.1M orthophosphoric acid. The homogenate was centrifuged. The supernatant was taken as the extract; 0.1 ml of the enzyme was added into 0.6 ml of the extract. 3.4 ml of the acetate buffer (pH 4.5) was added and stirred to mix. After which 0.2 ml of 0.5% chloramin-T and 0.6 ml of colour reagent were added and allowed to stand for 15 min. for colour development. The absorbance value was obtained at 605nm against a blank similarly prepared containing all reagents and 0.1 ml phosphate buffer added instead of KCN.

(iii) Calculation
The data from the standard were used to obtain a standard curve and its slope (b) by plotting absorbance values (Y-axis) against standard concentrations (X-axis). The unknown mean absorbance (A) and the weight of the sample (w) were used to calculate the residual cyanide, using the formula:

$$\text{Residual cyanide} = \frac{A \times 250 \times 0.4151}{b \times w}$$

the unit in mg HCN equivalent per kg sample.

2.3 Energy determination
The gross energy of the diets was calculated based on the Atwater factors in which the proximate composition and the conversion factors (protein, 4.0; carbohydrates 3.74; fat/oil, 9.0 and alcohol 7.0) were used. All the diets were approximately isocaloric by composition.

2.4 Feed intake
The daily feed intake (g/day) of the rats was determined for 7 days digestibility studies by subtracting the remainder ration from the initial for each of rats in a group and divided for the number of days to obtain the average feed intake.

2.5 Weight gain/loss
The daily average weight gain/loss of the rats was determined with a weighing balance during the 7 days digestibility studies. The daily measurement was

subtracted from the initial weight average for rats in a group and divided by the number of days. Weekly average weight gain/loss was similarly determined.

2.6 Feed conversion ratio
 The feed conversion ratio was determined by dividing the weight gain/loss by feed intake

2.7 Protein efficiency ratio (PER). PER was determined by dividing weight gain of the rats by total protein intake which is expressed by

$$\frac{\text{Final body wt} - \text{Initial body wt}}{(\text{Total dietary intake x 10\% protein composition})}$$

2.8 Hematological
 At the end of the feeding trials for 28 days, two of the rats were selected at random from each group and sacrificed by decapitation following the method of Obatomi *et al*. (1991). The blood was collected for analysis. 3.0 ml of the blood samples was put in EDTA treated bijou bottles. These were used to determine the packed cell volume (PCV), hemoglobin (Hb) count, erythrocyte sedimentation rate (ESR), white blood cell (WBC) count.

(i) Packed cell volume (PCV).
 A capillary tube was filled with the blood sample and one end was sealed with plasticine. This was placed in the haematocrit centrifuge and the cover was safely screwed. The lid was then closed and the centrifuged at 10,000 rpm for 5 min. The capillary tube containing the sample was brought out and placed in the groove of the reader. The PCV was then read off on the scale.

(ii) Erythrocyte sedimentation rate (ESR)
 The Westergren tube method was used. The blood sample was diluted by putting 0.5 ml of sodium citrate into 2 ml of the blood. The diluted blood was pipetted up to the zero mark of the Westergreen tube and placed firmly in the stand in an upright position. It was allowed to stand for one hour and the level of red cells read off.

(iii) Haemoglobin estimation
 The blood sample was diluted 1:200 (i.e. 0.02 ml) of blood to 4.0 ml of Arabkin solution in a test tube. This was allowed to stand for 10 min. The percent absorption was measured with a spectrophotometer at 520 nm (green filter) using a tube of Arabkin solution as blank. The percent absorption of a standard was also obtained at 520 nm. The haemoglobin was estimated using the calculation below.

$$\frac{\text{Hb of standard x OD of test}}{\text{OD of standard}} = \text{Hb of test}$$

(iv) Mean corpuscular hemoglobin (MCH).
 The mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) all derivatives of blood parameters were calculated as follows:

$$\text{MCH:} = \frac{\text{Hb in g\%} \times 100 \text{ (unit picogramme)}}{\text{No: of RBC}}$$

(v) Mean corpuscular hemoglobin concentration

$$\textbf{MCHC:} = \frac{\text{Hb in g \%} \times 100 \text{ (unit in \%)}}{\text{PCV}}$$

(vi) Mean corpuscular volume (MCV)

$$\textbf{MCV:} = \frac{\text{PCV} \times 10}{\text{No of RBC (unit in cubic microns)}}$$

(vii) White blood cell (WBC) total count
 The Wintrobe's method was used. The blood was diluted at a ratio 1:20 to give 0.02 ml of WBC diluting fluid. The counting chamber was initially cleaned and prepared by fixing the cover slip appropriately. A Pasteur pipette was used to draw the diluted blood and transfer it to the counting chamber. A low power objective (x 10) was used to count the cells in the 4 corner squares (each corner square contains sixteen squares) systematically.
 The WBC total count is then determined thus; for 1:20 dilution, 1 mm^3 of blood contains N x 2/1 x 20 cells.
 N = number of cells counted

(viii) White blood cell (WBC) differential count
 A thin blood film was made on a slide. The film was allowed to dry and was stained with Leishman's stain. This was allowed to drain and dry, and then examined with oil immersion objective lens. The different white blood cells were counted by examining the slide systematically. Counting was stopped after about 200 cells had been counted. The number of each type of cell counted was expressed as a percent of the total number of cells counted.

(ix) Red blood cell (RBC) count
 The sample was diluted 1:200 (i.e. 0.02 ml of blood in 4.0 ml of diluting fluid). The counting chamber was cleaned properly and cover slip placed properly in position. The diluted blood was mixed and a Pasteur pipette used to collect the diluted

blood. Holding the pipette in the 45° position, the chamber was charged. This was allowed to stand for 5 min. The chamber was then placed on the microscope stage and the red cells within the calibrated squares counted systematically using low power (x 10) objective.

The number of red blood cells was calculated as expressed below:

N = number of cells counted; A = area of small square = 1/400 mm^2; D = diameter of chamber = 1/10 mm; V = volume of fluid in the small square= 1/400 x 1/10 = 1/4000 mm^3; the volume of fluid in 80 small squares = 8/4000 mm^3

∴ 1 mm3 of diluted blood contained N x 4000/80 cells

Since dilution was 1:200,

Therefore: 1 mm^3 of blood contained N x 4000/80 x 2000= N x 10,000 cells.

2.9 Statistical Analysis

The data obtained from the study were subjected to analysis of variance (ANOVA) procedure of Steel and Torrie (1980) and the Fisher's least significant difference (LSD) used to separate significantly differing means as described by Roessler (1984).

3.0 Results
3.1 Proximate composition of diets for the per study

The proximate compositions of the formulated diets are shown in Table 2. The protein content of the control diet (29.15%) did not differ significantly (p> 0.05) from the rest of the diets. The highest mean ether extract (18.10%) was scored from Diet C, followed by diet D (18.10%) while diet E had the least 17.65%. There were no significant differences among the mean values of the fiber contents of all the diets. Diet B had the highest fiber (4.51%) while diet D had lowest 3.89%.The energy values as calculated for the Isocaloric diets averaged 1826.04 Kcal kg-1).

The feed intakes (g) per day of the albino rats are as shown in Figure 2. The rats administered diet A (control) maintained the highest feed intake per day and followed by diet E while the least feed intake (g) per day according to the trend is diet B The weight gain/loss per day of the rats is shown in Figure 1. The rats fed control diet A (corn starch) had the best performance 43 (g) for the 7th day of the PER study, which is followed by rats fed diet E (containing 72 h fermented gari) as shown by the trend in Figure 1. The least weight gain was recorded by the rats fed diet C (formulated with 24h gari).

The weekly weight gain/loss of rats fed various diets is shown in Figure 3. The rats fed gari free diet (control) had the highest weekly weight gain, averaging 63 g at the 4th week. The rats fed diet formulated 72h fermented gari component averaged at 36 g, the least weight gain was recorded for rats fed diet C (24 h fermented gari). The trends in figure 3 show growth response for the rats fed varied diets.

3.2 Residual cyanide content

The residual cyanide levels of gari used for the formulation of diets for the PER study is shown in Table 2. The zero hour fermented gari had the highest mean residual cyanide, 111.5 mg HCN/Kg, followed by 24h, 60.5 mg HCN/Kg while the 72h had the least, 18.6 mg HCN/Kg. These values differed significantly (p< 0.05) under the condition of study.

3.3 Hematological parameters of albino rats fed diets containing fermented gari and control (corn starch)

The result of hematological parameters of albino rats fed varying diet of gari is shown in Table 3.The red blood cell count (RBC) for rats fed diets A, B, C, D and E differed significantly (p< 0.05) among the groups. Rats diet A had the highest mean RBC count (3.6×10^6 /mm^3), which did not differ with the value obtained for diet E (3.4×10^6 /mm^3). Similarly the mean RBC obtained for diets B, C and D did not differ significantly at p> 0.05.

The highest packed cell volume (PCV) was obtained for diet B (unfermented gari) (37%) and followed by diet C (24 h fermented gari) (34%). The PCV for diets A, C, D and E did not differ at p > 0.05. The hemoglobin concentration (HB) of the rats differed significantly p< 0.05 among the groups with rats fed diet A having the highest, HB (10.4%), followed by diet E (9.6%) while diet B had the least 9.3%).

The mean corpuscular hemoglobin concentration (MCHC) of the rats administered diet B was low 28.1%, followed diet C, 28.5% while the control had highest, 30.5%. However, the MCHC of diet D and E were 30.1%and 31.1% respectively, and did not differ from the value obtained for the control at p< 0.05.

The mean corpuscular hemoglobin (MCH) showed that the control, diet A had the highest value 3,00pg, followed by diet E (2.94pg) while diet C had the least 2.76 pg. Similarly the control, diet A had the highest mean corpuscular volume (MCV) 10.30%, while diet C had the least 9.79%. However the highest white blood cell (WBC) count 6.6 $\times 10^3$/mm^3 was obtained for diets B and C respectively while the least WBC, 4.7 $\times 10^3$ /mm^3 was obtained for diet A. The erythrocyte sedimentation rate (ESR) of the blood of rats administered diet B (unfermented gari) was lowest, 2.0 mm/h while the control had the

highest, 5.5mm/h. The highest neutrophiles, were recorded for the blood samples of rats fed diet C (61%) and followed by diet D (60) while diet B had the least 54%. The eosinophiles occurred at very low percent 1.0%) for the control sample and the unfermented gari diet while diet C had zero%. Similarly the percent lymphocytes obtained from the blood of the rats were low and rats on diet C had the highest 2.0%. On the other hand, the basophiles obtained for all the blood samples were substantial. The blood sample from the rat group fed diet B had highest mean basophiles, 44%, followed by the control diet, 40%.while diet C had the least mean value of 37%.
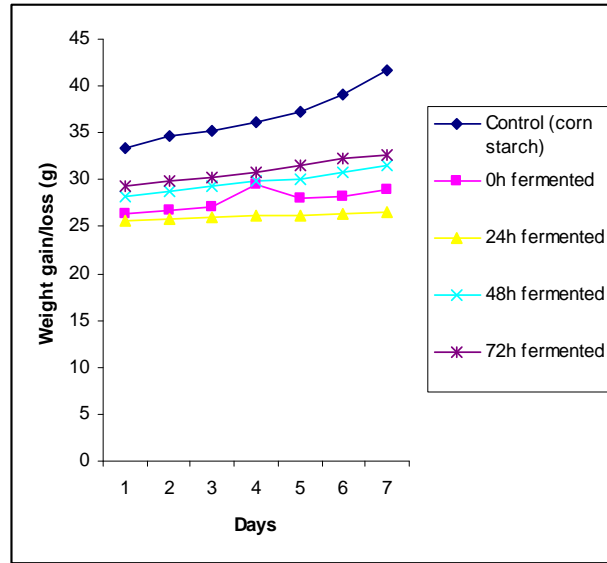

Figure 1 daily weight gain/loss of rats fed gari diets containing varying levels of dietary cyanide
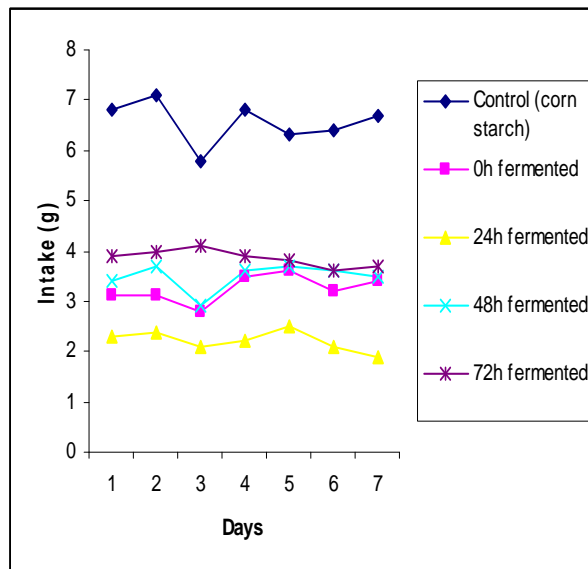

Figure 2. Feed intake of rats fed gari containing varying levels of dietary cyanide
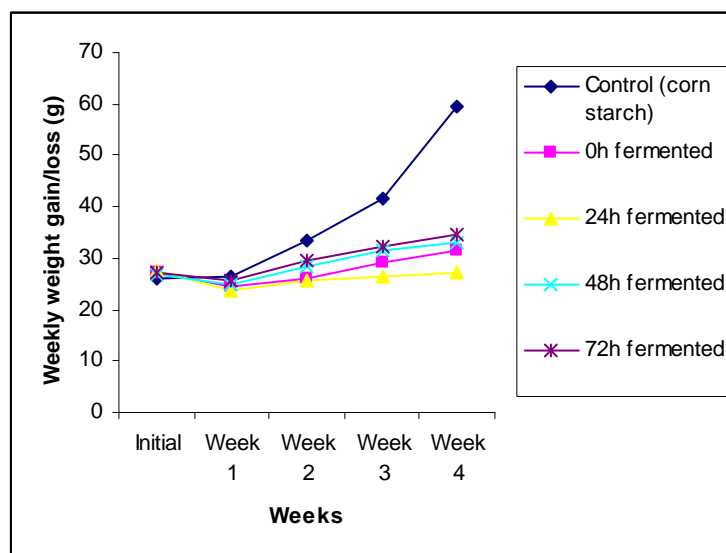
Figure 3 weekly mean weight gain/loss of rats fed gari diets containing varying levels of dietary cyanide.

Table 2: Proximate composition of the isocaloric diets fed to rats

Parameter	Diet A	Diet B	Diet C	Diet D	Diet E
Moisture	3.03	10.25	9.45	10.5	10.00
Crude protein	34.15 ± 0.84	28.26 ± 0.80	28.85 ± 0.93	27.00 ± 1.00	26.90 ± 0.10
Ether extract	18.5 ± 0.52	16.54 ± 0.56	17.15 ± 0.44	18.10 ± 0.17	16.45 ± 1.10
Crude fibre	3.56 ± 1.42	4.51 ± 0.92	4.00 ± 0.11	3.89 ± 0.19	4.00 ± 0.09
Ash	2.50 ± 0.36	3.25 ± 0.39	3.36 ± 0.41	3.40 ± 0.21	3.45 ± 0.56
Carbohydrate	38.71	37.19	40.55	37.11	39.20
Gross energy (calculated) (Kcl kg^{-1})	1826.04	1826.04	1826.04	1826.04	1826.04
Residual cyanide mgHCN/kg	Nil	115.6 ± 8.14[a]	60.5 ± 1.01[b]	22.7 ± 0.32[c]	18.6 ± 1.07[d]

Mean are values of triplicate determinations ±SD.

Key

Diet A: Control (corn starch);

Diet B: Formulated with gari fermented for zero h;

Diet C: Gari fermented for 24 h;

Diet D: Gari fermented for 48 h

Diet E: Gari fermented for 72 h

**Table 3:	Data obtained during 28 days study on Albino rats administered various diets formulated with gari**

Parameter	Diet A	Diet B	Diet C	Diet D	Diet E	LSD
Feed conversion (wt gain/feed consumption)	0.52 ± 0.02^a	0.38 ± 0.02^d	0.18 ± 0.04^e	0.42 ± 0.1^b	0.39 ± 0.1^c	0.0912
Protein efficiency (PER)	0.22 ± 0.01^a	0.15 ± 0.01^c	0.07 ± 0.01^d	0.18 ± 0.01^b	0.16 ± 0.01^c	0.0182
Mortality	0	0	0	0	0	

Mean are values of triplicate determinations. Only means with different superscripts across the row are significantly different at $p<0.05$

Table 4: Hematological values of Albino rats fed diets consisting corn starch (control) and gari fermented for 0, 24, 48, and 72h

Parameters	Diet A (control)	Diet B (0 h)	Diet C (24 h)	Fermented Diet D (48 h)	Diet E (72 h)
Erythrocytes (RBC) x 10^6/mm^3)	3.6 ± 0.24^a	3.1 ± 0.16^b	3.2 ± 0.15^b	3.2 ± 0.48^b	3.4 ± 0.62^a
Packed cell volume (PCV)%	30.5 ± 1.32^b	37 ± 1.74^a	33 ± 1.24^b	31.5 ± 1.48^b	31.6 ± 1.13^b
Hemoglobin concentration (HBC) %	10.4 ± 1.16^a	9.3 ± 1.10^c	9.4 ± 1.06^c	9.5 ± 0.87^{bc}	9.6 ± 1.24^b
Erythrocytes sedimentation rate (ESR) mm/h	5.5 ± 0.30	2.0 ± 0.46	4.0 ± 0.16	4.0 ± 0.25	4.1 ± 0.37
Mean corpuscular hemoglobin concentration (MCHC) %	30.5 ± 1.24	28.1 ± 1.44	28.5 ± 1.09	30.0 ± 1.29	31.4 ± 1.68
Mean corpuscular hemoglobin (MCH) Pg	3.0 ± 0.81	2.87 ± 0.95	2.76 ± 0.24	2.89 ± 0.19	2.94 ± 0.34
Mean corpuscular volume (MCV) %	$10.30\ 1.04^a$	$9.84 \pm 0.89a$	9.71 ± 0.96^a	10.00 ± 1.23^a	10.14 ± 1.43^a
Leucocytes (WBC) x 10^3/mm^3	4.7 ± 1.06^a	6.6 ± 0.98^a	6.6 ± 0.83^a	6.2 ± 0.82^a	$5.4 \pm 1.02a$
Haterophiles (Neutrophiles) %	58 ± 3.00	54 ± 3.00	61 ± 2.80	60 ± 3.18	59 ± 2.77
Eosinophiles %	1.00	1.00	0	1.00	1.00
Lymphocytes %	1.00	1.00	2.00	1.00	1.00
Basophiles %	Nil	Nil	Nil	Nil	0.00
Monocytes %	40 ± 1.84	44 ± 1.24	37 ± 2.80	38 ± 2.40	39 ± 1.78

Mean are values of triplicate determinations. Only means with different superscripts across the row are significantly different at $p<0.05$

4.0 Discussion

The different levels of residual cyanide obtained from the gari for the formulation of diets for PER study typify amounts left in gari when the fermentation regime was limited to durations adopted for the study. The amounts of residual cyanide found in the products cut across harmful to innocuous levels, going by the classification of Bolhius (1954). According to Balagoplan *et al*., (1988), cassava diets and forage plants have accounted for many instances of cyanide poisoning in humans and animals. Cyanide is a well-known rapid acting poison; the ion is rapidly absorbed from the gastric intestinal tract Jackson (1994) hypothesized that long- term exposure to sub-clinical cyanide to humans might impair their biological fitness.

The protein content of the diets did not differ among the diets suggesting that any manifestation of disease condition arising from intake of the diets on biological fitness of the experimental rats would not be attributed to protein energy malnutrition (PEM) rather on the varying levels of anti nutritional factors in the diets. Moreover, the protein content of the diets was above the amount recommended as adequate for animal protein requirements (AOAC, 1990). Previous pathological studies on gari diets by Ihedioha and Chineme (2001) in which protein content of diets were

restricted resulted to nutritional conditions in the rats that resemble protein energy mal nutrition.

The fibre and fat contents of all the diets were above the minimum recommendation by (AOAC, 1990). The energy value of the diets approximate to 1826.04 kcal kg-1. The isocaloric diets provided energy for the animal during the feeding trial such that manifestation of malnourishment might be associated with the toxic principle in diets. The least daily feed intake (2.34g) per day of rats fed diet C may be due to presence of high levels of cyanogenic glucoside intermediate – cyanohydrins (Conn, 1994). It may as well be suggested that the cyanohydrins is the potent stage of cyanogenic glucoside and readily interacts with β-glucodidase in the gut releasing cyanide in vivo which triggers wide range of biological effects, such as inhibition of metabolism (Solomonson, 1981). The daily average weight of the rats might be associated with feed intake going by the corresponding trend in weight increase with intake (g) per day. The poor performance in weight gain of the rats fed diets C might likely be due to high content of residual cyanide. Osaniyi and Eka (1978) showed that presence of gossypol, oxalic, phytic acid and tannins in horse bean led to depressed appetite and low digestibility in rats. The improved weight gain of rats on diet D and diet E might be that fermenting for 48 and 72h resulted in quantitative reduction of the toxic factor. Fermentation has been found to improve nutrient release and weight gain of rats fed food materials suspected to contain toxic components (Achinewhu, 1983a; Achinewhu and Isichei 1990; Isichei and Achinewhu, 1988). It may be suggested that high residual cyanide interferes with metabolism of rats, which influenced the feed conversion ratio (FCR) and the weight gain. Moreover, Achinewhu (1983) observed improvement of feed conversion ratio of rats fed fermented oil bean seed (which are known to contain toxic factors). That might be the reason why diet C with feed conversion ratio 0.18 had poor average weight gain.

The differences in PER for the rats on the various diets formulated with quality protein (casein) may be that amino acid in the diets was preferentially utilized by the rats system at different rates. The poor PER obtained from rats fed diet B and C with correspondingly high residual cyanide could be reasoned that sulphur component of the amino acid was used up in the detoxification mechanism of the rats liver in presence of rhodanese enzyme to form thiocyanate which is less toxic to the rats (Bourdoux et al., 1982). Thus the detoxification mechanism might have compromised the amino acid, which could have been used for growth and maintenance of the body. Hence fermenting for 48h and 72h

improved the PER 0.18 and 0.16 respectively. Similar improvement of PER with fermentation was observed by Achinewhu and Isichei (1990). However, Ikya et al., (2007) obtained PER, 1.75, for cassava diet compared to casein, 2.50. Moreover, none of the diets formulated with gari resulted to the mortality of the rats. That goes to suggest that individuals can subsist on cassava diets but may have to live with the health problems associated with cyanide and thiocyanate in humans.

The high values of erythrocytes (RBC) obtained from rats on diets A and E, which did not differ significantly, might suggest that fermenting for 48 and 72h, pose no problems to the erythropoietic centers (blood production center) (Nwosu, 1979; Okoli, et al., 2002).

The data obtained from the packed cell volume (PCV), which was high for diet B might be due to the presence of toxicant – hydrogen cyanide. PCV is the ratio of the red blood cell component to the total blood volume (Smith et al., 1974). Similarly, the high hemoglobin concentration (HBC) of rats on diet B may be associated with high concentration of residual cyanide in the diet. Oluyemi (1973) and Nwosu (1979) had suggested that high apparent PCV and high hemoglobin concentrations of blood samples of subjects might be due to inherent physiological factors involving the hemopoietic systems. Thus, the blood production system might be responding to presence of toxin by producing more blood to stabilize the subject. Okeudo et al. (2003) however, suggested that the superior values might be a dissipation of useless energy that can be used for productive purpose.

The MCHC was high for the control (Diet A) but did not differ from Diet D and diet E. The low MCHC for diet B and Diet C might be associated with high residual cyanide. The presence of cyanide may have interfered with uptake and utilization of minerals from the diet such as iron in the synthesis of hemoglobin.

Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were marginally decreased for the values obtained for diet C and these might be linked to the presence cyanohydrins (potent stage of cyanide break down), Erythrocyte sedimentation rate (ESR) is an indication of damage of blood cells (Smith et al., 1974). The low ESR value obtained for diet B with high residual cyanide might be that metabolites formed from cassava's cyanogenic glucoside breakdown exerted some physiological activity, which might have some beneficial effects in the rats (Balagoplan et al., 1988). Manning et al. (1972) found that cyanate from

cassava diet irreversibly inhibited the sickling of red blood cells in vitro.

The data obtained from the white blood differential show that the neutrophiles of rats' blood on the gari diets compared well with the control. However, the rats fed diet C (111.5 mg HCN/kg, which had 54%, may be responding to the high residual cyanide of the diet. Although the eosinophiles were present at very low percent for diets A, B and D, it was completely absent in diet C. In addition, the lymphocytes were obtained at 1% with exception of 2% for diet C. These white blood cell components are produced by the body to ward-off attacks by chemical or microbial substances invading the blood stream (Smith *et al*., 1974).

Conclusion

The animal studies have shown that the protein efficiency ratio, PER, of rats on control diet (0.22) performed better than those fed gari diets. However, the PER, improved as the fermentation duration of gari used for the formulation of diets increased, 0.18, for 48h and 0.16 for 72h respectively. This reaffirmed the need to ferment cassava mash for a period not less than 48h for gari production. The observed high average weight gain of the rats on control diet, 3.39, over the gari diets, which was found to increase with increase in fermentation duration justifies the importance of time in reduction of dietary cyanide. The poor PER and weight gain of rats on diets containing gari fermented for 0h and 24h have shown that residual cyanide which was high in these diets and its presence interfered with the absorption of food nutrients. Hematological study revealed that the erythrocyte of the rats on control diet, $3.6 \times 10^6/mm^3$, was higher than those on gari diets. The poorest erythrocyte of rats' blood on diet B (gari fermented for 0h), 2.1×10^6 /mm^3 which gradually increased to 3.4×10^6 /mm^3 for diet E (72h fermented gari) might suggest that high residual cyanide was responsible for reduction of erythrocyte concentration. These findings underscore need to reduce cyanide occurring in cassava products to safe level through fermentation or any other compatible processing method.

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