Asian Journal of Advances in Agricultural Research

2(1): 1-17, 2017; Article no.AJAAR.35297
ISSN: 2456-8864

The Use of Fermented Cassava (*Manihot esculenta*) and Ripe Plantain Peels (*Musa paradisiaca*) as Feed: Effects on Haematological and Histological Parameters of Albino Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author OFO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript and managed the analyses of the study. Author AOO managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJAAR/2017/35297
Editor(s):
(1) Iskender Tiryaki, Department of Agricultural Biotechnology, Faculty of Agriculture, Canakkale Onsekiz Mart University, Canakkale, Turkey.
Reviewers:
(1) Ngozi Franca Okoye, University of Port Harcourt, Nigeria.
(2) Mohamed Fadel, National Research Center, Egypt.
Complete Peer review History: http://prh.sdiarticle3.com/review-history/20380

Original Research Article

Received 5th July 2017
Accepted 2nd August 2017
Published 5th August 2017

ABSTRACT

Aims: The effects of fermentation of cassava and ripe plantain peels as feed *in-vivo* on the haematological indices and histological profile of albino rats.

Study Design: The diet was formulated from fermented and raw cassava and ripe plantain peel blendsvaried at different ratio (100:0, 70:30, 60:40, 50:50 and 0:100).

Place and Duration of Study: Sample: Department of Microbiology, Federal University of Akure, Ondo State between October 2015 and July 2016.

Methodology: Antinutrient analysis was carried out on the fermented and raw blends. Thirty three (33) albino rats were fed with the diets. Weight, fur, mortality and activeness of the rats were observed. Haematological and histology parameters of the rats were examined.

Results: A total number of fifteen (15) microorganisms were isolated during the fermentation of cassava-plantain blend; these comprise of four molds (*Penicillium notatum, Aspergillus niger,*

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Aspergillus flavus, Rhizopus stolonifer), ten bacteria (*Bacillus subtilis, Lactobacillus plantarum, Lactobacillus fermetum, Pedicoccus acidilactic, Lactobacillus delbrueckii, Micrcoccus luteus, Enterobacter cloacae, Bacillus cereus, Leuconostoc mescenteriodes* and *Staphylococcus aureus)* and a yeast (*Saccharomyces cerevisae).* The antinutrient content of the samples decreased significantly with fermentation. There was a significant increase in the Packed cell volume, Red blood cell, Haemoglobin concentration and Erythrocyte sedimentation rate, decrease in white blood cell and increase in the body weight gained of the rat fed with fermented feed. The histological parameters of the rats fed with fermented feed showed mild effect on the liver of the rats with no significant effect on the small intestine. The result obtained from this study shows that the fermented samples could be explored in animal feed formulation.

Keywords: Cassava peel; ripe plantain peel; antinutrients; necrosis; anaemia; vacoulation.

1. INTRODUCTION

In the agricultural industry, the level of survival between animals and humans has been so wide. Agricultural wastes are renewable resource of great variety of biotechnological potential [1]. In recent years, waste like rice straw, rice hulls, fruit wastes, cassava peels and starch residues have been used as substrates for growing microbes also in ruminant feeding which appears to be the available option left for farmers in addressing the problem of competition between human beings and animals for conventional feed ingredients [2]. Peels are the major by-products obtained during the processing of various fruits and these were shown to be a very good source of polyphenols, caroteinoids, dietary fibres, and other bioactive compounds which possess various beneficial effects on human health [3].

Cassava (*Manihot esulenta*) is primarily a source of carbohydrate and a staple food of the majority of people in Tropical Africa, Central and South America [4]. According to Hillocks [5], the reasons for the production of cassava are mainly for its response to drought, famine, hunger, efficient cheap food energy and resistance to pests and diseases. Cassava peel is a major by-product generated from the processing of cassava into its various uses. Although Cassava offers tremendous potentials as a cheap source of feed energy for livestock, but usually not good enough due to their high fibre and low protein contents [6]. For cassava peels to be used as a substitute for cereal in animal feeding, they must be supplemented, either with protein-rich oilseed, fish meals or by using microbial techniques [7]. The microbial enrichment process is relatively cheap and the enriched product can increase the potential of cassava as a feed [7].

Plantain belongs to the genus *Musa,* a major starch crop of importance in the human tropical zone of Africa, Asia, Central and South America. Plantain peels are rich in fibre, polyphenols and low in protein but their composition varies according to the species and the variety as well as the maturation [8]. Plantain peels contains higher levels of minerals such as calcium, iron and phosphorus. The amino acid components include; alanine, aminobutyric acid, glutamine, asparagine, histidine, serine, arginine and leucine. The ascorbic acid is high compared to that of banana. As a starchy staple food, plantain supply about 1 g protein/100 g edible portion [9]. The ripe, over ripe plantain peels and unripe (green) plantain peel has been used in various ways, majorly for soap making [10], animal feeds [11] and biogas production [12]. Research has been carried out on the use of plantain peels as inclusive in animal feed. [13] reported that Dried Plantain Peels could be included in broiler diet up to 15% while ripeness improved the performance of the birds.

The constraint to their use in livestock nutrition is majorly the loads of anti-nutrients like cyanide, tannins and phytates [14] and their effects on animals. Fermentation of food products helps to brings down the level of anti-nutrients [14]. Fermentation of these peels will help to reduce the anti-nutrients, decrease the rate of mortality and detriments in animal system, hence the need for this research.

2. MATERIALS AND METHODS

2.1 Collection of Samples

Fresh plantain (*Musa paradisiaca*) was obtained from “Oba” market in Akure, while fresh Cassava peels (*Manihot esculenta*) were provided by a Garri Processing Factory at Ilara-Mokin, Ondo State.

2.2 Processing and Formulation of Blends

The plantain and cassava peels were washed, sun dried, milled and formulated in the ratio of (cassava: ripe plantain).

A (50:50) = 50 gram of cassava peel and 50 g of ripe plantain peel
B (60:40) = 60 gram of cassava peel and 40 g of ripe plantain peel
C (70:30) = 70 gram of cassava peel and 30 g of ripe plantain peel
D (0:100) = 100 g of ripe plantain peel
E (100:0) = 100 g of cassava peel.

2.3 Fermentation of Blends

This was achieved by using solid state fermentation technique. A cooled sterile distilled water of about 90 ml was poured into 100 g of the blend in a sterile plastic and stirred. This was properly sealed in the laboratory at room temperature over a period of 96 hours for natural fermentation to occur. The fermented blends were subsequently analyzed daily.

2.4 Anti-nutrient Content Analysis

Alkaloid was determined according to Trease and Evans [15]. Tannin was determined according to Banso and Adeyemo [16]. Oxalate was determined according to Nguyen and Savage [17]. Saponin was determined according to to Obadoni and Ochuko [18]. Cyanogenic Glycosides was determined according to Dicenta et al. [19].

2.5 Formulation of Diets

The bagged fermented and raw cassava-plantain peels were used to formulate the experimental diets. Six experimental diets were formulated with the control having no cassava or plantain peel (Table 1).

2.6 Feeding Trials

A total of 33 male albino growing rats used for experiment were obtained from Vicom Farm Akure south. The rats were all weighed with an average weight of 63 g. They were then randomly assigned into ten groups in triplicate per treatment with each rat serving as a replicate in Randomized complete design experiment. The rats were housed individually on wood-wire cages. These feed and water were given to the rats 2 times daily. They were allowed to acclimatize to their environment and diet for seven days before the experimented feed was given to rats. The behavioral and physical changes such as skin and fur were observed. The weight of all the experimental groups and the control were recorded.

2.7 Haematogical Studies

At the 4th week of the experiment, blood samples were collected from the rats via the ear vein into EDTA (Ethylene Diamine Tetra Acetic Acid) bottles to prevent coagulation. The red blood cell and the white blood cell counts were determined using the new improved neubauer counting chamber while the packed cell volume (%) was determined using the haematocrit centrifugation technique (HCT) [20]. Haemoglobin concentration (g/dl) value was estimated using the cyanmethaemoglobin method with spectrophotometer [21]. The determination of the distribution of the various blood cells was done by shilling method of differential leukocyte counts [22].

2.8 Histology Evaluation

The histological processing was carried out and interpreted at the Animal Production and Health Laboratory, Federal University of Technology, Akure, Ondo State. Histopathological tests were carried out on the organs of the laboratory animals as follows: the organs of the animals were collected and fixed in 10% formalin to prevent decay. They were dehydrated in different percentage (50%, 70%, 80% and 100%) of alcohol 1 ½ hours each. After dehydration they were cleared with 100% xylene and left for 2hours to remove any remnant alcohol and impregnated in liquid wax for 2 hours for embedding. The embedded organs were sectioned using microtome and were stained with haematoxylin-eosin [23]. Excess stain was removed with tap water. After clearing in xylene, Canada balsam was added and cover slips placed on the slides [24]. The preparations were left in the oven at 40°C and then placed under the microscope with a digital camera connected to a computer system to be examined by an expert and take the photographs.

2.9 Statistical Analysis

All data obtained were carried out in triplicates and subjected to descriptive statistics, analysis of variance (ANOVA) and Duncan Multiple Range Test and the level of significance was set at $p \leq 0.05$.

Table 1. Composition of rats' diets

Ingredient (g)	Diets										
	RA	RB	RC	RD	RE	FA	FB	FC	FD	FE	CT
Cassava	50	60	70	0	100	50	60	70	0	100	0
Plantain	50	40	30	100	0	50	40	30	100	0	0
Groundnut	14	14	14	14	14	14	14	14	14	14	14
Palm kernel cake	15	15	15	15	15	15	15	15	15	15	15
Maize	20	20	20	20	20	20	20	20	20	20	20
Brewers dry grain	24	24	24	24	24	24	24	24	24	24	24
Fixed ingredient	27	27	27	27	27	27	27	27	27	27	27
Total	200	200	200	200	200	200	200	200	200	200	100

Fixed ingredients (g): corn bran- 8.25, rice bran -14, fish meal- 1, bone meal -2.5, salt- 0.5, premix -0.25, lysine- 0.25 and methionine- 0.25.

Keys: RA: Raw 50 gram cassava/ 50 gram plantain, RB: Raw 60 gram cassava/40 gram plantain, RC: Raw 70 gram cassava/30 gram plantain, RD: Raw 100 gram plantain, RE: Raw 100 gram cassava, FA: Fermented 50 gram cassava/ 50 gram plantain, FB: Fermented 60 gram cassava/40 gram plantain, FC: Fermented 70 gram cassava/30 gram plantain, FD: Fermented 100 gram plantain, FE: Fermented 100 gram cassava and CT: Control

3. RESULTS

3.1 Microbial Growth during Fermentation of Cassava and Ripe Plantain Peel Blends

Fifteen microorganisms were isolated from the fermented mash of cassava-ripe plantain peels which was confirmed by series of biochemical tests and identified as *Penicillium notatum, Saccharomyces cerevisiae, Aspergillus niger, Aspergillus flavus, Rhizopus stolonifer, Bacillus subtilis, B. cereus, Lactobacillus plantarum, Lb. fermetum, Lb. delbrueckii, Pedicoccus acidilactic, Micrcoccus luteus, Enterobacter cloacae, Leuconostoc mescenteriodes* and *Staphylococcus aureus.*

3.2 Antinutritional Composition of the Blend

3.2.1 Tannin content (mg/g) of the peel blends

The results of the tannin content of the peel blends are shown in Fig. 1. Tannin content recorded the highest value in Sample RE (100 g cassava) (0.82 mg/g) while the least was recorded in Sample FA (fermented 50 g cassava/50 g plantain) (0.48 mg/g).

3.2.2 Phytate content (mg/g) of the peel blends

The results of the phytate content of the peel blends are shown in Fig. 2. Phytate content recorded the highest value in Sample RD (100 g plantain) (21 mg/g) while the least was recorded in Sample RE (fermented 100 g cassava) (10.71 mg/g).

3.2.3 Oxalate content (mg/g) of the peel blends

Fig. 3 represent the results of the oxalate content of the blends. Blend RD (Raw 100 g plantain) was highest in oxalate content with a value of 3.60. Blend FA (fermented 50 g cassava and 50 g plantain) had the lowest in oxalate content with a value of 1.08 mg/g.

3.2.4 Alkaloid content (mg/g) of the peel blends

The results of alkaloid content of the blends are represented on Fig. 4. Sample RC (70 g cassava and 30 g plantain) had the highest in alkaloid content with a value of 2.19%. Sample FD (fermented 100 g plantain) had the lowest in alkaloid content with a value of 1.16%.

3.2.5 Saponin content (mg/g) of the peel blends

Fig. 5 represent the results of Saponin content of the blends. Sample RD (100 g plantain) had the highest in Saponin content with a value of 1.20%. Sample FC (fermented 70 g cassava/30 g plantain) had the lowest in saponin content with a value of 0.64%.

3.2.6 Hydrogen cyanide (mg/g) of the peel blends

Fig. 6 shows the results of hydrogen cyanide content of the flour blends. Sample RE (100 g cassava) had the highest in hydrogen cyanide content with a value of 0.03 mg/g. Sample FD (fermented 100 g plantain) had the lowest in hydrogen cyanide content with a value of 0.0142 mg/g.

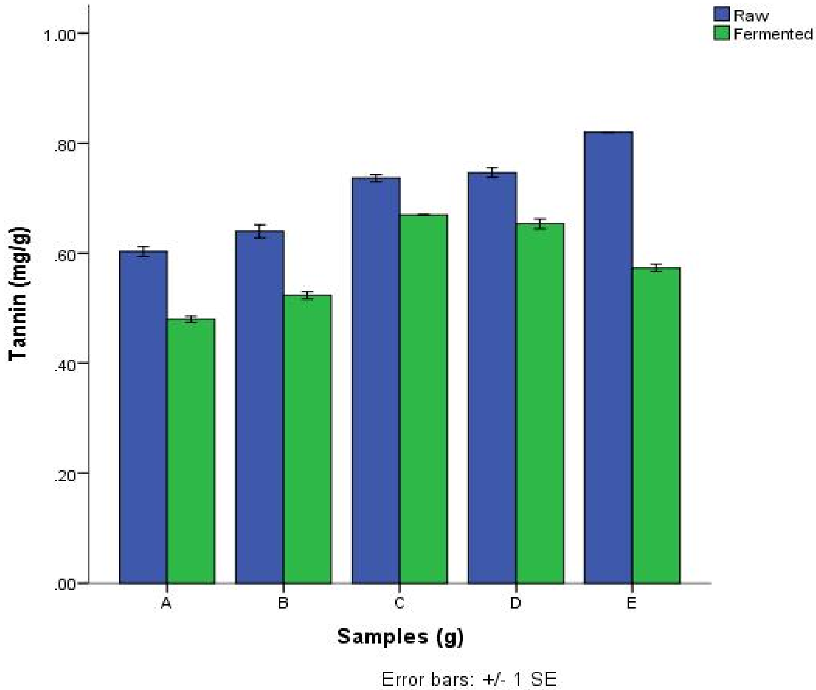

Fig. 1. Tannin content of fermented and raw cassava and ripe plantain peel blends
Keys: A: 50 g Cassava/50 g Plantain peel; B: 60 g Cassava/40 g plantain peel; C: 70 g Cassava/30 g Plantain peel; D: 100 g Plantain peel; E: 100 g Cassava peel

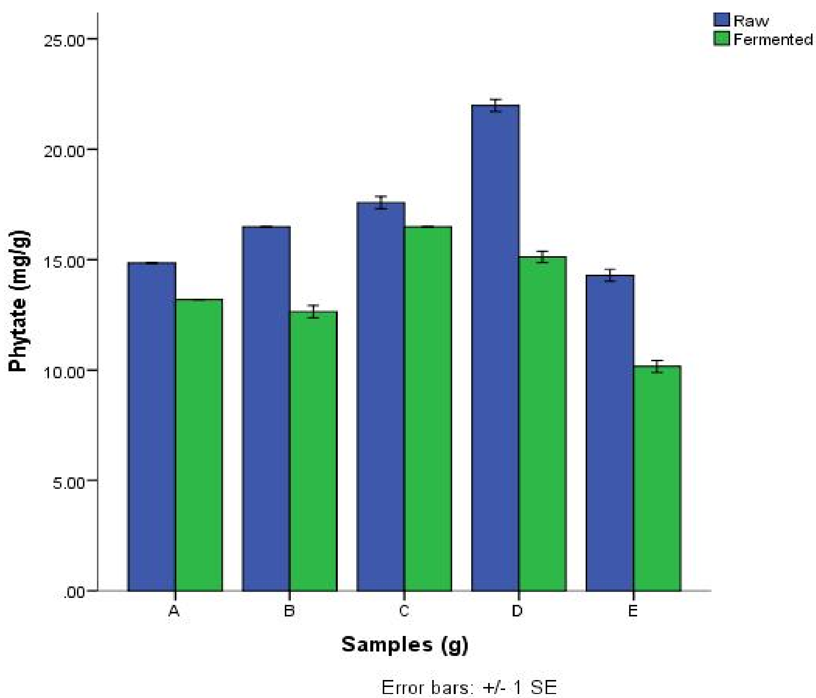

Fig. 2. Phytate content of fermented and raw cassava and ripe plantain peel blends
Keys: A: 50 g Cassava/50 g Plantain peel; B: 60 g Cassava/40 g plantain peel; C: 70 g Cassava/30 g Plantain peel; D: 100 g Plantain peel; E: 100 g Cassava peel

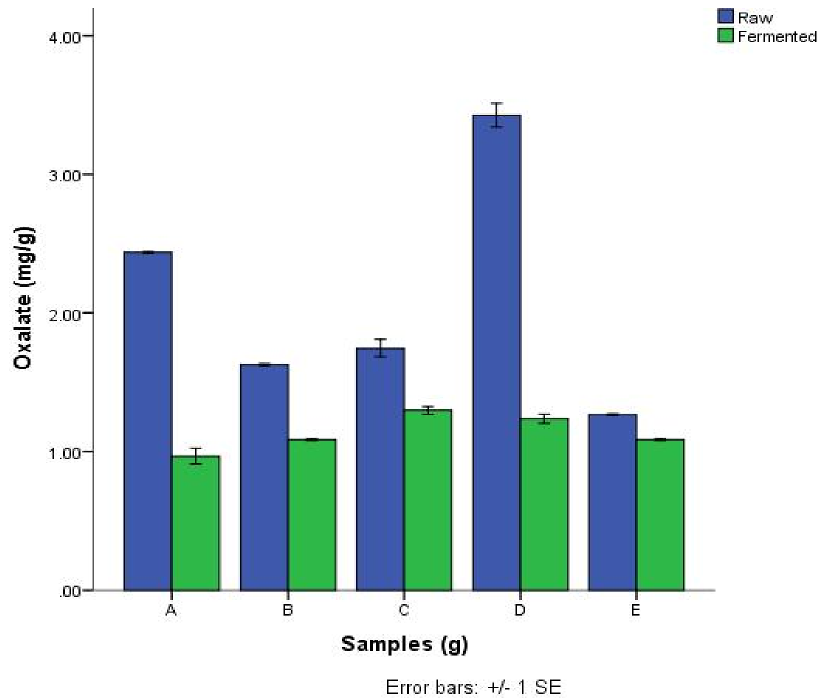

Fig. 3. Oxalate content of fermented and raw cassava and ripe plantain peel blends

Keys: A: 50 g Cassava/50 g Plantain peel; B: 60 g Cassava/40 g plantain peel; C: 70 g Cassava/30 g Plantain peel; D: 100 g Plantain peel; E: 100 g Cassava peel

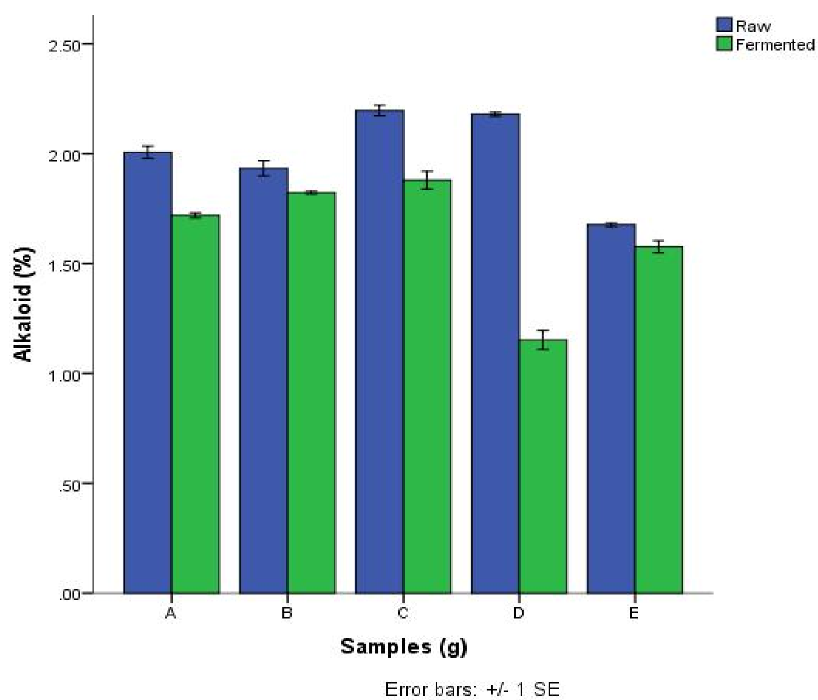

Fig. 4. Alkaloid content of fermented and raw cassava and ripe plantain peel blends

Keys: A: 50 g Cassava/50 g Plantain peel; B: 60 g Cassava/40 g plantain peel; C: 70 g Cassava/30 g Plantain peel; D: 100 g Plantain peel; E: 100 g Cassava peel

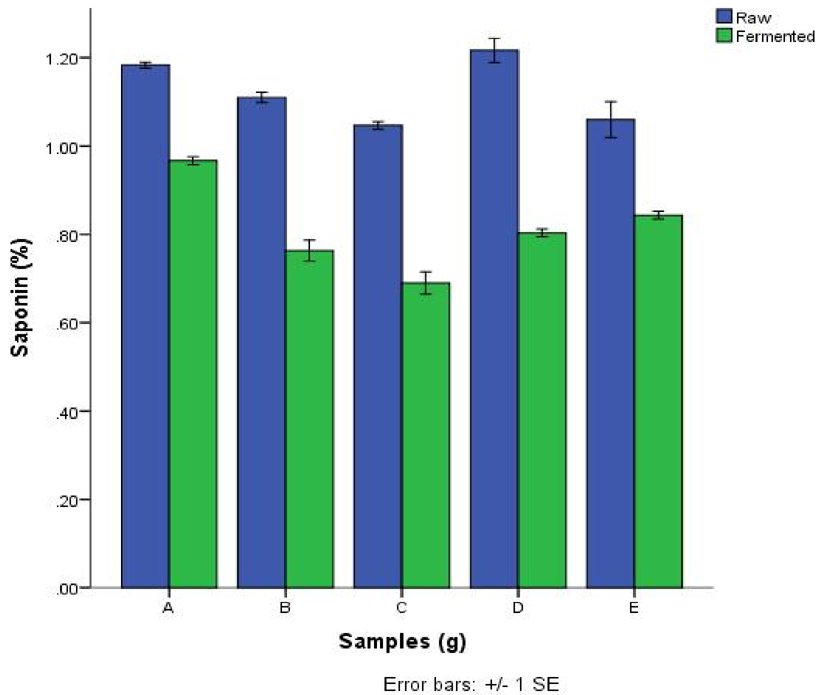

Fig. 5. Saponin content of fermented and raw cassava and ripe plantain peel blends
Keys: A: 50 g Cassava/50 g Plantain peel; B: 60 g Cassava/40 g plantain peel; C: 70 g Cassava/30 g Plantain peel; D: 100 g Plantain peel; E: 100 g Cassava peel

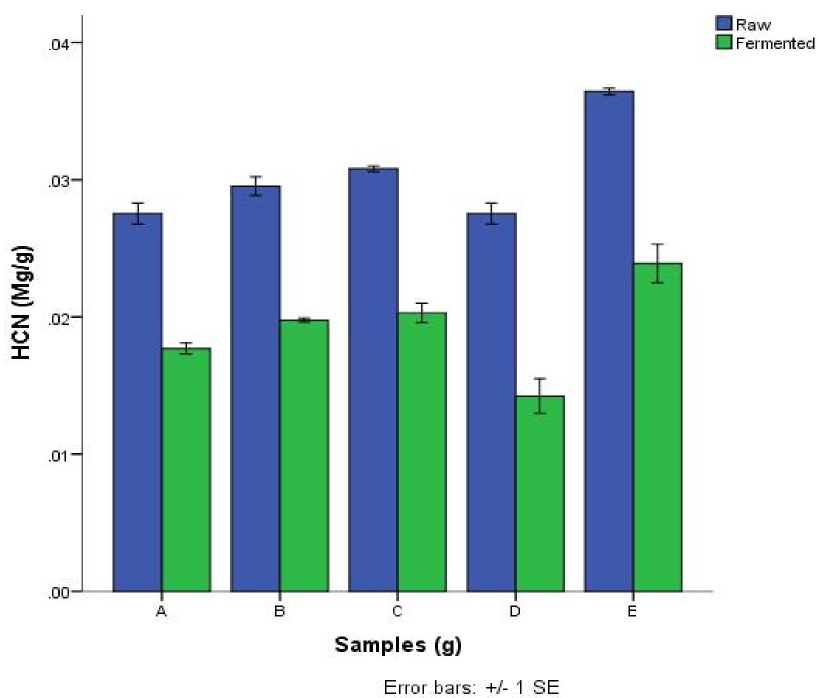

Fig. 6. Hydrogen cyanide content of fermented and raw cassava and ripe plantain peel blends
Keys: A: 50 g Cassava/50 g Plantain peel; B: 60 g Cassava/40 g plantain peel; C: 70 g Cassava/30 g Plantain peel; D: 100 g Plantain peel; E: 100 g Cassava peel

3.3 Effect of Diets on the Weight, Fur and Behavior of the Albino Rats Compared to the Control

The significant difference in average body weight gained, fur and behavior characterized by the rats were shown in Table 2. The highest body weight gain (41.00± 0.58) was recorded in the rats fed with FA (50g cassava/50g plantain) while the lowest body weight gain (3.53±0.60) was obtained in the rats fed with RE (100g) cassava.

3.4 Haematological Analysis

Table 3 and 4 shows the effect of fermented and raw diet on the hematological parameters of albino rats compared to control feed.

The Erythrocyte Sedimentation Rate (ESR) of rats fed with the fermented samples was significantly higher compared to the rats fed with control and raw diet. The highest ESR was recorded in rats fed with FA samples (fermented 50 g cassava/50 g plantain) with a mean valve of 7.77, and the lowest in rats fed with RA diet (raw 50 g cassava/50 g plantain) with a mean value of 5.10

The packed cell volume (PCV) of rats fed with fermented diet was higher than in rats fed with control diet except for rats fed with FE (fermented 100 g cassava) and FC diet (fermented 70% cassava and 30% plantain peels), the mean value of rats fed with raw diet was significantly lower compared to the rats fed with control diet. The highest PCV was recorded in rats fed with FE diet (fermented 50 g cassava/50% plantain) with a mean valve of 54.67, and the lowest in rats fed with RE diet (raw100 g cassava) with a mean value of 47.67.

White blood cell counts (WBC) was higher in the rats fed with raw diets compared to the rats fed with control. Rats fed with RE diet (raw 100g cassava peels) had the highest white blood cell of valve of 11.73, while rats fed with FB diet (fermented 60 g cassava/40 g plantain peels) had the lowest white blood cell of valve 6.44.

The red blood cell (RBC) of the rats fed with fermented diet was higher than the rats fed with control and raw diet except FE diet (fermented 100 g cassava peel) and FD (fermented 100g plantain peel), the valve of RBC in rats fed with raw diet was significantly lower compared to rats fed with control diet. The highest RBC was recorded in rats fed with FA diet (fermented 50g cassava/50 g plantain) with a valve of 6.56, and the lowest in rats fed with RC diet (raw 70% cassava/ 40% plantain) with valve of 4.46.

Table 2. Growth performance of rats fed with fermented and raw cassava and ripe plantain peel diet

Samples	Av. initial weight (g)	Av. final weight (g)	Av. weight Gained (g)	Mortality rate	Activeness	Fur
FA	45.00± 0.57[a]	85.00±0.57[i]	41.00± 0.58[g]	0	Very active	Shinny
FB	58.33±0.33[f]	89.33±0.67[j]	34.54±0.06[f]	0	Very active	Shinny
FC	58.33± 0.33[f]	71.00±0.57[e]	17.15±0.06[c]	0	Active	Moderate
FD	57.00±1.00[ef]	80.90±0.56[h]	23.90±1.30[d]	0	Active	Moderate
FE	53.33± 1.33[c]	63.88±0.56[c]	10.55±0.77[b]	0	Active	Moderate
RA	56.33±1.33[def]	77.67±0.67[f]	32.46±0.20[e]	0	Active	Moderate
RB	54.00±0.58[cd]	79.33±0.33[g]	32.97±0.03[e]	0	Active	Dull
RC	55.00±1.15[cde]	60.00±0.57[b]	11.70±0.03[b]	0	Less active	Dull
RD	57.00±0.57[ef]	67.67±0.33[d]	10.66±0.33[b]	0	Active	Moderate
RE	48.00± 0.57[b]	51.53±0.03[a]	3.53±0.60[a]	0	Less active	Dull
CT	59.00±0.00[f]	94.07±0.07[k]	35.07±0.07[f]	0	Very active	Shinny

**Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly different (P≤0.05)*

Keys: RA: Raw 50 gram cassava/ 50 gram plantain peel, RB: Raw 60 gram cassava/40 gram plantain peel, RC: Raw 70 gram cassava/30 gram plantain peel, RD: Raw 100 gram plantain peel, RE: Raw 100 gram cassava peel, FA: Fermented 50 gram cassava/ 50 gram plantain peel, FB: Fermented 60gram cassava/40 gram plantain peel, FC: Fermented 70 gram cassava/30 gram plantain peel, FD: Fermented 100 gram plantain peel, FE: Fermented 100 gram cassava peel, CT: Control

Table 3. Haematological analysis of rats fed with fermented cassava and plantain peel blends

Samples	ESR mm/hr	PCV (%)	WBC 1000/mm^3	RBC (mm^3)	HB (g/dl)	Lymp (%)	Neut (%)	Mono (%)	Eosin (%)	Baso (%)
FA	7.77±0.09^{c}	54.67±0.33^{c}	6.71±0.01^{b}	6.56±0.07^{c}	19.34±0.33^{d}	69.33±0.33^{b}	58.33±0.33^{d}	3.67±0.88^{a}	1.00±0.00^{a}	0.00±0.00^{a}
FB	7.21±0.06^{b}	54.33±0.33^{c}	6.43±0.01^{a}	6.53±0.03^{c}	17.00±0.58^{b}	71.33±0.67^{c}	54.66±0.01^{c}	4.33±0.88^{a}	1.67±0.33^{a}	0.00±0.00^{a}
FC	7.13±0.67^{b}	52.33±0.33^{b}	7.02±0.02^{c}	6.46±0.07^{c}	18.67±1.33^{d}	66.33±0.67^{a}	50.67±0.67^{a}	4.00±0.00^{a}	1.00±0.00^{a}	0.00±0.00^{a}
FD	7.30±0.06^{b}	54.67±0.33^{c}	7.17±0.02^{d}	6.17±0.05^{b}	18.22±0.11^{c}	67.00±0.58^{a}	50.00±0.33^{a}	3.33±0.88^{a}	1.33±0.33^{a}	0.33±0.33^{a}
FE	6.93±0.02^{a}	47.67±0.33^{a}	7.57±0.07^{e}	5.50±0.01^{a}	15.65±0.02^{a}	67.67±0.33^{ab}	52.00±0.00^{b}	2.67±0.33^{a}	1.00±0.00^{a}	0.00±0.00^{a}
CT	6.93±0.03^{a}	52.67±0.00^{b}	7.23±0.03^{d}	6.23±0.07^{b}	16.67±0.00^{b}	66.33±0.67^{a}	50.00±0.57^{a}	4.67±0.67^{a}	1.50±0.29^{a}	0.00±0.00^{a}

**Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly different (P≤0.05)*
Keys: FA: Fermented 50 gram cassava/ 50gram plantain peel, FB: Fermented 60gram cassava/40 gram plantain peel, FC: Fermented 70 gram cassava/30 gram plantain peel, FD: Fermented 100 gram plantain peel,
FE: Fermented 100 gram cassava peel, CT: Control

Table 4. Haematological analysis of rats fed with raw cassava and plantain peel diets

Samples	ESR (mm/hr)	PCV (%)	WBC (1000/mm^3)	RBC (mm^3)	HB (g/dl)	Lymp (%)	Neut (%)	Mono (%)	Eosin (%)	Baso (%)
RA	5.10±0.06^{a}	40.33±0.55^{b}	10.30±0.03^{d}	5.38±0.01^{c}	12.2±0.06^{e}	55.33±0.67^{c}	50.83±0.93^{bc}	4.00±0.58^{ab}	2.00±0.00^{a}	0.00±0.00^{a}
RB	6.76±0.03^{ef}	45.00±0.00^{c}	9.80±0.00^{c}	5.20±0.00^{b}	11.02±0.02^{d}	58.00±0.58^{d}	52.33±0.88^{c}	5.00±0.58^{b}	2.00±0.00^{a}	0.33±0.33^{a}
RC	5.33±0.03^{b}	41.00±0.57^{b}	11.24±0.01^{e}	4.46±0.01^{a}	8.3±0.01^{b}	51.00±0.58^{b}	46.67±0.88^{a}	5.00±0.58^{b}	2.00±0.58^{a}	0.33±0.33^{a}
RD	6.70±0.11^{d}	49.33±0.33^{d}	9.23±0.03^{b}	5.73±0.01^{d}	10.23±0.03^{c}	57.00±0.57^{c}	47.33±0.67^{a}	3.00±0.00^{a}	1.33±0.33^{a}	0.00±0.00^{a}
RE	6.30±0.06^{c}	31.33±0.33^{a}	11.73±0.03^{f}	5.17±0.00^{b}	7.38±0.01^{a}	44.16±0.44^{a}	51.00±0.58^{bc}	3.00±0.58^{a}	1.33±0.33^{a}	0.33±0.33^{a}
CT	6.93±0.03^{f}	52.67±0.33^{e}	7.23±0.33^{a}	6.23±0.67^{e}	16.67±0.00^{f}	66.33±0.67^{e}	50.00±0.00^{c}	4.67±0.67^{ab}	1.50±0.29^{a}	0.00±0.00^{a}

**Values are means of triplicate determinations ± SD. Means in the same column with different superscripts are significantly different (P≤0.05)*
Keys: RA: Raw 50 gram cassava/ 50 gram plantain peel, RB: Raw 60 gram cassava/40 gram plantain peel,
RC: Raw 70gram cassava/30 gram plantain peel, RD: Raw 100 gram plantain peel, RE: Raw 100 gram cassava peel, CT: Control

The haemaglobin concentration (HC) of the rats fed with fermented diet was higher than the rats fed with control diet; the valve of rats fed with raw diet was significantly lower compared to rats fed with control diet. The highest HC was recorded in rats fed with FA diet (fermented 50 g cassava/50 g plantain) with a valve of 20.33, and the lowest was recorded in rats fed with RE diet (raw 100 g plantain) with valve of 3.65.

The lymphocytes of the rats fed with fermented diet was slightly higher than the rats fed with the control diet; the mean valve of rats fed with raw diet was significantly lower compared to rats fed with control diets. The highest was recorded in rats fed with FB diet (fermented 60g cassava/40 g plantain peel blend) with a valve of 71.33 while the lowest was recorded in rats fed with RE diet (100 g plantain peel blend) with valve of 44.16.

There was significant difference in the values of neutrophils obtained. The mean percentage neutrophils obtained was significantly higher in the rats fed with fermented diet compared to the rats fed with control diet. The highest mean percentage neutrophils was obtained in rats fed with FA diet (50% cassava/50% plantain peel) with a value of 58.33 while the lowest was recorded in rats fed with RC diet (70% cassava/30% plantain peel) with a value of 46.67.

The mean values of Basophil and eosinophil and monocyte of the rats was insignificantly affected by the treatment (fermented and raw) compared to the rats fed with the control diet.

3.5 Histological Analysis

Plate 1- 10 showed the effect of the diets on the liver of the rats. The microscopic section of liver of rats fed with control diet showed normal liver tissues.This reveals that the fermented diet showed a very mild histological change on the liver compared with the control. Vacuole formation was observed in the liver of the rats fed with fermented diet. Necrotic effect was observed in the liver of rats fed with raw diet compared with that of rats fed with fermented and control diets.

There was no significant different between the treatments used and the control in the small intestines of the rats.

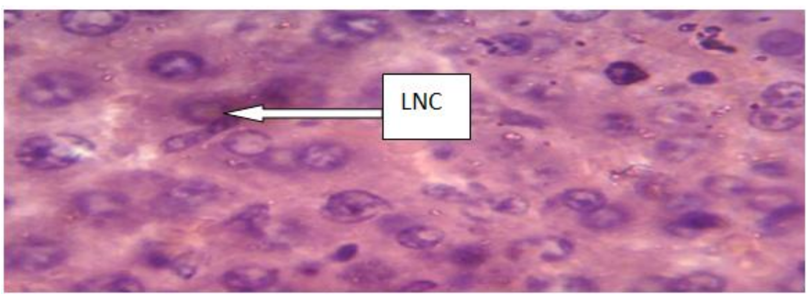

Plate 1. Photomicrograph of liver of albino rat fed with the control sample. (LNC) Liver with normal cells

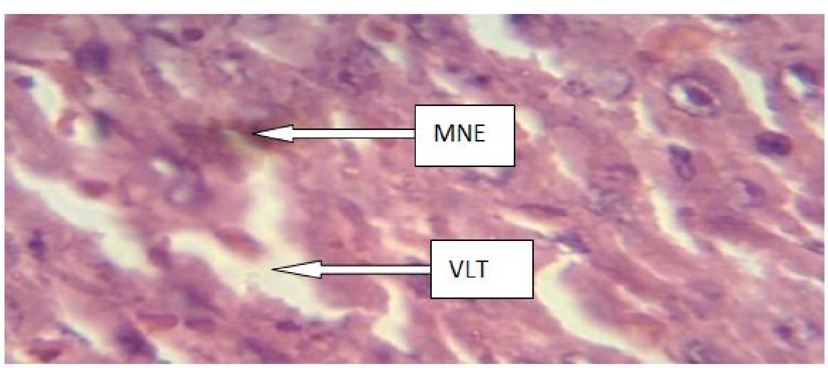

Plate 2. Photomicrograph of liver of albino rat fed with RA (Raw 50g cassava /50g ripe plantain peel blend) MNE-Mild necrotic effects on the liver , VLT- Vacuolization of liver

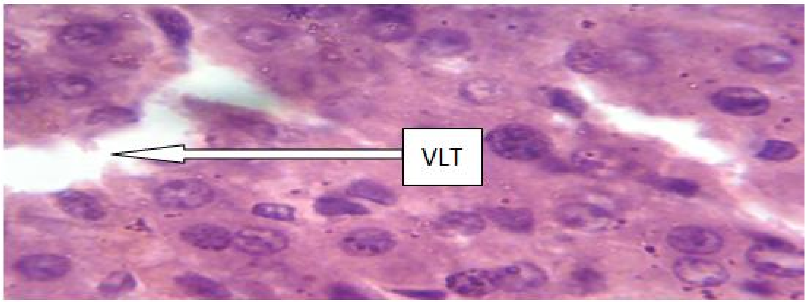

Plate 3. Photomicrograph of liver of albino rat fed with FA (fermented 50g cassava/50g plantain peel blend) VLT- Vacuolization of the liver tissue

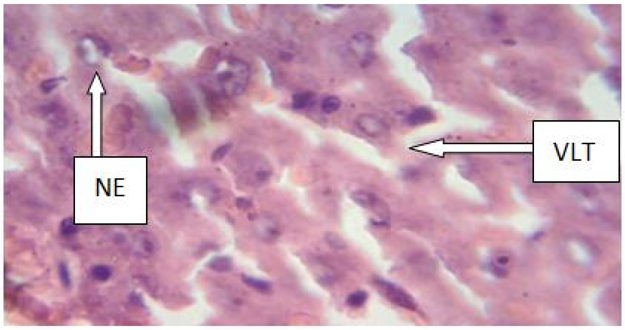

Plate 4. Photomicrograph of liver of albino rat fed with RB (Raw 60 g cassava /40g plantain peel blend) (NE)Necrotic effect and (VLT) Vacuolization of liver tissue

Plate 5. Photomicrograph of liver of albino rat fed with FB (fermented 60 g cassava /40g plantain peel blend) (VLT)Vacuolization of liver tissue

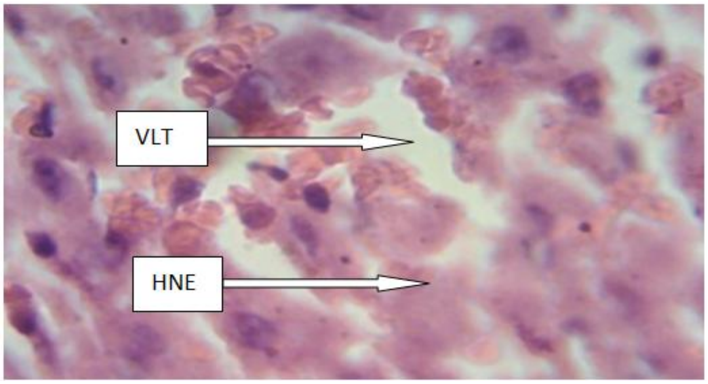

Plate 6. Photomicrograph of liver of albino rat fed with RC (Raw 70 g cassava/30 g plantain peel blend) (HNE)High necrotic effects and (VLT) Vacuolation of the liver tissue

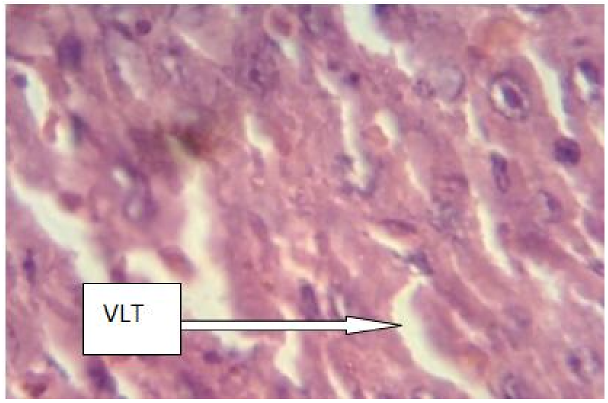

Plate 7. Photomicrograph of liver of albino rat fed with FC (fermented 70g cassava/30g plantain peels blend)(VLT) Vacuolation of liver tissue

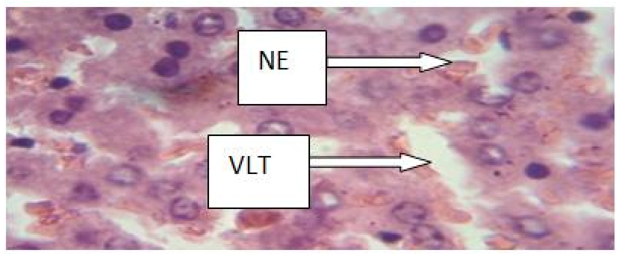

Plate 8. Photomicrograph of liver of rat fed with RD (Raw 100 g plantain peels blend) showing necrotic effect and VLT- Vacuolization of liver tissue

Plate 9. Photomicrograph of liver of rat fed with FD (fermented 100 g plantain peels blend) showing VLT- Vacuolization of liver tissue

4. DISCUSSION

At the end of fermentation, a significant decrease in all the antinutrient content was observed**.** The fermented cassava and plantain peels blend could be considered safe in terms of cyanide poisoning in view of the fact that the cyanide was below the deleterious level of 30 mg/kg [25] this could be due to the organism present in the breaking down of the cyanide. [26] reported that fermentation of cassava pulp with pure strains of *Saccharomyces cerevisiae, Lactobacillus delbruckii* and *Lb. coryneformis* resulted in decrease in cyanide content from 44.6mg/kg to 6.2 mg/kg.

A significant decrease in phytate content was observed in the fermented samples (10.71- 17 mg/g). The raw cassava- plantain peel blend had 15- 21 mg/g phytate content. A similar finding

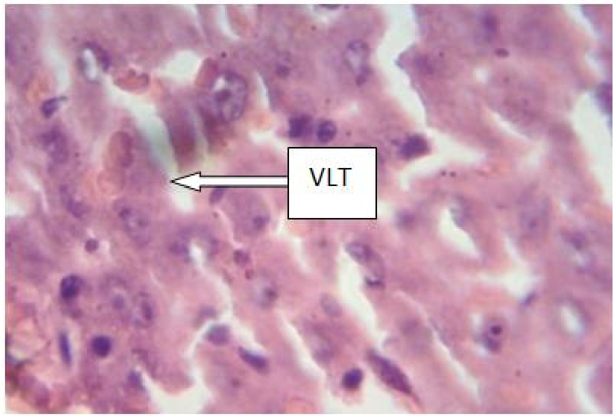

Plate 10. Photomicrograph of liver of rat fed with FE (fermented 100 g Cassava peel blend) showing VLT-Vacoulation in liver structure

was reported by [26] on the use of *Saccharomyces cerevisae* and *Lactobacillus spp in the* enrichment of cassava peels using solid media fermentation techniques. The decrease in phytate content could be as a result of possible secretion of the enzyme phytase by the microorganisms in the fermenting samples. This enzyme is capable of hydrolyzing phytate thereby decreasing the phytate content of the fermenting sample [27].

A decrease in the tannin content was observed in the fermenting samples (0.48-0.78 mg/g). The decrease in tannin may be as a result of the processing that the sample was subjected to couple with the activities of microbial enzymes involved in the fermentation. Similar report was observed by [28] during the fermentation of plantain and groundnut blend.

The oxalate value of the samples decreased with increase in the days of fermentation. The value ranged. This could be due to the activities of some microorganisms involved during the fermentation process. Similar report was observed by [28] during the fermentation of plantain and groundnut blend.

The observations of behavioral, weight, hematological and histological parameters have been employed in toxicological studies [29]. In this present study, there was significant difference in the average body weight of treatment groups when compared with the control group after 4 weeks. Increase in growth was observed in the rats fed with fermented diet, this could be due to the decrease in the antinutrient and increase in nutrients especially protein which was aided by fermentation. A slight increase in the body weight of rats fed with raw blends could either be due to the effect of the antinutrients especially hydrogen cyanide on the internal organs or to general discomfort resulting in lowered feeding in treated animals [30] and the low amount of protein in the raw diet.

Dietary content of feed usually affect the blood profile of healthy animals [31]. The result obtained from this study showed that the treatment had a significant effect on the hematological parameters of the rats except monocytes and basophils. Reduction in concentrations of erythrocytic parameters (such as PCV, RBC counts and Hb concentration) and elevation in white blood cell in the rats fed with raw diet are indications of anaemic condition. This was similar with the findings of [32] who reported a decrease in erythrocytic parameter when pigs are fed with cassava peels based diets supplemented with avizyme.

The fermented diets had less effect on the blood level of the rats, when compared raw diets. [33] had reported a normal packed cell volume (PCV) 36-55% of rats fed with brewery spent grains in dietary protein formulation. The observed decrease in PCV in the rats fed with raw diet might be due to decrease in red blood cell which agrees with [34]. [35] reported a decrease in PCV as a result of decrease in protein content of the birds fed with protein deficient diets.

An increase in Red blood cell was observed in the rats fed with fermented diets. [36] stated that increased red blood cell counts are associated with high quality dietary protein and with disease free animals. Decrease in RBC in the rats fed with raw diets may be due to presence of low amount of protein or iron when compared to the control diet and fermented diet. Increase in white blood due to presence of high amount of toxins or antinutrient might inhibit the amount of red blood cells in the blood system.

Haemoglobins are protein that carries oxygen from the lungs to different parts of the body. The haemoglobin concentration (HC) values of the rats fed with fermented diets was within the normal range of 11 –19.2 g/dl. This indicates that anti–nutritional factors which may be present in traces did not influence this hematological parameter. This result is similar to the findings of [37] who reported that processed cassava peel meal had no significant effect on the hemoglobin concentration of pullets. The decrease in value of HC in rats fed with raw diets ranges from 12.3 to 3.65 g/dl when compared with rats fed with control diet could be due to the destruction of red blood cell by the anti- nutritional factors.

White blood cells (WBC) are known to be the key actors in immune responses as they form the first line of defense against invading microorganism and the lowering of WBC values usually indicates fall in immune strength [38]. The value of white blood cell (WBC) obtained from this study showed that the rats fed with both fermented and raw diet was within the normal range of 6-18 ($1000/mm^3$). The slight decrease in WBC value of the rats fed the fermented diet compared to that of the rats fed with the control diets suggest that there was reduced or no elicitation of reaction for foreign body as seen in the rats fed with raw diets. An increase in WBC of the rats fed with raw diet may be as a result of response to foreign and toxic substances especially cyanide in the feed. This report is similar to the findings of [39] who reported an increase in white blood cell in rats fed gasoline and kerosene.

The mean percentage neutrophils obtained was significantly lower in rats fed with raw diets when compared with the control group. This is suggestive of low degree of infection. The immune system responds to this damages caused by oxidative stress conditions. During such responses, free radical is produced by the neutrophils the first-responders to inflammatory cells to remove damage cells. Being highly mobile, neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells and macrophages [40].

A significant increase in the lymphocytes of the rats fed with fermented diets was observed which was within the normal range of 65-85% [41]. The increase in lymphocytes may provide better level of immunity in the rats. The decrease in value of lymphocytes in rats fed with raw diets (44-58%) below the normal value of lymphocyte count suggests that some constituents in the feed may suppress the immune system leading to chronic digestion. Similar findings was observed by [42] that significantly lower lymphocyte count was an indication of a reduction in the ability of the experimental rats to produce and release antibiotics when exposed to toxic substances.

Eosinophils, basophils and monocytes values of the rats fed with fermented and raw diets were not significantly affected by the diets when compared to the rats fed with the control diets.

The liver is the first organ that encounters all absorbed materials from the gastrointestinal tracts. Liver play an important role in xenobiotic function. The result obtained from this study showed that the fermented cassava peel and ripe plantain peel blends caused vacoulation of the liver. The resulting vacuolation caused by fatty infiltration in liver which is a common pathological condition resulting from lipid metabolism as a result of toxicity due to natural non – nutrient toxins. This result was similar to the findings of [23]. Vacuole formation could also be as a result of delay in the bledding of rats after death or slight delay in the removal of the liver. The raw cassava and plantain peel blends revealed a higher toxicity causing liver necrosis and distortions. necrosis of the liver is a form of cell injury which results in the premature death of the cells. This may be caused by the high level of antinutrient present in the feed in which the liver is capable of detoxifying. The fall in values of red blood cell, packed volume cell, hemoglobin concentration and others due to the possible suppression of erythropoietic processes could result in liver necrosis and anemia [43]. This suggested a higher risk of damage on consumption of raw cassava and plantain peels by animals.

Small intestine helps in the absorption of water and digestion of food in the system. The result in this indicated that there was no significant difference in the treatments used in small intestine of the rats fed with fermented and raw diet when compared with the control.

5. CONCLUSION

Generally, the haematological results, nutritional parameters and the weight of rats obtained in this study indicate that the rats fed with fermented diets were healthy. This study revealed that the fermented cassava and ripe plantain peel blend can be used as alternative in the production of animal feed. From this research, it is evident that fermenting the cassava and ripe plantain blend will provide adequate nutrient in livestock feeding.

However, use of 50% of both cassava and plantain peel was found to be most suitable, which suggest its relevance in animal feed formulation for safe consumption and growth of animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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