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EFFECT OF FUNGAL FERMENTATION ON PROXIMATE COMPOSITION AND *IN VITRO* PERFORMANCE OF AGROWASTES USED IN ANIMAL FEED

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ABSTRACT

Large quantities of agricultural wastes generated annually from the processing of agricultural produce are disposed of indiscriminately in the environment, thus contributing to environmental pollution. Value addition to cassava (*Manihot esculenta* Crantz) peels, and a mixture of yam (*Dioscorea* spp.) and plantain (*Musa paradisiaca*) peels, which accumulate during manual peeling will help reduce this environmental impact in addition to being a source of income. The effects of fungal fermentation on their proximate composition and suitability for use as enriched animal feed were thus investigated in this study. Cassava peel and yam-plantain peel mixture were each fermented with *Aspergillus niger* and *Trichoderma* spp. at 32 °C at moisture content of 52% and 60% respectively for a week and the potential of the biomass was investigated in feeding trials of Wistar rats over a four-week period. After sacrifice, their sera and vital organs were analysed for key enzymes and metabolic products. It was observed that *Aspergillus niger*-fermented cassava peel (FCP) was the best treatment with increased protein content (from 6.73% to 19.3%) due to microbial biomass. While the carbohydrate content decreased following fermentation, the calorific value remained similar to that of the raw peel. No mortality was recorded in the experimental rats fed the FCP and standard commercial feed (CF) while 100% mortality was observed by the 4th week in the control group fed unfermented cassava peel (UCP). Organ-body weight ratio and some biochemical parameters e.g. cholesterol, high density lipoprotein (HDL), urea of FCP-fed rats were similar to those of the CF group. Lower amounts of some markers e.g. aspartate transaminase (ALP) and alanine transaminase (AST) were observed relative to UCP group. This research demonstrates the potential of microbially-detoxified food waste to replace commercial feed as a cheap alternative with minimal undesirable physiological effects in the animal models.

Keywords: Fungal Fermentation; Plantain Peel; Yam Peel; Food Wastes; *Aspergillus niger*; Wistar Rats

INTRODUCTION

Agricultural-based industries and homes release a large amount of untreated agro-wastes into the environment every year. This leads to environmental pollution and has a harmful effect on human and animal health. These untreated wastes also create different problems associated with climatic change by increasing a number of greenhouse gases (Sadh *et al.*, 2018).

The effective utilization of these agro-wastes such as creation of new products from the wastes (e.g. animal feed) require urgent attention as the recycling and reduction of wastes can reduce environmental pollution and the havocs caused by the pollution. The increasing costs and pressure concerned with disposal of wastes stress the need for a reappraisal of the utilization of wastes either directly or indirectly for livestock feeding (Ajila *et al.*, 2012), this can be achieved by biotransformation of wastes into commercially valuable products like protein-enriched products, single cell protein, mushroom production, etc. However, special efforts have been made to ensure that agricultural and industrial by-products, which are at times detrimental to man and animal health are utilized positively by converting them into value-added products. Some of these wastes include root tubers such as yam and cassava peels from food processing industries, and pineapple, citrus, banana, plantain and mango peels, coconut husks and shells, jatropha cake, corncobs, husks from fruit and vegetable processing industries and brans of cereals such as sorghum (Omojasola et *al.*, 2008; Sulaiman *et al.*, 2014; Aruna and Suneetha, 2016; Ahmed El-Imam *et al.*, 2019).

Cassava (*Manihot esculenta)* is an important tuber

crop that is widely grown and consumed in the tropics. Food Outlook, the report of the global food markets published biannually by the Food and Agricultural Organization of the United Nations, stated that Nigeria produced 55 million MT in 2017, making it the world's largest producer (FAO, 2017). While its tuber has a protein content of less than 3%, it has the highest amount of starch of all tropical tuber crops (Yafetto, 2018). It is boiled, fermented into garri, fufu and lafun and widely consumed. Yam (*Dioscorea* spp.) is also a local staple and Nigeria accounts for about 67% of the annual global total, producing over 44 million MT in 2016 (FDC, 2018). It is consumed boiled, fried or boiled and pounded into a thick paste and eaten with soup. Plantains (*Musa paradisiaca*) are also staple foods in tropical countries such as Nigeria. Before being consumed, cassava tubers, yam tubers and plantain are peeled and prepared for consumption by boiling, roasting, grilling, frying or pounding boiled tubers and plantain into dough. Roadside food sellers in many Nigerian cities fry yams and plantains and discard their peels in the drainages or just pile them by the roadside daily. While these sellers provide a quick and affordable meal for thronging commuters, the wastes constitute an environmental nuisance as they produce unpleasant smells and breed flies and vermin.

Solid state fermentation (SSF) refers to any biotechnological processes in which organisms grow on non-soluble material or solid substrates in the absence or near absence of free water (Bhargav *et al.,* 2008). Even though several studies have shown that the agricultural wastes can be used as ingredient for animal feeding (Bhalla and Joshi, 1994; Villas-Boas *et al.,* 2002; Sulaiman *et al*., 2014), however, the peels are still largely discarded. This could be due to the relatively low cost of food crops with a medium (4-5 kg) tuber of yam costing about N250 (about $0.70) in Ilorin City, North Central Nigeria. Converting these wastes to wealth will increase the profits of the entire agriculture chain and even lure more unemployed people to farming. However, these wastes commonly contain antinutritional factors, with cassava peels containing high amounts of cyanide while yam peel contains tannin, saponin, oxalate and phytate (Akinmutimi and Onen, 2008; Maniyan *et al.,* 2015). They are thus only consumed by hardy West African Dwarf goats in North Central and South Western Nigeria and not well accepted by many other domestic animals. In addition, they are poor in nutrients such as proteins and vitamins (Villas-Boas *et al.,* 2002). This work thus aims at determining the effects of solid-state fermentation by fungi on the nutritional profile of these wastes and the physiological responses of animal models to the resulting feed, while eliminating the usual step of sorting them into various waste types. The cassava peels were utilized including cuttings, while the yam and plantain peels were also collected together as they existed in the sellers' piles.

MATERIALS AND METHODS

Collection and Processing of Substrates

Cassava peels (CP) and the yam and plantain peel mixtures (about 70:30) (YPP) were obtained from roadside sellers in Ilorin, Kwara State, Nigeria. The various substrates were washed, sun-dried till constant weight and milled till they passed through a 5 mm mesh.

Isolation and Maintenance of Organisms

Aspergillus niger and *Trichoderma* spp. used for this research were isolated from a dry basidiomycetes conk found on the University of Ilorin campus. It was cultured on Malt Extract Agar plates at ambient room temperature for 7 days. The fungi were then maintained on Potato Dextrose Agar slants at 4 °C pending use.

Fermentation of the Substrates

Slants of the *Aspergillus niger* and *Trichoderma* spp. were flooded with 10 ml of sterile 0.1% Tween-80 solution which was swirled gently to collect spores. The suspension was diluted appropriately and the spore concentration estimated with the aid of a hemocytometer. Exactly 60 g of each substrate was weighed into glass bottles and moistened adequately with sterile basal medium (containing 2 g $NaNO_3$, 1 g K_2HPO_4, 0.3 g $MgSO_4.7H_2O$, 0.3 g KCl and 0.01 g $FeSO_4.7H_2O$ per liter) with no free running liquid, reaching about 52% moisture content for CP and 60% for YPP. The substrates were then sterilized at 121 °C for 15 minutes, cooled and inoculated with *Aspergillus niger* spores or *Trichoderma* spp.

spore suspension to a final concentration of 2 x 10^8 spores per gram dry weight, while uninoculated controls were also prepared. The jars were loosely capped and incubated at a temperature of 32 °C ± 3 °C for seven days; moisture loss, detected as a reduction in weight, was compensated for by daily replacement with sterile distilled water .All fermentations were carried out in triplicates.

Proximate Analysis of the Samples

Moisture content was determined by the constant weight method (AOAC, 2000). Ash content was determined by ashing the sample in a platinum crucible 550 °C for 3 hours (AOAC, 2000). Lipid content was determined by the soxhlet extraction technique described by Shirlaw and Gilchrist (1967) and AOAC (2000). Total amino nitrogen was determined by Kjeldahl method (AOAC, 2000).

To determine crude fiber, twenty grams (20 g) of sample was defatted with diethyl ether for 8 hours and boiled under reflux for 30 minutes with 200 ml of H_2SO_4. It was then filtered through cheese cloth, washed with boiling water to completely remove the acid. The residue was then boiled in a round bottom flask with 200 ml NaOH (1.25%) for another 30 minutes and filtered through a previously-weighed couch crucible. The crucible was then dried with samples in an oven at 100 °C, left to cool in a desiccator and weighed (AOAC, 2000).

The carbohydrate contents were estimated by deduction of the values of all the other components from 100.

Feed Formulation

The raw materials used to formulate the feed for the Wistar rats were procured from Oja-Oba market, Ilorin. The substrates were pounded and sieved with an approximately 60 mesh sieve and the powder mixed with other components (Table 1). A semi-solid mixture was formed by adding 650 ml of distilled water to the mixture. It was oven-dried at 40 °C until a constant weight was obtained. The feeds were compounded with fermented and the unfermented biomass labeled A and B respectively. The Wistar rats used for this study were obtained from animal house of Department of Biochemistry, University of Ilorin and were placed into two groups of three. They were housed in metabolic cages and acclimatized to laboratory conditions for a week where they were given clean water and commercial grower pellets twice daily. After acclimatization, the rats were fed *ad libitum* with the experimental diets for four weeks.

Table 1: Composition of the Experimental Diets

FEED COMPOSITION	FEED A (g)	FEED B (g)	CF (g)
Soybean	280	280	280
Soy oil	50	50	50
Sucrose	80	80	80
Corn husk	40	40	40
Vitamin mix	40	40	40
Methionine	4	4	4
Starch	0	0	506
Fermented biomass	506	0	0
Unfermented biomass	0	506	0
Total	1000	1000	1000

FEED A: Feed formulated with fermented food waste**;**
FEED B: Feed formulated with unfermented food waste
CF: Commercial feed

Preparation of Serum and Tissue Homogenates

At the end of the experiment, animals were anaesthetized with diethyl ether and sacrificed by an incision of the jugular vein and the blood samples were collected for serum analysis. The blood was left to stand at room temperature for 30 mins to clot, then centrifuged at 10,000 rpm for 10 mins. The serum was aspirated and stored in a refrigerator at 4 °C until further analysis.

Isolation and Homogenization of Tissues

The sacrificed rats were dissected and organs of interest (liver, heart and kidney), were removed, wiped with cotton wool to remove blood stains and weighed. They were then transferred into plastic bags and immediately stored on ice. Exactly 1 g of liver, kidney and heart tissues were homogenized with a mortar and pestle in four milliliters of ice-cold 0.25 M sucrose solution and the supernatant stored at -20 °C until further analysis.

Enzyme Assays and Substrates Concentration Determination

Aspartate transaminase (AST) was measured by monitoring the concentration of oxaloacetate hydrazones formed with 2,4-dinitrophenylhydrazine while alanine transaminase (ALT) was measured by monitoring the concentration of pyruvate-hydrazones formed with 2,4-dinitrophenylhydrazine. All enzyme assays were carried out in accordance with Reitman and Frankel method (1957). The triglycerides were determined after enzymatic hydrolysis with lipases. Urea in serum was hydrolysed with urease to ammonia which was then measured photo-metrically by Berthelot's reaction. Alkaline phosphatase was also determined spectrophotometrically based on the enzymes ability to catalyse the conversion of *p*-nitrophenylphosphate to phosphate and *p*-nitrophenol. The creatinine in serum, kidney and liver was determined according to previously reported method (Bartles *et al.*, 1972). Bilirubin was determined according to the colorimetric method described by Jendrassik and Grof (1938), total bilirubin was determined in the presence of caffeine by the reaction with diazotized sulphanilic acid. Albumin concentration was determined using the spectrophotometric measurement of the quantitative binding to the indicator bromocresol green. Total protein was determined by the Kjeldahl method as modified by Williams (1964).

Statistical Analysis

The group mean ± S.E.M were calculated and significant differences between the groups were analyzed using IBM SPSS Statistics 20 software. For all the measurements, one-way analysis of variance (ANOVA) and Duncan's multiple range test at 95% significance level (á=0.05) were used to assess the statistical significance of difference between the groups.

RESULTS/DISCUSSION

The yam and plantain peel mixture (CYP) had a carbohydrate content of about 73% (Table 2) which makes it a potentially good carbon source for constituting animal feeds. It was however found to be low in protein at 9.7% and this is slightly lower than 12.7% reported for pure yam peels by Akinmutimi and Onen (2008). This value was slightly improved by the *Aspergillus niger* fermentation process to about 11.5%. The raw cassava peel (CC) initially had a low protein content of 6.7%, but this was raised considerably to 19.3% (AC), which in addition to the increase to > 48% carbohydrate content, makes it a rich potential animal feed.

Table 2: Proximate Analysis of the Food Wastes

Sample	AC	BC	CC	AYP	BYP	CYP
Carbohydrates (%)	48.61 ± 4.99	68.12 ± 2.34	62.46 ± 0.21	75.84 ± 2.28	76.58 ± 3.53	73.05 ± 2.72
Total protein (%)	19.3 ± 3.10	7.01 ± 1.32	6.73 ± 0.59	11.47 ± 3.53	10.11 ± 2.72	9.69 ± 0.52
Calorific value (kg/100g)	1170.13 ± 40.51	1286.72 ± 26.23	1187.83 ± 28.24	1651.01 ± 34.23	1161.81 ± 32.25	1679.94 ± 21.23
Ash (%)	15.34 ± 0.07	8.34 ± 0.07	14.13 ± 0.29	8.43 ± 0.02	8.26 ± 0.03	8.85 ± 0.07
Crude lipids (%)	0.95 ± 0.24	0.85 ± 0.26	0.86 ± 0.39	1.15 ± 0.32	0.10 ± 0.23	1.65 ± 0.21
Crude fiber (%)	15.79 ± 2.19	15.67 ± 2.01	15.82 ± 0.91	4.89 ± 1.32	4.95 ± 1.28	4.98 ± 0.71
Dry matter (%)	37.34 ± 0.83	41.95 ± 0.52	41.22 ± 0.62	37.78 ± 0.72	39.46 ± 0.78	38.45 ± 0.71

Data expressed as Mean ± SD. KEY: **AC**: *Aspergillus niger*-fermented cassava peel; **BC**: *Trichoderma*-fermented cassava peel; **CC**: un-inoculated cassava peel; **AYP:** *A. niger*-fermented yam and plantain peel; **BYP:** *Trichoderma*-fermented yam and plantain peel; **CYP:** un-inoculated yam and plantain peel

This increase is higher than the just over 8% reported by Doughan and Dzogbefia (2018) in the fermentation of yam peels by *A. niger*. However, crude fiber decline reported by other authors (Adeleke, *et al.*, 2017; Doughan and Dzogbefia, 2018) was not observed in this work. The *Trichoderma spp* fermentation did not result in a similar improvement or alteration in the proximate composition of either of the waste peels (AYP and BYP). Consequently, feeds AC (fermented cassava peel) and CC (unfermented cassava peel) were investigated for their potential to support the growth of Wistar rats.

The fermented cassava peel and the unfermented cassava peels (now renamed FCP and UCP for simplicity) were used to compound feed as shown in table 1 and the rats were fed for the four week-duration of the experiment.

Weight Gain and Organ Weights

Their weights over the experimental period are presented in table 3 below.

Table 3: Weight Trend of Wistar Rats Fed Different Diets over a Four-Week Period

Week	FCP (g)	UCP (g)	CF (g)
One	129.0 ± 4.36^{a}	$129.33 \pm .5.69^{a}$	137.33 ± 10.50^{a}
Two	113.00 ± 19.16^{b}	88.67 ± 2.52^{a}	139.00 ± 11.00^{a}
Three	111.00 ± 19.16^{b}	86.00 ± 3.00^{b}	145.00 ± 9.54^{b}
Four	91.33 ± 19.43^{c}	N/A	155.33 ± 12.90^{c}

Values are expressed as means of five replicates ± SD. Means along a column with different superscripts differ significantly ($P < 0.05$). N/A = Not available, as the experimental rats died. FCP = Fermented cassava peel; UCP = Unfermented cassava peel; CF = Commercial feed.

A significant weight gain was observed in rats in the commercial feed group with a weight gain of 13% of their initial weight, while a significant weight retardation was recorded in the other test groups with the rats fed on *Aspergillus niger*-fermented cassava feed losing almost 30%. The feed may not encourage high food intake thus is less likely to cause obesity. The rats in the group fed the unfermented cassava peels died after three weeks confirming that the *Aspergillus niger* fermentation successfully detoxified the toxic cassava feed. This is similar to the report of

Sulaiman *et al.* (2014) in which an experimental rat fed with a feed based on unfermented jatropha seed cake died during the experiment while none died in the other groups. This also shows that the cassava peels are more toxic to Wistar rats than jatropha cake.

The weights of the individual organs are presented in table 4 below.

Table 4: Average Weight of the Organs of Experimental Rats Fed Various Feeds

GROUPS	LIVER (g)	KIDNEY (g)	HEART (g)
CF	6.50 ± 0.20^{a}	1.40 ± 0.20^{a}	0.53 ± 0.58^{a}
FCP	3.10 ± 1.35^{b}	0.966 ± 0.12^{a}	0.40 ± 0.10^{a}
UCP	7.93 ± 4.40^{c}	2.10 ± 1.00^{b}	0.80 ± 0.46^{b}

Values are expressed as means of five replicates ± SD. Means along a column with different superscripts differ significantly ($P < 0.05$). FCP = Fermented cassava peel; UCP = Unfermented cassava peel; CF = Commercial feed.

All the organs investigated were significantly larger in the UCP-fed group than the FCP- and the CF-fed groups; this implies hepatomegaly in the unfermented (7.93) when compared with the fermented (3.10). There was however no significant difference in the average weight of the kidney and the heart in these two groups (CF and the FCP). This result indicates that the inclusion of *Aspergillus niger* fermented cassava peel in feed may not be harmful to these vital organs.

Organ: Body Weight Ratios

The ratios of the sizes of the organs to the body weight were also calculated and presented in table 5 below.

Table 5: Percentage Organ Body Weight Ratios of Experimental Rats Fed Various Feeds

Group	Liver (%)	Heart (%)	Kidney (%)
CF	2.35 ± 0.29^{a}	0.66 ± 0.31^{a}	0.15 ± 0.05^{a}
FCP	2.14 ± 0.17^{a}	0.32 ± 0.05^{b}	0.18 ± 0.04^{a}
UCP	1.94 ± 0.28^{a}	0.29 ± 0.05^{b}	0.13 ± 0.04^{a}

Values are expressed as means of five replicates ± SD. Means along a column with different superscripts differ significantly ($P < 0.05$). FCP = Fermented cassava peel; UCP = Unfermented cassava peel; CF = Commercial feed.

There was no significant difference in the percentage organ: weight ratios of the liver and kidney among the groups of experimental rats. A significant decrease was however recorded in the percentage heart: weight in the UCP and FCP groups. This result suggests that the compounded feeds do not unfavourably increase the ratio of the rats' organs relative to body weight.

Enzyme/Metabolite Assay

Total Protein

Significantly lower protein was recorded in all samples tested in the FCP group except in the kidney where 2.95 mg/dl obtained was higher than the 2.48 mg/dl in the UCP group (Table 6). The concentration of total protein is a useful 'marker' of secretory, synthetic and excretory functioning of the liver (Yakubu *et al*., 2007). No significant alteration was recorded in the FCP when compared with the control. This suggests that the prolonged feeding of the FCP to the rats have no effect on the liver and the heart but may have a slight effect on the kidney.

ALP

Table 6 shows the ALP activity in various tissues. It was observed that there was a significant reduction in ALP activity in the serum of the group fed with fermented cassava peels feed compared with the unfermented peel group;

whereas, there was a significant increase in the heart and liver relative to the unfermented peel group. ALP is present in all tissues throughout the body, but is particularly concentrated in the liver, bile duct, kidney, bone, intestinal mucosa and the placenta (Wolf, 1999). ALP is a 'marker' enzyme for the plasma membrane and endoplasmic reticulum (Wright *et al.*, 1972) and is often used to assess the integrity of the plasma membrane (Akanji *et al.*, 1993). The increase in the ALP activity in the liver and the heart without a concomitant leakage into the serum may be due to increased activity of the enzyme.

Table 6: Activity/Amounts of Some Key Enzymes/Substrates in Serum and Organs of Experimental Rats Fed with Various Feeds

BIOCHEMICAL PARAMETERS	SERUM		LIVER			HEART			KIDNEY		
	CF	FCP	UCP	CF	FCP	UCP	CF	FCP	UCP	CF	FCP	UCP
Total protein (mg/dl)	1.09±0.35[a]	0.67±0.49[a]	3.23±3.42[b]	1.19 ±0.52[a]	1.05±0.74[a]	1.53±0.51[b]	3.15±0.02[a]	3.01±0.35[a]	3.29±0.21[a]	0.67 ±0.09[a]	2.95±1.94[b]	2.48±1.68[b]
Alkaline phosphate (ALP) activity (u/l)	241.96± 51.29[a]	155.48 ± 59.60[b]	278.76 ±115.03[c]	1112.08 ±165.94[a]	1077.32 ± 12.45[a]	1054.32 ±57.03[a]	184.92 ±75.13[a]	272.32 ±144.22[b]	158.24 ±26.81[c]	-	-	-
Aspartate transaminase (AST) activity (u/l)	3.91± 0.41[a]	2.51± 1.00[b]	4.49 ±1.72[c]	17.78 ±2.41[a]	17.57±0.58[a]	17.36±1.65[a]	2.83 ±1.33[a]	4.26 ±2.29[b]	2.96 ±0.99[a]	-	-	-
Alanine transaminase (ALT) activity (u/l)	6.35± 4.59[a]	4.21 ±1.48[b]	7.33 ±1.16[c]	27.89 ±3.90[a]	27.54 ±0.98[a]	27.45 ±2.38[a]	4.65 ±1.79[a]	6.91 ±3.78[b]	4.77 ±1.47[a]	-	-	-
Albumin (mg/dl)	2.80± 0.46[a]	2.37±1.92[a]	4.85±0.63[b]	3.42 ±0.43[a]	3.60±0.98[a]	4.97±1.38[b]	1.43 ±0.82[a]	1.53±0.44[a]	2.45±0.22[b]	-	-	-
Total bilirubin (mg/dl)	7.79± 1.82[a]	9.10 ± 1.17[b]	14.59 ±1.62[c]	2.73 ±0.67[a]	2.25 ±0.41[b]	3.69 ±0.21[c]	1.43 ±0.19[a]	1.18 ±1.21[a]	2.80 ±0.25[b]	-	-	-
Direct bilirubin (mg/dl)	1.87± 0.84[a]	0.76 ± 3.01[b]	3.96 ±2.11[c]	2.00 ±0.80[a]	2.06 ±0.09[a]	6.44 ±0.33[b]	0.46 ±0.17[a]	0.79 ±0.12[b]	0.84 ±0.10[b]	-	-	-
Y-glutamyl transferase(GGT) (mg/dl)	212.43±47.97[a]	225.42±16.79[a]	229.93±61.95[a]	335.56±23.99[a]	298.63±32.97[a]	336.85±25.52[a]	255.66±1.64[a]	324.49±49.41[b]	297.4±2.4.55[a,b]	-	-	-
Cholesterol concentration (mmol/l)	0.03± 0.00[a]	0.03 ± 0.01[a]	0.13 ±0.17[b]	0.05 ±0.00[a]	0.05 ±0.03[a]	0.09 ±0.02[b]	0.03 ±0.01[a]	0.03 ±0.00[a]	0.07 ±0.01[b]	-	-	-
HDL-cholesterol concentration (mg/dl)	0.06± 0.01[a]	0.04 ± 0.01[a]	0.01 ±0.02[b]	0.14 ±0.06[a]	0.16 ±0.11[a]	0.07 ±0.06[b]	0.06 ±0.36[a]	0.04 ±0.01[a]	0.02 ±0.06[a]	-	-	-
Triglycerides (mg/dl)	0.09± 0.02[a]	0.08 ± 0.01[a]	0.07 ±0.01[a]	0.23 ±0.03[a]	0.25 ±0.03[a]	0.32 ±0.02[b]	0.19 ±0.09[a]	0.16 ±0.02[a]	0.19 ±0.15[a]	-	-	-
Urea concentration (mg/dl)	0.02± 0.00[a]	0.03 ± 0.20[a]	0.03 ±0.00[a]	-	-	-	-	-	-	0.03. ±0.00[a]	0.03±0.01[a]	0.09 ±0.01[b]
Creatinine concentration (mg/dl)	0.13± 0.11[a]	0.22±0.033[b]	0.21±0.22[b]	-	-	-	-	-	-	0.10 ±0.09[a]	0.20±0.024[b]	0.18±0.32[b]

Values are expressed as means of five replicates ± SD. Means along a column with different superscripts differ significantly ($P < 0.05$). FCP = Fermented cassava peel; UCP = Unfermented cassava peel; CF = Commercial feed

AST

The AST activities in the sera were significantly lower among the FCP than the CF group with the highest AST activity being the one in the UCP group (Table 6). The AST activity was the same in the liver of all groups but elevated in the heart of the FCP group. The aminotransferases (ALT and AST) are 'markers' of liver damage and thus can be used to assess liver cytolysis (Pramyothin *et al.*, 2006; Sulaiman *et al.*, 2014). The decreased activity of AST in the serum and increased activity in the liver showed that the membrane of the tissue is not compromised by the feeds and prolonged diet have no deleterious effect on the liver.

ALT

ALT is a more sensitive biomarker of hepatotoxicity than AST (Pramyothin *et al.*, 2006). ALT levels in human serum has proven to be a valuable indicator of the liver function in clinical settings (Huang *et al*., 2006; Sulaiman *et al*., 2014). In the group fed fermented cassava peel-based feed, significantly lower ALT activity values were recorded in the serum but higher values in the heart and similar values in the liver when compared with that of the group fed the unfermented cassava peel (Table 6). This suggests a possible enzyme leakage from the liver or other tissues into the serum and this may destabilize the liver's integrity.

Albumin

Albumin is the most abundant blood plasma protein and it is produced in the liver and form large proportion of all plasma protein. There was a significant increase in albumin concentration in the serum, heart and liver of group fed UCP compared to others which weren't significantly different (Table 6). Low albumin is usually as a result of liver diseases or burns. The concentration of albumin is a useful 'marker' of secretory, synthetic and excretory functioning of the liver and kidney (Yakubu *et al*., 2007). Low albumin recorded in the serum as well as the high concentration in the liver indicates that the diet of unfermented cassava peels fed for prolonged period of time may have impaired albumin synthesis in the liver as well as mal-absorption of albumin.

Total Bilirubin

Bilirubin is made in the body when the haemoglobin protein in the old red blood cells is broken down. After circulating in the blood, bilirubin then travels to the liver. In the liver, bilirubin is conjugated, mixed into bile, and then excreted into the bile ducts and stored in the gall bladder. An increase in bilirubin concentration in the serum is called jaundice, which is a symptom of some forms of liver disease. There was no significant difference in total bilirubin content between the group fed commercial feed and that fed the fermented peels except in the serum where it was slightly higher. Whereas, similar to the findings in other parameters including albumin and total protein, the group fed unfermented cassava peels feed had significantly higher total bilirubin. Interestingly, the bilirubin content was even lower in the heart and liver of the FCP group than the CF group.

From the experiment, the increase in total bilirubin concentration in the serum of the UCP group may suggest inadequate bilirubin clearance, thus indicating that prolonged feeding might result in a liver disease or liver damage (Nuhu and Aliyu, 2008; Sulaiman *et al*., 2014).

Direct Bilirubin

The livers of the UCP-fed group had more than thrice the direct bilirubin content of those of other groups (Table 6). Similarly, fermentation also reduced the effect of cassava peel on serum bilirubin content to under a fifth of the original value of 3.96 mg/dl. Bilirubin is a tetrapyrole and a breakdown product of heme catabolism. Most bilirubin (70% - 90%) is derived from haemoglobin degradation and, to a lesser extent, from other hemo proteins. This decreased direct bilirubin level in the serum and heart suggests that *Aspergillus niger* fermentation of cassava peels may help eliminate any risks of toxicity to the organs of animals even if fed for a prolonged period of time.

γ-Glutamyl Transferase (GGT)

GGT in serum originates primarily from the hepatobiliary system. Therefore, GGT is elevated in all forms of liver diseases (Courtay *et al.,* 1992) and has being shown to be more sensitive in detecting obstructive jaundice, cholangitis and

cholecystitis (Berk and Korenblat, 2007). GGT concentration in the hearts of the rats in the UCP group was found to be significantly lower than in FCP while higher than in CF. Increased level of GGT in the liver than in the serum was observed in all groups which indicated an intact liver.

Cholesterol Concentration

Cholesterol is a sterol, a type of lipid molecule which is an essential component of all animal cells membrane and it is used in the synthesis of hormones and Vitamin D (American Heart Association, 1999). It is carried around the body by lipoproteins of which there are two types: high density lipoprotein (HDL) and low density lipoprotein (LDL). Table 6 shows that no significant differences were observed in the groups fed fermented and commercial feeds, while the UCP group showed significantly higher cholesterol in all tissues evaluated. Increased cholesterol levels cause atherosclerosis, so a diet containing unfermented cassava peels may result in cardiovascular diseases over time.

HDL-Cholesterol Concentration

High Density Lipoprotein (HDL) is one of the five major types of lipoprotein. It is termed "good" cholesterol because it removes cholesterol from other parts of the body and bringing it to the liver. The liver then removes the cholesterol from the body. Across the liver, heart and serum tested it was observed that HDL was lowest in the UFP-fed group. There was a significant difference in the control group and the rats fed with unfermented cassava peels feed, however there was no significant difference between the rats in commercial groups and the other groups. Increased levels of HDL in the serum, heart and liver indicates that the diets of fermented cassava peels feed helps in the removal of cholesterol from the body.

Triglycerides

Triglyceride content is related to cholesterol concentration, and if it surrounds the membrane of the heart, excessive triglycerides may cause cardiovascular diseases. The other feeds resulted in low concentration of triglycerides in the serum (Table 6), which indicates that it does not accumulate in the heart and hence, it may not cause cardiovascular disease.

There was no difference observed in the triglyceride contents across all organs and feeds, except a significant increase in the liver in the group fed unfermented cassava feed compared with the others.

Urea Concentration

Urea is the major end product of protein catabolism in animals and is the primary means of the removal of toxic ammonia from the body. It is primarily produced in the liver and excreted by the kidney. Urea determination is very useful for clinicians in accessing kidney function of patients. There was no significant difference in the urea contents of the sera of all the groups (Table 6), however the UCP group's kidneys revealed significantly higher urea. Increased urea levels are associated with nephritis, renal ischemia and urinary tract obstruction. Therefore, the consumption of fermented cassava feed is unlikely to cause uremia in animals.

Creatinine Concentration

Creatinine is a breakdown product of creatine phosphate in muscles. It is usually produced at fairly constant rate by the body and filtered out of the blood by the kidney. During the reaction involving creatine and phosphocreatine catalyzed by creatine kinase, spontaneous conversion to creatine might occur (Brosnan and Brosnan, 2007). Fermentation of the cassava peels didn't result in a significant difference in the serum or kidney creatinine levels in the FCP- and UCP-groups, which were both significantly higher than the control group fed the standard feed (Table 6). The rise in creatinine blood level suggests a deficiency in the filtering capacity of the kidney.

From this experiment, the creatinine production in the serum and kidney was found to be significantly higher in both UCP and FCP than the control, which indicates that diet of fermented cassava peel may have a deleterious effect on the functioning nephrons and prolonged diet may result in kidney impairment (Bartles *et al.,* 1972).

CONCLUSION

Solid state fermentation of cassava peels with *Aspergillus niger* for seven days resulted in improved nutritional profile of the CP which is an abundant agro-waste in Nigeria, suggesting it

could be used as a low-cost commercially-produced animal feed. A bioassay using Wistar rats demonstrated the effects of fermentation on cassava peel in a living system. Most of the biomarkers showed that fermentation significantly decreased the toxicological effect and improved the tolerance of this otherwise toxic agro-waste. This work thus shows that enriching waste cassava peels by *A. niger* fermentation could potentially generate increased value for the cassava value-chain while also ameliorating the environmental pollution caused by the indiscriminate disposal of the cassava peels. Subject to further investigations to confirm its effects on the filtering capacity of the kidney and develop mitigation strategies if found necessary, *Aspergillus niger*-fermented cassava peels are thus recommended for use as a protein-enriched carbohydrate source for various animal feeds.

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