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## Growth Performance, Genotoxicity and Histopathology of The Tissues of African Catfish (*Clarias Gariepinus*) Fed Fermented Ginger peel Meal

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### ABSTRACT

Fermented ginger peel meal (*Zingiber officinale*) was evaluated as a substitute for maize in the diet of *Clarias gariepinus* fingerlings over a period of 60 days. Ginger peels were fermented using pure cultures of *Aspergillus niger* fungus. The weekly weight gain was highest in fishes fed with 50% FGPM and was significantly higher ( $P>0.05$ ) than fishes fed with control, 10%, 20% and 30% FGPM. The digestibility results showed that the P value is greater than 0.05 ( $P>0.05$ ), there was no statistically significant difference between the control and all other digestibility parameters. Histological sections of the liver of all the fishes fed with the experimental diets, showed a liver with moderate fatty change, except with fishes fed with 30% FGPM which had a liver with severe fatty change. Also, the micronucleus test of the experimental fishes showed little nuclear abnormalities in fishes fed with 30% and 50% FGPM. This study indicated that fermented ginger peel meal could replace maize in the diet of *Clarias gariepinus* up until 50% inclusion without any adverse effect on the growth and nutrient utilization.

**Keywords:** Ginger peel, fermentation, micronucleus, histological.

### 1. Introduction

The scarcity and high cost of feed ingredients has resulted to an increasing cost of fish. This is because the conventional feedstuffs are equally consumed by man, an example is maize. This phenomenon has led to different researches into the possibility of replacing them with materials which are regarded as waste by man<sup>[1]</sup>. Several researches have been done to determine the possibility of replacing conventional feeds with unconventional feeds like: orange peels<sup>[2]</sup>, corn cob<sup>[3]</sup>, kola pod husk<sup>[4]</sup>, bread waste<sup>[5]</sup>, shrimp waste<sup>[1]</sup>, yam peels<sup>[6]</sup> etc. Because of the availability and easy digestibility, maize serves as a major source of dietary energy in most compounded diets for culturable fish. However, this ingredient is highly competed for by man and livestock<sup>[7]</sup>.

Ginger peels is generally considered as a household waste and it generally regarded as useless. Ginger is consumed as a spice in the domestic setting and it is used for beverage production in the industries. Ginger contains about 42% starch, 8% crude fibre, 1% of lime, oleoresins and essential volatile oils (1-3%). The volatile oil is yellowish, viscid liquid, sparingly soluble in water and contains the sesquiterpene zingiberene and its alcohol zingiberol ( $C_{15}H_{26}O$ ) which imparts the characteristic odour to the rhizome. The pungent principle of ginger is the oleoresin-zingerone which gives the sharp, bity taste. Zingerone is chemically 3-methoxy-4-hydroxy-phenyl- ethyl methyl-ketone ( $C_{11}H_{14}O_3$ )<sup>[8]</sup>.

Ginger contains several phytochemicals which are anti-nutrients. One of the processing methods that have been shown to significantly reduce or eliminate the level of anti-nutrients and also improve the nutritional quality of plant materials is solid fermentation using fungi<sup>9, 10, 11</sup>. Fermentation enhances the quantity, quality and bioavailability of nutrients, mineral elements, vitamins, essential amino acids and protein, by improving protein and fibre digestibility.

Fermentation gradually changes the characteristics of the food by the action of enzymes, produced by some bacteria, moulds and yeasts<sup>[12]</sup>.

This study aims to determine the growth performance, genotoxicity and histopathology of the tissues of African catfish (*Clarias gariepinus*) fed fermented ginger peel meal.

## 2. Materials and Methods

### 2.1 Substrate preparation

Fresh ginger rhizomes were purchased at Mile 12 market, Lagos state. They were washed with distilled water and allowed to air dry for few hours. A clean knife was used to scrape off the peels from the rhizomes after which the peels were oven-dried at 40 °C for 24 hours. The dried ginger peels were then blended to a powdery form, using a clean, dry electronic blender. The blended ginger peels were then stored in sterile polythene bags.

### 2.2 Fermentation of ginger peels

Three plates of pure cultures of *Aspergillus niger* was sub cultured to prepare 100 plates of pure cultures of *Aspergillus niger* fungus. The dried ginger peels was sterilized in an autoclave at 121 °C for 30 minutes<sup>[13]</sup>. The spores of the fungi was scraped from the plates into 1000ml sterile distilled water using a sterile spatula. The sterilized ginger peels was added to the water under an aseptic condition. The set up was sealed and placed inside a dark, sterile cupboard. It was allowed to ferment for 2 weeks and then dried in the oven at 45 °C for four hours.

### 2.3 Feed preparation

The fish feed was formulated at Sabo market Ikorodu Lagos. The feed ingredients was purchased at Champion feeds, Ikorodu. Five treatment groups were represented by 5 isocaloric and isonitrogenous diets, labeled A to E. The feed ingredients include: maize, wheat flour, soya bean cake, ground nut cake, fish meal, wheat offal, vitamin c, fish premix, bone meal, salt, palm oil, lysine and methionine. The fermented ginger peel was substituted for maize in the feed at 10%, 20%, 30% and at 50%. 5kg of fish feed was formulated for each concentration. The ingredients grinded into powdery form were thoroughly mixed together. The mixed feed ingredients were then fine crushed and made into pellets using a 2mm pelletizing machine. The pellets were dried to prevent the growth of microorganisms on it and later stored in jute bags.

### 2.4 Experimental procedure

150 fingerlings of *Clarias gariepinus* weighing average 0.3 g were procured from a reputable fish farm at Iyana-Ipaja, Lagos and transported to Environmental Biology Laboratory of Yaba College of Technology, Lagos. The fingerlings were acclimatized in the laboratory for two weeks, after which 150 fingerlings were randomly allotted into each of the five isocaloric and isonitrogenous diets: control, 10% FGPM, 20% FGPM, 30% FGPM and 50% FGPM, of the experimental feed and each treatment was replicated thrice in a completely randomized designed experiment. The fingerlings were starved for 60 hours before the feeding trial commenced. Ten fingerlings were allotted to each bowl and feeding was done *ad libitum* for eight weeks. Water supply was from Environmental Biology laboratory, stale water was drained off every day to prevent distortion of the water ecosystem and fouling. The fishes were weighed on a weekly basis and the feed was

adjusted accordingly. Faecal samples were collected in the fourth week and eight week and analyzed for digestibility. Unconsumed feed was siphoned using a sieve, sun dried and weighed. The weights were recorded and the feed was analyzed for digestibility. Mortality of the fishes was monitored weekly and recorded.

### 2.5 Analytical Procedure

Digestibility test of feed and faeces was carried out using Acid Insoluble Ash method (AIA), as described by Halver *et al*<sup>[14]</sup>. Proximate analysis of unfermented ginger peels, fermented ginger peels, the experimental diets and faeces were determined by Association of Official Analytical Chemists<sup>[15]</sup>. The phytochemical screening was done on the sample using methods as described by Sofowara<sup>[16]</sup>.

### 2.6 Micronucleus test

Micronucleus test was done using the method used by Alimba *et al*<sup>[17]</sup>.

### 2.7 Statistical analysis

Data obtained from the experiment was subjected to Analysis of Variance (ANOVA), using SAS<sup>[18]</sup>.

## 3. Results

**Table 1:** Growth Response and Nutrient Utilization of Experimental Fishes

	A	B	C	D	E
Initial weight	2.65	2.72	2.60	2.92	2.82
Final weight	4.81	5.69	6.51	8.77	9.77
Mean weight gain	4.10	3.97	5.11	5.96	6.96
Specific growth rate	0.432	0.567	0.582	0.795	0.897
No. of mortality	13	12	14	7	13
% mortality	43.33	40.00	46.67	23.33	43.33
% survival rate	56.7	60	53.3	76.7	56.7
Experimental period	60	60	60	60	60
Number of fish	30	30	30	30	30

Key:

- A = Control Treatment without Fermented Ginger peel
- B = Treatment With 10% Fermented Ginger Peel Meal
- C = Treatment With 20% Fermented Ginger Peel Meal
- D = Treatment With 30% Fermented Ginger Peel Meal
- E = Treatment With 50% Fermented Ginger Peel Meal

**Table 2:** Mean Weekly Weight Gain (G)

	Control	10% FGPM	20% FGPM	30% FGPM	50% FGPM
Week One	3.09±0.12 <sup>a</sup>	2.84±0.22 <sup>a</sup>	4.53±0.02 <sup>b</sup>	4.52±0.04 <sup>b</sup>	5.62±0.03 <sup>c</sup>
Week Two	3.56±0.06 <sup>a</sup>	3.60±0.03 <sup>a</sup>	4.64±0.07 <sup>b</sup>	4.81±0.07 <sup>b</sup>	5.77±0.06 <sup>c</sup>
Week Three	3.88±0.07 <sup>a</sup>	3.81±0.11 <sup>a</sup>	4.80±0.14 <sup>b</sup>	5.09±0.06 <sup>b</sup>	6.12±0.12 <sup>c</sup>
Week Four	4.09±0.07 <sup>a</sup>	3.86±0.09 <sup>a</sup>	4.91±0.01 <sup>b</sup>	5.29±0.01 <sup>b</sup>	6.42±0.06 <sup>d</sup>
Week Five	4.29±0.04 <sup>b</sup>	3.99±0.02 <sup>a</sup>	4.99±0.02 <sup>b</sup>	5.85±0.15 <sup>d</sup>	6.59±0.03 <sup>d</sup>
Week Six	4.48±0.03 <sup>a</sup>	4.06±0.01 <sup>a</sup>	5.15±0.02 <sup>b</sup>	6.58±0.43 <sup>c</sup>	6.94±0.12 <sup>c</sup>
Week Seven	4.63±0.02 <sup>a</sup>	4.45±0.08 <sup>a</sup>	5.39±0.06 <sup>b</sup>	7.63±0.07 <sup>c</sup>	8.84±0.08 <sup>d</sup>
Week Eight	4.42±0.51 <sup>a</sup>	5.66±0.04 <sup>b</sup>	6.60±0.09 <sup>c</sup>	8.67±0.25 <sup>d</sup>	9.83±0.06 <sup>c</sup>

Figures on the same row having the same superscript are not significantly different (p>0.05).

Keys:

- a = Control Treatment without Fermented Ginger Peel
- b = Treatment with 10% Fermented Ginger Peel Meal
- c = Treatment with 20% Fermented Ginger Peel Meal
- d = Treatment with 30% Fermented Ginger Peel Meal
- e = Treatment with 50% Fermented Ginger Peel Meal

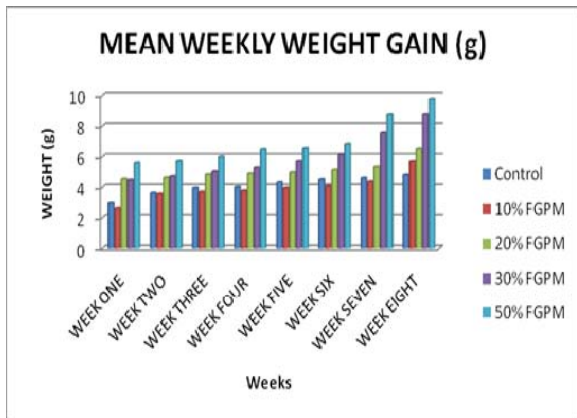
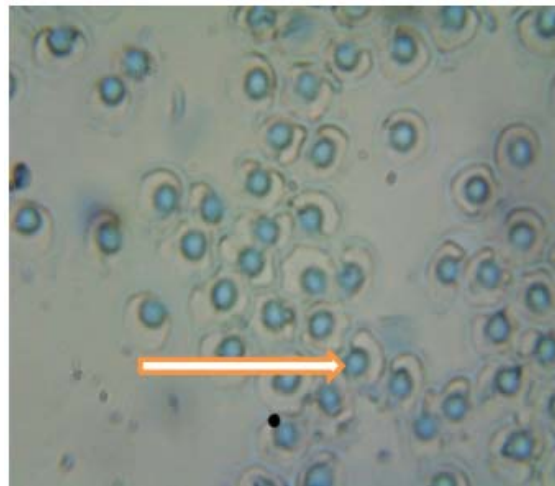


Fig 1: mean weekly weight gain (g)

Table 3: ANOVA of Digestibility Results Analysis of Variance

	Sum of Squares	Df	Mean Square	F value	p value
Between Groups	225.478	3	75.159	0.187	0.904
Within Group	6438.311	16	402.394		
Total	6663.789	19			

Since the p value (0.904) is greater than 0.05, there is no statistically significant difference between the control and all other digestibility parameters.



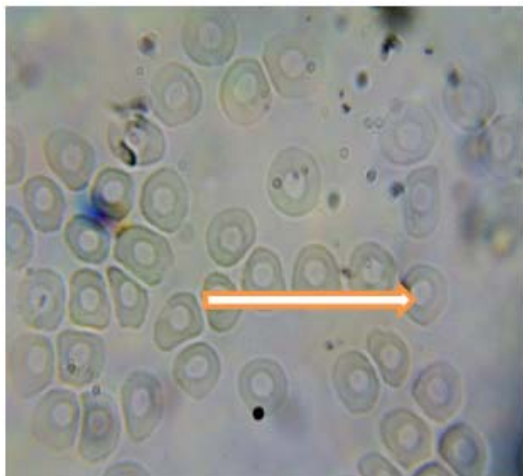
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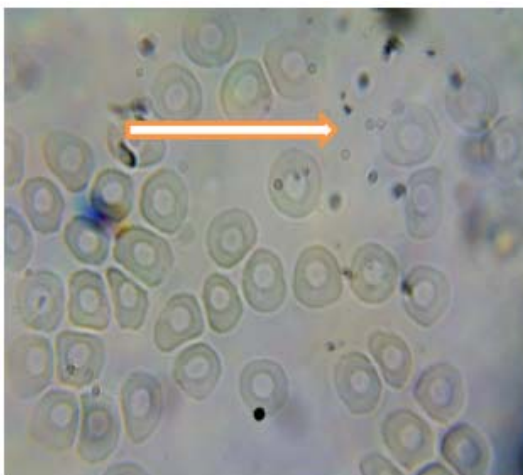
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Fig 2: Red blood cells abnormal and normal morphologies (arrowed) observed in catfishes fed varying diets of fermented ginger peel meal.

**Micronucleus test**



A



B

- A: showing normal nuclei (control)
- B: showing micronuclei (10% fgpm)
- C: showing notched nuclei (30% fgpm)
- D: showing bi-nuclei (50% fgpm)

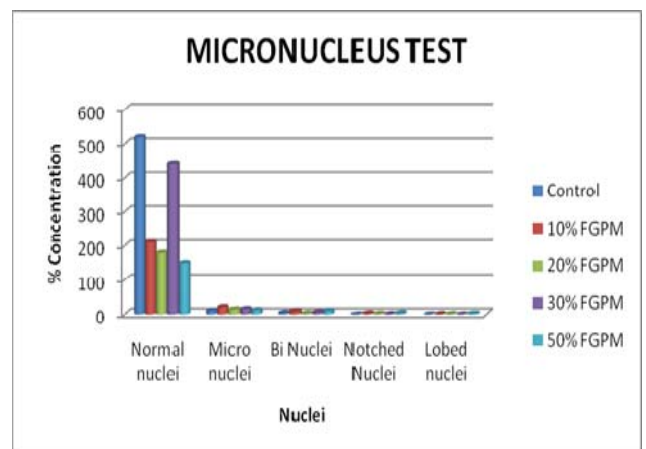
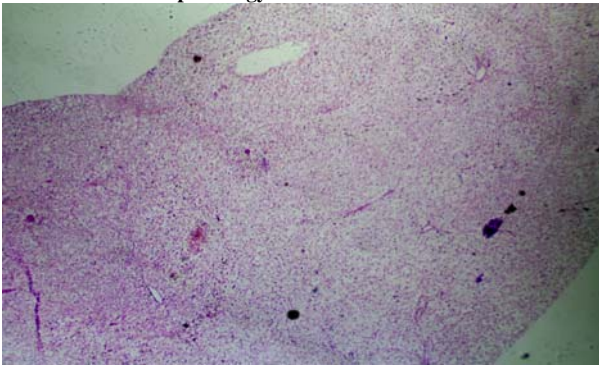


Fig 3: Graphical representation of Micronucleus Test

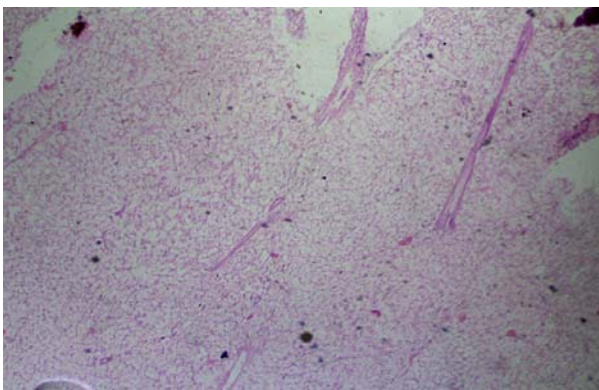
**Histopathology of the Liver of Catfish**



**Fig 4:** Histological sections of fish fed with control diet.

Fragments of liver tissue showing regular hepatocytes in cords and plates with normal portal tracts and central veins. There is white infiltration of the hepatocytes by tubules of matured adipocytes.

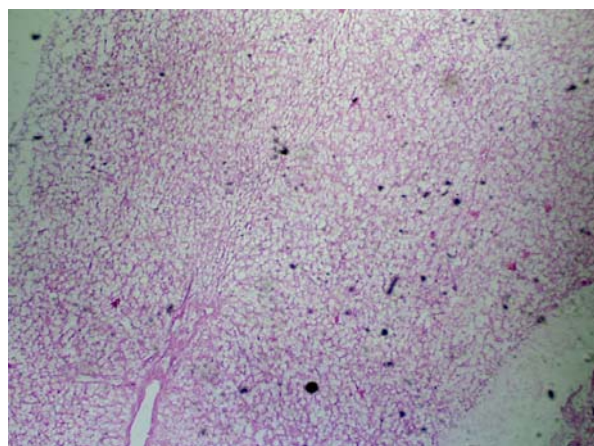
Conclusion: Normal liver with mild steatosis (fatty change)



**Fig 5:** Histological sections of fish fed with 10% FGPM

Liver tissue with regular hepatocytes in sheets and cords. There are regular portal tracts and central veins. Sections show moderate infiltration of the hepatocytes cytoplasm by tubules of matured adipocytes.

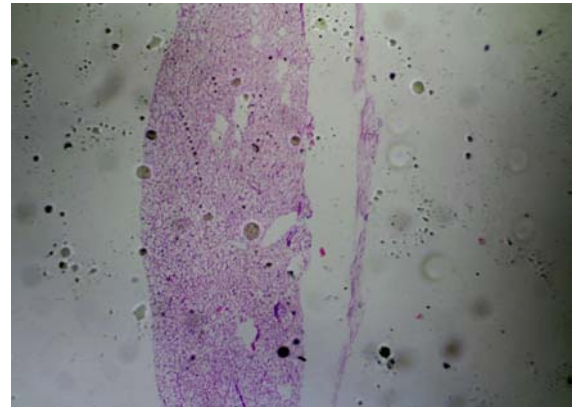
Conclusion: Normal liver with moderate fatty change.



**Fig 6:** Histological sections of fish fed with 20% FGPM

Liver tissue with sheet and cords of regular hepatocytes. Also seen are regular central veins and portal tracts. There is moderate infiltration of the hepatocyte cytoplasm by lobules of matured adipocytes.

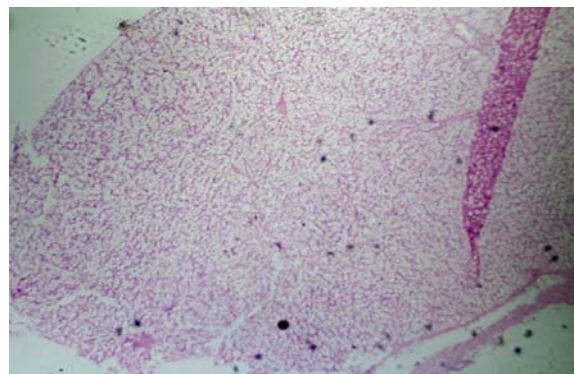
Conclusion: Normal liver with moderate fatty change.



**Fig 7:** Histological section of fish fed with 30% FGPM

Liver tissue with sheets and cords of regular hepatocytes as well as central veins and portal tracts containing the portal triads. There is intense infiltration of the hepatocyte cytoplasm by lobules of matured adipocytes.

Conclusion: Liver with severe fatty change.



**Fig 8:** Histological sections of fish fed with 50% FGPM

Histological sections show liver tissue composed of regular hepatocytes in sheets and cords.

Regular central veins and portal tracts containing the portal triads are also seen. Parts of section show hepatocytes with intra-cytoplasmic infiltration by matured adipocytes.

Conclusion: Liver with moderate fatty change.

**4. Discussion**

The major source of carbohydrate in the feed of most livestock fishes is maize. Other dietary carbohydrate sources already fed to some species of fish/ livestock includes cassava peel, orange peel, Almond fruit, Sorghum and wheat etc. In this study, five experimental diets were formulated with differently graded levels of fermented ginger peels, for *Clarias gariepinus*.

The increase in protein of the fermented ginger peels may be due to the fact that the fungus may have secreted extracellular enzymes in the peels which subsequently increase the protein content of the fermented sample. The significant reduction of the lipid content of the fermented ginger peel meal may be an indication that the fungus utilizes the lipid as a source of carbon and energy for growth and perhaps for the synthesis of protein.

Figure 1 revealed the mean weekly weight gain of the experimental fishes from week one to week eight. The graph showed that fishes fed with 50% FGPM have the highest weight ( $p>0.05$ ) from week one to week eight. This may be due to the low level of crude fibre (1.90%) in the fermented ginger peels, as against the 2.20% level

of crude fibre in maize. The metabolizable energy content in fermented ginger peel is also higher than that of maize. This may be the reason why the experimental fishes fed with 40% and 50% fermented ginger peels meal grew better than those fed with the control diet.

The survival rate was highest in 30% FGPM (76.7%) and lowest in 20% FGPM (53.3%). This may be due to the fact that 30% FGPM has the lowest level of crude fibre when compared with the crude fibre content of 20% FGPM. The level of mortality can be attributed to the environmental conditions of the animal house.

The digestibility results showed that the P value is greater than 0.05 ( $P > 0.05$ ). There was no statistically significant difference between the control and all other digestibility parameters. The graphical representation of the digestibility test showed that the NFE in faeces, NFE in feed, AIA values per feed and AIA values per faeces increased significantly from the control diet up to 50% FGPM. This study is in agreement with that of Agbabiaka *et al.*<sup>[19]</sup>, who concluded that tiger nut can be used as a replacement for maize in the diet of *Clarias gariepinus* at an inclusion level not beyond 50% for cat fish production.

Histological sections of fishes fed with control diet showed that the liver was normal with mild fatty change. The mild fatty change might be attributed to the high fat content of catfishes. Fishes fed with 10%, 20% and 50% FGPM had moderate fatty changes in their liver tissues. While fishes fed with 30% FGPM had severe fatty change in their liver tissues. This finding agrees with that of James *et al.*<sup>[20]</sup>.

The micro nucleus test revealed that there is minimal abnormality observed in all the experimental fishes. The normal nucleus count was highest in the control diet, while there was minimum micronucleus count in the control. Also, the normal nucleus count of fishes fed with other concentrations of feed is also high. This showed that the experimental feeds have no effect on the haematological parameters of fishes.

This study indicated that fermented ginger peel meal could replace maize in the diet of *Clarias gariepinus* up until 50% inclusion without any adverse effect on the growth and nutrient utilization.

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