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Histopathological effects of untreated ginger peel (*Zingiber officinale*) fish meal on the intestinal tissue profiling of African catfish (*Clarias gariepinus*)

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Abstract
Varied histopathology changes were observed in the intestine of *Clarias gariepinus* fed with unfermented ginger peel fish meal. The juvenile catfishes were exposed to different inclusion levels (10%, 20%, 30% and 50%) of unfermented ginger peel in meals over a period of 4weeks. Phytochemical analysis of the peel was shown the presence of tannins, terpenoids amongst others. Degeneration of the columnar epithelia, circular muscle fibre and intestinal submucosa were extensively observed, likewise phenomenal mortality counts. These aberrations could be due to some chemicals inherent in untreated ginger peel, therefore not considered suitable as substitute or additive in fish feed.

Keywords: Histopathology, intestine, unfermented ginger peel, *Clarias gariepinus*.

1. Introduction
The vigorous and unflinching efforts for substitute of fish feed ingredients has been recognized as a major stead out of the dark room enveloping aquaculture development in developing countries [1]. Consequently, the fish nutritionist world is consistently considering alternative protein and carbohydrate feed ingredients.
Ginger, a rhizome, is a delicacy and widely used in medicine. It is a perennial herb with irregular lobe and yellow – brownish in colour. The rhizome (*Zingiber officinale*) belongs to the family Zingiberaceae. There are different types of ginger, depending on the form of rhizomes. These include: African ginger, Indian ginger, Japanese ginger, Jamaican ginger [2].
In the choice of ginger, economical factor was considered, hence the use of what is meant to be waste was chosen, "Ginger Peel".
The study focused on the application of untreated ginger peel as a substitute for maize in the nutrition of *Clarias gariepinus*.

2. Material and Methods
2.1 Materials Required
Fresh ginger rhizomes, hot air oven, mercury in glass thermometer, P^H meter, transparent ruler, electric weighing balance, grinding machine, local pelletizing machine, 10 litre plastic bowl, 40 litres of water holding tank, sieve, net, measuring cylinder, latex medical hand gloves, knife, cotton wool, electric blender.

2.2 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 ^oC for 24 hours. The dried ginger peels were then blended with a powdery form, using a clean, dry electronic blender. The blended ginger peels were then stored in sterile polythene bags.

2.3 Feed Preparation
The fish feed was formulated at Sabo market Ikorodu, Lagos. The feed ingredients were 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 unfermented ginger peel was substituted for maize in the feed at 10%, 20%, 30% and 50%. 5 kg 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 2 mm pelletizing machine. The pellets were dried to prevent the growth of microorganisms on it and later stored in jute bags.

2.4 Phytochemical Analysis of Ginger Peel

1. Test for phlobatamin

Boil the extract, filtrate with 20% HCL solution. A red precipitate appears which shows the presence of phlobatamin.

2. Test for flavonoids

I added the extract to dilute NaOH and HCL (concentrated) was added. A yellow solution turns colourless which indicates the presence of flavonoids.

3. Test for steroids

2 ml of acetic anhydride was added to extract with 2 ml of H_2SO_4 was added. The colour changes from violet to blue or green in some samples, indicating the presence of steroids.

4. Test for glycosides

5 ml of each extract was treated with 2 ml of glacial acetic acid containing one chop of ferric chloride solution. This was underlay with 1ml of concentrated H_2SO_4 a brown ring of the interface indicate a deoxysugar characteristics of Cardenolides.

5. Test for terpenoids

5 ml of each extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colour of the interface was formed to show positive results for the presence of terpenoids

6. Test for reducing sugar

The extract was shaken with dilute distilled water and the filtrate was boiled with drops of Fehling solution A and B for minutes. An orange red precipitate indicate the presence of reducing sugar.

7. Test for alkaloids

The extract was warmed with 20% of H_2SO_4 for 2 minutes, filtered and few drops of dragendorff reagent were added. An orange- red precipitate indicates the presence of alkaloids.

8. Test for anthraquinones

The extracts were boiled with 10% HCL for a few minutes, in a water bath. It was filtered and allowed to cool. Also equal volume of CCL_3 was added to the filtrate.

Few drops of 10% NH_3 were added and heated. A formulation of rose-pink indicates the presence of anthraquinones.

9. Test for tannins.

To the extract filtrate, a few drops of 0.1% ferric chloride and observe for brownish green or a blue coloration.

10. Test for saponin

10 ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth.

2.5 Reagent Used

20% of Hcl, NaOH, concentrated HCL, acetic anhydride, H_2SO_4, glacial acetic acid, Ferric chloride(0.1%), chloroform, Fehling's solution A and B,2% of H_2SO_4, Drangindoff reagent,10% HCL , CCL_3,10% NH_3.

2.6 Experimental Procedure

The juvenile catfish was starved for 60 hours before the feeding trial commenced. Five *juvenile* catfish was allotted to each bowl and feeding was done in four *weeks*. Water supply was from the 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. Fecal 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.

2.7 Fish Allotment and Feeding

The fish samples were fed with different concentration of unfermented ginger peel fish meal from 10%-50% with a control of no ginger peel fish meal. After few weeks the fishes were dissected, then the intestine of each fishes were isolated and then taken to the histology laboratory where microscopic slide of each intestine were prepared.

2.8 Analytical Procedure

Digestibility test of feed and faeces was carried out using Acid Insoluble Ash method (AIA), as described by [3]. Proximate analysis of unfermented ginger peels, the experimental diets and feces were determined by Association of Official Analytical Chemists [4]. The photochemical screening was done on the sample using methods as described by [5].

2.9 Histopathological Analysis

Microscopic Examination

Each microscopic slide was viewed under a high resolution power with the help of a megapixel digital camera. The entire slides were observed under low and high resolution for their histological findings.

3. Results and Discussion

Table 1: Phytochemical screening of unfermented ginger peels

	Untreated Ginger Peels
Phlobatannins	-
Flavonoids	-
Steroids	-
Glycosides	++
Terpenoids	++
Reducing Sugars	++
Alkaloids	+
Tannins	++
Saponins	+
Anthraquinone	-

KEYS + = Present in Minute Amount, ++ = Present in Relative, Moderate Amount – = Absent

Table 2: Determined Proximate composition of experimental diets (%)

	Ufgpm	Maize
% Moisture	41.545±0.77[a]	15.605±0.86[b]
% Protein	1.775±0.21[a]	8.765±0.05[c]
% Ash	6.625±0.04[c]	2.31±0.16 [b]
% Crude fibre	2.22±0.17[a]	2.38±0.26[b]
% Fat	4.375±0.11[a]	4.18±0.54[a]
% NFE	44.29±0.07[a]	84.005±1.42[b]
% Dry matter	59.605±0.86[a]	85.615±0.87[b]
Metabolizable Energy (Kcal/Kg)	2233.2±17.68[b]	4087±103.24[c]

Table 3: Proximate composition of unfermented ginger peel meal and maize

	Control A	10% Ufgpm B	20% Ufgpm C	30% Ufgpm D	50% Ufgpm E
Moisture	6.6±0.85[a]	7.8±0.28[b]	9.25±1.06[c]	8.5±0.71[a]	11.45±0.78[a]
Ash	0.25±0.85[c]	0.218±0.05[a]	0.19±0.01[b]	0.159±0.03[b]	0.13±0.06[a]
Crude Fibre	7.9±0.28[a]	8.285±0.45[b]	9.3±0.28[a]	7.25±0.92[c]	7.9±0.57[b]
Crude Protein	2.055±0.35[a]	5.54±0.14	5.055±0.23	5.385±0.54[b]	4.54±0.41[a]
Fat	51.9±0.14[d]	9.85±0.21[c]	6.25±0.35[b]	16.87±1.60[a]	9.4±0.57[a]
Dry Matter	94.4±0.26[a]	92.2±0.28[a]	90.75±1.06[c]	91.5±0.71[b]	88.55±0.78[b]
Nfe	75.29±0.54	68.305±1.04	63.205±8.07	67.836±6.10	66.58±1.25
Metabolizable Energy Kcal/ Kg	3178.4	3178.4	3178.4	3178.4	3178.4

Means along rows with different superscript are significantly different from each other (P<0.05).

Histopathological Studies

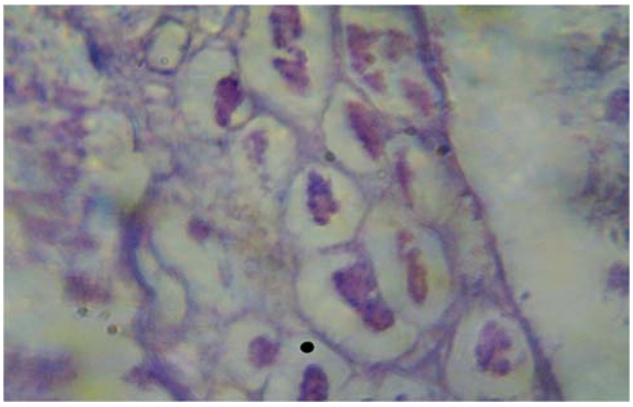

Fig 1: Control Histological section of *Clarias gariepinus* intestine control shows normal columnar epithelium circular muscle fibre and intestine.

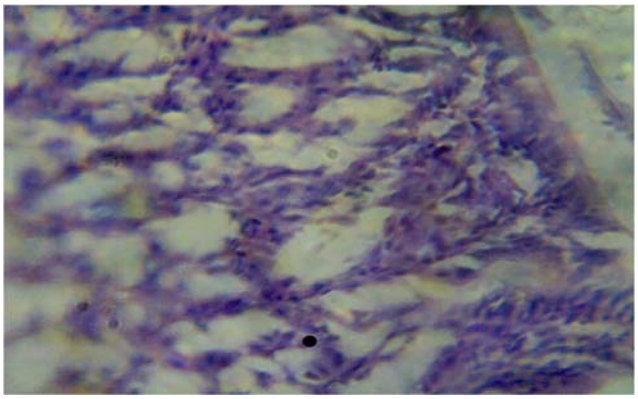

Fig 2: Integrity of intestine at 10% inclusion Photomicrography of *Clarias gariepinus* intestine shows mild degeneration on the columnar epithelium circular muscle fibre and intestinal submucosa.

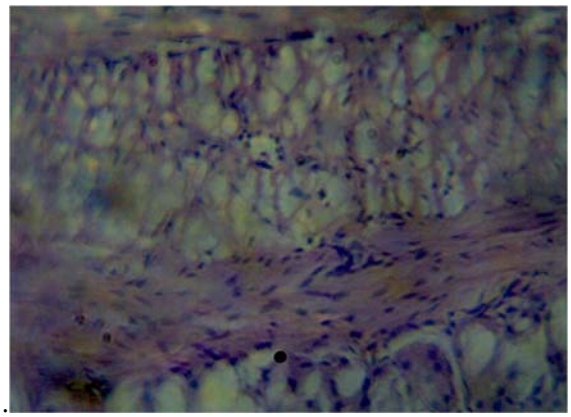

Fig 3: Integrity of intestine at 20% inclusion Photomicrography of *Clarias gariepinus* intestine (fig. 3) shows moderate degeneration on the columnar epithelium circular muscle fibre and intestinal submucosa.

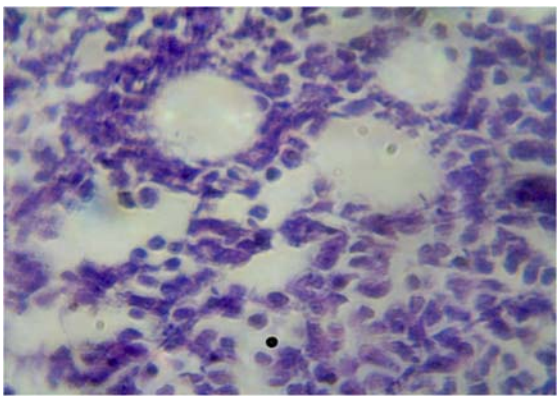

Fig 4: Integrity of intestine at 30% inclusion Photomicrography of *Clarias gariepinus* intestine (fig 4) shows severe degeneration on the columnar epithelium circular muscle fibre and intestinal submucosa.

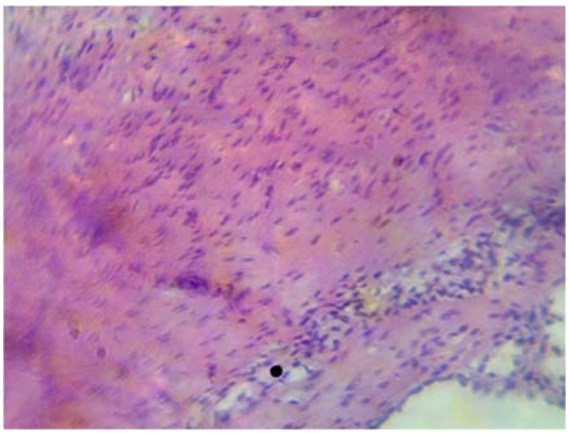

Fig 5: Integrity of intestine at 50% inclusion Photomicrography of *Clarias gariepinus* intestine (fig. 5) shows more severe degeneration on the columnar epithelium circular muscle fibre and intestinal submucosa.

This experiment was carried out to investigate the effect of unfermented Ginger peel fish meal on *Clarias gariepinus* catfish. The wall of the intestine is about four layers. This study revealed control sample (fig. 1) shows no negative effect on the intestinal submucosa as in line with [6] though the presence of columnar absorptive cells in catfish are exactly the same as found in other vertebrates, which secrete mucus to lubricate and protect the intestinal mucosa against physical and chemical damage and also some attacks which could be from microorganism found on the wall of the intestine. The presence of characteristic simple columnar epithelium in association with mucus producing goblet cells in the post – gastric intestinal mucosa have been observed in many fish and the first goblet cells can be determined early during the differentiation of the intestinal mucosa of fish. This contradicts the results obtained from the use of *Citrus sinensis* peel as a maize substitute [7].

Hoque MM *et al* [8] reported negative effect on *Puntius gonionotus*, *Barbodes gonionotus*, and *Clarias gariepinus* treated with diazinon and sumithion. According to them they observed irritation and destruction of the mucosa membrane of the intestine hamper absorption. This was corroborated by [9]. Also, epithelium degeneration and inflammatory cells infiltration in the submucosal oedema were seen in the intestine of tilapia fish exposed to carbofuran. [10] This re-affirmed the seemingly palatability and indigestibility factors associated with unfermented ginger peel feed substrate. It could be deduced that the intestinal lining of catfish rejects the unfermented ginger peel, hence the several, severe histopathology alterations.

4. Conclusion

The thrust of this study was to experimentally demonstrate the histopathology effect of unfermented ginger peel as substitute for maize as source of carbohydrate in fish feed and also to test how plant waste could be recycled for various needs of aquatic animal. The experiment shows that after few weeks of study the mortality rate of the fish increases with respect to the different concentrations showing the highest concentration having more mortality rate and intestinal distortion. This could be connected with Zingiberene present in the ginger, as well as the relative “moderate” presence of tannins, terpenoids and alkaloids.

5. Recommendation

In order to recommend ginger peel as a substitute or other source of carbohydrate in fishmeal, it is preferable to ferment toxic compounds of the ginger peel so as to free the fishes or other aquatic organisms from high mortality and improved palatability.

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