Dietary replacement of fishmeal by African catfish viscera meal: Effect on the distal intestinal health of African catfish (Clarias gariepinus)

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Abstract

In a 56-day feeding trial, the effects of feeding catfish visceral meal-based diets on the distal intestine health of African catfish (*Clarias gariepinus*) were examined using microbial load, diversity, microbial enzymes, and intestinal histology as indices. Catfish viscera meal protein substituted fishmeal protein in the control diet at the rates of 15 (D15T), 30 (D30T), 45 (D45T), and 60% (D60T). Each experimental diet was allotted at random to three tanks with catfish fingerlings (n =15 fish/replicate, 5.58±0.05g). The isolated microorganisms in the various gut regions of the fish fed the various dietary treatments examined were *Salmonella* spp., *Shigella* spp., *Staphylococcus* spp., *Enterobacter* spp., *Escherichia coli,* and *Aspergillus flavus*. Microbial diversity in the distal intestine was higher in the D15T-fed fish. Using the various diversity indicators such as Simpson Index (D), Simpson Dominance Index (1-D), Simpson Diversity Index (1/D). Shannon Weiner Index (H) and Margalef Richness Index, the D60T-fed group had the lowest microbial diversity in their hindgut. All of the dietary treatment groups have normal cellularity and morphological characterization of the distal intestine but a reduced number of villi was observed in the D30T-fed group, and cell infiltration was observed in the D45T-fed group, villi appeared longer in the D60T-fed group. These findings revealed that feeding catfish viscera meal to African catfish (*Clarias gariepinus*) did not harm the fish's distal intestinal health but could negatively impact distal intestinal villi structure as the level of the meal increases in the diet.

Keywords: viscera meal, intestinal microbiota, protease, *Clarias gariepinus,* lipase

Introduction

Aquaculture production, the fastest-growing food, foretells the ability to supply a greater proportion of man's animal-source protein needs . As a result, the recent development of aquaculture production is critical for long-term human nutrition . However, long-term expansion of aquaculture can be sustainable if there is a reduced reliance on forage fish. Since around two and a half decades ago, wild forage fish catch has been constantly falling year after year . Hua *et al*. (2019) projected that in some years to come (2025), extra 37.4 million tons of aquafeed would be needed to meet aquaculture production feed requirements. Hence, the need to research alternative feed ingredients to fishmeal beyond plant protein sources becomes imperative.

The inedible portion of fish, mainly fish viscera, discarded as waste from the fish processing industry could be used to replace fishmeal produced from forage fish which has recorded dwindling catches in recent years. Fish viscera are remnants obtained after fish processing for human consumption . According to , catfish processing could yield 25-35 % of waste

products. After cooking and drying the waste products, they can be reduced to a meal. Processing method, species, food intake, sex, age, seasonality, and other factors influence the chemical makeup of fish viscera (Huss, 1995). When compared to a fish meal generated from wild catches, viscera meal has poorer nutritional content, with lower crude protein (52–67 %), greater lipid (12–23 %), and ash content (7–14 %) .

According to Hua *et al.* (2019), there is a huge opportunity to enhance the volume of meals supplied from aquaculture by products globally, as aquaculture production is expected to reach 109 million tons by 2030. African catfish is the most widely cultivated fish species in Nigeria . Farmers use fresh fish viscera from fish processing plants to boost their fish feeding in earthen ponds. The degradation of uneaten feed in the earthen ponds could result in oxygen loss if there is no aeration . Many fish practical diets have effectively incorporated viscera meal from aquaculture and fishery industries . The utilization of fish viscera meal in the diet of African catfish (*Clarias gariepinus*, Burchell) was recorded by , , and in the recent time. It is vital to look at the influence of the meal on the distal intestine health as there has been no report on this as far as our knowledge can reach. reported that the gut of fish provides a conducive environment for the establishment of microbes owing to a large number of nutrients therein. The microbial community in the gastrointestinal tract of fish provides a synergistic role in the host's health, nutrition and development . Bacteria have been reported to constitute the majority of the gut colonizing microbiota . The abundance of nutrients in fish guts, according to , creates the perfect habitat for the development of microbes. However, reports of yeasts in fish guts have also been made . These intestinal yeasts have reportedly been found to boost freshwater fish immune systems . It has been observed that yeast can produce several chemicals with tremendous organic value, including enzymes and immunostimulants . Consequently, the host benefits greatly from the presence of microbes in the fish's intestines (Romero *et al*., 2014). reported that the enhanced microbial population in the hindgut of a host fish is essential to meeting its energy requirements (2002) in addition to this found out that the highest microbial load was found in the hindgut of Nile tilapia (*Oreochromis niloticus*) hybrid clariid catfish (*Clarias gariepinus* ♀ x *Heterobranchus bidorsalis* ♂). This study therefore investigated the effects of substituting fishmeal with catfish viscera meal in the diet of African catfish on the distal intestine health of the fish.

Materials and Methods

Preparation of viscera meal and diet preparation

Fresh fish offal, primarily viscera, was procured from a reputable fish processing facility in Ilorin metropolis and boiled for 25 minutes according to techniques of , to minimize fat content and eradicate potential pathogens. After 72 hours of oven drying at 50°C, the cooked offal was ground and sieved to make a visceral meal. The proximate composition of the final meal was determined and kept frozen (4 ^{0}C) until used. Based on the proximate composition of the basic feed ingredients as reported in our previous study , a fish meal based control diet and four test diets with catfish viscera meal substituting for the fishmeal

portion at 15%, 30%, 45%, and 60%, respectively, were produced (Table 1). These diets were designated as CTR, D15T, D30T, D45T, and D60T. A tool created by the Network of Aquaculture Center in Asian Pacific (NACA) was used for the feed formulation and estimated amino acid content

Table 1: Ingredients (g kg^{-1} as fed basis) and nutrient composition (g kg^{-1}) of the experimental diets containing viscera meal

	Experimental Diet					
Ingredient Composition (g kg^{-1})	CTR	D15T	D30T	D45T	D60T	
Fishmeal @ 68%cp	287.2	244.1	200.9	158.0	114.9	
Viscera meal@ 47.83cp	0.00	61.2	122.5	183.7	245.0	
SBM @ 38%Cp	450.0	450.0	450.0	450.0	450.0	
Maize @ 10%cp	100.0	100.0	100.0	100.0	100.0	
*Fish Premix	40.0	40.0	40.0	40.0	40.0	
Fish oil	5.0	5.0	5.0	5.0	5.0	
Veg Oil	5.0	5.0	5.0	5.0	5.0	
Starch	112.8	946	76.6	58.3	40.1	
Proximate Composition (g kg^{-1})						
Moisture	86.7	82.1	77.5	72.9	67.9	
Crude Protein	391.8	389.6	387.2	385.1	383.9	
Crude Lipid	143.1	142.7	142.4	142.0	141.2	
Ash	71.8	69.4	67.1	64.8	62.4	
Crude Fibre	20.2	21.5	22.8	24.1	25.5	
NFE	286.5	294.7	303.0	311.1	319.2	
Energy (kcal/g)	48.2	48.4	48.7	48.9	49.2	
Amino Acid Composition (g kg^{-1})						(**g/kg diet)
Arginine	30.3	28.8	27.3	25.9	24.3	10–12
Histidine	9.7	9.6	9.5	9.4	9.3	4–4.2
Isoleucine	18.6	17.7	16.8	15.8	14.8	6–7.3
Leucine	30.7	29.3	27.9	26.5	25.0	9–98
Lysine	28.7	27.4	26.0	24.6	23.2	13–14.3
Methionine	8.9	8.4	7.9	7.4	6.9	6–6.4
M+C	14.1	13.6	13.0	12.5	11.9	
Phenylalanine	17.8	17.1	16.4	15.6	14.9	12–14
P+T	30.9	29.5	28.1	26.7	25.2	
Threonine	18.2	17.2	16.2	15.2	14.1	5–5.6
Tryptophan	4.7	4.4	4.2	3.9	3.6	1.2–1.4
Valine	20.2	19.3	18.4	17.5	16.6	7.1–8.4

*1 kg Aero mix® fish premix contains Vitamin A 25,000,000 IU, Vitamin D3 2,000,000 IU, Vitamin E 200,000 IU, Vitamin K 8000 mg, Vitamin B2 20,000 mg, Vitamin C 500,000 mg, Niacin 150,000 mg, Pantothenic Acid 50,000 mg, Vitamin B6 12,000 mg, Vitamin B12 10 mg, Folic Acid 4000 mg, Biotin 800 mg, Choline Chloride 600,000 mg, Cobalt 2,000 mg, copper 4,000 mg, Iodine 5,000 mg, iron 40,000 mg, Manganese 50,000 mg, Selenium 200 mg, Zinc 40,000 mg, Antioxidant 100,000 mg, Lysine 100,000 mg, Methionine 100,000 mg manufactured by Aerobic Integrated Concept limited Km 130, Lagos Ibadan Expressway, Hossanah Bus Stop, opposite Islim Filling Station, P. O. Box 22109 UI post Office , Oyo State, Nigeria

M+C: Methionine + Cysteine
P+T: Phenylalanine + Tyrosine
** Amino Acid Requirement of Catfish (g/kg diet) (NRC 2011)

2.2 Experimental fish and system

African catfish fingerlings (average weight; 5.58g±0.05g) were obtained from a reputable hatchery in Ilorin, Kwara State, Nigeria (80 20'42"N, 40 31' 8" E), and acclimated for 14 days in an intermediate bulk container (IBC tanks) at the wet laboratory of the Department of Aquaculture and Fisheries University of Ilorin. During acclimatization, fish were fed a commercial diet (1.8 mm @Skretting). After acclimation, 225 fingerlings were randomly stocked into fifteen 60L rectangular glass containers (76 x 35 x 30cm). Tanks were assigned to experimental diets at random (n=3 tanks per treatment). Each tank was aerated continuously by the use air compressor and bubble stone. For 56 days, the fish were fed at 5%body weight per day in two equal halves at 0900 and 1700 h, with the feed amount adjusted fortnightly after periodic batch weighing., The average water quality conditions maintained during the culture period were Temperature at 27.3±0.26 °C; pH (6.57±0.42); and Dissolved Oxygen of 6.87±0.26 mg/l.

Isolation and characterization of microflora

The distal intestine sections of the fish in each treatment (n=3) were obtained after the feeding trial by aseptically dissecting the alimentary canal and placing in a sterile bottle containing 5 ml sterile distilled water which was vigorously shaken to allow the contents to separate into water. Prior to the commencement of microbial studies, the working table and the glassware were sterilized with 75% alcohol and at 160ºC for 90 minutes in an oven respectively. Freshly prepared Sabouraud Dextrose Agar (SDA) medium was used for fungi isolation and identification as described by . Bacteria were isolated and characterized using Nutrient Agar, and standard operating procedures were followed for gram reaction, morphology, motility, catalase and oxidase responses, citrate consumption, coagulase generation, starch hydrolysis, and sugar fermentation . The colonies that resulted were identified using 's criteria. After incubation, the bacteria colonies were enumerated and expressed in Colony Forming Units (CFU)/g.

Diversity Study

The diversity indices described in were employed for the diversity study of distal intestinal microbiota as follows;

$$Shannon\ Weiner\ Index\ (H) = -\sum_{i=1}^{s} PlnP$$

$$Simpson\ Diversity\ Index\ (1/D) = \frac{1}{\sum_{i+1}^{s} P^2}$$

Where $P = \sum_{i=1}^{s} \frac{n}{N}$

n = the total number of organisms of a particular species

N = the total number of organisms of all species

$$Margalef\ Richness\ Index = {}^{(S-1)}/_{\ln N}$$

where S= total number of species

N= total number of individuals in the sample

Distal intestine enzyme activities

Distal intestine enzyme activities were determined by homogenizing the sample (n=3) in cold 0.25 sucrose in a homogenizer. The homogenate was centrifuged at 5000 xg for 15 minutes at 4ºC. The resulting supernatant was frozen and stored at −20ºC until assayed for different digestive enzymes (protease and lipase). Protease activity was assessed using casein (Sigma-Aldrich, Shanghai, China) as a substrate using the procedure of Erban and Hubert (2010). Lipase activity was analyzed following the method of .

Distal intestine histology

The distal intestine (n=3 fish per treatment) was obtained by dissection, and fixed in 10% potassium buffered formalin. Dehydration in graded levels of alcohol followed using the method of . Sectioning of the tissues into thin sections (5-7µm) was done using a rotatory microtome (HS2205, China) after embedding in malted wax. The sections were stained with Harris haematoxylin-eosin (H&E). The stained slides were observed under a light microscope (SW380T) (x40).

Chemical Analysis

The proximate composition of feed and feed components was determined using methods. Dry matter was determined by placing samples in an oven (DHG 9053A, Axiom Medical Ltd) at 105°C for 24 hours. After acid digestion, crude protein concentration was determined using a protein auto-analyzer (Foss Tecator KjeltecTM 8400) and a factor of 6.25 to convert nitrogen to crude protein. The Soxhlet extraction method was used to analyze crude lipid using the Foss Tecator SoxtecTM 8000 while the crude fibre was determined using a Foss Tecator Fibertec 2010 analyzer. The ash concentration was determined by burning the samples for 4 hours at 600°C in a furnace (Krupp Widia-Fabrik).

Ethical Statement

Animal research ethics were strictly adhered to, as stated in the University of Ilorin's research policy on the use and care of animals in Ilorin, Nigeria.

Statistical Analysis

After passing the Levene test of homogeneity of variance, data were reported as mean ± SE and analyzed using one-way analysis of variance (ANOVA). Duncan multiple range tests were employed to distinguish treatment means with a

significant difference ($p<0.05$).

Results

Distal intestine microbial load

Table 2 presents gut microbial load (log CFU/ml) in the hindgut of *Clarias gariepinus* fed diets containing catfish viscera meal replacing fishmeal. Fish fed D60T had significantly lower ($p<0.05$) gut total bacteria load than fish fed other diets. The total bacteria count in the gut of fish fed regular fish meal (CTR) was the highest but was not significantly different ($p>0.05$) from that of test diets up to 45% replacement level. Total coliform, Enterobacteriaceae and salmonella shigella count were significantly higher ($p<0.05$) in the gut of fish fed 15% substituted viscera meal. Fish fed fish meal only had fungus load in their guts while the fungi load were below detection levels in others. Similarly, coliforms were not detected among fish fed viscera meal substitution of 30% up to 60%. The total salmonella shigella count was below detection level in the gut of fish fed 60% replaced viscera meal.

Table 2. Microbial load (log CFU/ml) in the hind gut of *Clarias gariepinus* fed diets containing African catfish viscera meal replacing fishmeal

	CTR	D15T	D30T	D45T	D60T
Total Bacteria Count	6.87 ± 0.07^{a}	6.73 ± 0.09^{a}	6.76 ± 0.03^{a}	6.83 ± 0.03^{a}	5.50 ± 0.20^{b}
Total Coliform Count	2.93 ± 0.03^{a}	2.98 ± 0.14^{a}	ND	ND	ND
Total Enterobacteriaceae Count	4.45 ± 0.05^{b}	4.90 ± 0.06^{a}	4.19 ± 0.04^{b}	3.83 ± 0.13^{c}	3.15 ± 0.25^{d}
Total Salmonella Shigella Count	4.19 ± 0.04^{b}	4.67 ± 0.05^{a}	4.19 ± 0.04^{c}	4.16 ± 0.05^{b}	ND
Total Fungi Count	2.15±0.15	ND	ND	ND	ND

Values (means with standard errors, n=3) within the same row with different superscripts are significantly different ($p<0.05$) using Duncan Multiple Range Test (DMRT) ND not detected; CTR Control (Fishmeal Based Diet); D15T diet with 15% viscera meal replacement; D30T diet with 30% viscera meal replacement; D45T diet with 45% viscera meal replacement; D60T diet with 45% viscera meal replacement

Distal intestine microbial diversity

Microbial occurrence and diversity in the hindgut section of *Clarias gariepinus* fed diets containing fish viscera meal are presented in Table 3. The isolated organisms in the different gut sections among the fish fed the dietary treatments were *Salmonella* spp, *Shigella* spp, *Staphylococcus* spp, *Enterobacter* spp, *Escherichia coli,* and *Aspergillus flavus.* Distal intestine microbial diversity was higher among the fish group fed 15% visceral meal replaced diet. The group fed 60% visceral meal replaced diet had the lowest microbial diversity in their hindgut using the different diversity indices.

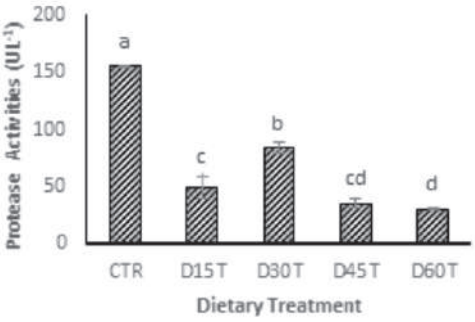
Table 3. Microbial occurrence and diversity in the hindgut section of *Clarias gariepinus* fed diets containing African catfish viscera meal replacing fishmeal

Bacteria	CTR	D15T	D30T	D45T	D60T
Salmonella spp.	0 (0.00)	1 (0.20)	1 (0.33)	1 (0.25)	0 (0.00)
Shigella spp.	1 (0.25)	1 (0.20)	1 (0.33)	1 (0.25)	0 (0.00)
Staphylococcus spp.	0 (0.00)	1 (0.20)	0 (0.00)	1 (0.25)	0 (0.00)
Enterobacter spp.	1 (0.25)	1 (0.25)	1 (0.33)	1 (0.25)	1 (1.00)
Escherichia.coli	1 (0.25)	1(0.25)	0 (0.00)	0 (0.00)	0(0.00)
Fungi					
Aspergillus flavus	1 (0.25)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Diversity Indices					
Simpson Diversity Index (1/D)	4.00	5.00	3.00	4.00	1.00
Shannon□Weiner Index(H)	1.39	1.61	1.10	0.35	0.00
Margalef's richness	2.16	2.49	1.82	2.16	0.00

The figure in parentheses are proportion

Distal intestine enzyme activities

Protease and lipase activities in the gut of fish fed the different treatments are as presented in Figure 1. Protease activities were significantly higher ($p<0.05$) in the control group while the group fed 60% visceral meal replaced diet recorded the lowest protease activities that were not significantly different ($p>0.05$) from the 45% viscera replaced fed group. The lipase activities of fish fed 60% visceral meal replaced diet were significantly higher ($p<0.05$) than in other fed groups. No significant variation was recorded in the lipase activities among fish fed CTR, D15T, and D30T.



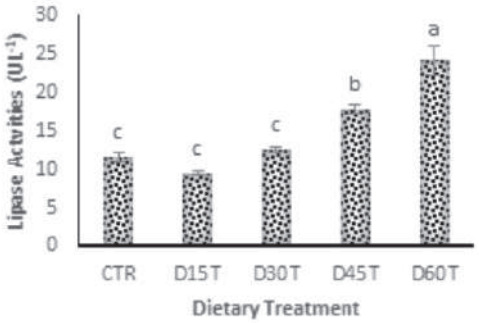


Figure 1: Distal intestine enzyme activities (U L^{-1}) of *Clarias gariepinus* fed diets containing catfish viscera meal replacing fishmeal. Bar values (means with standard errors, n = 3) with different letters are significantly different (Duncan test; $p<0.05$).

Distal intestine histology

The photomicrographs (H&E stained; x40) of the hindgut of *Clarias gariepinus* fed diets containing catfish viscera meal replacing fishmeal are as presented in Figure 2a–e. The cellular and morphological delineation appear normal in all the dietary treatment groups. A reduced number of villi was observed in the intestine of fish fed D30T and villi of the D60T fed group appeared longer. Cell infiltration was observed in D45T fed groups..

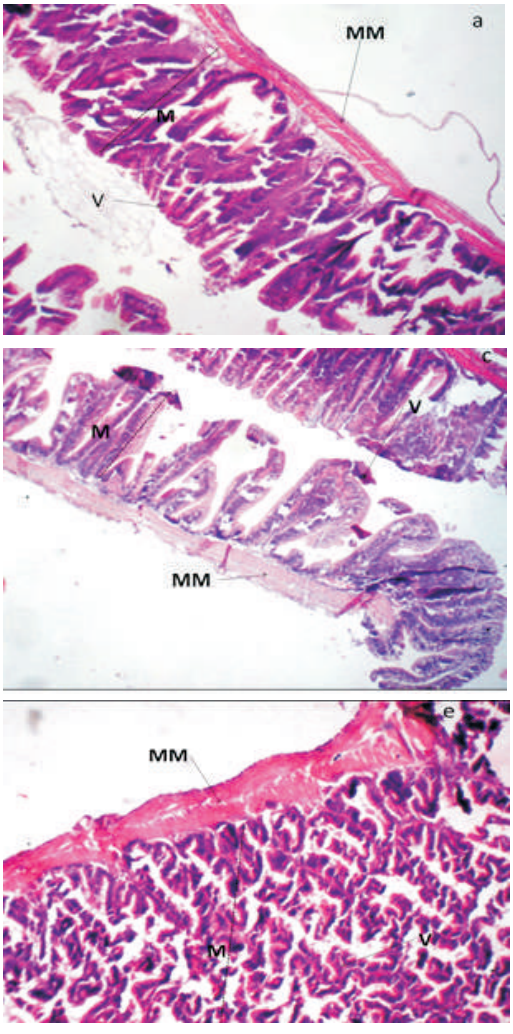


Figure 2a–e: Photomicrographs (x40; H&E) of the hindgut of *Clarias gariepinus* fed diets containing catfish viscera meal replacing fishmeal. (MM – Muscularis propria, M – Mucosa, V– villi)

Discussion

Some intestinal microbiota strengthens the immune and digestive systems in fish but is relatively less investigated . The distal intestine of fish host the highest microbial load in their gut . Bacteria are the most abundant in this study. The total bacteria count in the gut of the control group was the highest with statistical similarity up to 45% replacement level. Total coliform, enterobacteriaceae and salmonella shigella count were significantly higher in the gut of fish fed 15% visceral meal replaced diet. A synergistic relationship between dietary ingredients may plausibly account for this. For instance, dietary fibre and other complex polysaccharides are acted upon by gut microbes producing short chain fatty acids (SCFAs) that lower the gut **pH thus inhibiting the growth of harmful bacteria producing toxins** and conversely, serving as precursors to developing certain beneficial gut bacteria that could offer probiotic effects . The isolated organisms in the distal intestinal sections such as enterobacters are known to produce exogenous enzymes such as proteases which complement the digestive function in fishes . This perhaps informs why higher protease activities were recorded among the control fed groups. Gram negative bacteria such as *Salmonella* spp. and *Escherichia coli* can produce exogenous enzymes that can digest complex carbohydrates . Additionally, intestinal microflora could destroy antinutrients in feed, thereby improving feed utilization . It has been established that these gut microbes have the capacity for modulation of fish physiology and immunity through diverse activities which includes, but are not restricted to, the production of organic acids, hydrogen peroxides and exclusion of adhesion sites . The low microbial diversity recorded among the group fed 60% visceral meal replaced diet could be attributed to dietary treatment . Structural molecules that preserve the integrity of intestinal epithelium are sensitive to dietary manipulation of , amino acids, , antinutrients , organic acid or vitamins . A reduced number of villi that was observed in the group fed 30% visceral meal replaced diet; cell infiltration that was observed in group fed 45% visceral meal replaced diet and longer villi that were observed in the group fed visceral meal replaced diet were all manifestations of the effect of the dietary treatments on the distal intestine. Previous studies by found that dietary chitosan included in feed formulation on the intestinal morphology of sea bass (*Dicentrarchus labrax*) enhanced the villus height in response to dietary chitosan. also pointed out that feeding fish vegetables affected the histology of the intestine of sea bream (*Sparus aurata*). observed histological alterations in the intestine of Asian catfish, *Clarias batrachus* fed with different types of fats through semi purified diets. These data suggest that dietary treatments can affect the morphology of intestinal villi. However, the integrity of distal intestinal health of the fish groups in this study was not adversely affected by feeding fish viscera meal as cellular and morphological delineation of the gut of the fish appear normal in all the dietary treatment groups.

Conclusion

Feeding catfish viscera meal to African catfish (*Clarias gariepinus*) did not harm the fish's distal intestinal health but could negatively impact on distal intestinal villi structure as the level of the meal increases in

the fish diets. The total enterobacteriaceae and salmonella shigella counts were significantly higher in the gut of fish fed D15T. Similarly, the distal intestine microbial diversity was higher among the fish group fed D15T fed group.

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