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Influence of orange fleshed sweet potato (*Ipomea batatas)* peels meal in the diet of the African catfish (*Clarias gariepinus*) fingerlings on the histology of the liver, small intestine and gills

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Abstract:

This research assesses the histological changes in the liver, small intestine and gills of *Clarias gariepinus* fingerlings fed varying levels of orange fleshed sweet potato (*Ipomea batatas*) peels meal. Six different diets containing orange fleshed sweet potato (*Ipomea batatas*) peel inclusion levels of 0%, 20%, 40%, 60%, 80%, and 100% were compounded. Histological examinations of the liver, small intestine, and gills were conducted through standard hematoxylin-eosin staining procedures. The findings revealed no histological changes in the small intestine across all the treatment groups, which is an indication that orange fleshed sweet potato (*Ipomea batatas*) peel does not impair intestinal integrity. The liver, however, had varying levels of histopathological changes with increase in the level of orange fleshed sweet potato (*Ipomea batatas*) peel inclusion. Mild hepatocellular degeneration with nuclear degeneration and cytoplasmic vacuolations were observed at 60% inclusion, with increased damage at (80% and 100%) levels of inclusion, including focal cellular infiltration and central venous congestion. Low to moderate levels of orange fleshed sweet potato (*Ipomea batatas*) peel (20% and 40%) did not cause serious damage in the gills, while high concentrations (60%, 80%, and 100%) caused mild lamellar hypertrophy, interlamellar space occlusion, and severe lamellar cell depletion. These findings indicate that orange fleshed sweet potato (*Ipomea batatas*) peel could be included in the diet of *C. gariepinus* fingerlings up to 20% without affecting liver, small intestine, or gill health. This research shows the potential for orange fleshed sweet potato (*Ipomea batatas*) peel as an affordable and sustainable aquaculture feed ingredient at 20% level of inclusion.

Keywords: Orange fleshed sweet potato (*Ipomea batatas)* peel, *Clarias gariepinus,* fingerlings, Histology.

Introduction

Aquaculture is a critical component of international food security, with global demand for fish as a high-quality animal protein continuing to grow (FAO, 2020). The expensive nature of conventional fish feed is one of the greatest challenges confronting the industry, which has a significant effect on the profit

margin and sustainability of farm fish production (Tacon & Metian, 2008). Therefore, scientists around the globe have focused on discovering other cost-effective, sustainable, and alternative feed sources that can ensure fish health and growth while minimizing production costs (Omeru, & Solomon, 2016). Orange fleshed sweet potato is a source of fiber, vitamins, and minerals and has emerged as a potential substitute feed ingredient in aquaculture (Sajjadi *et al*., 2016). Orange fleshed sweet potato has high nutritional value and is suitable for food fortification or functional food development. (Neela & Fanta, S2019). Thus, are expected to be useful in fish nutrition and health. African catfish (*Clarias gariepinus*) is one of the most economically valuable fish in aquaculture because it has a rapid growth rate and can withstand a wide range of environmental conditions (Hecht & Verheust,1996). Despite this, there is limited research on its health and organ morphology when subjected to varying levels of incorporation of orange fleshed sweet potato peel into its diet.

Histopathological examination is a reliable technique for evaluating the effects of dietary treatments on the structure and function of fish organs, because it can detect subtle cellular and tissue alterations that may not be apparent through gross morphological observations or growth performance measurements (Roberts, 2012). This research seeks to assess the histological impacts of feeding *C. gariepinus* fingerlings diets with different levels of orange fleshed sweet potato peel, focusing particularly on the liver, small intestine, and gills. The findings will aid in the knowledge of the use of orange fleshed sweet potato peels as a fish feed ingredient and give insights into the risks that can be linked to its use.

Materials and Methods

Study Location

The study was carried out in the Aquaculture Laboratory, Hydrobiology and Fisheries unit, Department of Zoology, Faculty of Natural Sciences, University of Jos.

Preparation of orange fleshed (*Ipomea batatas*) peels meal

Fresh orange fleshed *Ipomea batatas* were collected from local farmers. The peels were washed, sun-dried for two weeks, and then milled into fine powder using a hammer mill. The powdered peels were sieved to remove coarse particles, and a fine uniform powder was produced. The dry orange fleshed *Ipomea batatas* peel powder was subsequently incorporated into experimental diets at varying inclusion levels.

Formulation of Experimental Diets

Six experimental diets were formulated using fish meal, orange fleshed *Ipomea batatas* peel meal, soybean cake, maize, cassava flour, palm oil, blood meal, mineral and vitamin mix, The diets included

different levels of orange fleshed sweet potato (*Ipomea batatas)* peel meal at 0%, 20%, 40%, 60%, 80%, and 100% respectively. These diets were prepared by thoroughly mixing the ingredients in a stainless-steel pot and pelleting the mixture using a meat mincer (Omoregie *et al*., 2009). The pellets were made with a diameter of 2mm to suit the size of the fingerlings.

Experimental Design

Eighteen plastic tanks of 20-litres capacity were used in a completely randomized design, with three tanks per diet treatment. Each tank contained ten fish. The fish were fed their respective diets at 5% of their body weight, twice a day. Growth performance indices (mean final weight and mean weight gain) and feed utilization parameters were measured bi-weekly. Water quality parameters such as temperature, pH, dissolved oxygen, and alkalinity were monitored and excess feed and feces were removed daily for water quality maintenance. The experiment lasted for 16 weeks.

Histological assessment of the liver, intestine and gills of *Clarias gariepinus*

Preparation of Samples

The abdominal walls of the fish were dissected open through a mid-ventral abdominal incision. The positions of the organs were carefully noted in situ. The individual liver, intestine and gills were carefully dissected. The organs are stored in 10% formalin solution in the specimen containers.

Tissues Processing, Staining and Histopathological Studies

The preserved organs (gills, liver, gonads) were used for histopathological studies. The organs were removed from the formalin solution and rinsed in normal saline solution. Samples of the gills, liver, testes and ovary were later fixed in Bouin's fluid fixative for 72 hrs. Each of the tissue samples taken from the liver, intestine and gills were immersion fixed separately in 10% neutral buffered formalin at room temperature. The specimens were left in the fixative for 24 h. Following fixation, the tissues were rinsed in running water, dehydrated by immersing in ascending grades of ethyl alcohol, cleared in two changes of absolute xylene, impregnated with molten paraffin wax in hot oven and embedded in paraffin blocks at room temperature. The paraffin blocks were sectioned with microtome at 5-6µ thickness. Ribbons containing every 8th to 10th sections were collected and gently floated on a tissue floatation bath at 40°C and put on glass microscopic slides. Before staining, the tissue sections were deparaffinized with xylene and hydrated with ethanol. After rinsing the slides with distilled water, sections were stained regressively with Haematoxylin for 10 min. The sections were washed in tap water and dip into 1% of acid alcohol for differentiation and to remove excess stain. The sections were rinsed briefly in running tap water to

remove excess acid and halt distain. The slides were then placed in saturated sodium bicarbonate solution for 3 min, and counterstained in 1% alcoholic eosin for 1 min. The Haematoxylin and Eosin-stained sections were dehydrated by increasing concentration of ethanol and cleared in xylene and were mounted using DPX mountant and glass cover slips. Microscopic slides were examined under light microscope. Photomicrographs of selected slides of each of the organs under study were taken using Digital Photo Camera mounted on a binocular compound microscope (Axiostar MWIB, US).

RESULTS

Histological assessment of the liver, intestine and gills of *Clarias gariepinus* fed diets with varying levels of orange fleshed sweet potato (*Ipomea batatas)* peels. The results of the histological examination are as presented in plates 1 to 3. Histology of the liver of the African catfish (*Clarias gariepinus*) fingerling fed diets with varying levels of orange fleshed sweet potato (*Ipomea batatas)* peels. The results of the liver of the African catfish (*Clarias gariepinus*) fingerlings fed diets with varying levels of orange fleshed sweet potato (*Ipomea batatas)* peels is presented in plates 1. Diet A which is the control (0%) showed normal liver histo-morphological appearance as evidenced by intact hepatocytes (black arrow) and central vein (asterisk). Furthermore, B (20%) and C. (40%) shared similar histological appearance of normal liver cells and intact central vein with A. In D (60%), there is a moderate hepatocellular degeneration characterized nuclear degeneration (long arrow) and cytoplasmic vacoulations (black arrowhead). In addition, E (80%) showed moderate hepatocellular degeneration (black arrow) and focal cellular infiltration (red arrowhead) and F (100%) had mild central venous congestion (asterisk) and diffuse perivascular hepatocellular degeneration (black arrowhead) (plate 1).

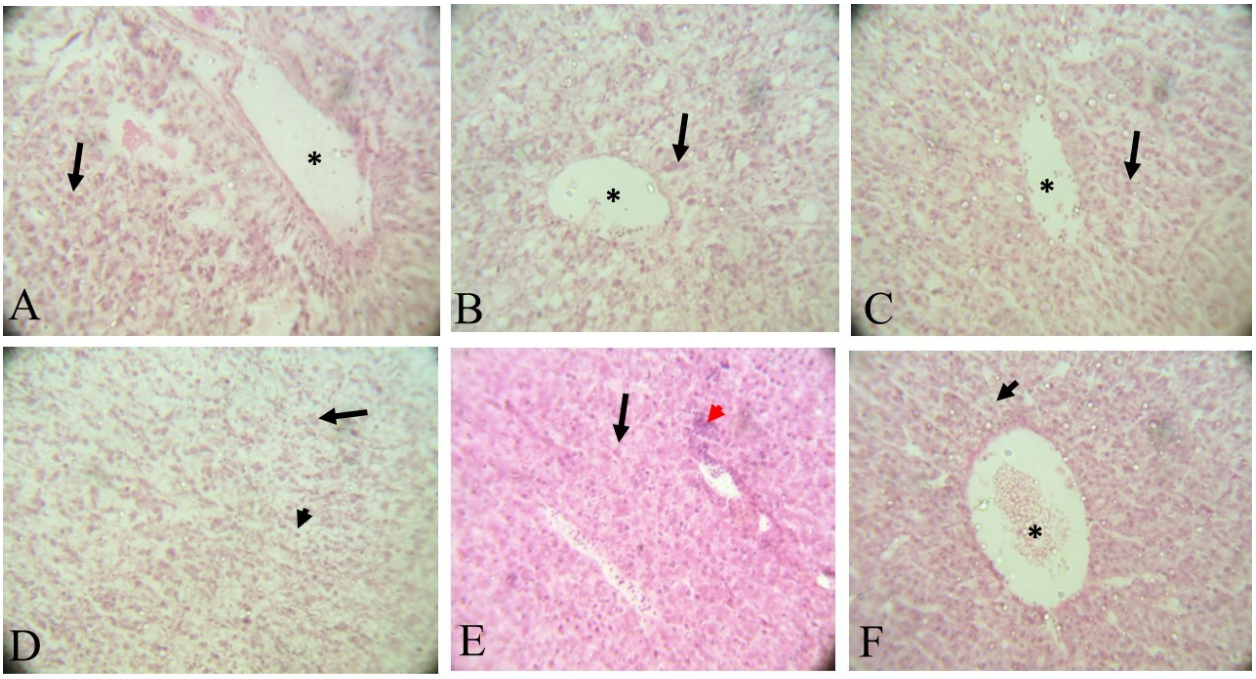


Plate 1: Photomicrographs of the liver of the African catfish (*Clarias gariepinus*) fingerling fed diets with varying levels of orange fleshed sweet potato (*Ipomea batatas)* peels. Stain: Haematoxylin-Eosin; Magnification: X400.

Histology of the small intestine of the African catfish (*Clarias gariepinus*) fingerling fed diets with varying levels of orange fleshed sweet potato (*Ipomea batatas)* peels

The small intestine of fingerling the African catfish (*Clarias gariepinus*) fingerlings fed diets with varying levels of orange fleshed sweet potato (*Ipomea batatas)* peels is presented in plate 2. In the control diet A (0%), there were no visible lesion in the small intestinal parenchyma as revealed by intact epithelial mucosa (red arrowhead), submucosa (black arrowhead), muscular coat (black double arrowhead) and serosa (black arrow) tunics, diets B-F (20%-100%) shared similar histo-architectural appearances with the control diet A (0%).

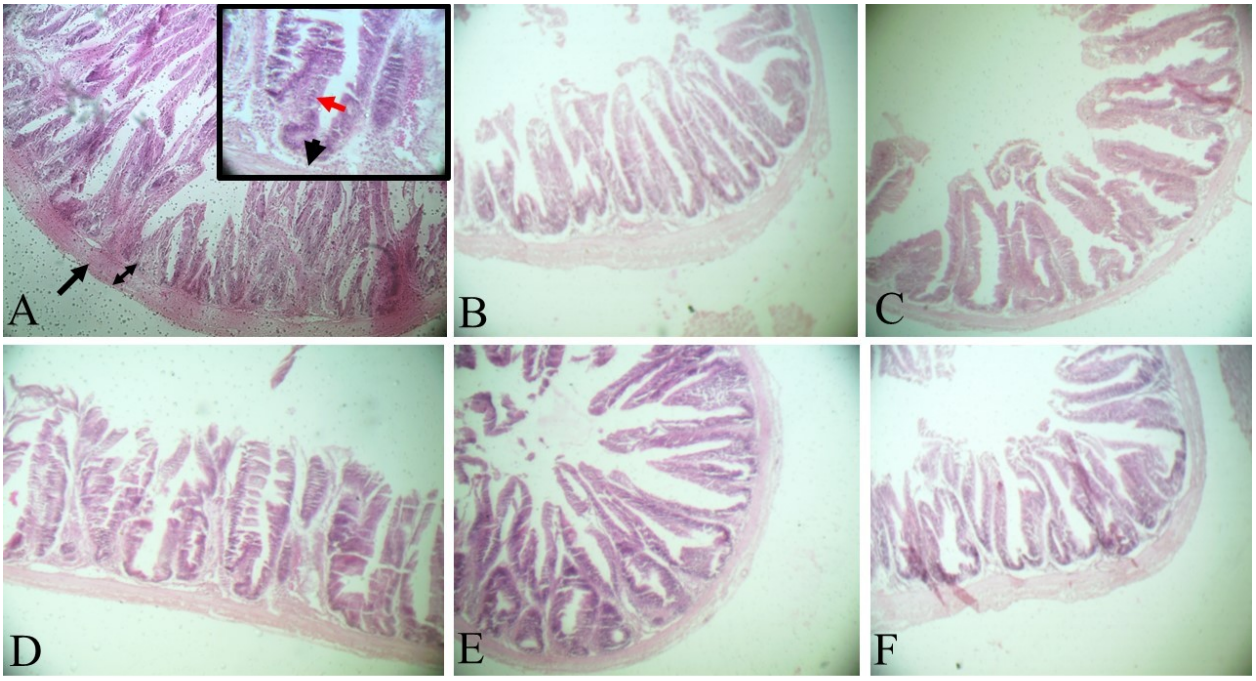


Plate 2: Photomicrographs of the small intestine of the African catfish (*Clarias gariepinus*) fingerling fed diets with varying levels of orange fleshed sweet potato (*Ipomea batatas)* peels: Stain: Haematoxylin-Eosin; Magnification: X400.

Histology of the gills of the African catfish (*Clarias gariepinus*) fingerling fed diets with varying levels of orange fleshed sweet potato (*Ipomea batatas)* peels

The gills of African catfish (*Clarias gariepinus*) fingerling fed diets with varying levels of orange fleshed sweet potato (*Ipomea batatas)* peels is presented in plate 3. In the control diet A (0%), there is no visible lesion in the gill parenchyma as shown by the presence of intact secondary lamellae (black arrow) and patent interlamellar space/water channel (star) and diet B (20%) shared similar gill histomorphology with the control. With the exception of mild lamellar hypertrophy, there is no visible lesion in the gill parenchyma in diet C (40%). However, there is marked occlusion of the interlamellar space in diet D (60%). Additionally, diets E (80%) & F (100%) showed severe lamellar cell depletion (black arrow) and gill filament cartilage disruption (black arrowhead) respectively (plate 3).

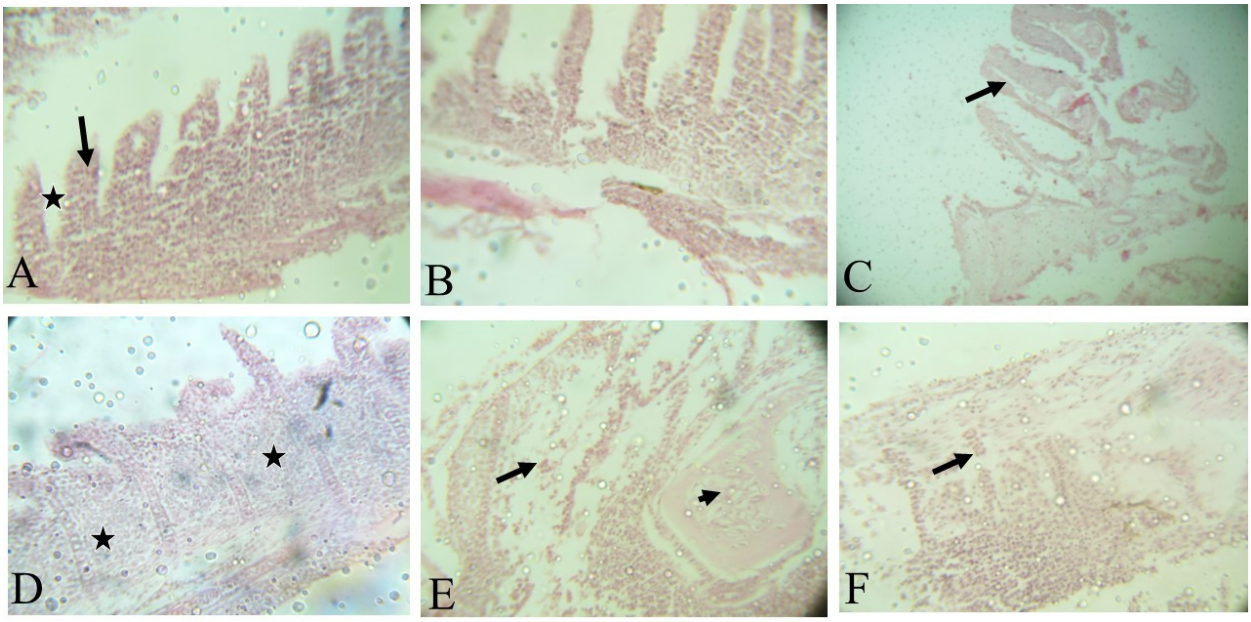


Plate 3: Photomicrographs of the gills of African catfish (*Clarias gariepinus*) fingerling fed diets with varying levels of orange fleshed sweet potato (*Ipomea batatas)* peels. Stain: Haematoxylin-Eosin; Magnification: X400.

DISCUSSION

Histopathological studies have been established as effectual and sensitive tools for monitoring fish health. Histologically, treatments D1, D2, and D3 resembled a normal liver in that they had central veins and intact hepatocytes. This suggests that the inclusion of orange fleshed sweet potato peel meal to the diets of the fish in these treatment groups resulted in only minor changes to the liver histomorphology. However, fish in treatment D4 had moderate level of hepatocellular degeneration as revealed by cytoplasmic vacuolations and nuclear degeneration. This result is an indication that increased levels of orange fleshed sweet potato peel meal in the diets of *C. gariepinus* fingerlings may have a deleterious impact on the integrity of the hepatocyte (van Dyk, Cochrane & Wagenaar, 2012). In addition, treatment D5 revealed focused cellular infiltration and mild hepatocellular deterioration, which is an indication of a more pronounced effect on liver health compared to treatments D1–D4. This indicates that the inclusion of orange fleshed sweet potato peels meal in the diets of *C. gariepinus* fingerlings could cause hepatocellular injury as similarly noted by Raimi *et al*. (2021) when they fed *Clarias gariepinus juveniles* with *Morinda lucida* (Oruwo leaf). Additionally, treatment D6 had diffuse perivascular hepatocellular degeneration and mild central venous congestion, which is an indication of a greater deterioration in liver histology. This study's findings are consistent with the reports of Anayeokwu, Obarombi, Ogbe, Wintola, Dauda, and Dasuma (2023), who fed *C. gariepinus* fingerlings leftover sorghum from a locally fermented

beverage called Buruutu. The abnormalities observed in the liver as the level of inclusion of orange fleshed sweet potato peels meal increases, could be due to the high level of carbohydrate in the diets. This is similar to the report of NRC (2011), which stated that high level of carbohydrate in diets produce liver abnormalities, poor growth and high mortalities in salmonids.

There must be normal histological features as well as a good integrity of the intestinal wall's underlying layers and epithelial cells for fish to absorb nutrients optimally, get a healthy immune system, and possess overall digestive health (Tran-Ngoc *et al*., 2019). The absence of evident lesions or abnormalities in the small intestine parenchyma shows that the inclusion of orange fleshed sweet potato peel meal in the diets had no negative effect on the integrity or morphology of the intestinal wall. It has been acknowledged that feedstuffs high in dietary fibre, antioxidants, and bioactive substances increase fish gut integrity and optimize nutritional utilization (Maas, *et al*., 2018). The absence of obvious histological alterations in the small intestine of *C. gariepinus* fingerlings fed diets with varying levels of orange fleshed sweet potato peel meal is an indication that the diet is compatible with the intestinal health of the fingerlings. *C. gariepinus* fingerlings fed diets with varying levels of orange fleshed sweet potato peels meal did not reveal any obvious histological changes in their small intestine which is an indication that the inclusion in the diets did not cause any significant structural alterations or pathological lesions in the intestinal tissue. The preservation of intestinal integrity and function is demonstrated by the intact serosa tunics, muscular coat, epithelial mucosa, and submucosa seen in all treatment groups as well as the control. This outcome is consistent with the findings of Muhd, Kang'ombe and Orire (2024) who reported that adding processed M*oringa oleifera* seed kernel to the diet of *C. gariepinus* fingerlings led to histological alterations of the intestine.

The normal appearance which was characterized by intact secondary lamellae and patent interlamellar spaces of the gill in treatments D1 and D2 is an indication of optimal gill structure and function. This observation is an indication that low to moderate levels of orange fleshed sweet potato peels inclusion in the diet of *C. gariepinus* fingerlings did not cause remarkable histological modifications in the gill tissue as noted by Yildirim *et al*., (2006); Zhang *et al*., (2021). However, treatment D3 to D6 showed different levels of histopathological aberrations in the gill tissue, which included mild lamellar hypertrophy in D3, an indication of compensatory response to environmental stressors or dietary factors, while the marked occlusion of interlamellar spaces in D4 is indicative of impairment of respiratory surface area and gas exchange efficiency as similarly reported by Ekwueme, Iroegbu, Eze and Udedibie (2020).

Treatments D5 and D6 showed severe lamellar cell depletion and disruption of gill filament cartilage, indicating significant gill damage and structural impairment that could compromise respiratory efficiency and overall physiological performance of the fish. Similar gill histopathological alterations have been

reported in fish exposed to nutritional and environmental stressors, where changes in lamellar structure, epithelial integrity, and filament organization were used as indicators of impaired gill function. (Bernet *et al*., 1999). This could be due to the increase in fat content of the diet as the level of inclusion of orange fleshed sweet potato peels meal increased. High dietary lipid intake has been reported to induce gill inflammation, epithelial hyperplasia, epithelial swelling, and structural damage in fish, suggesting that excessive dietary fat negatively affects gill integrity and immune function (Jiang *et al*., 2024).

Similarly, dietary and environmental factors can influence gill morphology and function, as the gills are highly specialized organs responsible for gas exchange, osmoregulation, acid–base regulation, and other physiological processes; alterations in gill structure may therefore impair respiratory efficiency and overall fish health (Evans *et al*., 2005).

CONCLUSION

The histological examination of the small intestine of *C. gariepinus* fingerlings fed diets containing varying levels of orange fleshed sweet potato peels meal demonstrated the absence of marked histological changes. The intact structural integrity of the small intestine across all dietary treatments suggests the compatibility of orange fleshed sweet potato peels meal as a feed ingredient without compromising digestive health in *C. gariepinus* fingerlings. There was however, marked histological changes in the gills and liver as the level of inclusion of orange fleshed sweet potato peels meal in the diets increased, with, D3 to D6 showing moderate to severe alterations. Based on histopathological analysis of the liver, small intestine, and gills, orange fleshed sweet potato peels can be fed up to 20% to *C. gariepinus* fingerlings without harming the fish.

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