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Effect of feeds on histopathology of gills, skin, liver and kidneys of *Clarias gariepinus* (Burchell, 1822) reared in plastic tanks

Sulem Yong Nina Nindum[1,2], Tchiedjo Marie Laure D.[1,2], Essoh Etouke Adrien M.[2], Mengue Ngadena Y. Sandrine[3,4], Owona P. Emmanuel[4], Etchu A. Kingsley[2], Nola Moïse[1], Zebaze Togouet Serge H. [1, *]

[1]Hydrobiology and Environment, Department of Animal Biology and Physiology, Faculty of Sciences, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon.
[2]Institute of Agricultural Research for Development, P.O. Box 2123, Yaoundé, Cameroon.
[3]Unit of Biology, Department of Psychology, Faculty of Arts and Human Sciences, University of Yaoundé I, P.O. Box 755, Yaoundé, Cameroon
[4]Animal Physiology, Department of Animal Biology and Physiology, Faculty of Sciences, University of Yaoundé I, P.O. Box 812, Yaoundé, Cameroon

*** Corresponding author: zebasehu@yahoo.fr**

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ABSTRACT

The aim of this study was to assess histopathological alterations in the gills, skin, liver, and kidneys of the African catfish (*Clarias gariepinus*) fed with industrially manufactured extruded (Le), locally pelleted (Lpe) and locally extruded (Lex) feeds and reared in intermediate bulk containers (IBC) tanks. Nine hundred juveniles (15.15± 3.48 g; 128.37± 9.67 mm) were stocked in 9 IBC tanks (1 m^3) at a density of 100 fish/tank in triplicate and fed with the studied feeds thrice a day to satiation for 16 weeks. At the end of the experiment, 5 fish/treatment were harvested and the studied organs were extracted for histopathological assessment using standard procedures. Results revealed that no pathological alterations were observed in the gills, skin, liver, and kidneys of fish fed with "Le" feed. Hyperplasia of interlamellar epithelia, fusion of lamellae, curling of secondary lamellae, edema of primary lamellae, and erosion of secondary lamellae were observed in the gills of fish fed with "Lpe" and "Lex" feeds. Skin tissues indicated the presence of mucous cell proliferation and hypertrophy, erosion of epithelial surfaces, and thickening of the epidermal layer in fish fed with "Lpe" and "Lex" feeds. Mild diffuse and moderate vacuolation of hepatocytes were observed in the liver of fish fed with "Lpe" and "Lex" feeds respectively. Moderate diffuse vacuolar degeneration of the epithelial cells of the tubules with mild fatty change was observed in the kidneys of fish fed with "Lpe" and "Lex" feeds. The lesions observed in the local feeds were categorized as mild to moderate and could be reversible indicating that these feeds could be used in out-of-pond holding systems.

INTRODUCTION

Freshwater catfishes amongst which the African catfish (*Clarias gariepinus*, Burchell, 1822) are ranked the 6th aquaculture species according to the top 10 aquaculture species groups by quantity in world aquaculture production (**FAO, 2021).** This species is the most cultivated freshwater fish in Cameroon (**Worldfish, 2005**; **Yong-Sulem *et al*., 2006; Pouomogne and Pemsl, 2008**; **Tiogué *et al*., 2017**). The progressive dominance of this

species is due to its fast growth rate, high resistance to diseases, tolerance to environmental extremes and high consumers` preference (**Haylor, 1991; de Graaf and Janssen, 1996**; **Kestemont *et al*., 2007**). However, the major constraint to its production expansion and growth remains the need to produce cost-effective and nutritionally adequate feeds by using locally available ingredients so as to reduce the cost of production which generally accounts for about 40-70 % of the running cost (**Gabriel *et al*., 2007**; **Hetch, 2013**; **Limbu, 2020**).

In recent years, fish nutrition has improved with the development of cheaper and readily available balanced diets that promote optimal growth and health of fish (**Ahmed and Ahmed, 2020**). The advent of these improved feeds led to a tremendous increase in intensive small scale fish farming (100-500 fish/1 m^3 of water) using out of pond holding systems (concrete, tarpaulin, plastic tanks etc.) especially in the urban areas of Cameroon. With this level of intensive culture, there is therefore the need to monitor the health status of cultured fish to prevent the outbreak of devastating diseases (**Akinrotimi *et al*., 2011**).

Histology and histopathology are useful tools in assessing health status of fish as they provide information on the severity of tissue damage, injuries, and organ functionality (**Raibeemol *et al*., 2020**). Histology of fish species organs such as the liver, gut and kidney are important in the understanding of the pathological changes related to endogenous and exogenous xenobiotics which source could be nutritional (**Van Dyk *et al.*, 2009**). The use of histopathology as a parameter of evaluating pathological changes related to nutritional sources in various fish species (tilapia, turbot, sharp snout sea bream, carp, rainbow trout, etc.) is well documented (**Pereira *et al*., 2002; Mérida *et al.*, 2010; Bian *et al.*, 2017; Ali *et al.*, 2018; Parmar and Shah, 2020**).

As for *Clarias gariepinus*, the use of histopathological parameters as indices of assessment of the effect of formulated diets was documented by **Bamidele *et al.* (2015)** who worked on "*Moringa oleifera*" seed meal-based diets and used liver and kidneys histology as parameters of assessment, **Jimoh *et al.* (2015)** who worked on *Chrysophyllum albidum* based-diet and used kidney histology as the parameter of assessment and **Wasiu (2021)** who worked on toasted Semase-based diets and used liver histology as the index of assessment.

Till date, there is paucity of information on the effect of using industrially manufactured and farm-made feeds on histopathology of *C. gariepinus* reared in plastic tanks using gills, skin, liver and kidneys as indices of assessment. This study attempts to assess the histopathological changes in the gills, skin, liver and kidneys of the African catfish (*Clarias gariepinus*) fed with industrially manufactured and farm-made diets and reared in plastic tanks.

MATERIALS AND METHODS

Experimental fish

This study was carried out at the grow-out production unit of the Fish Farming Demonstration Association (FIFADA) Yaoundé, Cameroon. A total of nine hundred juveniles of mean initial weight (15.15±3.48 g) and length (128.37 ± 9.67 mm) were acclimated in 03 IBC (Intermediate Bulk Container) tanks (1x1x1 m^3) containing 800L domestic water for two weeks before the commencement of the experiment and fed with an industrially manufactured extruded feed (Le Gouessant).

Experimental diets

Locally available feed ingredients (fishmeal, soybean cake, groundnut cake, wheat bran, cassava flour, palm kennel cake, premix, L-lysine (FoodChem©), DL-methionine (FoodChem©) and refined palm oil) were purchased for formulating the locally pelleted feed (Lpe) using Pearson square method. The ingredients were processed, mixed and pelleted using a flat die pelletizer (Capsfeed Ltd), sun-dried and stored in airtight containers at room temperature until further use. Locally extruded floating (Lex) and industrially manufactured extruded floating (Le) feeds (Le Goussant) were purchased respectively from a respectable feed producer (VicFeeds) and a retailer (AgroBio) in Yaoundé, Cameroon. Proximate analyses carried out according to the methods of **AOAC (2005)** revealed the following: industrially manufactured extruded feed (42.00% crude protein, 11.00% crude fats and 2.30% crude fibre), locally pelleted feed (32.55% crude protein, 5.15% crude fats and 6.21% crude fibre) and locally extruded feed (33.57% crude protein, 5.57% crude fats and 3.90% crude fibre).

Experimental design

The fish were randomly stocked in 9 IBC tanks at a density of 100 juveniles per tank in triplicate and reared for 16 weeks. The juveniles were hand-fed experimental diets thrice daily to satiation. Domestic water (tap) that was well de-chlorinated (left for 48 h) and oxygenated was used. Water level in each tank was maintained at 800L throughout the study period and the top of the tanks were covered with screen nets to prevent fish from jumping out. One third of water from each tank was replaced daily and completely changed every fortnight throughout the experimental period to maintain a relative uniform water quality and prevent fouling from feed remnants and metabolic waste. Water parameters of importance to aquaculture were measured fortnightly (between 5:00 am to 6:00 am) according to **Boyd (1979)** and **APHA (2000)**. All the parameters were within standard limits for aquaculture as recommended by **Boyd (1979)** except for dissolved oxygen which had low values (<3 mg/L). This could be attributed to the time of sampling **(Godoy *et al*., 2021)**.

Histopathological analysis

Five fish from each dietary treatment were harvested randomly and their weights and lengths measured to the nearest “g” and “mm” respectively. Mean weight and length obtained for fish fed with “Le” were 693.40±38.55 g and 426.10±7.79 mm respectively. Those fed with “Lpe” were respectively 261.54±22.08 g and 302.50±11.28 mm and those fed with “Lex” were respectively 297.27±25.27 g and 322.30±6.42 mm.

The fish were later dissected and the gills, skin, liver and kidneys carefully removed from the body of the fish so as to avoid damage and fixed in labelled Eppendorf bottles containing Bouin`s solution for proper tissue preparation and slide production. The preserved tissues were transported to the Animal Physiology Laboratory of the University of Yaoundé I, Cameroon for microscopic observations.

The tissues were cut into small pieces of about 4 mm thick into pre-labelled cassettes. They were further immersed in 70° alcohol bath for 1 h corresponding to the first step of

dehydration. The tissues were allowed to pass through increasing concentrations of alcohol baths: 70 % (1 h), 95 % (1 h), 95 % (1 h 30), 100 % (1 h), 100 % (1 h 30), 100 % (2 h), then passed through 2 consecutive baths of xylene and finally transferred to a wax (paraffin) bath for 4h30min in a tissue embedding centre (Microm Heidelberg). Each processed tissue was given a solid support medium (paraffin wax). The molten paraffin wax was dispensed into a metal mould and the tissue was buried. A pre-labelled cassette was placed on it and was transferred to a cold plate to solidify.

The blocks were trimmed to 7µm using a rotary microtome (Reichert-Jung 2030) in order to expose the tissue surface. The trimmed sections were floated on a water bath set at 45^0C and were picked using clean labelled slides. The slides were dried at 45^0C in an oven for 24h before staining with Haematoxylin and Eosin. The stained slides were observed under a light microscope at varying X400 magnification, sections were examined and photographed using a Scientico STM-50 microscope fitted with a camera (Celestron 4442) and connected to a laptop to obtain microphotographic shots which were later interpreted.

RESULTS

Histopathology of gills

Histological changes in gills of *Clarias gariepinus* fed with industrially manufactured extruded and locally manufactured feeds are illustrated in **Fig. (1)**. Gills of fish fed with industrially manufactured extruded feed showed no alterations in the arrangement of the gill lamellae with the secondary lamellae (SL) projecting and clearly interspaced. In the core (central axis) was a visible cartilaginous supporting rod (C) and blood vessels. Pathological changes such as, hyperplasia of interlamellar epithelia (HILE), fusion of lamellae, curling of secondary lamellae (CL), oedema of primary lamellae and erosion of secondary lamellae were observed in gills of fish fed with locally pelleted feed. These changes were more pronounced in gills of fish fed with locally extruded feeds as illustrated by the gradual distortion of the lamellae with primary and secondary lamellae overlapping, eroded secondary lamellae and epithelial lifting (EL). In addition, there was shrinkage of cartilaginous supporting mass resulting in a decrease in size of the gills of fish fed with locally extruded feed.

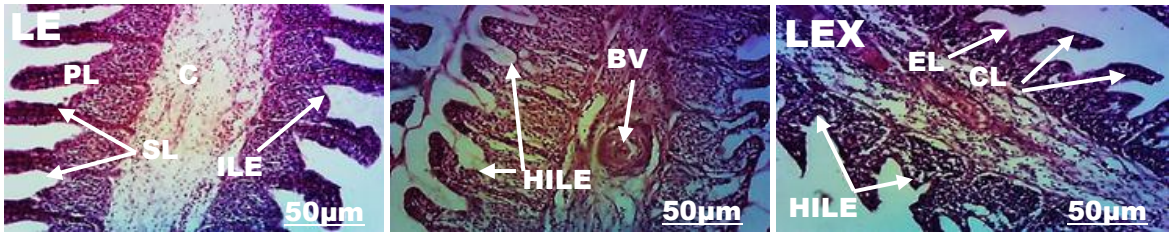

Fig. 1. Photomicrograph of a section of the gill of *C. gariepinus* fed with experimental feeds: **Le**: industrially manufactured extruded feed, **Lpe**: locally pelleted feed, **Lex**: Locally extruded feed, **PL**: Primary lamella, **SL**: Secondary Lamella, **ILE**: Interlamellar epithelia, **HILE**: Hyperplasia Interlamellar epithelia, **BV**: Blood vessel, **CL**: Curling of lamellae, **C**: Cartilaginous rod, **EL**: Epithelial lifting.

Histopathology of skin

Effect of industrially manufactured extruded and locally manufactured feeds on the histological changes on the skin of *Clarias gariepinus* is illustrated in **Fig. (2)**. No

histological changes were observed in fish fed with industrially manufactured extruded feed. Mucous cells proliferation and erosion of epithelia surfaces were observed in skin of fish fed with locally manufactured feeds. These alterations were pronounced in fish fed with locally extruded feed as illustrated by mucous cells hypertrophy and thickening of epidermal layer.

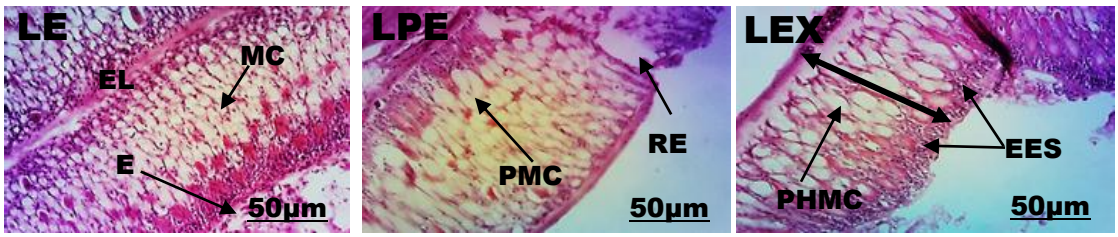

Fig. 2. Photomicrograph of a section of the skin of *C. gariepinus* fed with experimental feeds: **Le**: industrially manufactured extruded feed, **Lpe**: locally pelleted feed, **Lex**: Locally extruded feed, **EL**: Epidermal layer, **MC**: Mucous cell, **E**: Epithelium, **RE**: Ruptured epithelial cells, **PMC**: Proliferated mucous cells, **EES**: Eroded epithelial surface, **PHMC**: Proliferated and hypertrophied mucous cells, **double arrow**: thickened epidermal layer.

Histopathology of liver

Histological changes in liver of *Clarias gariepinus* fed with industrially manufactured extruded and locally manufactured feeds are illustrated in **Fig (3)**. No pathological changes were observed in liver of fish fed industrially manufactured extruded feed (Le) as illustrated by the normal hepatocytes (H) in cords and plates, sinusoids (S) making continuous communication as they are seen converging into the central vein (CV). Mild diffuse vacuolation (fatty inclusion) of hepatocyte was observed in fish fed with locallly peletted feed (Lpe) while moderate vacuolar degeneration of hepatocyte was observed in fish fed with locally extruded feed (Lex). Areas of hepatic necrosis and fatty degeneration were observed in the liver of fish fed with “Lpe” and “Lex” feeds.

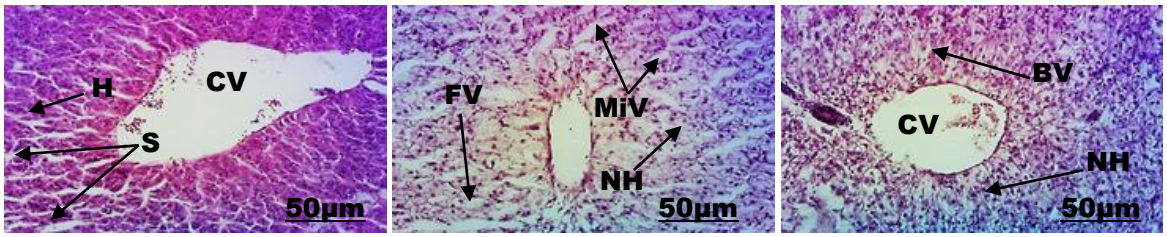

Fig. 3. Photomicrograph of a section of the liver of *C. gariepinus* fed with experimental feeds: **Le**: Industrially manufactured extruded feed, **Lpe**: locally pelleted feed, **Lex**: Locally extruded feed, **CV**: Central vein, **BV**: Blood vessel, **H**: Hepatic plate, **S**: Sinusoids, **MiV**: mild vacuolation of hepatocytes **FV**: Fat vacuole, **NH**: Necrotic hepatocyte.

Histopathology of kidneys

The histological sections of the kidneys of fish fed with experimental diets are illustrated in **Fig. (4)**. Fish fed with industrially manufactured extruded (Le) feed revealed kidney tissues with regular epithelial cells, glomeruli and no physical damage was done to the tissues. Moderate diffuse vacuolar degeneration of the epithelia cells of the tubules with mild fatty change were observed in kidneys of fish fed with locally pelleted (Lpe) and extruded (Lex) feeds. Necrosis of renal tubules was also observed with these feeds.

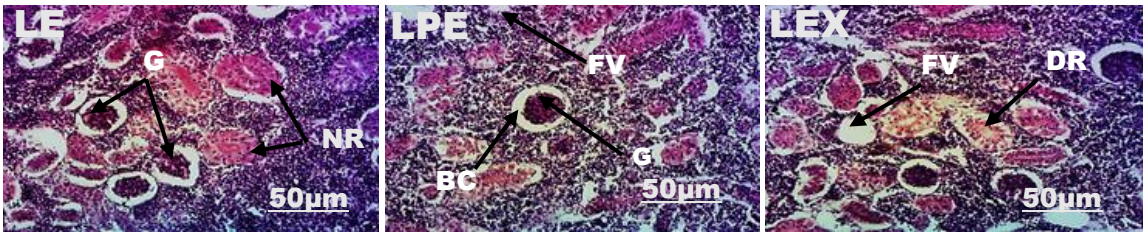

Fig. 4. Photomicrograph of a section of the kidney of *C. gariepinus* fed with experimental feeds: **Le**: Industrially manufactured extruded feed, **Lpe**: locally pelleted feed, **Lex**: Locally extruded feed, **G**: Glomeruli, **BC**: Bowman`s capsule, **NR**: normal renal tubules, **FV**: fatty vacuole, **DR**: Degenerated renal tubule.

DISCUSSION

Histopathology of gills

Gills are the primary corridor for molecular exchange between the internal milieu of fish and their external environment **(Olson, 1996)**, such as gas transfer, acid-base regulation and ionic regulation **(Eddy**, **1982**). Histological study of the gills of fish fed with industrially manufactured extruded feed showed a typical structural organization of the lamellae (**Olojo *et al*., 2005; Abubakar *et al*., 2019**). Hyperplasia was the major gill lesion observed in fish fed with experimental local feeds. **Roberts (2001)** describes hyperplasia as a fish's response to ward off or block something that irritates its tissue whether externally or internally. It may have been an early response of the gills to harmful substances in the feed or water. This agrees with the works of **Agbebi *et al*. (2013)** who also observed hyperplasia in gills of *C. gariepinus* fed different dietary treatments. Fusion of secondary lamellae, which is a result of hyperplasia, could cause a decrease in free gas exchange, thus affecting the general health of the fish **(Skidmore and Tovel, 1972)**. Curling of the secondary epithelium, lamellar fusion, oedema and epithelial lifting observed in this study were a defensive attempt by the fish to reduce available brachial surface area to stressors **(Mallat, 1985; Figueirodo-Fernandes *et al*., 2007; Mabika & Barson, 2013; Parmar and Shah, 2020)**. According to the degree of tissue change used to ascertain the nature and severity of lesions in gills of exposed fish as described by **Poleksic and Mitrovic-Tutundzic (1994)** and modified by **Abalaka *et al*. (2015)**, the lesions observed in this study did not alter the normal functioning of the gills and were classified as Stage I lesions.

Histopathology of skin

The skin of catfishes (normally not keratinized) is covered by a layer of slimy mucous **(El-Sayyad *et al*., 2010)** and act as an accessory respiratory organ **(Bruton, 1988)**. The proliferation of mucous cells observed in fish fed local feeds could be attributed to the fact these tissues were challenged. In fact, when challenged, catfish secretes mucous which helps to clean up the respiratory surfaces in facilitating the removal of trapped toxicants **(Chandra and Banerjee, 2004)** given that it also respires through the skin on its dorsum **(Bruton, 1988)**. Proliferation and hypertrophy of mucous cells, erosion of epithelia surfaces observed in fish fed with local feeds could be attributed to toxicants present in their culture media. Several authors **(Das and Mukherjee, 2003; Chandra and Banerjee, 2004; Abalaka *et al*., 2015; Utete *et al*., 2019; Okey *et al*., 2021)** observed that prolonged exposure to toxicants reduces mucous secretion resulting in severe erosion of superficial cells and widening of the epidermal and dermal layers. In this study, the lesions observed did not alter the normal

functioning of the skin and were classified as Stage I lesions according to the degree of tissue change used to ascertain the nature and severity of lesions in skin of exposed fish as described by **Poleksic and Mitrovic-Tutundzic (1994)** and modified by **Abalaka *et al.* (2015).**

Histopathology of Liver

The liver is one of the vital organs in the body that plays a major role in carbohydrates, proteins and fats metabolism. According to **Hinton and Lauran (1990)** the liver is the main detoxification centre of the body which is carried out by the hepatocytes, and alteration in liver cells is the main indicator of a toxic environment. The presence of diffuse vacuolar degeneration of hepatocytes of fish fed with locally pelleted and locally extruded feeds could be as a result of the excessive work required by the fish`s liver to get rid of the plant toxicant from its body during the process of detoxification **(Adegbesan *et al.*, 2018)**. This corroborates the works of many authors (**Bamidele *et al.*, 2015; Ibidunni *et al.*, 2017; Raimi *et al.*, 2021)** who revealed similar effects on the fish liver when fed varying diets. Nevertheless, vacuolar degeneration is considered a reversible injury and cells can recover their normal functions (homeostasis) when the stress is removed **(Szende and Suba, 1999)**. Necrosis observed might be due to the inability of fish to regenerate new liver cells **(Abubakar *et al.*, 2019)**.

Histopathology of Kidney

The primary function of the kidney tubules is to remove excess of water, salts, waste material and foreign substances from the blood **(Gordon and Zanjani, 1970)**. That fish fed with industrially manufactured extruded feed exhibited no visible change in their kidney histology shows that the studied fish could tolerate this feed. This agrees with the observations of **Adegbesan *et al.* (2018)** and **Jimoh *et al.* (2020)** who working on the same species revealed no visible changes in the histological sections of the kidney of fish fed with varying dietary doses of *Aloe barbadensis* and *Jatropha curcas* seed meal-based diets respectively. Necrosis of tubular epithelium observed in kidneys fed with locally pelleted and extruded feeds might be due to toxin, injury and infection, which could have led to unregulated cell death (**Agbebi *et al.*, 2013; Adegbesan *et al.*, 2018; Shahida *et al.*, 2021).**

CONCLUSION

The study revealed that gills, skin, liver and kidney of fish fed industrially manufactured extruded feed exhibited no pathological alterations. Fish fed locally pelleted and locally extruded revealed lesions in the gills, skin, liver and kidney which could be categorized as mild to moderate and could be reversible. This implies that the studied feeds can be used to grow-out *Clarias gariepinus* in out of pond holding systems without any harmful effect to the fish. However, affordable locally technological processing methods should be developed to reduce the anti-nutritional factors that could have been present in the local feeds.

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