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Production Agriculture and Technology is a journal which is published biannually (June and December) by the Faculty of Agriculture, Nasarawa State University, Keffi, Lafia Campus. Its fundamental aim is to publish and disseminate scientific and technological information of practical relevance to those involved with the development of tropical Agriculture.

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PAT June, 2026; 22(1):11-20, ISSN: 0794-521
Online copy available at https://www.patnsukjournal.org/
Publication of Nasarawa State University, Keffi

ORIGINAL ARTICLE

HISTOPATHOLOGICAL EXAMINATION OF TISSUES FROM *Clarias gariepinus* AND *Heterobranchus longifilis* FED METHIONINE-SUPPLEMENTED GROUNDNUT CAKE–BASED DIETS

***[1]Awolumate, S.; *[2]Agbo, A. N.; * [3]Bankole, A. S.**
* Department of Animal and Fisheries, Faculty of Agricultural Science,
National Open University of Nigeria
Corresponding author: sawolumate@noun.edu.ng

ABSTRACT
The increasing use of plant protein ingredients in aquafeeds necessitates adequate amino acid supplementation to maintain fish health and tissue integrity. Groundnut cake is a readily available plant protein source; however, its utilization is limited by deficiencies in essential amino acids, particularly methionine. This study evaluated the hepatic histopathological responses of Clarias gariepinus and Heterobranchus longifilis fed groundnut cake–based diets supplemented with graded levels of methionine. Six experimental diets were formulated, comprising a fishmeal-based control diet (GFM), an unsupplemented groundnut cake diet (GM0), and four groundnut cake–based diets supplemented with methionine at 0.25, 0.50, 0.75, and 1.00 g/kg diet (GM1–GM4). Fish were fed the experimental diets for 56 days under controlled conditions. At the end of the feeding trial, liver tissues were collected and processed for histopathological examination using standard haematoxylin and eosin staining techniques. Liver sections from fish fed the unsupplemented groundnut cake diet exhibited marked histopathological alterations, including hepatocellular vacuolation, inflammatory cell infiltration, and disruption of hepatocyte cord arrangement, indicating compromised hepatic architecture under methionine-deficient conditions. In contrast, fish fed methionine-supplemented diets showed progressive improvement in liver tissue organization, with reduced pathological alterations at higher supplementation levels. The most preserved hepatic architecture was observed in fish fed diets supplemented with 0.75 and 1.00 g methionine/kg diet. Comparative assessment indicated that hepatic alterations were generally more pronounced in Heterobranchus longifilis than in Clarias gariepinus at lower methionine inclusion levels, although both species exhibited improved liver organization at higher supplementation levels. The findings indicate that methionine supplementation mitigates diet-associated hepatic histopathological alterations when groundnut cake is used as a major plant protein source in catfish diets. Dietary inclusion of 0.75–1.00 g methionine/kg diet appears adequate to support normal liver tissue organization in Clarias gariepinus and Heterobranchus longifilis under the conditions of this study.
KEYWORDS: *African catfish, fish nutrition, organ histopathology, protein diets.*

INTRODUCTION

Aquaculture continues to play a critical role in meeting the increasing demand for fish and other aquatic products, particularly in developing countries where fish constitutes a major source of affordable animal protein (FAO, 2014). In Nigeria and other parts of sub-Saharan Africa, aquaculture development is strongly linked to the availability of nutritionally balanced and economically affordable feeds. Feed cost remains the single largest operational expense in fish farming, accounting for over 60% of total production cost, with dietary protein sources contributing the highest proportion (Madu *et al*., 2003; Ayinla, 2007). African catfishes, notably *Clarias gariepinus* and *Heterobranchus longifilis*, are among the most widely cultured fish species in Nigeria due to their fast growth rate, high market value, tolerance to adverse environmental conditions, and ability to utilize a wide range of feed ingredients (Olaoye *et al*., 2012; Fagbenro and Ajani, 2013). These species are omnivorous and possess digestive adaptations that enable efficient utilization of plant-based protein sources (Akinwole and Fagbenro, 2012). However, the increasing cost and limited availability of conventional protein sources such as

fishmeal have necessitated the search for alternative plant protein ingredients in aquafeed formulation. Fishmeal is traditionally regarded as the primary protein source in aquafeeds because of its high crude protein content and balanced amino acid profile. Nevertheless, continued reliance on fishmeal is increasingly unsustainable due to declining capture fisheries, rising global demand, and escalating market prices (Olaifa *et al*., 2020). Consequently, several plant protein ingredients such as soybean meal, groundnut cake, Bambara nut meal, and other oilseed by-products have been investigated as partial or complete replacements for fishmeal in aquafeeds (Enyidi and Mbenka, 2014; Jimoh *et al*., 2024). Among these alternatives, groundnut cake is widely available in many tropical regions, relatively inexpensive, and contains appreciable levels of crude protein, making it a promising plant protein source for aquaculture feeds (Ayinla, 2007). Despite its nutritional potential, groundnut cake is limited by imbalances in essential amino acids, particularly methionine. Methionine is a sulphur-containing essential amino acid required for protein synthesis, methyl-group donation, antioxidant defence, immune function, and normal tissue development in fish (Wang *et al*., 2023). Diets deficient in methionine have been associated with poor growth performance, reduced feed efficiency, increased oxidative stress, and metabolic disturbances in fish (Elesho *et al*., 2021; Wu *et al*., 2022). To address these limitations, supplementation of plant-based diets with synthetic methionine has become a common strategy to improve the nutritional adequacy of fishmeal-replacement diets (Du *et al*., 2024; Sun *et al*., 2025). Several studies have shown that appropriate amino acid supplementation enhances nutrient utilization and supports normal physiological function when plant proteins are used at high inclusion levels (Reda *et al*., 2021). Beyond conventional growth performance indices, increasing attention has been directed toward evaluating the internal health status of fish fed alternative diets. Histopathological examination of vital organs such as the liver, intestine, and gills is recognized as a sensitive tool for detecting subclinical nutritional stress and tissue-level alterations resulting from dietary imbalances (Rastgar *et al*., 2025). Structural changes in these organs often reflect disturbances in metabolism, detoxification processes, and nutrient absorption, thereby providing insight into the biological suitability of experimental diets (Liu *et al*., 2021; Su *et al*., 2025).

Previous studies have reported that excessive replacement of fishmeal with plant protein ingredients without adequate amino acid supplementation can induce histological abnormalities, including hepatocellular vacuolation, intestinal epithelial degeneration, and gill lamellar damage (Liu *et al*., 2021). Conversely, appropriate supplementation of limiting amino acids such as methionine has been shown to alleviate such pathological changes and support normal tissue architecture (Khodadoust *et al*., 2024; Yu *et al*., 2024; Aguillón-Hernández *et al*., 2025). Despite these findings, information on the histopathological responses of African catfish species fed methionine-supplemented groundnut cake–based diets remains limited. Furthermore, comparative data between *Clarias gariepinus* and *Heterobranchus longifilis* regarding tissue-level responses to amino acid-supplemented plant protein diets are scarce. Given the metabolic and physiological differences between these species, understanding their histopathological responses to dietary methionine supplementation is essential for developing nutritionally safe and cost-effective feeds. Therefore, the present study was conducted to evaluate the hepatic histopathological responses of *Clarias gariepinus* and *Heterobranchus longifilis* fed groundnut cake–based diets supplemented with graded levels of methionine. Specifically, the study aimed to assess diet-related alterations in liver tissue architecture and to determine methionine inclusion levels that support normal hepatic organization when groundnut cake is used as a major plant protein source in catfish diets.

MATERIALS AND METHODS

Experimental and Diets

The experiment was conducted at the National Open University of Nigeria (NOUN) Teaching and Research Farm, Rigachikun, Igabi Local Government Area of Kaduna State, Nigeria. The area lies within latitude 9°34′N and longitude 8°17′E and is characterized by Guinea savannah vegetation. The climate is of the Köppen Aw type with distinct wet (April–October) and dry (November–March) seasons, which is suitable for catfish culture (Ayinla, 2007).

Six experimental diets were formulated using groundnut cake as the major plant protein source. A fishmeal-based diet served as the control, while five groundnut cake-based diets were supplemented with graded levels of methionine to correct amino acid imbalance.

The dietary treatments were designated as:

GFM: Fishmeal-based control diet
GM0: Groundnut cake diet without methionine supplementation
GM1: Groundnut cake diet supplemented with 0.25 g methionine/kg diet
GM2: Groundnut cake diet supplemented with 0.50 g methionine/kg diet
GM3: Groundnut cake diet supplemented with 0.75 g methionine/kg diet
GM4: Groundnut cake diet supplemented with 1.00 g methionine/kg diet
All the diets were formulated to be approximately isonitrogenous using standard aquaculture feed formulation procedures (Ayinla, 2007). Feed ingredients were thoroughly mixed, pelleted using a 3-mm die, air-dried, and stored in airtight containers until use.

Experimental Fish and Acclimatization
Juvenile *Clarias gariepinus* and *Heterobranchus longifilis* were obtained from a reputable commercial fish farm in Kaduna State. Fish were acclimatized for 14 days in holding tanks and fed a commercial catfish diet to stabilize physiological conditions prior to the feeding trial (Akinwole and Fagbenro, 2012). Before the start of the experiment, fish were starved for 24 hours to allow gut evacuation (Fagbenro and Ajani, 2013).

Experimental Design and Feeding Trial
The experiment was arranged in a completely randomized design consisting of six dietary treatments, each replicated three times with fifteen fish per replicate tank. A total of 270 fish were used per species. Fish were fed their respective diets at 5% of body weight per day, divided into two equal meals administered in the morning and evening. Feeding rates were adjusted fortnightly based on batch weight measurements, and the feeding trial lasted for 56 days. This feeding regime is consistent with established practices in catfish nutrition studies (Olaoye *et al*., 2012). Culture water was partially replaced twice weekly to maintain suitable water quality conditions throughout the experimental period (Ayinla, 2007).

Liver Tissue Sampling
At the end of the feeding trial, three fish were randomly selected from each dietary treatment for histopathological examination. Fish were humanely euthanized by rapid spinal severance prior to tissue collection. Liver tissues were carefully excised, rinsed in physiological saline, and fixed in 10% neutral buffered formalin for 24–48 hours.

Histological Processing
Fixed liver tissues were processed following routine histological procedures. Samples were dehydrated through graded ethanol series, cleared in xylene, and embedded in paraffin wax. Tissue sections of approximately 5 µm thickness were cut using a rotary microtome, mounted on glass slides, and stained with haematoxylin and eosin (H&E) as described for standard fish histopathological studies (Liu *et al*., 2021).

Microscopic Examination
Prepared liver sections were examined under a light microscope at a magnification of ×250. Histopathological features assessed included hepatocellular vacuolation, hepatocytic degeneration, inflammatory cell infiltration, dilation or congestion of the central vein, and overall organization of hepatocyte cords. Histopathological alterations were evaluated descriptively based on the frequency and extent of tissue changes observed across examined sections. Lesion severity terms such as slight, moderate, and marked were used comparatively to describe relative differences among dietary treatments, following qualitative approaches commonly applied in fish nutrition histopathology (Liu *et al*., 2021; Rastgar *et al*., 2025). No numerical lesion scoring system was applied. Representative photomicrographs were captured to illustrate observed hepatic alterations.

Statistical Analysis
Histopathological observations were evaluated descriptively. No quantitative lesion scoring, biochemical, or molecular analyses were conducted; therefore, no statistical comparison of treatment means was performed. Interpretations are based solely on qualitative assessment of hepatic tissue architecture following established histopathological evaluation practices (Ayinla, 2007).

RESULTS

Histopathological Responses of Clarias gariepinus

Histological examination of liver tissues of *Clarias gariepinus* revealed clear diet-related structural variations across the dietary treatments (Plates I–VI). Fish fed the fishmeal-based control diet (GFM) exhibited largely preserved hepatic architecture. The central vein was surrounded by well-organized cords of hepatocytes, with only scanty hepatocellular vacuolation observed, indicating normal liver structure (Plate I). In contrast, liver sections from fish fed the unsupplemented groundnut cake diet (GM0) showed notable histopathological alterations. These included congestion of the central vein, hepatocellular vacuolation, and infiltration of mononuclear inflammatory cells, suggesting compromised hepatic integrity under methionine-deficient conditions (Plate II). Fish fed the diet supplemented with 0.25 g methionine/kg (GM1) exhibited inflammatory cell infiltration that partially obscured hepatocyte cords. Congestion of the central vein and portal areas was also evident, indicating persistence of hepatic stress despite supplementation at this level (Plate III). At 0.50 g methionine/kg diet (GM2), liver sections showed degenerative changes in hepatocytes accompanied by fatty vacuolation. Although some hepatocyte organization was retained, cellular alterations were still apparent (Plate IV).

Fish fed the 0.75 g methionine/kg diet (GM3) exhibited extensive dilation of the central vein with infiltration of inflammatory cells that partially masked hepatocyte cords. These observations suggest ongoing hepatic response to dietary treatment at this inclusion level (Plate V). Liver tissues from fish fed the highest methionine supplementation level (1.00 g/kg; GM4) showed comparatively improved hepatic organization. Hepatocyte cords appeared more distinct, with only mild hepatocytic swelling and scanty lymphocyte clusters surrounding the central vein (Plate VI).

Histopathological Responses of Heterobranchus longifilis

Histological sections of liver tissues from *Heterobranchus longifilis* also showed diet-dependent alterations in hepatic structure across treatments (Plates VII–XII). Fish fed the fishmeal-based control diet (GFM) exhibited near-normal liver architecture. The central vein and surrounding hepatocyte cords appeared well organized, although mild fatty vacuolation was occasionally observed (Plate VII). In fish fed the unsupplemented groundnut cake diet (GM0), liver sections showed more pronounced pathological changes. These included hepatocellular degeneration, cytoplasmic vacuolation, and inflammatory cell infiltration, indicating marked hepatic disruption under methionine-deficient conditions (Plate VIII). At 0.25 g methionine/kg diet (GM1), liver tissues showed extensive dilation of the central vein surrounded by heavy clusters of lymphocytes. Hepatocyte cords appeared disrupted, suggesting persistent inflammatory response at this supplementation level (Plate IX). Fish fed the 0.50 g methionine/kg diet (GM2) exhibited infiltration of numerous lymphocytes, dilated central vein, and fatty vacuolation. These changes indicate partial but incomplete improvement in hepatic structure (Plate X). At 0.75 g methionine/kg diet (GM3), liver sections showed dilated central vein, vacuolation, and altered hepatocyte cords. Although some structural organization was evident, pathological features were still present (Plate XI). Fish fed the highest methionine supplementation level (1.00 g/kg; GM4) showed largely preserved hepatic architecture. The central vein and most hepatocytes appeared normal, with only scanty clusters of lymphocytes observed, indicating improved liver integrity at this supplementation level (Plate XII).

Comparative Observations between the Species

Across dietary treatments, both species exhibited hepatic deterioration when fed the unsupplemented groundnut cake diet. However, *Heterobranchus longifilis* generally showed more pronounced inflammatory infiltration and hepatocellular disruption than *Clarias gariepinus* under methionine-deficient conditions. Progressive improvement in liver tissue organization was observed in both species with increasing levels of methionine supplementation, with the highest supplementation level producing near-normal hepatic architecture.

Plates: Histological Photomicrographs of Liver Tissues

Representative liver histopathological changes observed across dietary treatments are illustrated in Plates I–VI for *Clarias gariepinus* and Plates VII–XII for *Heterobranchus longifilis*.

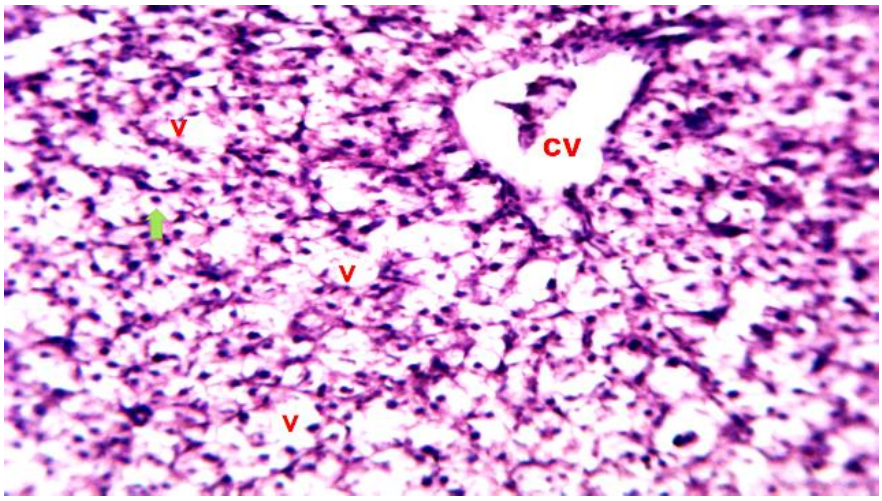


Plate I: Photomicrograph of liver section of *Clarias gariepinus* fed fishmeal-based diet (GFM), showing preserved hepatic architecture. The central vein (CV) is surrounded by radially arranged hepatocyte cords (green arrow) with scanty hepatocellular vacuolation (V). H&E stain, ×250

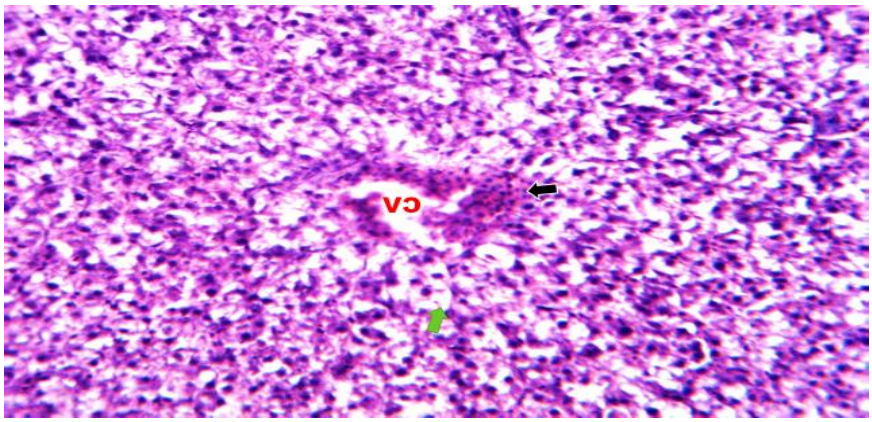


Plate II: Photomicrograph of liver section of *Clarias gariepinus* fed unsupplemented groundnut cake diet (GM0), showing congested central vein (CV) infiltrated with mononuclear cells (black arrow) and hepatocellular vacuolation (V). H&E stain, ×250.

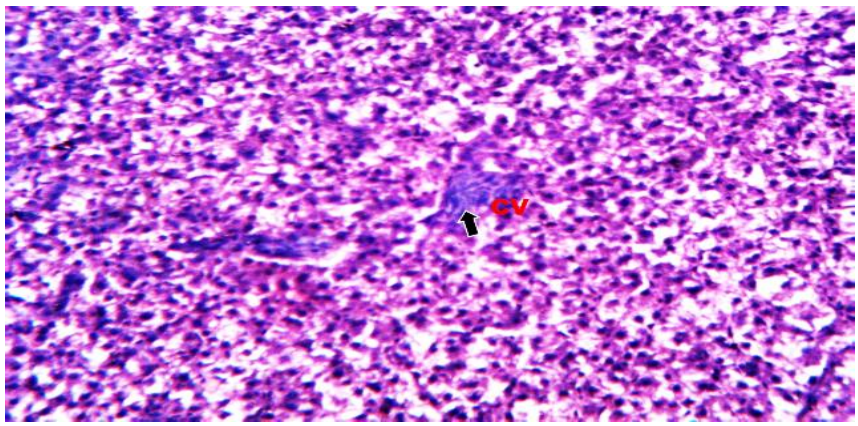


Plate III: Photomicrograph of liver section of *Clarias gariepinus* fed methionine-supplemented diet (GM1), showing inflammatory cell infiltration partially obscuring hepatocyte cords and congestion of the central vein (CV). H&E stain, ×250.

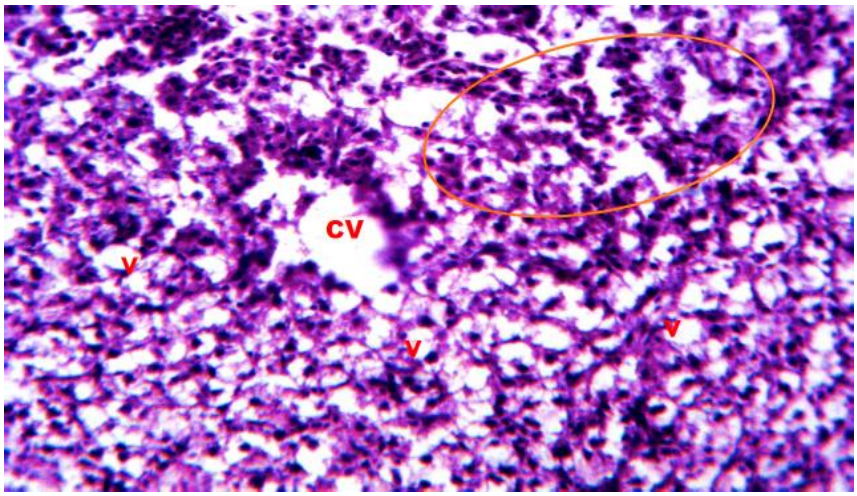


Plate IV: Photomicrograph of liver section of *Clarias gariepinus* fed methionine-supplemented diet (GM2), showing degenerative changes in hepatocytes with fatty vacuolation (V) and altered hepatic architecture (circle). H&E stain, ×250.

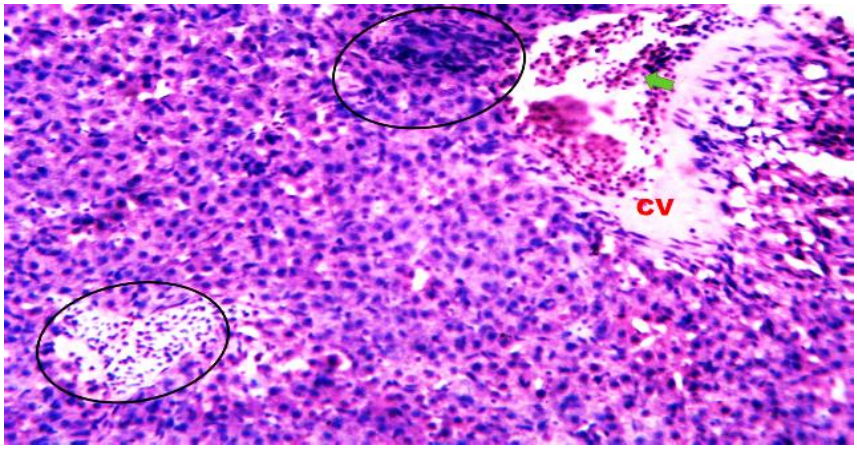


Plate V: Photomicrograph of liver section of *Clarias gariepinus* fed methionine-supplemented diet (GM3), showing extensively dilated central vein (CV) with infiltration of inflammatory cells masking hepatocyte cords (arrows). H&E stain, ×250.

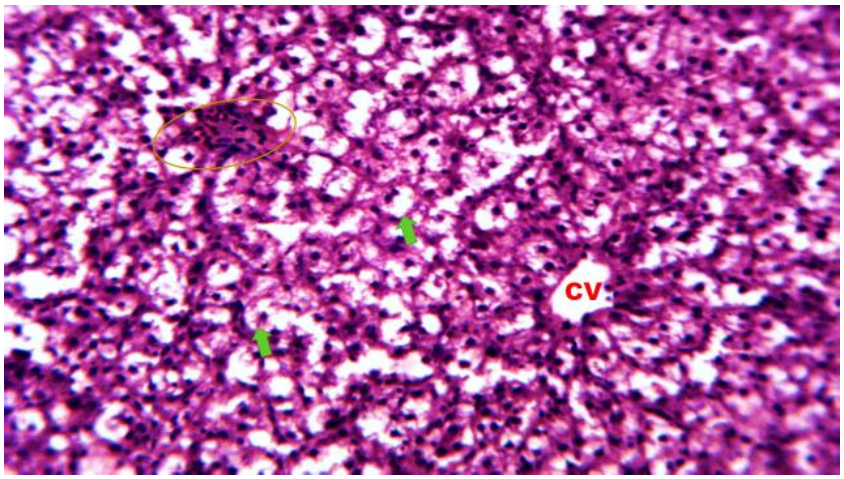


Plate VI: Photomicrograph of liver section of *Clarias gariepinus* fed methionine-supplemented diet (GM4), showing improved hepatic organization with mild hepatocytic swelling (green arrow) and scanty lymphocyte clusters around the central vein (CV). H&E stain, ×250.

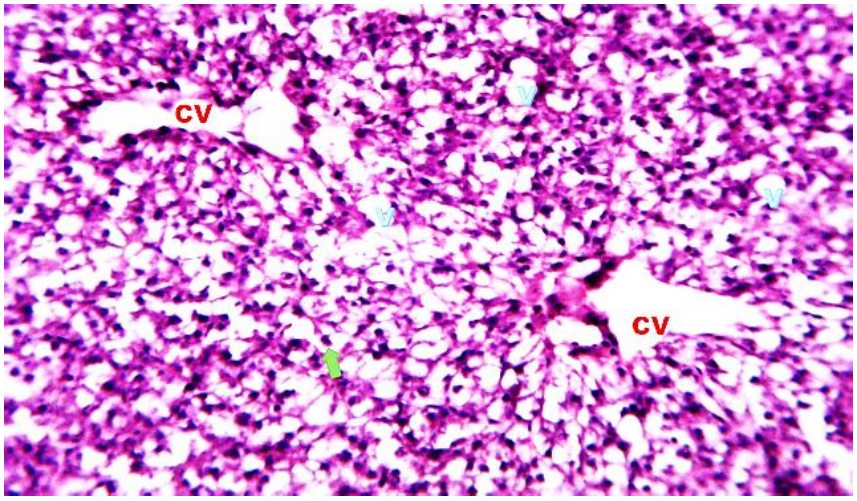


Plate VII: Photomicrograph of liver section of *Heterobranchus longifilis* fed fishmeal-based diet (GFM), showing preserved hepatic architecture with central vein (CV) and surrounding hepatocyte cords, with mild fatty vacuolation (V). H&E stain, ×250.

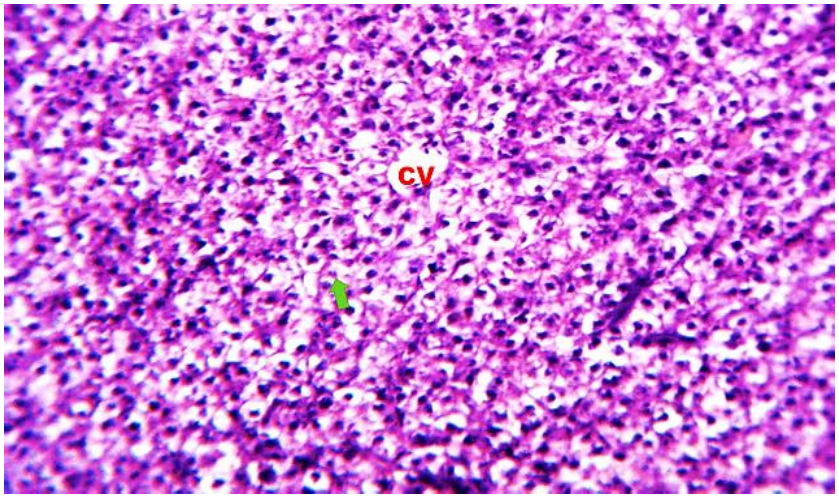


Plate VIII: Photomicrograph of liver section of *Heterobranchus longifilis* fed unsupplemented groundnut cake diet (GM0), showing hepatocyte cords surrounding the central vein (CV) with early cellular alterations. H&E stain, ×250.

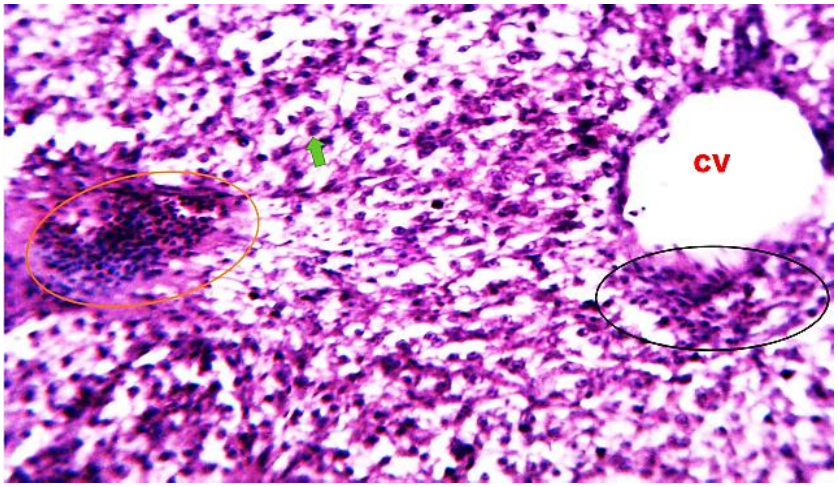


Plate IX: Photomicrograph of liver section of *Heterobranchus longifilis* fed methionine-supplemented diet (GM1), showing extensively dilated central vein (CV) surrounded by heavy clusters of lymphocytes (arrows). H&E stain, ×250.

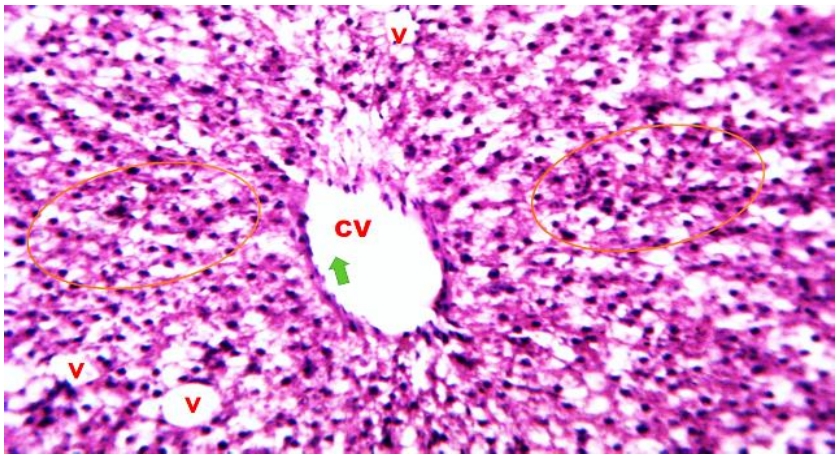


Plate X: Photomicrograph of liver section of *Heterobranchus longifilis* fed methionine-supplemented diet (GM2), showing infiltration of numerous lymphocytes (circles), dilated central vein (CV), and fatty vacuolation (V). H&E stain, ×250.

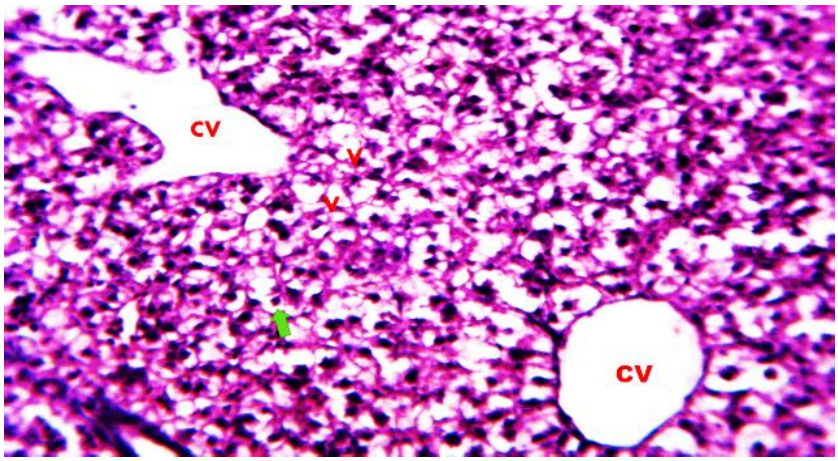


Plate XI: Photomicrograph of liver section of *Heterobranchus longifilis* fed methionine-supplemented diet (GM3), showing dilated central vein (CV), hepatocellular vacuolation (V), and altered hepatocyte cords. H&E stain, ×250.

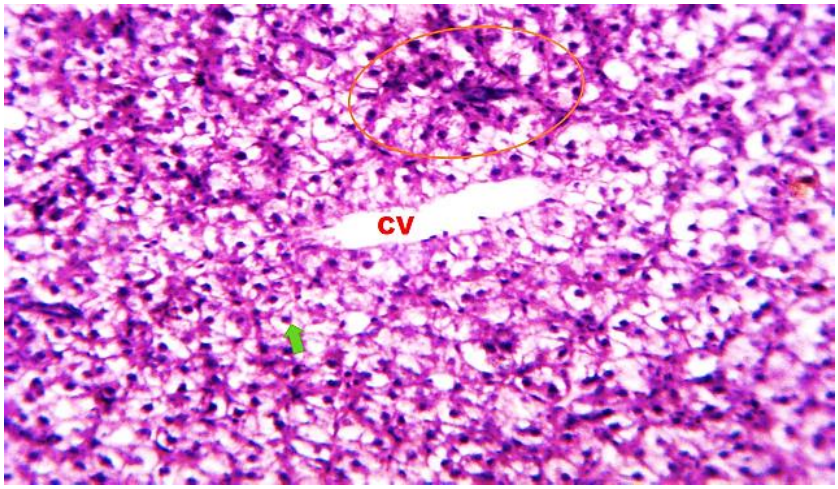


Plate XII: Photomicrograph of liver section of *Heterobranchus longifilis* fed methionine-supplemented diet (GM4), showing relatively preserved hepatocyte cords and central vein (CV) with scanty lymphocytic aggregates. H&E stain, ×250.

DISCUSSION

The present study evaluated the hepatic histopathological responses of *Clarias gariepinus* and *Heterobranchus longifilis* fed groundnut cake–based diets supplemented with graded levels of methionine. Liver histopathology provides a sensitive indicator of nutritional adequacy and metabolic stress in fish, as the liver plays a central role in protein metabolism, detoxification, and energy regulation (Ayinla, 2007; Liu *et al*., 2021). Fish fed the fishmeal-based control diet (GFM) in both

species exhibited preserved hepatic architecture, characterized by well-organized hepatocyte cords radiating from the central vein with minimal vacuolation. This observation is consistent with previous studies reporting that fishmeal-based diets support normal liver structure due to their balanced amino acid composition (Olaifa *et al*., 2020; Reda *et al*., 2021). The preserved hepatic organization observed in the control groups confirms the suitability of fishmeal as a reference protein source for maintaining liver integrity in African catfish.

In contrast, fish fed the unsupplemented groundnut cake diet (GM0) exhibited marked hepatic alterations, including hepatocellular vacuolation, inflammatory cell infiltration, and disruption of hepatocyte cord arrangement. These alterations are indicative of hepatic stress and impaired metabolic function associated with essential amino acid imbalance. Similar liver pathologies have been reported in fish fed plant protein–based diets deficient in methionine, where inadequate amino acid supply compromises protein synthesis and hepatic metabolism (Wu *et al*., 2022; Wang *et al*., 2023). Methionine supplementation resulted in progressive improvement in hepatic tissue organization. At lower supplementation levels (GM1 and GM2), liver sections still showed inflammatory infiltration and hepatocytic degeneration, suggesting that these inclusion levels were insufficient to fully mitigate the hepatic effects of the groundnut cake diet. However, at higher supplementation levels (GM3 and GM4), liver tissues exhibited comparatively reduced pathological alterations, with improved hepatocyte cord organization and reduced vacuolation. These findings agree with earlier reports that adequate methionine supplementation alleviates hepatic degeneration and supports liver integrity in fish fed plant protein–based diets (Reda *et al*., 2021; Du *et al*., 2024).

Comparative assessment between species indicated that *Heterobranchus longifilis* generally exhibited more pronounced hepatic alterations than *Clarias gariepinus* at lower methionine inclusion levels. This suggests species-specific sensitivity to methionine deficiency, possibly related to differences in metabolic rate, nutrient utilization efficiency, or protein turnover. Similar interspecific variations in dietary amino acid requirements have been reported among African catfish species (Akinwole and Fagbenro, 2012; Fagbenro and Ajani, 2013). Nevertheless, both species showed improved liver organization at higher methionine supplementation levels, indicating that the beneficial effects of methionine are broadly applicable across catfish species. It is important to note that histopathological assessment in this study was qualitative and conducted at the end of the feeding trial, without baseline liver histology prior to dietary exposure. Therefore, the observed differences among treatments represent relative hepatic tissue organization associated with dietary methionine levels rather than direct tissue recovery. This approach is consistent with descriptive histopathological studies in fish nutrition research (Liu *et al*., 2021; Rastgar *et al*., 2025). Overall, the findings of this study reinforce existing evidence that methionine is a critical limiting amino acid in plant protein–based diets and that its supplementation is essential for maintaining normal hepatic structure in fish. By demonstrating liver-specific histopathological responses in *C. gariepinus* and *H. longifilis*, this study provides additional support for the nutritional safety of methionine-fortified groundnut cake diets in African catfish culture.

CONCLUSION

The present study demonstrated that feeding *Clarias gariepinus* and *Heterobranchus longifilis* with groundnut cake–based diets without methionine supplementation resulted in marked hepatic histopathological alterations, indicating compromised liver tissue organization associated with essential amino acid imbalance. In contrast, graded methionine supplementation was associated with progressive improvement in liver histoarchitecture in both species. Liver tissues from fish fed higher methionine supplementation levels (0.75 and 1.00 g methionine/kg diet) exhibited comparatively preserved hepatic organization, with reduced hepatocellular vacuolation and inflammatory infiltration relative to the unsupplemented groundnut cake diet. Although *Heterobranchus longifilis* appeared more sensitive to methionine deficiency than *Clarias gariepinus* at lower supplementation levels, both species responded positively to increased dietary methionine inclusion. These findings indicate that methionine supplementation is necessary to support normal liver tissue organization when groundnut cake is used as a major plant protein source in catfish diets. Dietary inclusion levels between 0.75 and 1.00 g methionine/kg diet are considered adequate to mitigate diet-associated hepatic histopathological alterations in *Clarias gariepinus* and *Heterobranchus longifilis* under the conditions of this study.

REFERENCES

Aguillón-Hernández, M., Martínez-García, R., Hernández-Martínez, M. and Cruz-Suárez, L.E. (2025). Effects of amino acid supplementation on intestinal morphology and health status of fish fed plant-based diets. *Aquaculture Nutrition* 31(2): 1–12.

Akinwole, A.O. and Fagbenro, O.A. (2012). Utilization of plant protein sources in the diets of African catfish (*Clarias gariepinus*). *Journal of Aquaculture in the Tropics* 27(1): 1–12.

Ayinla, O.A. (2007). Analysis of feeds and fertilizers for sustainable aquaculture development in Nigeria. *FAO Fisheries Technical Paper* No. 497. Food and Agriculture Organization of the United Nations, Rome, Italy.

Du, J., Zhang, Y., Li, X. and Wang, J. (2024). Methionine supplementation improves growth performance, antioxidant capacity and liver health of fish fed plant protein-based diets. *Aquaculture* 576: 739810.

Elesho, F.E., Adewole, A.M. and Omitoyin, B.O. (2021). Effects of dietary amino acid imbalance on growth performance, blood indices and stress response of African catfish. *Aquaculture Reports* 19: 100620.

FAO (2014). *The State of World Fisheries and Aquaculture*. Food and Agriculture Organization of the United Nations, Rome, Italy.

Fagbenro, O.A. and Ajani, E.K. (2013). Food and feeding habits of African catfish. In: *Sustainable Aquaculture Development in Nigeria*. Nigerian Institute for Oceanography and Marine Research, Lagos, Nigeria, pp. 121–138.

Jimoh, W.A., Abdullahi, S.A. and Balogun, A.M. (2024). Evaluation of alternative plant protein ingredients in aquafeeds: Implications for fish health and sustainability. *Journal of Applied Aquaculture* 36(1): 45–60.

Khodadoust, S., Hosseini, S.A. and Hajirezaee, S. (2024). Amino acid supplementation improves intestinal histology and nutrient absorption in fish fed plant-based diets. *Fish Physiology and Biochemistry* 50: 377–389.

Liu, Y., Zhang, Q., Li, S. and Xu, C. (2021). Histopathological responses of liver, intestine and gills of fish fed diets with different protein sources. *Aquaculture Research* 52: 3451–3463.

Olaifa, F.E., Olagunju, F.I. and Ayeloja, A.A. (2020). Fishmeal scarcity and the quest for alternative protein sources in aquaculture feeds. *Nigerian Journal of Fisheries* 17(2): 205–215.

Olaoye, O.J., Ashley-Dejo, S.S., Fakoya, E.O. and Ikeweinwe, N.B. (2012). Assessment of catfish farming practices in Nigeria. *Aquaculture Research* 43: 1076–1086.

Rastgar, S., Sadeghi, A. and Zare, M. (2025). Histopathological biomarkers as indicators of nutritional stress in aquaculture species. *Environmental Science and Pollution Research* 32: 11245–11260.

Reda, R.M., Ibrahim, R.E. and Ahmed, E.N. (2021). Methionine supplementation alleviates oxidative stress and liver damage in fish fed plant protein diets. *Aquaculture Nutrition* 27(5): 1595–1605.

Su, J., Chen, L., Wang, Y. and Li, Z. (2025). Intestinal morphological responses of fish to amino acid-balanced and imbalanced diets. *Aquaculture Reports* 28: 101440.

Sun, Y., Zhao, H., Liu, X. and Zhang, D. (2025). Dietary amino acid balance reduces physiological stress and improves gill morphology in cultured fish. *Fish and Shellfish Immunology* 134: 108580.

Wang, X., Li, M., Chen, Y. and Zhou, Q. (2023). Methionine deficiency affects protein metabolism and antioxidant capacity in fish fed plant-based diets. *Aquaculture Nutrition* 29(3): 1–10.

Wu, P., Tang, L., Jiang, W. and Feng, L. (2022). Methionine deficiency induces hepatic metabolic disorder and tissue damage in fish. *Fish Physiology and Biochemistry* 48: 1289–1302.

Yu, H., Luo, Z., Liu, Y. and Chen, Q. (2024). Effects of amino acid supplementation on gut integrity and immune response in fish fed low-fishmeal diets. *Aquaculture* 565: 739095.