Journal of Science of Agriculture, Food Technology and the Environment Vol. 21 No. 1, 2023 & 2024 133-147
Available online: jsafeacademicjournal.com

DIETARY EFFECT OF FERMENTED *Annona muricata* LEAF MEAL ON THE HISTOLOGY OF LIVER AND INTESTINE OF *Heterobranchus bidorsalis* FINGERLINGS

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ABSTRACT

This study evaluated the effects of fermented Annona muricata leaf on the histology of liver and intestine in *Heterobranchus bidorsalis* fingerlings by using electron microscope. A total of One hundred and fifty (150) fingerlings with an average weight of 27.1g and length 13.23cm, were exposed to five different concentrations of fermented *Annona muricata* leaves: 0.00g (0%), 1.61g (5%), 3.21g (10%), 4.82g (15%) and 6.42g (20%) over a period 56 days. Histological analysis showed that the control group (0.00g) and the 1.61g (5%) group exhibited normal liver and intestinal structures. However, with higher concentrations, changes such as hepatocyte infiltration by lymphocytes forming lymphoid follicles in the liver, oedematous mucosa with increased lymphocytes in the intestinal mucosa, and sloughing of intestinal villi were observed. The findings indicate that concentrations of *Annona muricata* leaf above 5% (1.61g) were toxic to the fish. Therefore, its use should be approached with caution.

Keywords: Annona muricata leaves, *Heterobranchus bidorsalis*, Liver, Intestine

INTRODUCTION

Fisheries and aquaculture provide livelihoods for approximately 800 million people and supply 3.1 billion people with 20% of their animal protein (FAO, 2015), along with essential micronutrients and fatty acids crucial for cognitive and physical development (HLPE, 2014). In many low-income food-deficit countries, where fish accounts for over a one-third of the animal protein in the diet, it is often the most affordable and accessible source of animal nutrition (Kawarazuka and Bene, 2011; Belton and Thilsted, 2014). However, despite its importance as a key nutrient source, fish consumption has not been fully incorporated into strategies to address undernutrition and the nutritional aspects are not adequately reflected in aquaculture and fisheries policies (Thilsted *et al.*, 2016). The nutritional value of fish extends beyond basic dietary diversity, as it is a nutrient-dense food particularly beneficial for vulnerable groups, such as children and pregnant and lactating women. Fish is one of the few animal-based foods with strong evidence supporting its positive health benefits (Ezzati and Ribboli, 2013). Diets rich in fish are associated with a lower risk of non-communicable diseases compared to conventional diets (Tilman and Clark, 2014). Aquaculture holds significant potential to increase fish production and improve nutrition and food security in

developing nations (World Bank, 2013) with aquaculture growth rates surpassing those of land-based animal foods (FAOSTAT, 2014), production will need to double by 2030 to meet the rising demand for fish, especially in developing countries (FAO, 2014).

Heterobranchus bidorsalis is a commercially valuable fish species in Nigeria and other developing countries due to its fast growth rate, low feed conversion ratio, high stress and disease resistance, and adaptability to artificial diets. This omnivorous species, with accessory breathing organs, can survive in harsh aquatic environments where other fish species cannot (Fagbenro*et al.,* 1991; Fagbenro, 1992; Agbebi*et al.,* 2009; Owodeinde and Ndimele, 2011).

Most research on the histology or histopathology of fish organs has focused on identifying tissue abnormalities in order to determine appropriate treatment methods for fish health. The use of antibiotics and chemotherapeutics for disease prevention and treatment in aquaculture has faced significant criticism due to their harmful effects (Onuoha, 2009). As a result, there is growing emphasis on the use of natural plant-based products with immuno-modulatory and antimicrobial properties for disease management (Adewole *et al.,* 2009; Chukwuma *et al.,* 2011). Immune stimulants are compounds that enhance the non-specific immune response, either on their own or when combined with an antigen, to improve the animal's resistance to microbial and parasitic infections. The leaves of *Annona muricata* (Soursop) are known for their treatment of hypoglycemia and inflammation, as well as their antispasmodic effects (Pathak *et al.,* 2010). These properties are attributed to bioactive compounds in the plant, such as saponins, alkaloids, tannins, and other active metabolites (Vijajameena*et al.,* 2013).

Annona muricata is a tall, evergreen tree that can grow up to 9.1 meters. Belonging to the Annonaceae family, it is native to the Caribbean and Central America but is now widely cultivated in tropical and subtropical regions globally (CABI, 2018). Its leaves are used to treat conditions such as insomnia, diabetes, HIV-1, herpes, and inflammation, thanks to their anti-inflammatory and virucidal properties, which are attributed to polyphenols (Moghadamlousi*et al.,* 2015).*Soursop* is widely used in traditional medicine to treat various ailments, including headaches, insomnia, rheumatism, arthritic pain, fever, and heart and liver diseases (Adewole and Caxton-Martins, 2006; de Souza *et al.,* 2009; Mishra *et al.,* 2013). The plant's leaves, roots, and bark are used for their anti-inflammatory, hypotensive, smooth muscle-relaxant, and anti-plasmodic effects. Over 120 acetogenins have been identified from the leaves, stems, bark, pulp, and fruit peel of *Annona muricata*, with approximately 46 acetogenins found specifically in the leaves (Adewole and Caxton-Martins, 2006; de Souza *et al.,* 2009; Mishra *et al.,* 2013).

The use of antibiotics and chemotherapeutics for prophylaxis and treatment in fish farming has been widely criticized for its negative impact. Issues associated with the use of antibiotics in disease management should therefore focus on the use of natural plant products that possess immuno-modulatory and antimicrobial activities. *A. muricata* is cheap and available. For instance, there are sporadic cultivation of the plant in the southern part Nigeria specifically in communities around the study area (Federal University of Technology, Owerri) and other parts of Imo state. Therefore, this study aimedto investigate the effect of various dietary inclusion levels of fermented *A. muricata* (Soursop) leaf meal on the histology of liver and intestine of *H. bidorsalis* fingerlings.

Figure 1: Plant of *A. muricata* Plant leaves with the fruit.

MATERIALS AND METHODS

Study Area

The experiment was conducted at the Department of Fisheries and Aquaculture Technology Research Farm, Federal University of Technology, Owerri, Imo State. A completely randomized design was used for the study, with five treatments, each replicated three times. The treatments were as follows: Treatment 0 (control) at 0%, Treatment 1 at 5%, Treatment 2 at 10%, Treatment 3 at 15%, and Treatment 4 at 20% inclusion of fermented *Annona muricata*.

Source of Fingerlings and Feed Ingredients

A total of 150 *Heterobranchus bidorsalis* fingerlings, with an average weight of 27.1±0.3g and a total length of 13.23±0.2cm, were used in the experiment. The fingerlings were sourced from a reputable fish farm, acclimatized for 14 days, and fed vital feed at the Fisheries and Aquaculture farm prior to the start of the experiment. The *Annona muricata* leaves were identified and harvested from Eziobodo, Owerri, Imo State. These leaves were fermented in water for three days, and air-dried until crisp, making them suitable for grinding. The dried leaves were ground into a fine powder. Other feed ingredients were

purchased from the Relief Market in Owerri, Imo State.

Preparation of Experimental Diets

Five iso-nitrogenous diets, each containing 40% crude protein, were formulated with varying levels of fermented *Annona muricata* leaf meal as a replacement for soybean meal. The treatments were as follows: T1 (0%), T2 (5%), T3 (10%), T4 (15%), and T5 (20%) fermented *Annona muricata*. The diets were prepared by mixing the ingredients to ensure uniform distribution, and gelatinized with hot water. The resulting mixture was pelleted, sundried, and packaged in polyethylene bags, which were labeled according to their respective treatments.

Table 1: Nutritional Composition of some Major Ingredients in Comparison with *Annona muricata* Leaf Meal.

INGREDIENTS	%ASH	%MOISTURE	%PROTEIN	%CHO	%LIPID	%FIBRE
Annona muricata	4.39	12.73	26.25	8.48	9.50	40.79
Bone Meal	85.99	1.75	2.63	4.59	1.05	4.075
Corn Flour	0.60	3.16	8.75	83.99	3.15	0.35
Soybean Meal	4.92	5.71	45.94	12.89	15.6	14.94
Fish Meal	30.05	3.92	56.00	0.68	7.80	1.55

Table 2: Composition of experimental Feed at different inclusion levels of *Annona muricata* Leaf Meal.

INGREDIENTS	T0 (0%)	T1 (5%)	T2 (10%)	T3 (15%)	T4 (20%)
Annona muricata	0.00	1.61	3.21	4.82	6.42
Soybean meal	32.14	30.53	28.93	27.32	25.72
Fishmeal	32.14	32.14	32.14	32.14	32.14
Corn meal	23.72	23.72	23.72	23.72	23.72
Lysine	0.50	0.50	0.50	0.50	0.50
Methionine	1.00	1.00	1.00	1.00	1.00
Vit min Premix	1.00	1.00	1.00	1.00	1.00
Vit C	0.50	0.50	0.50	0.50	0.50
Palm Oil	2.00	2.00	2.00	2.00	2.00
Bone Meal	3.50	3.50	3.50	3.50	3.50
Common Salt	1.00	1.00	1.00	1.00	1.00
Corn Flour (starch)	2.50	2.50	2.50	2.50	2.50
Total	100	100	100	100	100
Proximate Composition (%)					

%Ash	12.12	15.6	12.45	15.94	14.22
%Moisture	5.58	7.02	5.40	8.55	11.47
%Protein	40.05	40.45	41.05	40.19	40.67
%CHO	31.89	23.00	28.88	17.96	25.45
%Lipid	5.38	8.08	8.25	10.88	5.29
%Fibre	0.32	2.30	1.52	6.48	0.90

Laboratory Method

The liver and intestines of the fish from each treatment group were collected for analysis and taken to the laboratory. Histological examination was conducted by preparing tissue samples, which were sectioned into thin slices using a microtome, stained, and mounted on microscope slides. The tissue specimens were then examined under an electron microscope. Histological stains were used to enhance the visualization of different biological structures, making it easier to distinguish them, especially those that are adjacent or in close contact. Histotechnology involves a series of techniques that enable the observation of tissue and cellular features under the microscope, allowing for the identification of specific structural changes associated with diseases (Bancroft and Stevens, 1996).

RESULTS AND DISCUSSION

The liver is considered a key indicator of nutritional health due to its role in metabolizing and detoxifying substances from the digestive tract (Raskovic and Poleksic, 2011). In this study, normal liver structure was observed in the control group (Plate I) and at the 5% inclusion level (Plate II). At higher concentrations, the liver exhibited alterations: at 10% (Plate III), 15% (Plate IV), and 20% (Plate V), the liver showed changes typical of liver toxicity. In Plate V (20% concentration), normal hepatocytes (appearing black) were heavily infiltrated by lymphocytes, forming lymphoid follicles (blue). This lymphocytic infiltration is part of the immune response, triggered by the increasing concentration of *Annona muricata* inclusion. These findings are consistent with those of Wang *et al.* (2006), who noted that lymphoid follicles are organized lymphoid structures whose formation can be stimulated by luminal factors to generate immune responses. The histological changes observed in the liver suggest that higher concentrations of *Annona muricata* lead to inevitable liver toxicity.

The intestine, a tubular organ responsible for food digestion and nutrient absorption (Nasruddin *et al.*, 2014), showed normal structure at lower inclusion levels. The coiled intestine aids in nutrient absorption (Okuthe and Bhomela, 2020). At 5% and 10% inclusions (Plates VI and VII), the intestinal architecture remained normal, with healthy villi and a well-formed muscularis propria, in line with the findings of Sugiura and Ferraris (2004) and Kasozi *et al.* (2017), which indicated normal intestinal structure suited for absorption. Similar results were found in studies by Roskovic and Poleksic (2011), who reported no pathological changes in the intestines of fish fed diets with up to 30% soybean meal replacement. Grisdale-Helland *et al.* (2000) also observed no intestinal pathology in Atlantic halibut with up to 36% diet replacement.

However, at higher inclusion levels (10% and 15%), signs of intestinal toxicity became apparent. In Plates VIII and IX, the intestinal villi were sloughed, indicating the shedding of dead tissue, a clear sign of toxicity from *Annona muricata* at 10% (3.20g) and 15% (4.80g) concentrations. At the 20% inclusion level (Plate V), the intestine showed an oedematous mucosa with moderate lymphocyte infiltration, suggesting swelling and hyperplasia due to the higher concentration of *Annona muricata*.

In terms of growth performance, *Heterobranchus bidorsalis* showed a decrease in growth as the inclusion level of *Annona muricata* leaf meal increased. This reduction in growth could be attributed to the high fiber content of the leaf meal, along with anti-nutritional factors that make the feed less palatable and harder to digest, particularly at higher inclusion levels. This led to a reduction in feed intake, as reflected in the study's growth data. This is in contrast to the findings of Kuka and Anayo (2017), who observed enhanced growth in African dwarf goats when fed *Soursop* leaf meal. However, since *Heterobranchus bidorsalis* is a carnivorous fish, it found it difficult to fully utilize the leaf meal, unlike ruminants which can digest high-fiber foods more effectively (Fagbenro*et al.*, 1992).

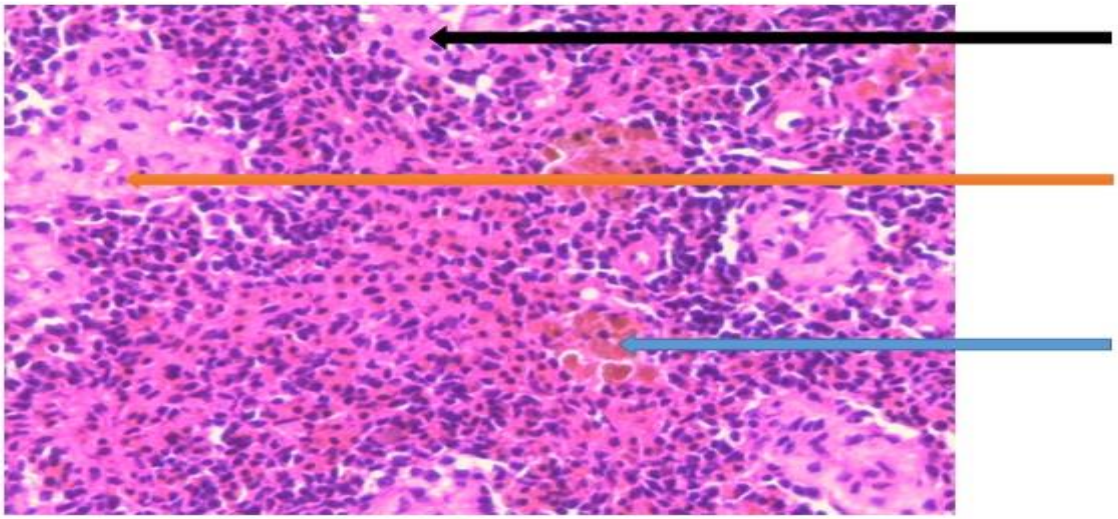

Plate I: Section of the liver of *Heterobranchus bidorsalis* (×400 magnification) fed with formulated feed at 0% *Annona muricata* inclusion level– Control, showing normal hepatocytes (black), normal veins (red) and normal ducts containing bile (blue).

Staining: Haemotoxylin counterstained with Erosin.

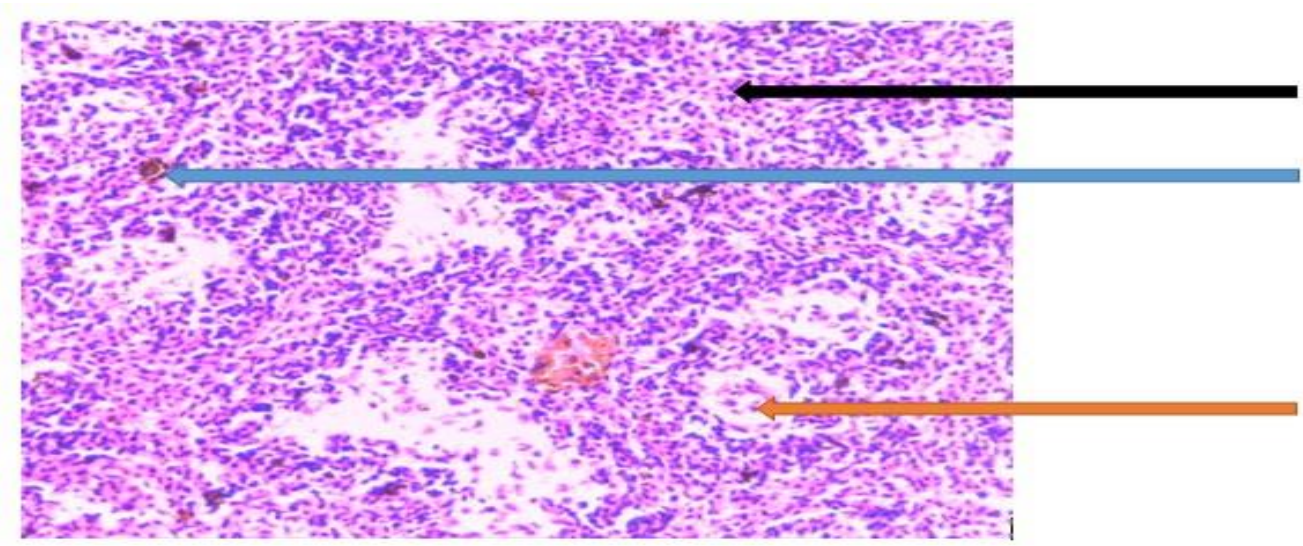

Plate II: Section of the liver of *Heterobranchus bidorsalis* (×400 magnification) fed with formulated feed at 5% *Annona muricata* inclusion level, showing normal hepatocytes (black), normal veins (red) and normal ducts containing bile (blue).

Staining: Haemotoxylin counterstained with Erosin.

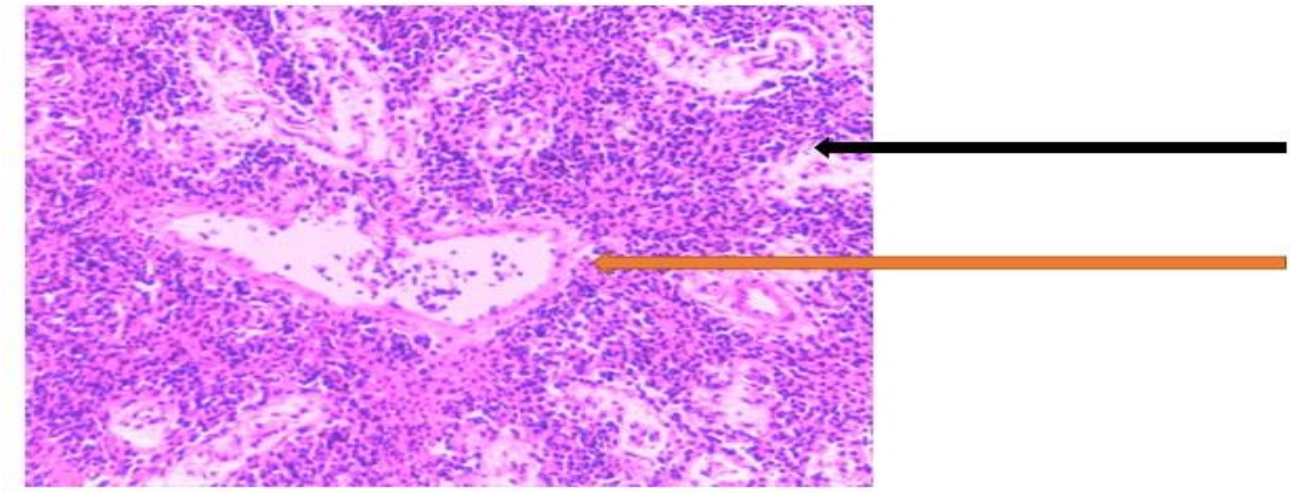

Plate III: Section of the liver of *Heterobranchus bidorsalis* (×400 magnification) fed with formulated feed at 10% *Annona muricata* inclusion level, showing normal hepatocytes (black) and normal veins (Orange).

Staining: Haemotoxylin counterstained with Erosin.

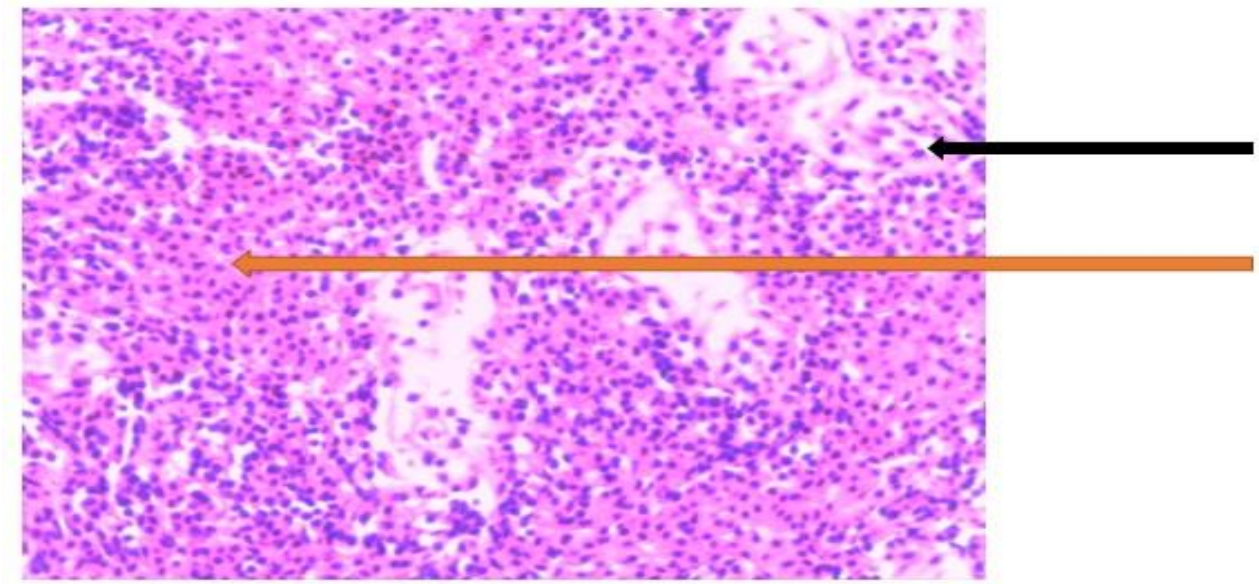

Plate IV: Section of the liver of *Heterobranchus bidorsalis* (×400 magnification) fed with formulated feed at 15% *Annona muricata* inclusion level, showing shows normal hepatocytes (black) and normal veins (orange).

Staining: Haemotoxylin counterstained with Erosin.

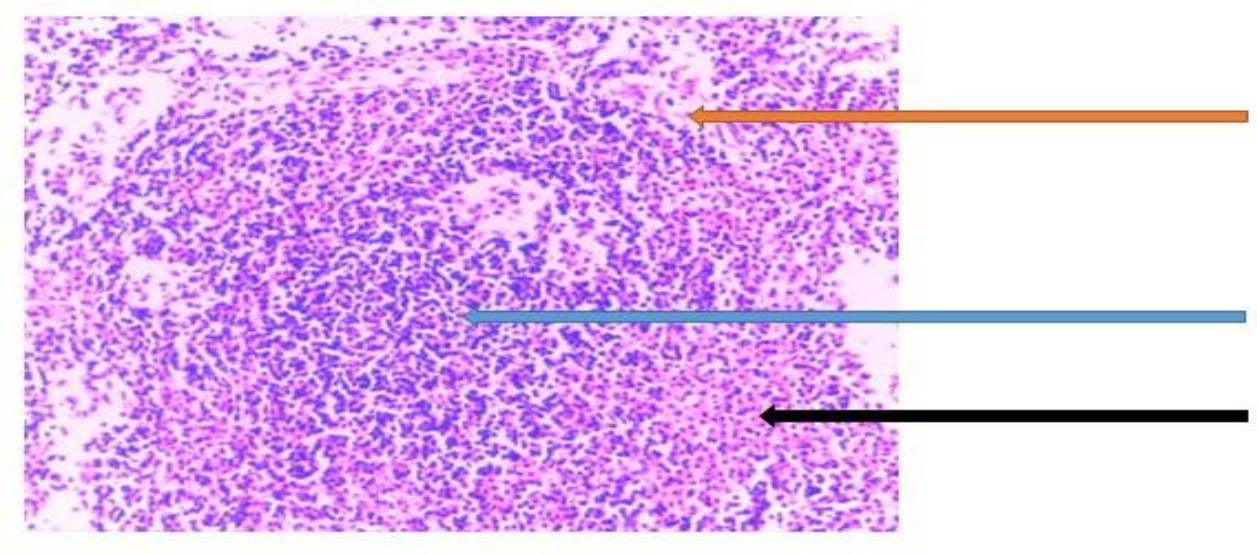

Plate V: Section of the liver of *Heterobranchus bidorsalis* (×400 magnification) fed with formulated feed at 20% *Annona muricata* inclusion level, showing normal hepatocytes (black) heavily infiltrated by lymphocytes that form lymphoid follicles (blue) and normal veins (orange).

Staining: Haemotoxylin counterstained with Erosin.

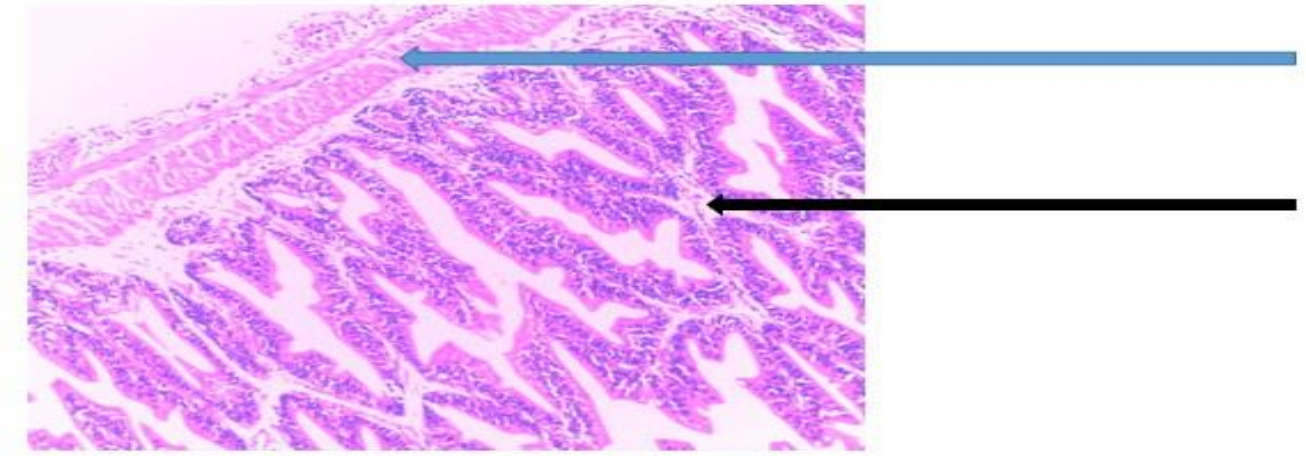

Plate VI: Section of the intestine of *Heterobranchus bidorsalis* (×400 magnification) fed with formulated feed at 0% *Annona muricata* inclusion level– Control, showing normal intestinal villi (black) and normal muscularis propia (blue).

Staining: Haematoxylin counterstained with Erosin.

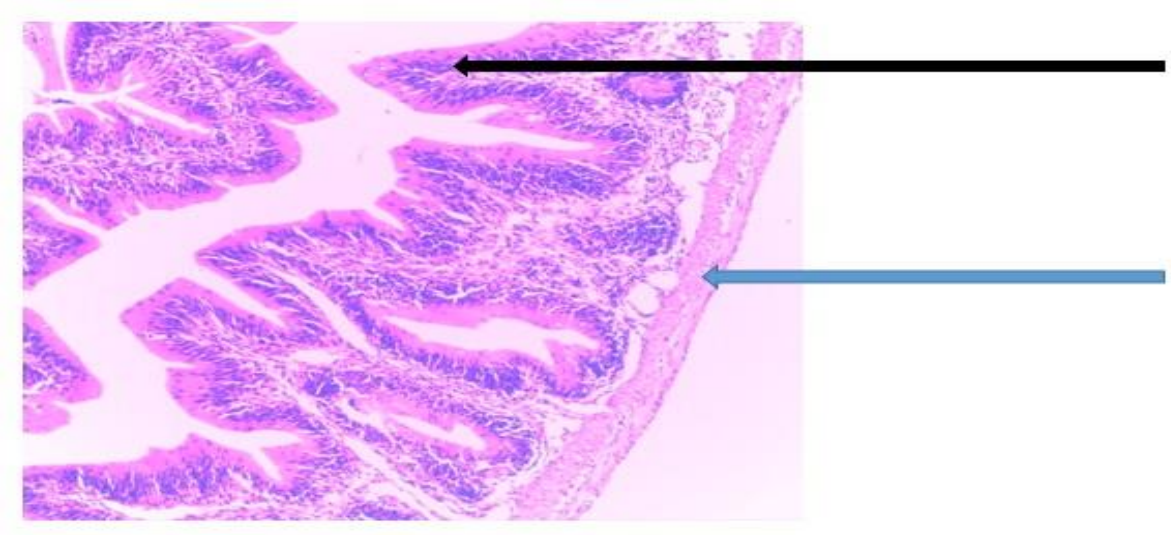

Plate VII: Section of the intestine of *Heterobranchus bidorsalis* (×400 magnification) fed with formulated feed at 5% *Annona muricata* inclusion level, showing normal intestinal villi (black) and normal muscularis propia (blue).

Staining: Haemotoxylin counterstained with Erosin.

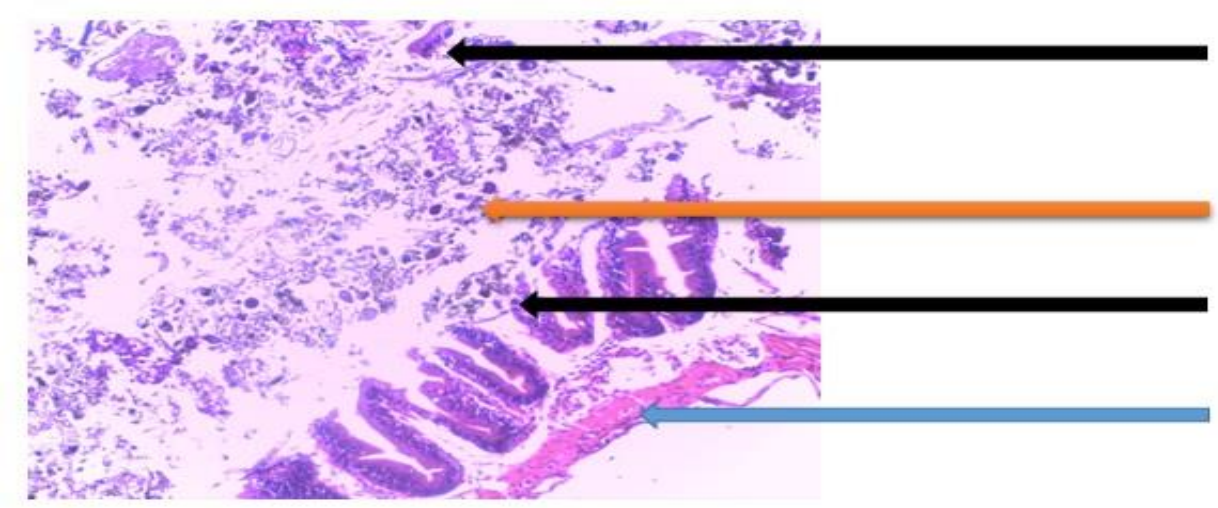

Plate VIII: Section of the intestine of *Heterobranchus bidorsalis* (×400 magnification) fed with formulated feed at 5% *Annona muricata* inclusion level, showing sloughed intestinal villi (black), faecal material (orange) and normal muscularis propia (blue).

Staining: Haemotoxylin counterstained with Erosin.

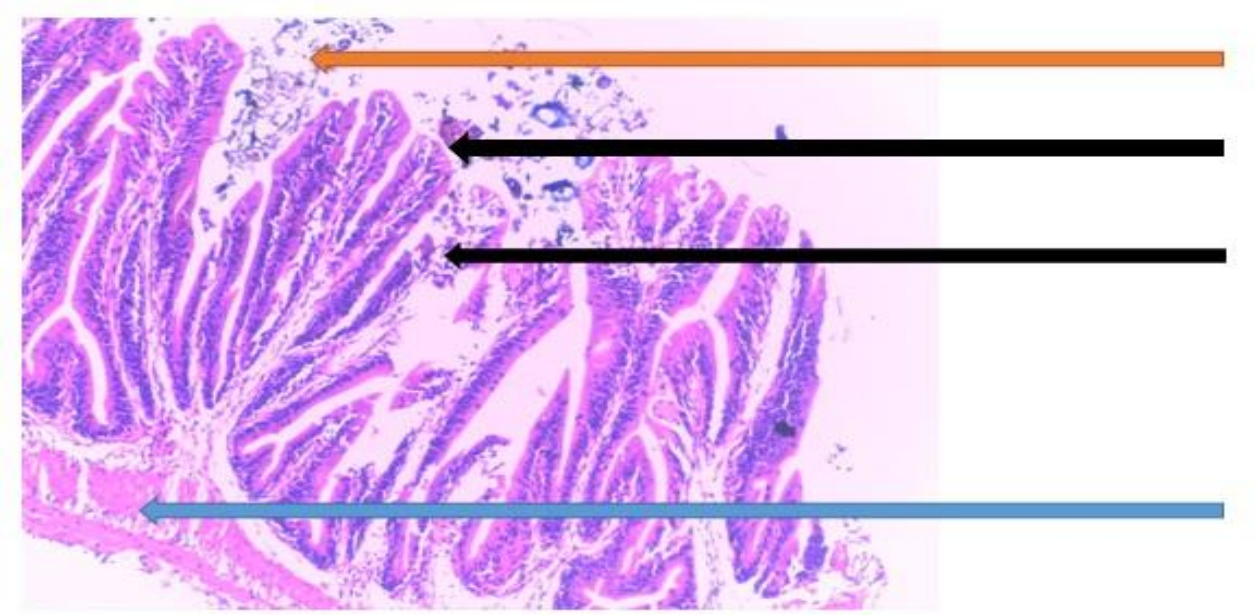

Plate IX: Section of the intestine of *Heterobranchus bidorsalis* (×400 magnification) fed with formulated feed at 5% *Annona muricata* inclusion level, showing sloughed intestinal villi (black), faecal material (orange) and normal muscularis propia (blue).

Staining: Haemotoxylin counterstained with Erosin.

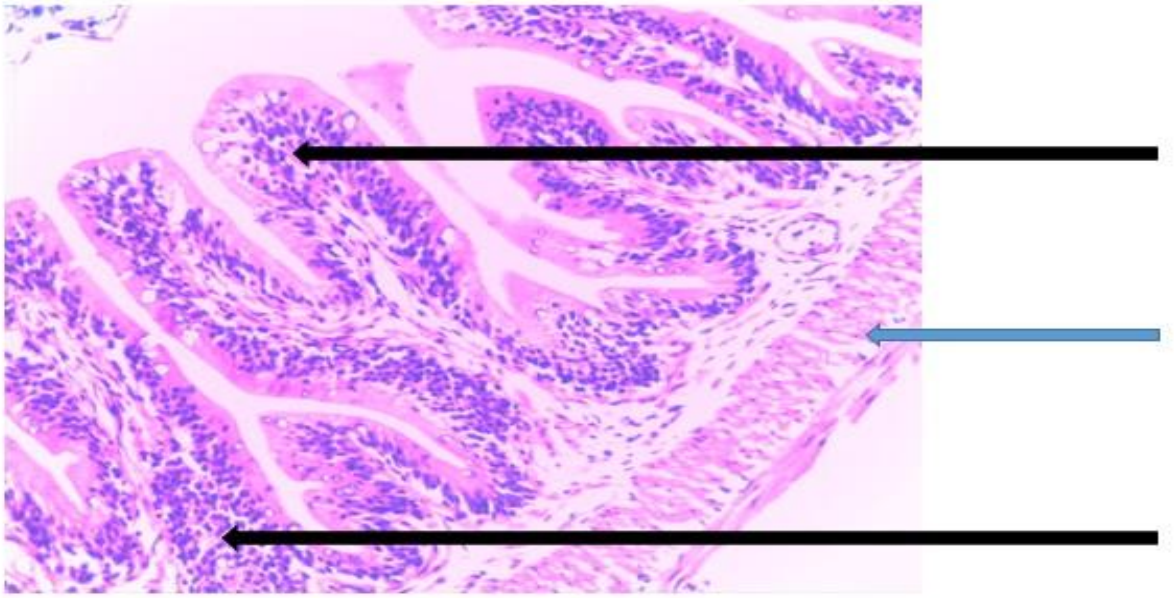

Plate X: Section of the intestine of *Heterobranchus bidorsalis* (×400 magnification) fed with formulated feed at 5% *Annona muricata* inclusion level, showing oedematous mucosa with moderate increase in the lymphocytes in the mucosa (black) and normal muscularis propia (blue).

Staining: Haematoxylin counterstained with Erosin.

CONCLUSION AND RECOMMENDATION

This study examined the histology of the liver and intestine of *Heterobranchus bidorsalis* fingerlings treated with fermented *Annona muricata* leaf meal. While normal liver and intestine structures were observed at lower concentrations (up to 5%), increasing levels (10%-20%) led to significant histological changes, including lymphocyte infiltration in the liver and intestinal villi sloughing, along with oedematous mucosa and hyperplasia in the intestine.

The findings recommend that high inclusion levels of *Annona muricata* above 10% could pose toxic risks to fish. Inclusion levels above 5% could introduce toxic substances into the aquaculture system, leading to serious negative consequences for the fish internal organs. Therefore, the use of *Annona muricata* in aquaculture should be limited to lower concentrations to avoid harmful effects.

REFERENCES

Adewole, S. O. & Caxton-Martins, E. A. (2006). Morphological changes and hypoglycemic effects of Annona muricata Linn. (Annonaceae) leaf aqueous extract on pancreatic B-cells of streptozotocin-treated diabetic rats. *Afr. J. Biomed. Res.*

Adewole, S. O., Olayiaki, L. A. &Wosu, N. (2009). Annona muricata Linn: Annonacae from Nigeria: A Review of its ethnomedicinal, Phytochemical and Pharmacological Uses. African Journal of tradition, Complementary and Alternative Medicines, 17(3):1-20.

Bancroft, J. D. & Stevens, A. (1990). Theory and practice of histological techniques, Churchill livingstone inc. 1990. Edinburgh. London, Melbourne and New York.

Belton, B. & Thilsted, S. H. (2014). Fisheries in transition: Food and nutrition security implications for the global south. Global Food Security 3(1):59–66. Belton B, van Asseldonk

CABI (2018). Huisache (*Acacia farnesiana*). Invasive Species Compendium, CABI Publishing, Wallingford, UK.

Chukwuma, K. S., Kheloa, J. O., Okonkwo, I. O., Moody, J. O. and Makinde, T. A. (2011). Advances in applied Science research,2(4):37-48.

De Souza, R., Benassi, E., da Silva, R. R., Afonso, S. &Scarminio, I. S. (2009). Enhanced extraction yields and mobile phase separations by solvent mixtures for the analysis of metabolites in *Annona muricata L. Leaves. J. Sep. Sci.*

Ezzati M. & Riboli E. (2013). Behavioral and dietary risk factors for non-communicable diseases. *The New England Journal of Medicinen* 369(10):954–64.

Fagbenro, A. O., Balogun, A. M. & Anyanwu, C. N, (1992). Optimal Dietary Protein Level of *Heterobranchus bidorsalis* fingerlings fed Compounded died. Israel journal of Aquaculture. Bamidach, 44:87-92.

Fagbenro, O. A., Olaniran, T. S. & Esan, A. O. (1991). Some aspects of the biology of the catfish, Heterobranchusbidorsalis,GoeffroySaintHilaire,1809(Clariidae)inriverOgbese, *Nigeria.* J. Afr. Zool., 105:363-372.

FAOSTAT (Food and Agriculture Organization of the United Nations) FAOSTAT database. (2014). Retrieved from http://faostat3.fao.org/home/E

Food and Agriculture Organization of the United Nations [FAO] (2014). The state of world fisheries and aquaculture: Opportunities and challenges. Rome: FAO.

Food and Agriculture Organization of the United Nations [FAO] (2015). Voluntary guidelines for securing sustainable small-scale fisheries in the context of foodsecurity and poverty eradication. Rome: FAO.

Goeffroy St. Hilaire (1809). In Owena Reservior, Southern Nigeria. Trop. Zool., 5:11-17.

Gonzalez, J., Casals, F. & Ruiz, A. (2004). Duplicative and conservative transpositions of larval serum protein 1 genes in the genus Drosophila. Genetics 168(1): 253--264.

Grisdale-Helland, S., Beaverfjard, G. & Berge, G. (2002): Full-fat soyabean meal in diets for Atlantic haliburt: growth, metabolism and intestinal histology. Aquaculture Nutrition 8:265-270

High Level Panel of Experts on Food Security and Nutrition [HLPE] (2014). Sustainable fisheries and aquaculture for food security and nutrition. A report by the High-Level Panel of Experts on Food Security and Nutrition of the Committee on World Food Security, Rome.

Belton, I. J. M.& Thilsted, S. H. (2014). Faltering fisheries and ascendant aquaculture: Implications for food and nutrition security in Bangladesh. Food Policy 44:77–87.

Kuka, T. T. &Anayo, P. (2017). Soursop (Annona muricat) leaf Meal Enhanced Growth of West African Dwarf Goats. Nigerian Journal of Animal Production. 44 (5): 239-243.

Kasozi, N., Degu, G. I., Mukalazi, J., Kato, C. D., Kissekka, M., Wadunde, A. O., Kityo, G. and Namulawa, T. (2017). Histomorphological Description of the Digestive system of Pebbly fish, Alestesbaremoze (Joannis, 1835). The scientific world Journal Volume 2017, Article ID 8591249,9 pages

Kawarazuka, N. &Béné, C. (2011). The potential role of small fish in improving micronutrient deficiencies in developing countries: Building the evidence. Public Health Nutrition 14(20):1–12.

Mishra, S., Ahmad, S., Kumar, N. & Sharma, B. K. (2013). *Annona muricata* (the cancer killer): A review. *Glob. J. Pharm. Res.*

Nasruddin, N. S., Azmai, M. N. A., Ismail, A., Saad, M. Z., Daud, H. M. & Zulkifli, S. Z. (2014). Historical features of the Gostrointestinal track of wild Indonesian shortfin Eel,Anguillabicolorbicolr (McClelland, 1844),Captured in Peninsular Malaysia. *The Scientific world Journal* Volume 2014, Article ID 312670,8 pages.

Nogales, M. S., Tomas-Vidal, A. Martinez-Liorens S., & Jover, C. M. (2010). Sunflower meal as apartial substitute in juvenile Sharpsnout sea bream (Diplodus, puntazzo) diets: amino acid retention, gut and liver histology. Aquacture 298-275-281

Okuthe, G. E. &Bhomela, B. (2020). Morphology, history and historichemistry of the digestive tract of the Banded Tilapia Sparrmanii (Perciformes: Cichlidae). An international journal for zoology. Zoologia 37:1-4

Onuoha, G. G. (2009). Fundamental Principles of Fisheries Science,New edition,pp1-171.

Owodeinde, F. G. &Ndimele, P. E. (2011) Survival, growth and feed utilization of two Clariid catfish (*Clarias gariepinus*, Burchell 1822 and *Heterobranchus bidorsalis*, Geoffroy, 1809) and their reciprocal hybrids. *Journal of Applied lcthyology* 27: 1249-1253.

Pathak, P, Saraswati, V. A. &Savaj,J. (2010). Antimicrobial activity and phytochemical analysis of the leaves of Annona muricata, International Journal of Pharma Research and Development,Vol.2(5).

Roskovic, B. &Poleksic, V. (2011). Historical methods in the assessment of different feed effects on the liver and intestine of fish, Journal of Agricultural sciences Balgrade. Dol 10 2296/JAS 11 067R

Thilsted, S. H., Thorne-Lyman, A., Subasinghe, R., Webb, P., Bogard, J. R., Phillips, M. J. & Allison, E. H. (2016). Sustaining healthy diets:The role of capture fisheries and aquaculture for improving nutrition in the post-2015 era. Food Policy 61:126–31.

Tilman, D. & Clark, M. (2014). Global diets link environmental sustainability and human health. Nature 515(7528):518–22.

Vijajameena,C.G., Subhashini,M.,Lonanayagi& Ramesh, B. (2013). Phytochemical screening and assessment of antibacterial activity for bioactive compounds in Annona muricata. Int.j.curr.Microbio.App.Sci.2(1): 1-8.

World Bank (2013). Fish to 2030: Prospects for fisheries and aquaculture. World Bank Report No. 83117-GLB. Washington, DC: World Bank.