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Histology of African Catfish *Clarias gariepinus* Fingerlings Fed Processed *Moringa oleifera* Seed Kernel Diets

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ABSTRACT

Cost of feed is threatening sustainability of aquaculture, use of non-conventional feed ingredients is becoming necessary, but their effects on both humans and animal health are equally important. The present study investigated the effects of processed moringa seed kernels (MSK) (raw, toasted, boiled, autoclaved and ethanol soaked) on the histology of *Clarias gariepinus* fingerlings. The experimental fish were fed processed MSK diets at levels of 0%, 25%, 50%, 75% and 100% respectively. Plastic bowls of thirty five litres were used for fifty six days feeding trials, twenty fish were stocked in completely randomize designed. The experimental fish with average weight of 2.50g were used. The findings of this study indicated that *Clarias gariepinus* fingerlings could tolerate up to 50% inclusion level of boiled MSK without any negative effect. It can also be concluded that raw, ethanol soaking, toasting and autoclaving of MSK can cause structural abnormalities in the histological organs (liver and kidney) resulting in severe physiological problems. These conditions can cause even death of the fish. It is therefore recommended to use boiled MSK at 50% inclusion level when use in aquaculture to maintain healthy conditions for the better survival of fishes.

Key words: Histology, moringa, processed, *Clarias gariepinus* and fingerlings.

INTRODUCTION

High costs of feeds have marginalized or even nullify the profit of the fish farming production and lowering the yield in terms of quantity and quality. The Kidney, liver and gills played vital role in maintenance of an organism internal environment being the key to regulation of volume of the extracellular fluid and composition as well as acid base balance. The toxic chemicals target the internal organs and disrupt their functions and cause permanent or temporary

rearrangement of homeostasis (Miller *et al*., 2002). Poisonous plants are known to cause decrease in dissolved oxygen and physiological changes in fish which can eventually leads to the death of fish (Morah *et al*., 2005). Kidney for example, is a vital organ playing a crucial role in elimination of metabolic nitrogen waste, maintenance of homeostasis and synthesis of essential hormones. Owing to significant blood flow circulating through the kidney tissues, and large amount of toxin afflux, the kidney is the primary toxicant - target organ (Oduola *et al*., 2010). Hence exposure to environmental pollutants increases the risk of kidney disease. As evidenced by failure of certain organ processes following the accumulation of toxicants in the nephron and particularly in the renal tubules. When the concentration of toxic substance is higher than what the homeostasis of aquatic organism can control, it results in organ damages. In fish, organs such as opercular, skin, liver, small intestine and gills could be impaired (Oyedapo, 2011). The effects of waste water discharged into water bodies can be acute that occurs rapidly and are clearly defined as fatal and rarely reversible or chronic which normally have lingering effects after long period of exposure and may ultimately cause death (Adewoye, 2005). The need to enhance growth performance on feed efficiency and disease resistance of cultured organism is substantial for various sectors of this industry (El- Haroun *et al*., 2006).The knowledge that harmful compounds elicit both toxic and advantageous biological responses has given rise to several investigations in recent times as to their possible physiological implications in various biological systems. Toxicity is expressed generally as a dose-response relationship, involving the quantity of substance to which the organism is exposed to. Furthermore, it is well known that many plant products contain some toxic substances which when consumed could accumulate gradually in the body and later cause some damages to cells, tissue or organs. The toxic effects of environmental toxins and drugs on the human and animal system have become a major health concern (El-Haroun *et al*., 2006). It is noteworthy that treated moringa seed kernels have not been used in fish diets. Hence, this study aims at determining histology of *Clarias gariepinus* fingerlings fed different inclusion levels of treated moringa seed kernels diets.

MATERIALS AND METHODS

Study locations

The study was carried out in Muhd I.U farms (Gidan kifi plaza), Guringawa quarters, located in Kumbotso Local Government Area of Kano State, Nigeria. Kumbotso is located within the Kano

metropolitan area. It lies at the geographical coordinates of 11^0 53' 17' N, 8^0 30' 10" E. (NPC, 2006). Histopathological examinations were carried out on the liver and kidney of the fish at Ultramedikx pathology laboratory, Kano.

Experimental Setup

The fish *Clarias gariepinus* fingerlings (average weight 2.50 g) were collected from Muhd I.U Farms (Gidan kifi plaza) in the local government area of Kumbotso, Kano state. Twenty (20) fishes were randomly stocked and replicated three times in seventy-five (75) plastic containers of 35 litres capacity using completely randomized designed (CRD). Before stocking, the initial weight and length of the fish were measured and recorded. The feeding trial lasted for 56 days. The feed was pelleted into 2mm pellet size. The formulated feeds were given at a 5% body weight of the fish twice daily till the end of the feeding trials. At the end of the study, histological examination of the livers, and kidney were examined on *Clarias gariepinus* fed experimental diets.

Collection of *Moringa oleifera* and processing

The plant materials *Moringa oleifera* seed kernels were purchased from the moringa plantation garden in Guringawa quarters, Kumbotso local government area, Kano state Nigeria. It lies at the geographical coordinates of 11^0 53' 17' N, 8^0 30' 10" E. (NPC, 2006).

The moringa seeds were obtained from matured dried pods. The collected seeds were shelled manually to obtain the cream-coloured kernels. The seeds were dehulled, clean of dirts by hand picking and winnowing. The size of the seeds were reduced first with a pestle and mortar, before been milled to give a fine powder and sieved using a sieving material to keep in a polythene bag until analysis. In this study, the following processing methods were used: raw, toasting, boiling in water, autoclaving, and soaking in ethanol.

Processing of moringa seed kernel meal (MSKM)

Five processing methods were used, including ethanol-soaked, boiled in water, toasted, autoclaved, and raw moringa seed kernel meal.

Raw moringa

The moringa seeds were collected from matured dried pods and manually shelled to obtain the cream-coloured kernels seed, the seeds were cracked using a pestle and mortar, and the meals were packaged in a cellophane bag before use (Muhd *et al*., 2018).

Toasting

The clean seed kernels were toasted using a gas cylinder until they turned brownish before the cracking point. The kernels of the toasted seeds were milled and the samples were packaged in a cellophane bag before the start of the experiment (Muhd *et al*., 2018).

Boiling

Four (4) liters of clean water was heated to the boiling point at 100^0C and while boiling, moringa seed kernels were poured into it and allowed for 5 minutes before they were removed with a colander (sieve) to drain off the water. The drained seed kernels were air dried until a constant weight was obtained. The dried seed kernels were milled and packed in cellophane bags before using the samples (Vadivel *et al*., 2008) modified.

Autoclaving

The recommended fifteen (15) minutes of Sulehria *et al.* (2011) at 121-124°C (200 kPa) were used for sterilization in an autoclave. The temperature was used to control and monitor the process; the pressure was mainly used to obtain the required steam temperature as described by WHAR Resolution of the World Health Assembly WHAR.10.

Ethanol Soak

Fifty grams (50g) of powdered sample were soaked in 500 ml of absolute ethanol and allowed to stand for 12 hours. The mixture was stirred occasionally. After twelve (12) hours, the samples were double-filtered using a muslin cloth and collected in a conical flask. The filtrate was dried in hot-air oven at temperature of 45^0C (Handa *et al*., 2008) modified.

Dissection of fish

At the beginning and end of the feeding trial, the fishes were sampled and sacrifice, the liver and kidneys were excised using scissors and forceps as described by the method of Tseng. (1982).

Histopathological analysis

One (1g) of each organ was put in 10ml of formal saline for proper fixation, and after the tissues have been processed and embedded in paraffin wax, sections of about 5 µm – 6 µm thick were made and stained with haematoxylin and eosin for histopathological examination (Avwioro, 2010).

Grossing

The tissues were observed and cut into small pieces of not more than 4mm thick into pre-labeled cassettes. These were further immersed in 10% formal saline for 24 hours to fix.

Tissue processing

This was done automatically using automatic tissue processor (Leica TP1020). The tissues were allowed to pass through various reagents including; stations 1 and 2 containing 10% formal saline, station 3 to station 7; alcohol (70%, 80%, 90%, 95%, absolute 1 and absolute 11) for the purpose of dehydration. The tissues continued to pass through station 8 and station 9 containing two changes of xylene for the purpose of clearing and finally transferred into three wax baths for infiltration/impregnation. The machine has been programmed to run for 12 hours; tissues stayed in each station for 1hour.

Embedding

Each processed tissue was given a solid support medium (paraffin wax) and this was done using a semi-automatic tissue embedding center. The molten paraffin wax was dispensed into a metal mold and the tissue was buried and oriented in it, a pre labeled cassette was placed on this and was transferred to a cold plate to solidify. The tissue block formed was separated from the mold.

Microtomy

The blocks were trimmed to expose the tissue surface using a rotary microtome at 6micrometer. The surfaces were allowed to cool on ice before sectioning. The tissues were sectioned at 4micrometer (ribbon section)

Floating

The sections were floated on water bath 0 (Raymond lamb) set at 55^0C and these were picked using clean slides. The slides were labeled.

Drying

The slides were dried on a hotplate (Raymond lamb) set at 60 c for 1hour. Permanent slides were prepared and photomicrographs taken, using a Carl Zeiss (Axioskope 40) Trinocular Photo micrographic microscope with digital camera for comparison with tissues obtained from those taken before feeding trials.

Staining

The staining technique used was Haematoxylin and Eosin technique.

RESULTS

Histology of the fish before experiment

Figures 1 and 2 presented below the photo-micrograph of the kidney and liver of the experimental fish before the commencement of feeding trial.

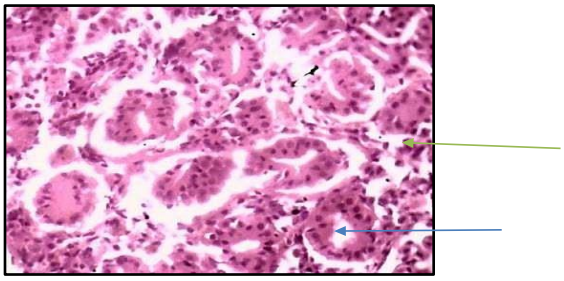

*Figure 1***:** Photomicrograph of the kidney cells of *Clarias gariepinus* fingerlings showing section of normal kidney tissue. This exhibits unremarkable tubules (blue arrow) surrounded by fibrous interstitium (green arrow) Hematoxylin and Eosin.

The figure 2 below represents photo-micrograph of the liver of the fish before the experiment.

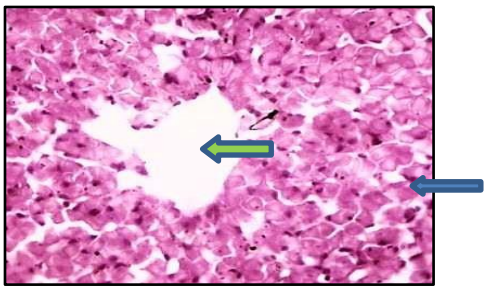

Figure 2: Photomicrograph of the liver cells of *Clarias gariepinus* showing section of normal liver tissue. This exhibits unremarkable hepatocytes (blue arrow) arranged around a central vein (green arrow). Hematoxylin and Eosinx40.There was no inflammation or abnormality seen.

Both liver and kidney were normal before the commencement of feeding trials.

Histology of fish after the experiment, fed processed moringa seed kernels meal (MSKM) based diets at different inclusion levels

Figures 3-8 show photo-micrographic of liver and kidney cells of *Clarias gariepinus* fingerlings fed experimental diets.

Kidney of the fish fed boiled moringa seed kernels meal (MSKM) based diets

The figure 3 below represents photo-micrograph of the kidney of some selected fish fed boiled MSKM based diets.

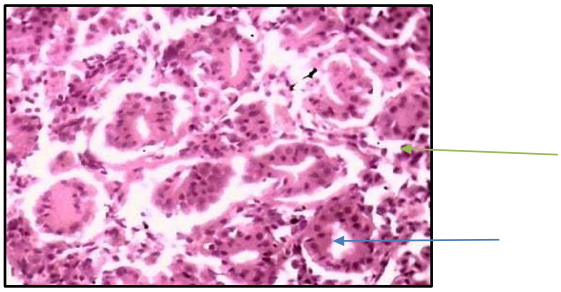

Figure 3: Photomicrograph of the kidney cells of *Clarias gariepinus* fed 0%, 25 and 50% boiled MSKM showing section of normal kidney tissue. This exhibits unremarkable tubules (blue arrow) surrounded by fibrous interstitium (green arrow). Hematoxylin and Eosin. In the kidney of fish fed the different dietary treatments (boiled), there was no major inflammation or abnormality in all the three selected diets.

Liver of the fish fed boiled MSKM based diets

The figure 4 below represents photo-micrograph of the liver of some selected fish fed boiled MSKM based diets and control.

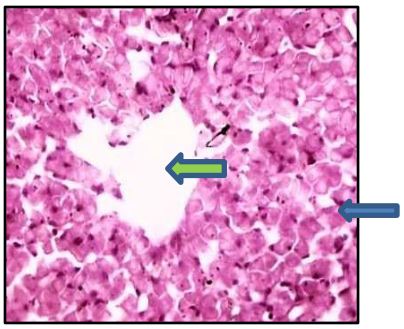

Figure 4: Photomicrograph of the liver cells of *Clarias gariepinus* fed 0%, 25% and 50% boiled diets showing section of normal liver tissue. This exhibits unremarkable hepatocytes (blue arrow) arranged around a central vein (green arrow). Hematoxylin and Eosinx40.There was no inflammation or abnormality seen.

Kidney of the fish fed raw MSKM and ethanol soaked based diets

The figure 5 below represents photo-micrograph of the kidney of some selected fish fed experimental diets.

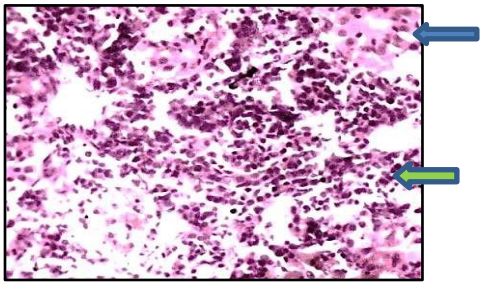

Figure 5: Photo-micrograph of the kidney cells of *Clarias gariepinus* fed 25, 50,100% raw MSKM diets and 25, 50 and 75% ethanol soaked MSKM diets showing section of kidney tissue. This exhibits focally intact tubules (blue arrow) surrounded by intensely inflamed interstitium (green arrow). Hematoxylin and Eosin x40.

Liver of the fish fed raw MSKM and ethanol soaked based diets

The figure 6 below represents photo-micrograph of the liver of some selected fish fed experimental diets.

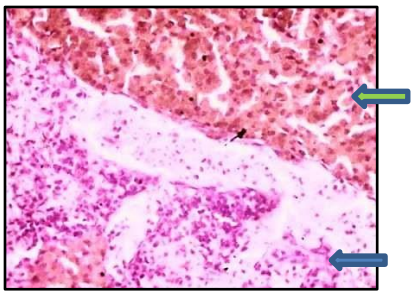

Figure 6: Photo-micrograph of the liver cells of *Clarias gariepinus* fed 25, 50 and 100% raw mkm and 25, 50 and 75% ethanol soaked diets showing section of liver tissue. This exhibits extensive necrosis and inflammation (blue arrow) and adjacent preserved hepatocytes containing golden brown intra-cytoplasmic pigments (green arrow). Hematoxylin & Eosin x40.

Kidney of the fish fed autoclaved MSKM and toasted based diets

The figure 7 below represents photo-micrograph of the kidney of some selected fish fed experimental diets.

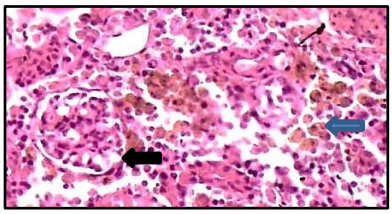

Figure 7: Photo-micrograph of the kidney cells of *Clarias gariepinus* fed 25, 75 and 100% autoclaved and 50, 75 and 100% toasted diets showing section of kidney tissue. This exhibits focal tubules with golden brown intra-cytoplasmic pigments (blue arrow) and adjacent preserved glomerulus (Black arrow). Hematoxylin & Eosin x40.

Liver of the fish fed autoclaved MSKM and toasted based diets

The figure 8 below represents photo-micrograph of the liver of some selected fish fed experimental diets.

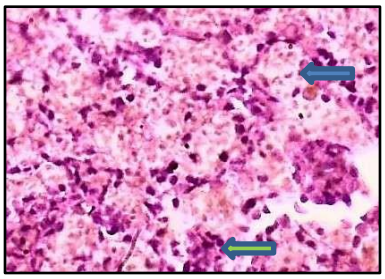

*Figure 8***:** Photomicrograph of the liver cells of *Clarias gariepinus* fed 25, 75 and 100% autoclaved and 50, 75 and 100% toasted diets showing section of liver tissue. This exhibits extensively necrotic tubules (blue arrow) surrounded by moderately inflamed interstitium (green arrow) Hematoxylin and Eosin x40.

DISCUSSION

The histology of the liver and kidney of the experimental fish *Clarias gariepinus* fingerlings fed 0%, 25% and 50% (Figures 3 and 4) of boiled moringa seed kernels (MSKM) based diets did not affect the histology of the liver, or kidney when compared with liver and kidney of the fish before the experiments. This is similar to results of Merida *et al.* (2010) and Pereira *et al.* (2002) whom reported rainbow trout fed diets with partial substitution of Brassica by-products likewise, (Hansen *et al.,* 2006). According to Ogunji *et al.* (2007), including *M. domestica* larva meal in tilapia diets had no stress on tilapia metabolism because it appears to lack a compound capable of producing reactive oxygen for oxidative stress. Lock *et al.* (2016) and Renna *et al.* (2017) found no difference in the histo-micrographs of fish fed control and insect meal diets. This is consistent with the findings of the present study. The experimental fish *Clarias gariepinus* fed (figures 5 and 6) 25, 50 and 75% ethanol based diets and 25, 50, and 100 raw mkm based diets as well as fish fed 25, 75 and 100% autoclaved and 50, 75 and 100% toasted based-diets (figures 7 and 8) showed various histological alterations in the liver and kidney which exhibited intensely inflammation and necrosis. Necrosis is an advanced and irreversible stage of degeneration and is characterized by dead hepatic cells (Pal *et al.,* 2006). This may be attributed to the excessive work required by the liver to get rid of the toxicants in the diets from it is body during the process of detoxification. Ervnest. (2004) reported liver as the main organ for detoxification, suffer serious morphological alterations in fish exposed to chemicals. Marchand *et al.* (2009) also reported that liver tissue damage occur when it is constantly exposed to toxicants. Sarkar *et al.* (2005): Camarago *et al.* (2007): Pathan *et al.* (2010) and Radhakrishnan *et al.* (2010) also reported necrosis of liver hepatocytes in different fishes following stress of starvation or xenobiotics. In the present study, necrosis appears to have caused damage and death of hepatic cells in the liver, though liver has its own regenerating capacity (Ayoola *et al.,* 2008). Under the influence of processing methods and untreated seeds kernels based-diets which may still contain excess

anti-nutrients, it appears that liver hepatocytes lose their integrity (due to necrosis) and hence the energy reserves in the form of glycogen and lipids stored in hepatocytes may apparently get depleted. Since glycogen and lipids stored in liver hepatocytes are the only source of energy for the fish during starvation, their pronounced depletion provoked by presence of toxicants that were not eliminated by the processing methods, lands the fish in extremely worst condition. Therefore it is the combined effect of necrosis and intensely inflamed interstitium which seemingly appear to result in overall degeneration of the cellular architecture of liver tissue. The total degeneration of the liver tissue may definitely results in disruption of various metabolic processes including erythropoiesis and it is functional efficiency.

Microscopic examination of the kidney of the experimental fish *Clarias gariepinus* fed 0%, 25% and 50% (Figure 4) of boiled moringa seed kernels based diets were not affected when compared with the kidney before the experiments. The absence of visible changes in the histological sections of the kidney of fed aforementioned diets could be as a result of the tolerability of the diets to the fish kidney. This agrees with the findings of Bamidele *et al.* (2015) who found similar result on the kidney of *Clarias gariepinus* fed *M.oleifera* seed meal. This portends the fact that inclusion levels of 25-50% of boiled moringa seed kernels based diets in the present study is tolerable by fish metabolic organs. The kidney cells of the experimental fish fed 25, 50 and 75% ethanol based diets and 25, 50, and 100 raw MSKM based diets as well as 25, 75 and 100% autoclaved and 50, 75 and 100% toasted based-diets exhibit focally intact tubules surrounded by intensely inflamed interstitium and focal tubules with golden brown intra-cytoplasmic pigments with preserved glomerulus. The kidney tissue was observed to undergo total degeneration with loss of normal architecture of tubules. Such histological variations observed in kidney tissue are in conformity with the findings of Velmurugan *et al.* (2007): Mohamed. (2009) and Prashanth. (2011) in fishes when exposed to different periods of starvation. Degenerative changes in kidney tissue as observed presently can be due to the effect of differently processing methods. This may result in disruption in the blood forming efficiency of kidney and hence inhibits further release of normal erythrocytes into the general circulation. Thus degrades the normal architecture of the fish kidney thereby affecting its functional efficiency which has a direct influence on physiology of fish. The response of histological profiles of *Clarias gariepinus* fed (figures 5 and 6) 25, 50 and 75%

ethanol based diets and 25, 50, and 100 raw MSKM based diets as well as fish fed 25, 75 and 100% autoclaved and 50, 75 and 100% toasted based-diets (figures 7 and 8) indicated both processing methods and inclusion levels can have negative effects on the fish as a sustainable feedstuff.

CONCLUSION AND RECOMMENDATION

From the result of this study *Clarias gariepinus* fingerlings could tolerate up to 50% inclusion level of boiled *M.oleifera* seed kernels meal (MSKM) without any negative effect. It can also be concluded that raw, ethanol soaking, toasting and autoclaving of MSKM can caused structural abnormalities in the histological organs (liver and kidney) resulting in severe physiological problems. These conditions can cause even the death of the fish. The findings of the present histological investigations demonstrate a direct correlation between processing methods and histopathological disorders observed in the tissues. It is therefore recommended to boil MSKM to 50% inclusion level when use in aquaculture to maintain healthy conditions for the better survival of fishes. However, more research should be carried out on the use of MSKM with different processing methods to this research, in order to reduce the anti-nutritional factors for better utilization of the plant.

REFERENCES

Adewoye, S. O., Fawole, O. O. and Owolabi, O. D. (2005). Toxicity of cassava waste Water effluent to AFR. Catfish Clariasgariepinus. Ethiop. Journal sci.28(7):189-194.

Ayoola .S.O. (2008) “Histopathological effects of glyphosate on juvenile African catfish (*Clarias gariepinus*)”, Ameri-surasian *J. Agric. Environ.Sci* 4: (3) 362-367.

Avwioro, O.G. (2010). Histochemistry and tissues pathology, principle and techniques, claverianum press, Nigeria

Bamidele. T.O, Aremu .M. O. and Ibrahim H. (2015) Physicochemical Characteristics of the Oils Extracted from Some Nigerian Plant Foods – A Review, *Chemical and Process Engineering Research* www.iiste.org 32: 36-52.

Camarago M.M.P. and C.B.R. Martinez (2007). "Histopathology of gills, kidney and liver of a Neotropical fish caged in an urban stream" *Neotropical Ichthyology* 5: (3): 327-336.

E L Haroun, E.R., Goda, S., Kabirchiowdhury, M.A. (2006). Effect of dietary probiotic biogenssupplementation as growth promoter on a growth performance and feed utilization of Nile tilapia Oreochromisniloticus. Aquao Res 37:1473-1480

Ervnest Hodgson (2004) A Textbook of Modern Toxicology. 3rd Edition, John Wiley and Sons, Hoboken.

Handa, S.S., Khanuja, S.P., Longo, G. and Rakesh, D.D. (2008). *Extraction Technologies for Medicinal and Aromatic Plants*. United Nations Industrial Development Organization and the International Centre for Science and High Technology 1:60-66.

Hansen, J., M. Sato, R. Ruedy, K. Lo, D.W. Lea, and M. Medina-Elizade, (2006). Global temperature change. *Proc. Natl. Acad. Sci.*, 103: 14288-14293, doi:10.1073/pnas.0606291103.

Lock E.R., Arsiwalla T., Waagbø R (2016). Insect larvae meal as an alternative source of nutrients in the diet of Atlantic salmon (Salmo salar) postsmolt. *Aquaculture Nutrition,* 22, 1202-1213.

Marchand M.J.J, Van Dyk C, Pieterse GM, Barhoorn IEJ & Borman MS (2009). Histopathological alterations in the liver of the sharptooth catfish Clarias gariepinus from polluted aquatic ecosystems in South Africa. *Environmental Toxicology* 24(2): 133-147.

Merida S.N, Ana. T-V, Silvia M-L, Miguel J. C. (2010) Sunflower meal as a partial substitute in juvenile sharpsnout sea bream (*Diplodus puntazzo*) diets: Amino acid retention, gut and liver histology. *Aquaculture* 298(3-4): 275-281.

Miller, G. G., Sweet, L. I., Adams, J. V., Omann, G. M., Passino-reader, D. R. and Meier, P.G.

(2002): Invitro toxicity and interactions of environmental contaminants (Arochlor 1254 and mercury) and immu n omo d u l a t o r y a g e n ts (lipopolysaccharide and cortisol) on thymocytes from lake trout (Salvelinusnamaycush). Fish & Shellfish Immunology, 13(1): 11-26.

Mohamed .F.A.S. (2009) "Histopathological studies on *Tilapia zilli* and *Soleo vulgaris* from lake Qarun, Egypt", *World Journal of Fish and Marine Sciences* 1: (1) 29-39.

Morah, F.N.I., Ekanem, P. and Ashipu, L. B. (2005): Effect of Mormordicacharanti leaf on water parameters and survive rate of Clariasgariepinus. International journal of current research in life science. In press

Muhd I.U., Indee, A.M., Mohammed R.A., and Garba, A.A (2018). Proximate analysis and anti nutritional composition of processed and raw bambaranut seed meal, *Journal of Agriculture,Food security and sustainable environment*, 1(2): 3635-3547.

Nigeria National Population Census. National Population Council (2006).

Oduola, T., Bello, I., Adeosun, G., Ademosun, A.W., Raheem, G. and Avwioro , G (2010). Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to Morinda lucida leaf extract. North Am. J. Med. Sci., 2: 230-233.

Ogunji J.O., Nimptsch J., Wiegand C., Schulz C. (2007). Evaluation of the influence of housefly maggot meal (magmeal) diets on catalase, glutathione S-transferase and glycogen concentration in the liver of Oreochromis niloticus fingerling. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 147:942-947.

Oyedapo, F. and Akinduyite, I. (2011). Acute toxicity of aqueous Morinda lucida leaf extract to Nile tilapia Oreochiomisniloticus (Linnaeus 1857). Proceeding of the ninth international symposium on tilapia in aquaculture Shanghai, China. April 22nd-24th, 52-59.

Pal. M, (2006). "Bioassay studies under the ecotoxicological stress of chromium tri-oxide in a

freshwater food fish, *Channa punctatus* (Bloch)". Ph.D. Thesis,Department of Zoology, University of Lucknow, Lucknow.

Pathan T.S, S.E. Shinde, P.B. Thete, and D.L. Sonawane (2010). "Histopathology of liver and kidney of *Rasbora daniconius* exposed to paper mill effluent", *Research Journal of Biological Sciences* 5 (5): 389-39.

Pereira, T. G. Oliva-Teles, A. (2002). Preliminary evaluation of pea seed meal in diets for gilthead seabream (*Sparus aurata*) juveniles. *Aquacult. Res*. 33 (14): 1183-1189.

Prashanth .M.S. (2011) "Histopathological changes observed in the kidney of freshwater fish, *Cirrhinus mrigala* (Hamilton) exposed to cypermethrin" *Recent Research in Science and Technology* 3(2): 59-65.

Radhakrishnan M.V. and S. Hemalatha (2010) "Sublethal effects of cadmium chloride to liver of freshwater fish *Channa striatus* (Bloch)", AmericanEurasian *Journal of Toxicological Sciences* 2 (11): 54-56.

Renna M., Schiavone A., Gai F., Dabbou S., Lussiana C., Malfatto V., Prearo M., Capucchio M.T., Biasato I., Biasibetti E., De Marco M., Brugiapaglia A., Zoccarato I., Gasco L. (2017). Evaluation of the suitability of a partially defatted black soldier fly (Hermetia illucens L.) larvae meal as ingredient for rainbow trout (Oncorhynchus mykiss Walbaum) diets. *Journal of Animal Science and Biotechnology, 4*8-57.

Sarkar B, A. Chatterjee, S. Adhikari, and S. Ayyappan, (2005). "Carbofuran and cypermethrin induced histopathological alterations in the liver of *Labeo rohita* (Hamilton) and its recovery", *J. Appl. Ichthyol* 21: 131 -135.

Sulehria A.Q, Yasser M, B, Altaf, H, Mehwish F, Humera A and Tayyab M. (2011). Enumeration of coliform bacteria in drinking water of Mughalpura, Lahore. *Biologia (Pakistan)* 57 (1;2); 75-80.

Tseng, W. Y. and Chan, K. L. (1982). The reproductive biology of the rabbit fish in Hongkong.

J. word MariculSoc 13(1-4): 313-321.

Vadivel, V., Pugalenthi, M., and Megha, S. (2008). Biological evaluation of protein quality of raw and processed seeds of gila bean (Entada scandens Benth.). *Tropical and Subtropical Agroecosystems* 8(2):125–133.

Velmurugan. B. M. Selvanayagam, E.I. Cengiz, and E. Unlu (2007) **"**The effects of fenvalerate on different tissues of freshwater fish *Cirrhinus mrigala*", *J. Environ. Sci. Heal* 42:157-163.