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Research article

EVALUATION OF ORGANOSOMATIC INDICES AND HISTOPATHOLOGICAL RESPONSE OF *CLARIAS GARIEPINUS* JUVENILES FED DIETS CONTAINING GRADED LEVELS OF MECHANICALLY EXTRACTED SUNFLOWER (*HELIANTHUS ANNUUS*) SEED MEAL

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ABSTRACT: Histopathological studies provide useful information on diet quality and metabolism as well as reflect fish nutritional and physiological status. This study assessed the effects of replacing soybean meal (SBM) with mechanically extracted sunflower seed meal (MESSM) on the organosomatic indices and histopathological alterations in the liver, kidney and intestine of *Clarias gariepinus* juveniles for fifteen weeks. MESSM was substituted for SBM at 0, 20, 40, 60, 80 and 100% in formulating six isonitrogenous and isocaloric diets. Diets were fed twice daily to 360 *C. gariepinus* juveniles inside eighteen rectangular tanks (in triplicate treatments). Finally, three fish specimens per treatment were dissected and their livers, kidneys and intestines removed and processed for histopathological examinations. Their tissues were fixed, washed and dehydrated with graded alcohol, embedded in paraffin, sectioned, stained and examined using photomicrography. Data were analysed using descriptive statistics and ANOVA at P = 0.05. The results revealed that hepatosomatic index (HSI) was insignificantly ($p>0.05$) superior (1.87%) in the fish fed 20% MESSM diet and lowest (1.19%) in those fed 100% inclusion. Kidney-somatic index (KSI) and intestino-somatic index (ISI) were insignificantly ($p>0.05$) highest (0.65% and 3.63%) in fish fed 0% MESSM inclusion and least (0.38% and 2.42%) in those fed 40% inclusion respectively. Fish fed 0% and 20% MESSM inclusions maintained structurally normal liver, kidney and intestine without visible lesions. However, fish fed above 20% MESSM inclusion exhibited extensive fatty infiltration, central portal venous congestion and several large cytoplasmic vacuolations of the hepatocytes. Kidney tubules showed moderate swelling, depletion of haemopoietic and tubular compartments, conspicuous degeneration and necrosis of tubule epithelia and epithelial cells with pyknotic nuclei. Intestinal changes included hyperplastic villi, pronounced necrosis and erosion of villi and enterocytes at villi's tips. The study showed that inclusion of mechanically extracted sunflower seed meal above 20% in *C. gariepinus*' diet could cause severe physiological alterations, predispose fish to disease and consequently lower aquaculture profitability.

Key words: *Clarias gariepinus*, Mechanically extracted sunflower seed meal, Histology, Organosomatic indices, Anti-nutritional factors, Photomicrography.

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INTRODUCTION

The success of aquacultural operation partly depends on the quality and quantity of feed which constitutes about 70% of the total production cost while protein is the most essential and expensive component of aquaculture diets (Omitoyin, 2007; Garza de Yta, 2012). The formulation and production of commercial feeds for cultured aquatic animals have traditionally been based on fishmeal as the principal protein source due to its high protein content and balanced essential amino acid profile. Fishmeal is also a good source of essential fatty acids, digestible energy, minerals and vitamins. However, the availability of fishmeal as the main protein component in fish feeds can no longer be guaranteed because the capture fisheries are levelling off (FAO, 2011). Also, there has always been much demand for fishmeal, hence its supply is inadequate and it is relatively expensive. As a result, the price of fishmeal continuously rises and adversely affects the profitability of aquaculture enterprises (Sintayehu *et al*., 1996). Besides, the quality of fish meal is often compromised and does not always meet the requirements for proper growth and development of cultured fish. This has necessitated the aquaculture industry to constantly source for and explore alternative protein-rich dietary supplements that are cheap, locally available and nutritionally safe for use as fishmeal replacers in aquafeeds. The decrease in the global production of fishmeal clearly indicates that the growth and sustainability of this industry will largely depend on the sustained supply of plant proteins for aquafeeds.

Soybean meal has been the main plant protein source used in animal feeds as a replacement for fishmeal because of its high protein content and relatively well balanced amino acid profile (Sintayehu *et al*., 1996). However, soybean meal has been increasingly commercialised and variously used in human, livestock and poultry dietary formulations, hence its utilisation as the main protein source in fish feeds may longer be economically viable (Siddhuraju and Becker, 2001). Therefore, this has necessitated the need to focus on using less expensive, less competitive and readily available alternative plant protein sources such as sunflower seed meal to replace soybean meal without reducing the nutritional quality of the feed (Barros *et al.*, 2002).

Sunflower (*Helianthus annuus* Linnaeus) seed is one of the important annual crops of the world grown for oil. It has a nutritional quality comparable to most other oilseed proteins including soybean and other conventional legumes (Sanz *et al*.,1994; Sintayehu *et al*., 1996) and its potential as a dietary protein source in animal feeds is well recognized (Olvera-Novoa *et al*., 2002). Studies into the use of sunflower seed meal in the feeds of livestock, poultry birds and some other monogastric animals including fish are not as extensive as for soybean meal. However, for a plant protein ingredient to be included in aquafeeds, its utilisation should be tested in different fish species because fish species differ in their sensitivity and response to anti-nutrients present in plant protein sources (Francis *et al*., 2001; Gatlin *et al*., 2007; Chaudhuri *et al*., 2012).

Clariid catfishes are the second most important group of cultured fish in the world (Fasakin *et al*., 2003). They feed on a wide range of natural and artificial food items, exhibit high growth rates and always tolerate poor water quality parameters (Amisah *et al*., 2009). High activities of protease, lipase and amylase enzymes in the digestive tract of *C. gariepinus* often indicate its ability to utilise both animal- and plant-based feed resources (Hlophe *et al*., 2014). The intestine and liver are major organs responsible for digestion and absorption of nutrients from food while the kidney performs excretion of metabolic wastes; therefore, the monitoring of these organs is imperative in nutritional studies (Raskovic *et al*., 2011).

Histopathological changes have been widely used as biomarkers in the assessment of fish health status after they have been exposed to various ontaminants in the laboratory (Thophon *et al*., 2003) and field studies (Schwaiger *et al*., 1997; Teh *et al*., 1997). One of the main advantages of using histopathological assessment is that the markers allow us to study the target organs, such as kidney, gill and liver, which are responsible for important physiological functions, such as deposition and bio-magnification of chemicals as well as excretion in fish (Gernhofer *et al*., 2001). The histopathological changes recorded are generally simpler to identify than functional changes (Fanta *et al*., 2003) and serve as signs of deleterious effects on animal health (Hinton and Laurén, 1990). Histological studies provide information on diet quality and metabolism as well as indicate the nutritional status of a fish (Segner and Braunbeck, 1988; Caballero *et al.*, 2004).

Exposure of fish to pollutants and anti-nutritional compounds usually stimulates lesions in different organs to varying degrees. Gills, liver and gut are suitable organs for histological examination to determine the effects of pollution, especially in laboratory experiments (Capkin *et al*., 2009). For an accurate and effective assessment of the effects of xenobiotic and anti-nutritional compounds in field and experimental studies, the proper monitoring of histological changes in fish liver is a highly sensitive and accurate approach (Shalaka and Pragna, 2013). The inspection of liver is pertinent as it plays an important role in the metabolism and excretion of xenobiotic compounds (Rocha and Monteiro, 1999).

Fish kidney is an important organ which performs endocrine, reticulo-endothelial, haematopoietic and excretory functions. The major function of the kidney in fish is the osmotic regulation of salts and water other than the excretion of nitrogenous wastes as in the case of mammals. The histological alterations in the kidney tissues of vertebrates subjected to experimental dietary treatments are useful bio-markers in the assessment of the effects of such dietary treatments. Assessment of histological tissues of fish kidney is a method required to establish the possible effects of various nutrient raw materials of plant and animal origin (Akhilesh *et al*., 2014). The lesions in kidneys alone are not sufficient to reveal the effects of the contaminants and they must be supported by the histopathological results obtained from the other tissues (Mishra and Mohanty, 2008).

Organosomatic indices also constitute a useful tool in correlating the weight of the visceral organs, such as liver, kidney and intestine, with the body weight of fish. For instance, hepatosomatic index (HSI) of fish has been used as an indicator of environmental risk (Pinkney *et al*., 2001; Yang and Baumann, 2006). They found a positive correlation between HSI and the concentration of polycyclic aromatic hydrocarbon (PAH) metabolites in fish. Thus, the aim of this study was to evaluate the effects of substituting mechanically extracted sunflower seed meal (MESSM) for soybean meal (SBM) on the organosomatic indices and histopathological alterations in the liver, kidney and intestine of *Clarias gariepinus* juveniles.

MATERIALS AND METHODS

Collection of organs

Effects of dietary treatments on histology of liver, kidney and intestine of *C. gariepinus* juveniles were investigated. At the completion of the feeding trial, three fish samples were taken from each dietary treatment, weighed individually and injected with benzocaine at a concentration of 50 mg/L (Coyle *et al*., 2004) to anaesthetize them before dissection. The fish were dissected using a dissecting kit and images of internal organs were taken by means of a digital camera (Olympus CH XSZ-107BN) during dissection. After gross examination of the internal organs, the entire liver, kidney and intestine of each fish sample were removed, weighed separately and recorded for evaluation of organosomatic indices.

Determination of organosomatic indices

The ratio of the weight of the liver, kidney and intestine in relation to the body weight of fish was calculated separately from the following organosomatic index formula as described by Ali (2001):

$$\text{Organosomatic index (\%)} = \frac{\text{Organ weight (g)}}{\text{Fish body weight (g)}} \times 100$$

This formula was used to calculate organosomatic indices of the liver, kidney and intestine respectively as follows:

$$\text{Hepatosomatic index (HSI)} = \frac{\text{weight of liver (g)}}{\text{fish body weight (g)}} \times 100$$

$$\text{Kidney-somatic index (KSI)} = \frac{\text{weight of kidney (g)}}{\text{fish body weight (g)}} \times 100$$

$$\text{Intestino-somatic index (ISI)} = \frac{\text{weight of intestine (g)}}{\text{fish body weight (g)}} \times 100$$

Histopathological analysis

Histopathological examinations were carried out to assess possible alterations in the intestines, livers and kidneys of the fish fed with the different experimental diets. The examinations were carried out at the Department of Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, University of Ibadan, Nigeria, following Lynch's medical laboratory procedures. At the end of the experiment, three fish samples from each dietary treatment were used for the diagnostic histological analysis.

The fish were injected with benzocaine at a concentration of 50 mg/L (Coyle *et al*., 2004) to anaesthetize them before dissection. They were then dissected using a dissecting kit and their whole intestines, livers and kidneys were carefully removed, washed with distilled water to remove blood stain and immediately pre-fixed in Bouin's fixative solution and later in 10% formalin solution for 48 hours. The organs were dehydrated in periodic acid Schiff's reagent (PAS) following the method of Hughes and Perry (1976) in graded levels of 50%, 70%, 90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding. The organs were then cleaned and embedded in melted wax and carefully sliced into thin sections with a rotatory microtome (5µm thick).

The cut sections were again cleaned by placing them in warm water (38°C) from where they were transferred into clean slides and oven-dried at 58°C for 30 minutes to melt the wax and stained with Harris' haematoxylin–eosin (H and E) stain (Bancroft and Cook, 1994). The slides containing sectioned tissues were cleaned using xylene and graded levels of 50%, 70%, 90%, 95% and 100% alcohol for two minutes each. The sections were again stained in haematoxylin-eosin for ten minutes and mounted in dipterex on glass slides. To obtain their photomicrography, the stained sections were examined and photographed at different magnifications (x40, x100 and x400) by means of a binocular light microscope (Olympus Japan 312545) fitted with a digital camera (Olympus CH XSZ-107BN), a photographic attachment (Olympus C35 AD4) and an automatic light exposure unit (Olympus PM CS5P).

Data analysis

Histopathological description of morphological changes and statistical analysis of indices were used to present the research findings. All data obtained in this work are presented as mean± standard deviation. Comparisons were made between the control and experimental groups. One-way ANOVA and Duncan's multiple range test (Duncan, 1955) were used on SPSS statistical software (Version 16.0 for Windows; SPSS Inc., Chicago, USA) to detect the significant differences among the control and experimental groups. Differences were considered to be statistically significant at probability levels below 0.05 (i.e. $p<0.05$) (Zar, 1984).

RESULTS

Organosomatic indices

The values of organosomatic indices of the liver, kidney and intestine of *C. gariepinus* juveniles are shown in Table 1. Hepatosomatic index (HSI) was highest (1.87%) in the fish fed 20% MESSM-based diet and least (1.31%) in the fish fed 60% MESSM-based diet. However, no significant difference ($p>0.05$) existed in the HSI values among the fish in the dietary treatments. Kidney-somatic index (KSI) was highest (0.65%) in the fish fed 0% MESSM-based diet and least (0.38%) in the fish fed 40% MESSM-based diet. However, the values of KSI did not show significant differences ($p>0.05$) in the fish among the dietary treatments. Intestino-somatic index (ISI) was highest (3.63%) in the fish fed 0% MESSM-based diet and lowest (2.42%) in the fish fed 40% MESSM-based diet. The fish fed with 0%, 40% and 80% MESSM-based diets had ISI values which significantly differed ($p<0.05$) from the values recorded for the fish fed with the other MESSM-based diets.

Table 1: Organosomatic indices of *C. gariepinus* juveniles fed graded levels of mechanically extracted sunflower seed meal-based diets for 15 weeks

Organosomatic indices (%)	Experimental dietary inclusions					
	MESSM 1 (0%) (Control)	MESSM 2 (20%)	MESSM 3 (40%)	MESSM 4 (60%)	MESSM 5 (80%)	MESSM 6 (100%)
Liver (HSI)	1.67±0.71^{a}	1.87±0.80^{a}	1.55±0.15^{a}	1.31±0.31^{a}	1.51±0.13^{a}	1.19±0.29^{a}
Kidney (KSI)	0.65±0.09^{a}	0.60±0.15^{a}	0.38±0.04^{a}	0.46±0.18^{a}	0.63±0.21^{a}	0.49±0.17^{a}
Intestine (ISI)	3.63±0.82^{a}	3.13±0.38^{ab}	2.42±0.16^{b}	3.27±0.16^{ab}	3.61±0.57^{a}	3.31±0.40^{ab}

The above values are means of triplicate data. Mean values in each row with similar superscripts are not significantly different ($p>0.05$). MESSM = mechanically extracted sunflower seed meal HSI - Hepatosomatic index KSI - Kidney-somatic index ISI - Intestino-somatic index

Gross and photomicrographic examination of liver, kidney and intestine

At the end of the feeding experiment, the liver, kidney and intestine samples appeared externally normal as no visible deformity was observed and they retained their normal colour appearance. However, microscopic examination of these organs revealed varying degrees of histological changes as a result of dietary treatments (Table 2 and Plates 1 to 18). Photomicrographs of sections of the livers of the fish fed 0% (control diet) and 20% MESSM-based diets showed moderate diffuse cytoplasmic vacuolations in their hepatocytes (Plates 1 and 2) while the fish fed 40% MESSM-based diet revealed multiple foci of large cytoplasmic vacuolations of the hepatocytes (Plate 3). Moderate diffuse vacuolar change and fatty infiltration were observed in the liver sections of the fish fed 60% MESSM-based diet (Plate 4). The livers of the fish fed 80% MESSM-based diet revealed moderate periportal vacuolar change (thin arrow), extensive fatty infiltration and central portal venous congestion (thick arrow) (Plate 5) while those fed 100% MESSM-based diet had severe diffuse cytoplasmic vacuolations (arrows) and central portal venous congestion (Plate 6).

Table 2: Histopathological observations on *C. gariepinus* juveniles fed graded levels of mechanically extracted sunflower seed meal-based diets for 15 weeks

	Tissues of organs examined		
Dietary inclusions	**Liver**	**Kidney**	**Intestine**
MESSM 1 (0%) (Control)	Moderate diffuse cytoplasmic vacuolations in the hepatocytes.	No visible lesion as both tubular and haemopoietic compartments appeared normal in proportion.	No visible lesions as enterocytes and villi appeared normal in numbers and architecture.
MESSM 2 (20%)	Moderate diffuse cytoplasmic vacuolations in the hepatocytes.	No visible lesions. Prominent haemopoietic compartments occurred in the midst of the tubular compartments.	No visible lesions observed.
MESSM 3 (40%)	Multiple foci of large cytoplasmic vacuolations of the hepatocytes.	No visible lesions as haemopoietic and renal tubular compartments retained relative normal proportions.	No visible lesions except for numerous villi.
MESSM 4 (60%)	Moderate diffuse vacuolar change and fatty infiltration.	Moderately swollen tubules. Haemopoietic compartments were slightly reduced.	Very long villi appearing slightly hyperplastic.
MESSM 5 (80%)	Moderate periportal vacuolar change, extensive fatty infiltration and central portal venous congestion.	Marked degeneration and necrosis of renal tubular epithelia. A few of the epithelial cells had pyknotic nuclei	Moderate sloughing off/erosion of villi.
MESSM 6 (100%)	Moderate diffuse vacuolar change and fatty infiltration.	Depletion of haemopoietic and renal tubular compartments	Marked necrosis and sloughing off (erosion) of enterocytes at the tips of villi.

MESSM = mechanically extracted sunflower seed meal

Photomicrograph of sections of the kidneys of the fish fed 0% MESSM-based diet (control diet) showed no visible lesions as both tubular and haemopoietic compartments appeared normal in proportion (Plate 7). Sections of the kidneys of the fish fed 20% MESSM-based diet revealed prominent haemopoietic compartments (arrow) in the midst of the renal tubular compartment (Plate 8). A section of the kidney of the fish fed 40% MESSM-based diet showed no visible lesion as haemopoietic and renal tubular compartments retained their relative normal proportions (Plate 9). There were moderately swollen tubules (big arrow) and slightly reduced haemopoietic compartment (thin arrow) in the section of the kidney of the fish fed 60% MESSM-based diet (Plate 10). Sections of the kidneys of the fish fed 80% MESSM-based diet showed marked degeneration and necrosis of renal tubular epithelia while a few of the epithelial cells had pyknotic nuclei (arrows) (Plate 11). Depletion of haemopoietic and renal tubular compartments were observed in the kidney sections of the fish fed 100% MESSM-based diet (Plate 12).

Photomicrograph of a section of the intestine of the fish fed 0% MESSM-based diet (control diet) showed no visible lesions as enterocytes and villi appeared normal in numbers and architecture (Plate 13). There were no visible lesions in the section of the intestine of the fish fed 20% MESSM-based diet (Plate 14). A section of the intestine of the fish fed 40% MESSM-based diet showed numerous villi without any visible lesions (Plate 15). A section of the intestine of the fish fed 60% MESSM-based diet was observed to show very long villi appearing slightly hyperplastic (Plate 16). There was moderate sloughing off/erosion (arrow) of villi in the section of the intestine of the fish fed 80% MESSM-based diet (Plate 17). A section of the intestine of the fish fed 100% MESSM-based diet revealed marked necrosis and sloughing off of enterocytes at the tips of villi (circle) (Plate 18).

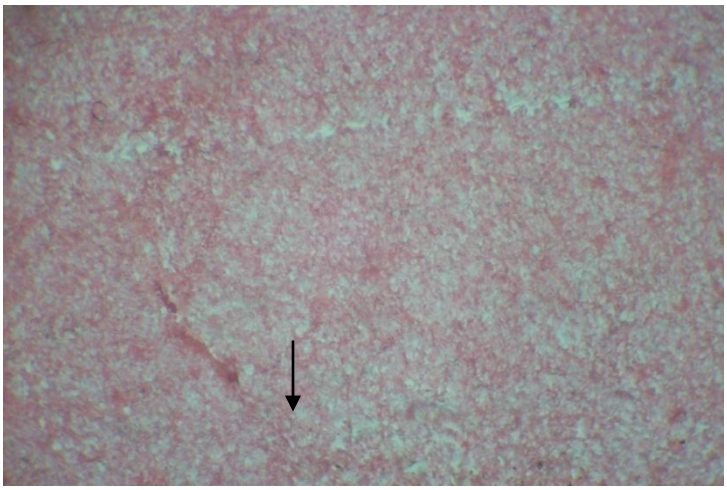

Plate 1: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 0% MESSM-based diet (control diet) showing moderate diffuse cytoplasmic vacuolations (arrow) in the hepatocytes (H and E; x40).

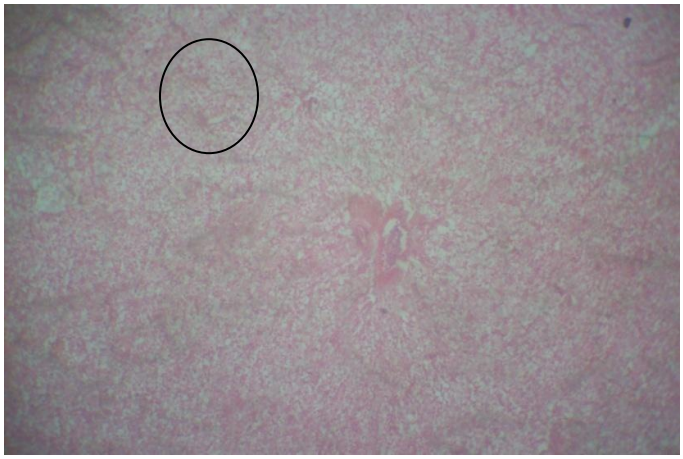

Plate 2: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 20% MESSM-based diet showing moderate diffuse cytoplasmic vacuoles (circle) in the hepatocytes (H and E; x100).

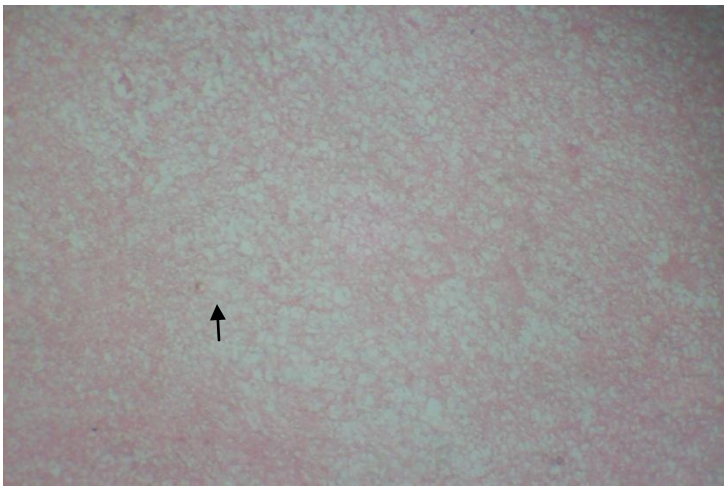

Plate 3: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 40% MESSM-based diet showing multiple foci of large cytoplasmic vacuolations (arrow) of the hepatocytes (H and E; x40).

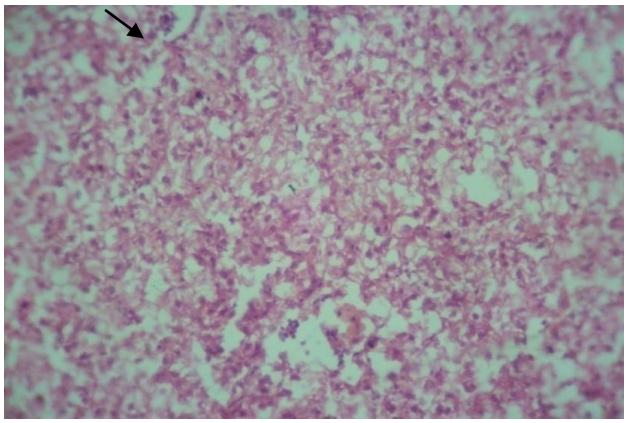

Plate 4: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 60% MESSM-based diet showing moderate diffuse vacuolar change and fatty infiltration (arrow) (H and E; x100).

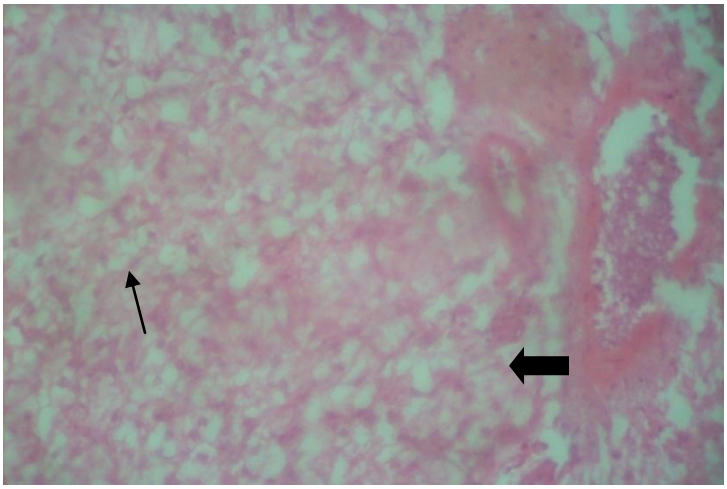

Plate 5: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 80% MESSM-based diet showing moderate periportal vacuolar change (thin arrow), extensive fatty infiltration and central portal venous congestion (thick arrow) (H and E; x400).

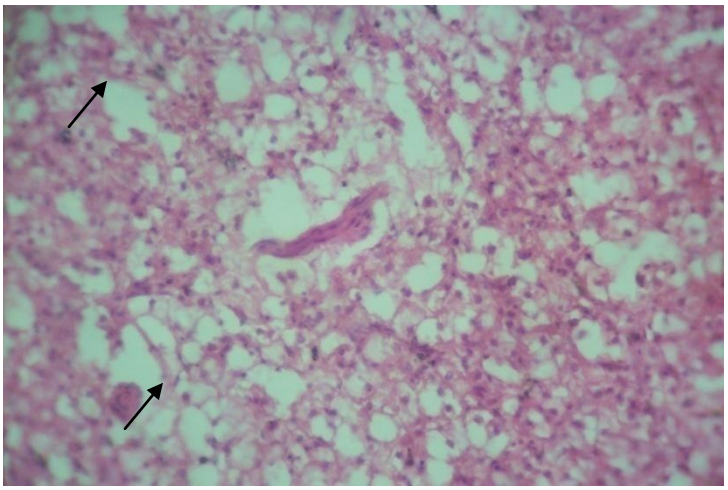

Plate 6: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 100% MESSM-based diet showing severe diffuse cytoplasmic vacuolations (arrows) and central portal venous congestion (H and E; x100).

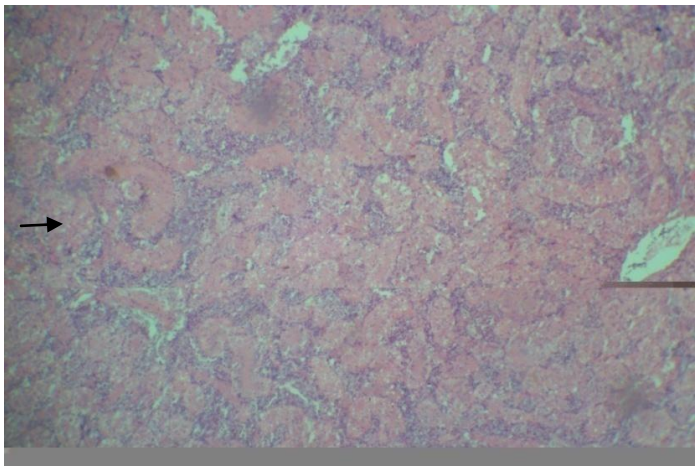

Plate 7: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 0% MESSM-based diet (control diet) showing no visible lesion as both tubular and haemopoietic (arrow) compartments appeared normal in proportion (H and E; x100).

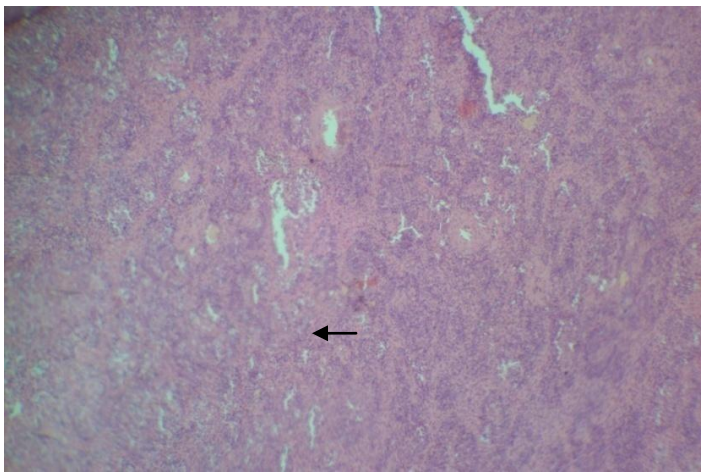

Plate 8: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 20% MESSM-based diet showing prominent haemopoietic compartments (arrow) in the midst of the tubular compartment (H and E; x100).

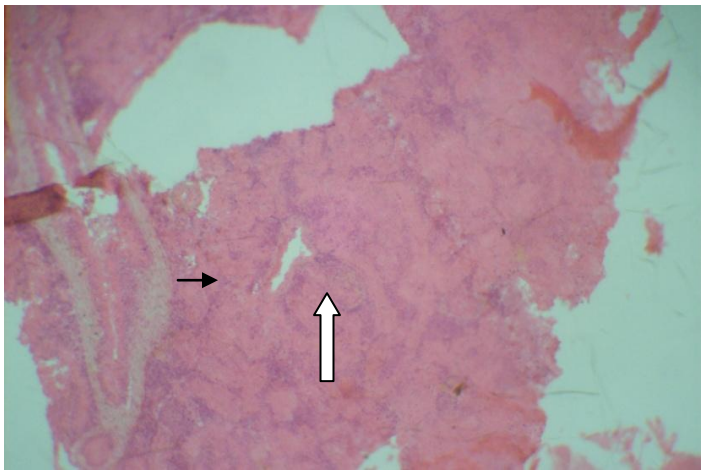

Plate 9: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 40% MESSM-based diet showing no visible lesion as haemopoietic (thin arrow) and renal tubular compartments (white arrow) retained relative normal proportions (H and E; x100).

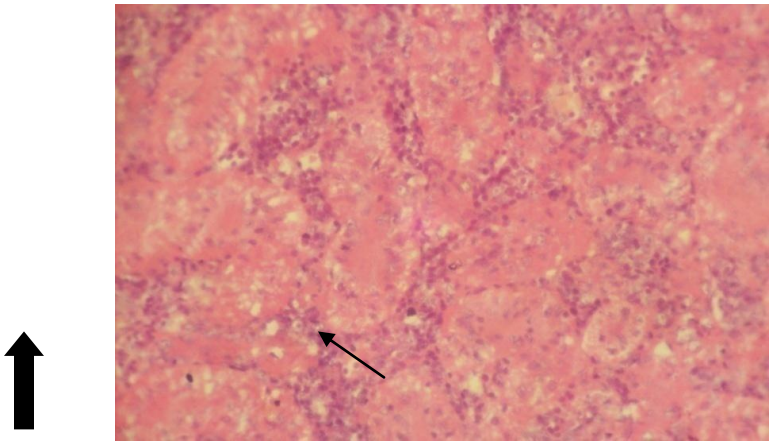

Plate 10: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 60% MESSM-based diet showing moderately swollen tubules (big arrow); the haemopoietic compartment is slightly reduced (thin arrow) (H and E; x400).

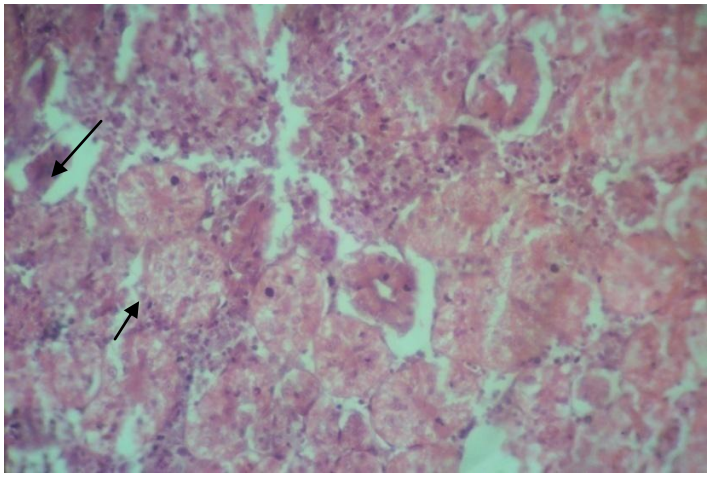

Plate 11: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 80% MESSM-based diet showing marked degeneration as well as necrosis of renal tubular epithelia. A few of the epithelial cells have pyknotic nuclei (arrows) (H and E; x400).

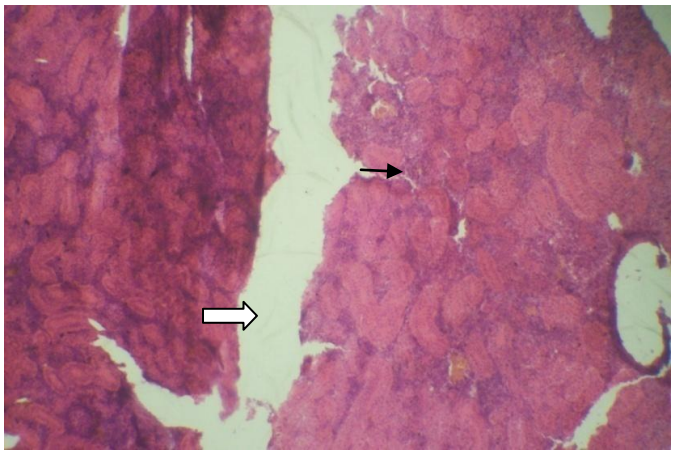

Plate 12: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 100% MESSM-based diet showing depletion of haemopoietic (thin arrow) and renal tubular (big arrow) compartments (H and E; x40).

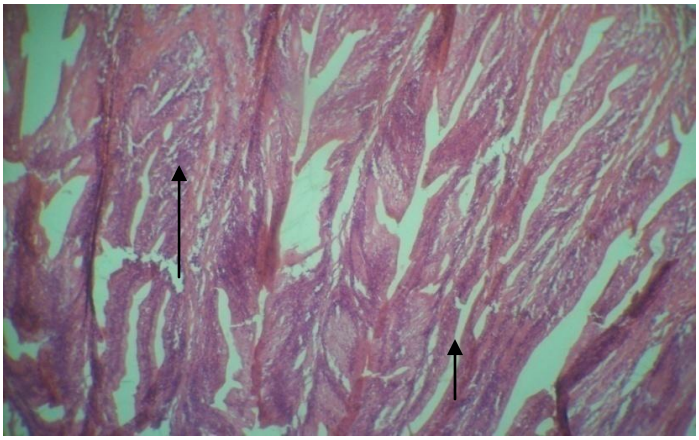

Plate 13: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 0% MESSM-based diet (control diet) showing no visible lesions as enterocytes and villi (arrows) appeared normal in numbers and architecture (H and E; x100).

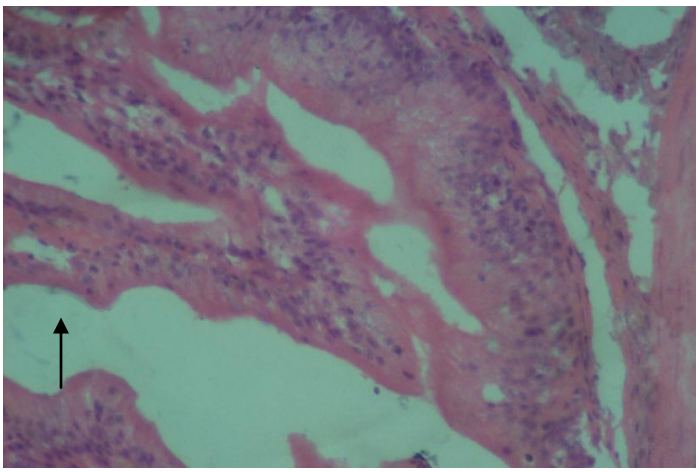

Plate 14: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 20% MESSM-based diet showing no visible lesion (arrow) (H and E; x400).

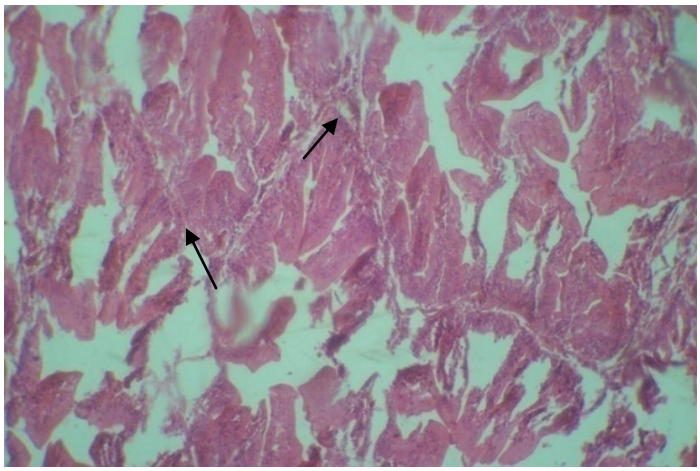

Plate 15: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 40% MESSM-based diet showing numerous villi (arrows) without any visible lesions (H and E; x100).

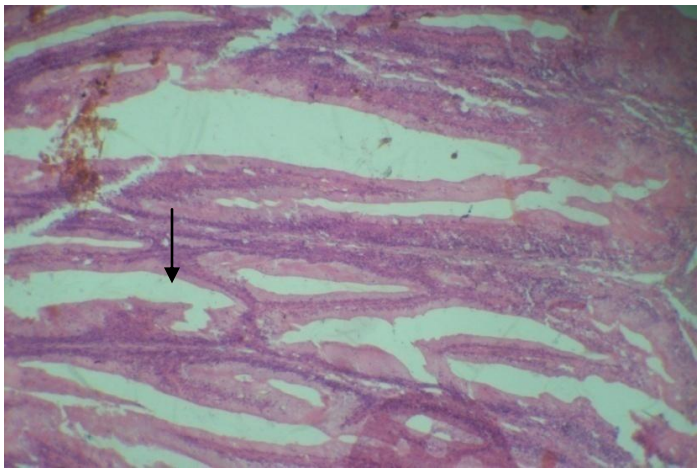

Plate 16: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 60% MESSM-based diet showing very long villi (arrow) appearing slightly hyperplastic (H and E; x100).

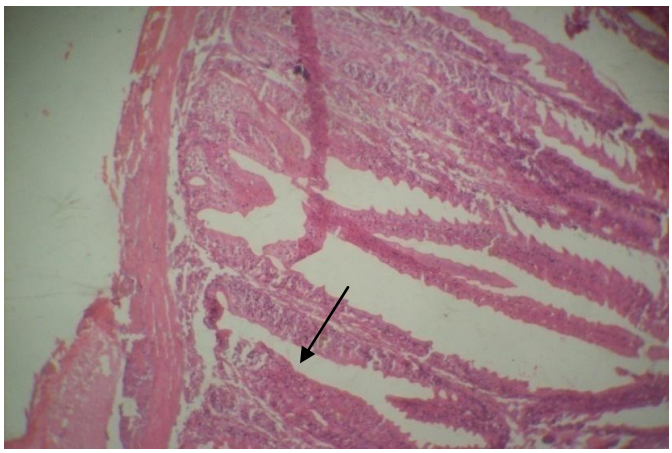

Plate 17: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 80% MESSM-based diet showing moderate sloughing off/erosion (arrow) of villi (H and E; x100).

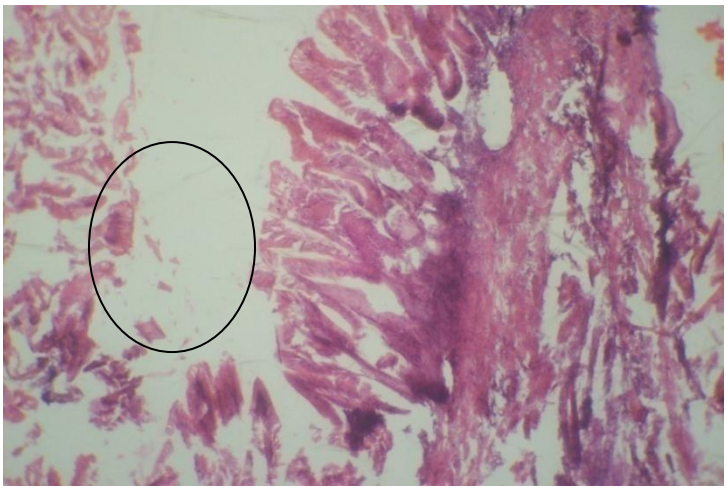

Plate 18: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 100% MESSM-based diet showing marked necrosis and sloughing off (erosion) of enterocytes at the tips of villi (circle) (H and E; x100).

DISCUSSION

Hepatosomatic index (HSI) of the liver is a considerable potential tool used by fish biologists to assess the toxicity situation of the exposure of fish to any toxicant as well as a management tool for evaluating growth or health status of various fish species in different environments (Hoque *et al.*, 1998). HSI is also a useful biomarker to detect the hazardous effects of the environmental stressors (Pait and Nelson, 2003). The increase in HSI value in an ideal environment is related to normal liver growth but, in cases of pollution, liver enlargement is associated with hyperplasia (Hoque *et al.*, 1998). The higher values of hepatosomatic index (HSI) observed in the fish fed 0, 20 and 40% MESSM-based diets could be attributed to higher feed take at these inclusion levels and indicated normal liver growth resulting from dietary treatment. Enlargement of organs, such as liver, kidney and heart, has been associated with dietary factors especially if such diets contain toxins, anti-nutrients or heavy metals (Adejinmi, 2000). High HSI values have been reported for male *Fundulus heteroclitus* (Killifish) induced by selected estrogenic compounds (Pait and Nelson, 2003) and Crucian carp (*Carassius carassius*) exposed to treated sewage effluent (Diniz *et al.*, 2005). Barse *et al.* (2006) also reported superior HSI values for *Cyprinus carpio* subjected to 4-*tert*-butylphenol while Abdel-Hameid (2007) reported elevated HSI values for *Oreochromis aureus* juveniles due to phenol intoxication and stated that the observed hepatomegaly might partially reflect the enhancement of the liver size due to destructive changes. In the same vein, Figueiredo-Fernandes *et al*. (2006) also obtained increased values of HSI in male and female tilapias, *Oreochromis niloticus*, exposed to paraquat.

However, the progressively reduced HSI values recorded for the fish fed 60% to 100% MESSM-based diets could be linked with lesser feed intake which was probably associated with the presence of anti-nutrients in the mechanically extracted sunflower seed meal incorporated in the diets at these higher levels. Akerman *et al*. (2003) also found a decrease in HSI values after nine weeks in rainbow trout, *Oncorhynchus mykiss*, injected with paraquat. Histopathological biomarkers are useful as indicators of the general health of the fish and are considered as a mirror that reflects the exposure of fish to a variety of anthropogenic pollutants (Van der Oost *et al*., 2003).

The lack of statistical variation in the values of kidney-somatic index (KSI) recorded for fish in all the treatments suggests that this index was not affected by both the varying MESSM inclusion levels and the presence of anti-nutrients in the diets. It can also be suggested that the non-significant variation in the KSI values indicates that the weight of the kidney and total body weight were not correspondingly affected by the increasing inclusion level of mechanically extracted sunflower seed meal in the diets. Intestino-somatic index (ISI) was observed to exhibit an irregular pattern of variation and was not correspondingly affected by the increasing inclusion level of mechanically extracted sunflower seed meal in the diets. This result disagrees with that of Abdel-Hameid (2007) who obtained reduced ISI values for *O. aureus* juveniles and attributed them to a reduction in the total body weight as a result of reduced appetite caused by phenol intoxication.

Histopathological observations on *Clarias gariepinus* fed graded levels of MESSM diets

In this study, histopathological examination of the liver, kidney and intestine was carried out because of their physiological importance during absorption and metabolism of nutrients and chemicals (Roberts, 1989). Evaluation of histological structure of digestive organs in fish fed new dietary ingredients provides valuable information about their digestive capacity as well as potential health effects of such new diets (Caballero *et al*., 2003; Diaz *et al.*, 2006). Substitution of different inclusion levels of mechanically extracted sunflower seed meal (MESSM) for soybean meal in the diets has resulted in varying degrees of histopathological changes in the liver cells (hepatocytes) of *C. gariepinus* juveniles. Such changes included mild/moderate diffuse vacuolations, periportal congestion, central venous congestion, mild periportal vacuolar degeneration, severe fatty infiltration, extensive hepatic degeneration and overlapping of liver tissue. These observations closely support the finding of Hlophe and Moyo (2014) who, in a related feeding trial, observed that *C. gariepinus* fed high moringa leaf meal inclusion levels (>50%) showed an increase in the number of degraded hepatocytes with irregularly shaped cells, small dark pyknotic nuclei, poor fatty deposition and isolated necrosis. The present observations also agree with those of Uwachukwu *et al.* (2003) who reported that diets containing raw beans caused extensive periportal necrosis with some mononuclear cell infiltration in the livers of broilers while the centrilobular areas showed vacuolation and degeneration of hepatocytes. Vacuolated hepatocytes are usually accumulated with glycogen and have little or no degenerative and regenerative ability (Nayak *et al*., 1996) and the excessive vacuolation of the liver cells would result in abnormal functioning of such liver cells, for instance, accumulation and immobilization of fat, which could consequently result in fatty infiltration of the hepatic parenchyma (Adeyemo, 2005). Despite similar protein and energy levels in the experimental diets in the present study, liver histology showed that *C. gariepinus* juveniles fed higher MESSM inclusion levels had necrotic signs associated with poor nutritional status (Ostaszewska *et al*., 2005; Tusche *et al*., 2012). The malnutrition signs observed in *C. gariepinus* fed higher levels of MESSM might be due to non-availability of protein and amino acids that have bound with or have formed indigestible complexes with the anti-nutritional compounds in the sunflower seed meal. As a result of the poor digestibility, a substantial portion of the essential dietary nutrients was not available to the fish and was subsequently excreted. This could be responsible for the nutritional necrosis observed in the hepatocytes.

Wade *et al.* (2002) earlier reported that after a 96-hour toxicity bio-assay of cassava (*Manihot esculenta* Crantz) effluent on the Nile tilapia, histopathological examination of the liver of the treated fish indicated vacuolation and necrosis of the liver cells. Adeyemo (2005) also made similar observations in *C. gariepinus* fed cassava mill effluent. Similarly, Jha (2004) reported remarkable lesions in the liver of *Clarias batrachus* exposed to surf and Omitoyin *et al.* (2006) observed similar trends in *C. gariepinus* exposed to Lindane. Ayoola (2008) observed similar effects of glyphosate in *C. gariepinus* and opined that vacuolation of liver cells is an evidence of fatty degeneration of the cells. In this study, histological changes observed in the liver might have been caused by the ingestion of a high percentage of MESSM-based diets which probably imposed stress on the organ above its physiological capacity to cope with. The lesions observed in the liver might probably have resulted from the excessive work load done by the liver of the experimental fish during the processes of detoxification and removal of toxicants from its body. Hepatocytes in the periportal areas have been reported to suffer most from toxicants. In this situation, anti-nutritional substances present in sunflower seed meal must have been responsible for the observed histopathological changes in the liver sections.

Metelev *et al*. (1971) stated that the liver as the primary organ for detoxification of organic xenobiotics is often prone to various pollutants and other toxic by-products which tend to accumulate in high concentrations within it and thereby suffer from harmful effects. Alterations in the liver serve as useful markers of exposure to environmental stress. Both the liver and kidney have been identified as the sites that are mostly affected by toxic substances in man and various clinical signs have been associated with liver detoxifying and kidney removing these toxic substances in man (Benjamin, 2009). The results obtained indicated a sign of toxicity of the diets to the fish at higher inclusion levels and therefore necessitated further research to explore better and more effective processing methods that will significantly reduce the levels of anti-nutritional components in sunflower seeds as an alternative feed ingredient.

Substitution of mechanically extracted sunflower seed meal for soybean meal in the diets also caused some histological alterations in the kidney of *C. gariepinus* such as marked degeneration and necrosis of renal tubular epithelia at 80% MESSM inclusion as well as depletion of haemopoietic and renal tubular compartments at 100% MESSM inclusion. Olasunkanmi (2011) earlier reported a marked congestion in the kidneys of *C. gariepinus* fed raw, cooked and toasted mucuna seed meal diets and associated the histological changes in the kidney with ingestion of a high percentage of mucuna seed meal which probably imposed stress on the organ's physiological capacity.

In a related study, Olasunkanmi (2015) also observed that *C. gariepinus* fed higher inclusion levels of processed velvet beans showed a marked congestion of the kidney cells which usually impairs their maintenance of constant homeostatic conditions, thus implying that fish fed with the diets containing higher inclusion levels of processed velvet bean meal and mechanically extracted sunflower seed meal (in the present study) might have some difficulty with maintaining a constant osmoregulatory mechanism. Benjamin (2009) stated that congestion of the kidney tubules is the first stage in the development of kidney disease while Wade *et al*. (2002) and Adeyemo (2005) earlier observed histological changes such as oedema in the kidneys of *Oreochromis niloticus* and *C. gariepinus* fed cassava mill effluents respectively.

Fish kidney is one of the most susceptible organs being affected by contaminants in water bodies (Thophon *et al*., 2003). Most common alterations found in the kidneys of fishes are tubule degeneration, dilation of capillaries in the glomerulus and reduction of Bowman's capsular space (Takashima and Hibya, 1995). Exposure to chemicals often causes alterations in the glomerulus and renal tubules as described by Thophon *et al.* (2003) for the perch (*Lates calcarifer*). In more severe cases, the degenerative process can lead to tissue necrosis (Takashima and Hibya, 1995**)**. The occurence of pronounced tubule disruption, degeneration and necrosis of renal tubular epithelia as well as depletion of haemopoietic and renal tubular compartments observed in the kidneys of fish fed higher MESSM inclusions in the present study indicates that the kidneys must have suffered some damage which could be attributed to the presence of anti-nutritional compounds in the MESSM. Lesions observed in kidneys are not enough to verify the level of contamination or effects of dietary treatments. They must be supported by the histopathological data of the other organs. The histopathological findings commonly observed in kidneys are necrosis, fibrin and haemorrhage (Lawrence *et al*., 2003; Uçar and Atamanalp, 2008).

The examined sections of the intestines of *C. gariepinus* juveniles fed mechanically extracted sunflower seed meal-based diets revealed very mild histological changes except for moderate erosion of villi at 80% MESSM inclusion as well as pronounced necrosis and erosion of enterocytes at the tips of villi at 100% MESSM inclusion. This result corroborates that of Hlophe and Moyo (2014) who, in a related study, observed that the intestine histology of *C. gariepinus* fed diets containing higher moringa leaf meal inclusion levels (>50%) showed significantly shorter villi. The longer villi found in fish fed lower levels of sunflower seed meal in the diet indicate a larger surface area and consequently higher efficiency of the intestine in the absorptive process (Caballero *et al*., 2002; Da Silva *et al*., 2012). This was corroborated by the better growth performance of fish fed with these diets at lower inclusion levels as reported by Adesina *et al*. (2013). The decrease in villi height resulted in reduced surface area for nutrient absorption (Da Silva *et al*., 2012). Necrosis and mucosal degeneration may affect the permeability and absorption of substances across the stomach (Roberts, 1878) and intestinal walls. Other authors have reported a widening of the central stroma within the mucosal folding, higher amounts of connective tissue and an infiltration of inflammatory cells in the *lamina propria* (Krogdahl *et al*., 2000; Refstie *et al*., 2000). This may suggest that *C. gariepinus*, being an omnivore, is more capable of utilising plant diets than carnivorous fish.

CONCLUSION

Histopathological examinations of the thin sections of the liver, kidney and intestine of *C. gariepinus* fed graded levels of MESSM-included diets have revealed changes ranging from mild to severe lesions and few anatomical alterations, particularly at the higher levels of inclusion of mechanically extracted sunflower seed meal in the formulated diets. From histological analysis, it was clearly observed that, as the level of inclusion of mechanically extracted sunflower seed meal in the diets increased, *C. gariepinus* was subjected to more stress. The presence of residual traces of anti-nutritional factors (tannin, oxalate and phytate) in the mechanically extracted sunflower seed meal could be most probably responsible for the poor performance of the MESSM-based diets at higher levels of inclusion. It is suggested that the specific roles that each of these anti-nutrients plays in the utilisation of nutrients be further investigated. The results of this study showed a disruption of normal physiological activities in *C. gariepinus* which was due to the level of processing adopted in this study that had not completely detoxified the inherent anti-nutrients in the sunflower seed meal for safe and maximum consumption and utilisation by *C. gariepinus*. It is therefore concluded that further processing methods should be explored before incorporating sunflower seed meal in fish diets.

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