https://dx.doi.org/10.4314/ijs.v19i1.9
Ife Journal of Science vol. 19, no. 1 (2017)

ASSESSMENT OF ORGANOSOMATIC INDICES AND HISTOPATHOLOGICAL CHANGES IN VITAL ORGANS OF *CLARIAS GARIEPINUS* JUVENILES FED GRADED LEVELS OF BOILED SUNFLOWER (*HELIANTHUS ANNUUS*) SEED MEAL

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(Received: 9th March, 2017; Accepted: 12th June, 2017)

ABSTRACT

This study investigated the effects of substituting boiled sunflower seed meal (BSSM) for soybean meal (SBM) on the organosomatic indices and histopathological changes in the liver, kidney and intestine of *Clarias gariepinus* juveniles for fifteen weeks. BSSM was substituted for SBM at 0, 20, 40, 60, 80 and 100% in formulating six isonitrogenous (40% crude protein) and isocaloric (4651.5 Kcal/kg) diets. Diets were fed twice daily to triplicate groups of 360 *C. gariepinus* juveniles inside eighteen rectangular tanks (20 fish per tank). The result showed that hepatosomatic index (HSI) was significantly highest (3.11%) in the fish fed 0% BSSM diet and lowest (0.87%) in those fed 100% BSSM inclusion. HSI values progressively decreased with increasing inclusion level of BSSM. Kidney-somatic index (KSI) was highest (0.74%) in fish fed 20% BSSM inclusion and least (0.32%) in those fed 100% inclusion. Intestino-somatic index (ISI) was superior (3.82%) in fish fed 100% BSSM inclusion and least (3.16%) in those fed 40%. The liver, kidney and intestine of fish fed 0 and 20% BSSM inclusions showed no visible signs of damage. However, fish fed higher BSSM inclusions (>20%) revealed extensive hepatic degeneration, periportal congestions and severe diffuse cytoplasmic vacuolations of hepatocytes. Kidney tubules of fish fed higher BSSM inclusions exhibited diffuse interstitial congestions, diffuse swelling, epithelial vacuolations and degeneration as well as necrosis. Intestinal changes in fish fed higher BSSM inclusions included reduced sub-mucosal width, markedly stunted villi, moderate erosion of villi's epithelia, hyperplastic villi, necrosis of enterocytes and inflammatory cells in villi's *lamina propria*. Inclusion of boiled sunflower seed meal beyond 20% in *C. gariepinus*' diet could cause adverse histological alterations.

Keywords: *Clarias gariepinus*,Sunflower seed meal, Histopathology, Hepatosomatic index, Anti-nutrients.

INTRODUCTION

The success of fish farming partly depends on the quality and quantity of feed which accounts for about 70% of the total production cost while protein is the most essential and expensive component of aquaculture diets (Garza de Yta, 2012). The formulation and production of commercial feeds for cultured fish have usually been based on fishmeal as the major protein source due to its high protein content and balanced essential amino acid profile. However, the availability of fishmeal in fish feeds can no longer be guaranteed because the capture fisheries are levelling off (FAO, 2011). Besides, excessive demand for fishmeal has made its supply 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). This has compelled fish culturists to constantly consider and explore alternative protein-rich dietary supplements that are cheap, locally available and nutritionally safe for use as fishmeal replacers in fish diets.

Similarly, soybean meal has always been the principal plant protein source used in animal feeds as a replacement for fishmeal owing to 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 extensive utilisation as the main protein source in fish feeds may longer be economically viable (Siddhuraju and Becker, 2001). Therefore, less competitive and locally available plant protein sources such as sunflower seed meal should be considered as an alternative to replace soybean meal without reducing the nutritional quality of the feed.

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 (Sintayehu *et al.*, 1996) and its potential as a dietary protein source in animal feeds is well recognized (Olvera-Novoa *et al.*, 2002). Studies involving the utilisation 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 which differ in their sensitivity and response to anti-nutrients present in such plant protein sources (Chaudhuri *et al.*, 2012).

Histopathological changes have been widely used as biomarkers in the assessment of fish health status after their exposure to various chemicals/contaminants in the laboratory (Thophon *et al.*, 2003). One of the main advantages of using histopathological assessment is that the markers enable us to study the target organs, such as kidney, gill and liver, which perform important physiological functions, such as deposition and bio-magnification of chemicals as well as excretion in fish (Gernhofer *et al.*, 2001). Histological studies provide information on diet quality and metabolism as well as indicate the nutritional status of a fish (Caballero *et al.*, 2004). 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) has been used as an indicator of environmental risk and Yang and Baumann (2006) found a positive correlation between HSI and the concentration of polycyclic aromatic hydrocarbon (PAH) metabolites in fish.

Environmental contaminants have potentially toxic effects on the aquatic ecosystems and fish are often significantly exposed to such toxic effects. The effect of pollution on fish is determined according to mortality, organosomatic indices, pathological symptoms, haematology and blood biology (Ali *et al.*, 2014). 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 approach (Shalaka and Pragna, 2013). Liver also shows specific histological changes with pollution and therefore it is a very good bio-indicator of the effects of pollutants (or anti-nutritional compounds in the present study) on fish health status (Abdel-Moneim *et al.*, 2012).

Fish kidney performs endocrine, reticulo-endothelial, haematopoietic and excretory functions. 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). Therefore, this study investigated the effects of substituting boiled sunflower seed meal (BSSM) for soybean meal (SBM) on the organosomatic indices and histopathological changes in the liver, kidney and intestine of *Clarias gariepinus* juveniles after fifteen weeks of feeding trial.

MATERIALS AND METHODS

Experimental design and formulation of boiled sunflower seed meal–based diets in replacement for soybean meal

Six isonitrogenous diets (at 40% crude protein level) were formulated and prepared using Pearson's square method. Boiled sunflower seed meal (BSSM) was incorporated in the six diets at graded levels of 0%, 20%, 40%, 60%, 80% and 100% in replacement for soybean meal (SBM) with 0% being the control diet (Table 1). Boiled sunflower seed meal was prepared at 100 ^{0}C for 15 minutes in a pressure cooker (Qlink Model No. 9000), oven-dried in a Gallenkamp oven at 60 ^{0}C for 6 hours. Other ingredients included fish meal, groundnut cake, vitamin and mineral premix, bone meal, oyster shell, maize, cassava starch, salt and palm oil. The ingredients were measured and mixed together to formulate a 40% crude protein diet. Each diet mixture was extruded through a 3 mm die pelleting machine (Hobart A-200T GmbH, Rhen-Bosch, Offenbug, Germany) to form noodle-like strands, which were manually crumbled into a suitable size for the *C. gariepinus* juveniles. The pellets were sun-dried, packed in labeled polythene bags and stored in a cool dry place to prevent fungal growth. The gross composition of the experimental diets is shown in table 1 and diet 1 served as the control with no sunflower seed meal supplementation. Six graded levels (0%, 20%, 40%, 60%, 80% and 100%) of boiled sunflower seed meal (BSSM) were substituted for soybean meal (SBM) in dietary treatments 1, 2, 3, 4, 5 and 6. The six dietary treatments contained the following percentage composition of boiled sunflower seed meal and soybean meal respectively:

Treatment 1: 0% BSSM : 100% SBM
Treatment 2: 20% BSSM : 80% SBM
Treatment 3: 40% BSSM : 60% SBM
Treatment 4: 60% BSSM : 40% SBM
Treatment 5: 80% BSSM : 20% SBM
Treatment 6: 100% BSSM : 0% SBM.

Experimental set-up and fish feeding trial

The experiment was carried out using fifteen plastic tanks (60 cm × 45 cm × 30 cm) for 15 weeks in the research laboratory of the Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria. Each tank was supplied with well water up to 70% capacity which was replaced every three days to maintain relatively uniform physico-chemical parameters and prevent fouling from feed residues. The tanks were well aerated using air stones and aerator pumps. There were six dietary treatments and each had three replicates with 20 fish per replicate. The fish were weighed, distributed into experimental tanks and allowed to acclimatize for 14 days before the experiment. The experiment lasted for 15 weeks during which the fish were fed at 5% body weight (in two equal portions of 2.5%) twice daily. Weight changes were recorded weekly and feeding rates adjusted to the new body weight.

Table 1: Gross ingredient composition (g/100 g diet) of boiled sunflower seed meal diets in substitution for soybean meal at graded levels for *Clarias gariepinus* juveniles

Ingredients	**BSSM 1 0%(Control)**	**BSSM 2 (20%)**	**BSSM 3 (40%)**	**BSSM 4 (60%)**	**BSSM 5 (80%)**	**BSSM 6 (100%)**
Sunflower seed meal	____	7.88	15.75	23.63	31.51	39.38
Soybean meal	39.38	31.51	23.63	15.75	7.88	____
Fish meal	20.19	20.19	20.19	20.19	20.19	20.19
Groundnut cake	20.19	20.19	20.19	20.19	20.19	20.19
Yellow maize	14.50	14.50	14.50	14.50	14.50	14.50
Vitamin/mineral premix*	2.00	2.00	2.00	2.00	2.00	2.00
Bone meal	1.00	1.00	1.00	1.00	1.00	1.00
Oyster shell	0.50	0.50	0.50	0.50	0.50	0.50
Palm oil	0.75	0.75	0.75	0.75	0.75	0.75
Salt	0.50	0.50	0.50	0.50	0.50	0.50
Cassava starch	1.00	1.00	1.00	1.00	1.00	1.00
Total (%)	100.00	100.00	100.00	100.00	100.00	100.00
Calculated crude protein content (%)	40	40	40	40	40	40

BSSM: Boiled sunflower seed meal
Vitamin/mineral premix*:
Vit. A: 1,000,000 IU; Vit. B_1: 250 mg; Vit. B_2: 1750 mg; Vit. B_6: 875 mg; Vit. B_{12}: 2500 mg; Vit. C: 12,500 mg; Vit. D_3: 600,000 IU; Vit. E: 12,000 IU; Vit. K_3: 15 mg; Calcium D-pantothenate: 5000 mg; Nicotinic acid: 3750 mg; Folic acid: 250 mg; Cobalt: 24,999 mg; Copper: 1999 mg; Iron: 11,249 mg; Selenium (Na_2SeO_3. $5H_2O$): 75 mg; Iodine (Potassium iodide): 106 mg; Anti-oxidant: 250 mg.

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 (Adesina *et al*., 2013), 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 determination of organosomatic indices.

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 (Ali, 2001):

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

These formulae were 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 & 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 tests (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. $p<0.05$).

RESULTS

Organosomatic indices

Values of organosomatic indices of the liver, kidney and intestine of *C. gariepinus* juveniles are shown in table 2. Hepatosomatic index (HSI) was highest (3.11%) in the fish fed 0% BSSM-based control diet and lowest (0.87%) in those fed 100% BSSM-based diet. HSI values progressively decreased with increasing inclusion level of BSSM in the diets. Fish fed 0% BSSM-based control diet significantly differed ($p<0.05$) from those fed higher inclusion levels (⩾ 80%). No significant difference ($p>0.05$) existed among fish fed 20%, 40% and 60% BSSM-based diets. Kidney-somatic index (KSI) was highest (0.74%) in fish fed 20% BSSM-based diet and least (0.32%) in those fed

100% BSSM-based diet. Fish fed 20% BSSM-based diet significantly differed ($p<0.05$) in KSI value than from those fed 100% BSSM-based diet and other diets. No significant difference ($p>0.05$) occurred in those fed 0%, 40%, 60% and 80% BSSM-based diets. Intestino-somatic index (ISI) ranged between 3.16% and 3.82%. Fish fed 100% BSSM-based diet had the highest value (3.82%) while those fed 40% BSSM-based diet had the least value (3.16%). However, ISI values exhibited no statistical difference ($p>0.05$) between the control and the other dietary treatments.

Table 2: Organosomatic indices of *C. gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets for fifteen weeks

Organosomatic indices (%)	Experimental dietary inclusions					
	BSSM 1 0% (Control)	BSSM 2 20%	BSSM 3 40%	BSSM4 60%	BSSM 5 80%	BSSM 6 100%
Liver (HSI)	3.11 ± 2.30^{a}	1.73 ± 0.69^{ab}	1.78 ± 0.35^{ab}	1.17 ± 0.39^{ab}	0.95 ± 0.53^{b}	0.87 ± 0.34^{b}
Kidney (KSI)	0.56 ± 0.31^{ab}	0.74 ± 0.06^{a}	0.56 ± 0.13^{ab}	0.55 ± 0.07^{ab}	0.63 ± 0.21^{ab}	0.32 ± 0.09^{b}
Intestine (ISI)	3.67 ± 0.81^{a}	3.77 ± 0.16^{a}	3.16 ± 1.09^{a}	3.45 ± 0.92^{a}	3.49 ± 0.79^{a}	3.82 ± 0.59^{a}

The above values are means of triplicate data. Mean values in each row with similar superscripts are not significantly different ($p>0.05$).

BSSM – Boiled sunflower seed meal; HSI - Hepatosomatic index; KSI - Kidney-somatic index; ISI - Intestino-somatic index

Gross and microscopic examination of internal organs

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 3 and Plates 1 to 18). Photomicrograph of a section of the liver of *C. gariepinus* juveniles fed 0% BSSM-based diet (control diet) showed no visible lesion except mild diffuse cytoplasmic vacuolations of the hepatocytes (Plate 1). Fish fed 20% BSSM-based diet showed fine moderate cytoplasmic vacuolations (arrow) in their hepatocytes (Plate 2). The liver of fish fed 40% BSSM-based diet had extensive hepatic degeneration and severe diffuse cytoplasmic vacuolations of hepatocytes (Plate 3). There were slight cytoplasmic vacuolations of hepatocytes in the liver of fish fed 60% BSSM-based diet (Plate 4). Some foci of mild diffuse cytoplasmic vacuolations of hepatocytes and periportal congestion (arrows) were observed in the liver of fish fed 80% BSSM-based diet (Plate 5). Fish fed 100% BSSM-based diet had severe diffuse cytoplasmic vacuolations (arrow) likely due to fatty infiltration of hepatocytes (Plate 6).

Photomicrographs of sections of the kidneys of fish fed 0% and 20% BSSM-based diets showed no visible lesions in their renal tubules (Plates 7 and 8). The kidneys of fish fed 40% BSSM-based diet revealed diffuse interstitial congestions as well as swelling and degeneration of the epithelia of the kidney tubules (arrows) (Plate 9). The kidney sections of fish fed 60% BSSM-based diet showed diffuse swelling and degeneration of the epithelia of the kidney tubules as well as necrosis; the affected degenerate tubular epithelia also contained vacuoles (arrow) (Plate 10). There were some foci of tubular epithelial degeneration in the kidneys of fish fed 80% and 100% BSSM-based diets (Plates 11 and 12).

Photomicrographs of sections of the intestines of fish fed 0% and 20% BSSM-based diets showed no visible lesions (Plates 13 and 14). The intestine of fish fed 40% BSSM-based diet showed reduced sub-mucosal width and short villi (Plate 15). There were very long hyperplastic villi (Plate 16) in the intestines of fish fed 60% BSSM-based diet. Markedly reduced and stunted villi were observed in the intestines of fish fed 80% BSSM-based diet (Plate 17). The intestines of fish fed 100% BSSM-based diet showed moderate sloughing off/erosion of the epithelia of villi, necrosis of enterocytes and presence of moderate amounts of inflammatory cells in the *lamina propria* of the villi (Plate 18).

Table 3: Histopathological observations on *C. gariepinus* juveniles fed graded levels of boiled sunflower seed meal-based diets for fifteen weeks

Dietary inclusions	**Organ tissues examined**		
	Liver	**Kidney**	**Intestine**
BSSM 1 (0%) (control)	No visible lesion except mild diffuse cytoplasmic vacuolations of hepatocytes.	No visible lesions observed in the renal tubules.	No visible lesions observed.
BSSM 2 (20%)	Fine moderate cytoplasmic vacuolations of hepatocytes.	No visible lesions observed in the renal tubules.	No visible lesions observed.
BSSM 3(40%)	Extensive hepatic degeneration and severe diffuse cytoplasmic vacuolations of hepatocytes.	Diffuse interstitial congestions as well as swelling and degeneration of the epithelia of the kidney tubules.	No visible lesions observed except for reduced sub-mucosal width and short villi.
BSSM 4 (60%)	Slight cytoplasmic vacuolations of hepatocytes.	Diffuse swelling and degeneration of the epithelia of the kidney tubules. Necrosis of kidney tubules. Affected degenerate tubular epithelia also contained vacuoles.	Very long hyperplastic villi were observed.
BSSM 5 (80%)	Some foci of mild diffuse cytoplasmic vacuolations of hepatocytes and periportal congestion.	Some foci of degeneration of epithelia of kidney tubules.	Markedly reduced and stunted villi were observed.
BSSM 6 (100%)	Severe diffuse cytoplasmic vacuolations (arrow) likely due to fatty infiltration of hepatocytes.	Some foci of degeneration of epithelia of kidney tubules.	Moderate sloughing off/erosion of the epithelia of villi, necrosis of enterocytes and presence of moderate amounts of inflammatory cells in the *lamina propria* of the villi.

BSSM – Boiled sunflower seed meal

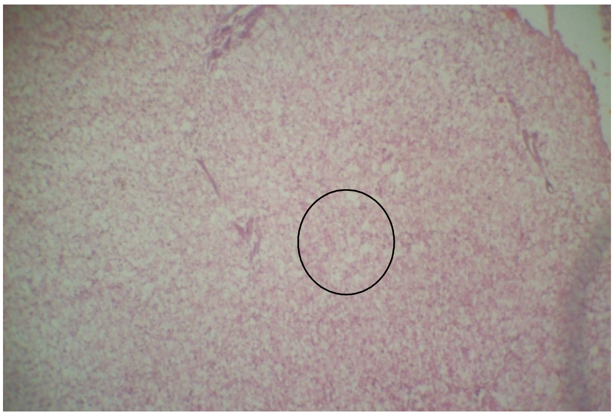

Plate 1: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 0% BSSM-based diet (control diet) showing no visible lesion except mild diffuse cytoplasmic vacuolations of the hepatocytes (circle) (H and E; x40).

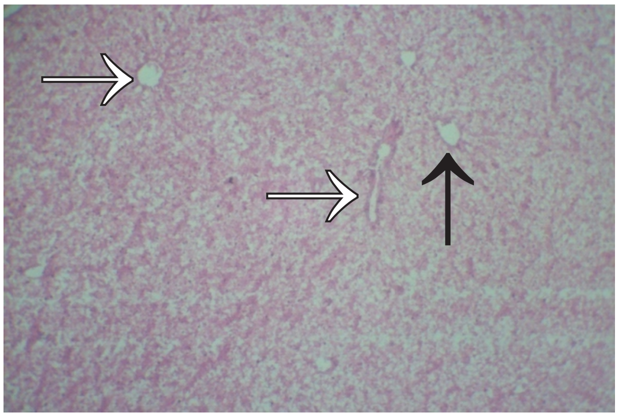

Plate 2: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 20% BSSM-based diet showing fine moderate cytoplasmic vacuolations (black arrow) in the hepatocytes. Central veins (white arrows) are normal (H and E; x100).

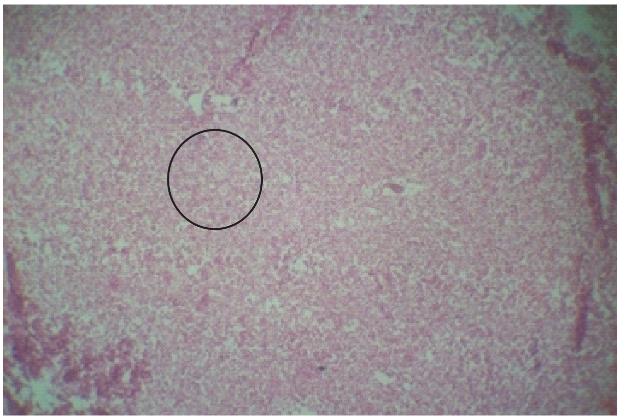

Plate 3: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 40% BSSM-based diet showing extensive hepatic degeneration and moderate diffuse cytoplasmic vacuolations of hepatocytes (circle) (H and E; x40).

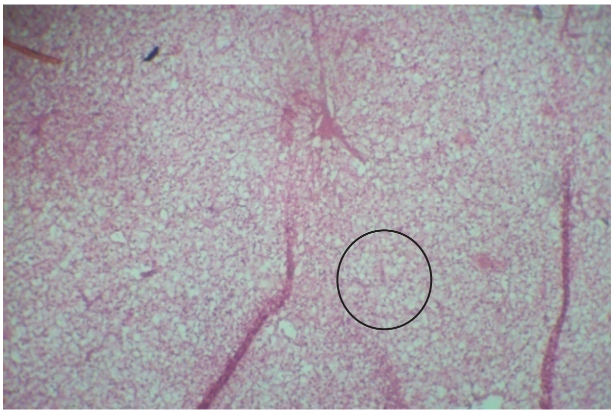

Plate 4: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 60% BSSM-based diet showing no visible lesion except for slight cytoplasmic vacuolations of hepatocytes (circle) (H and E; x100).

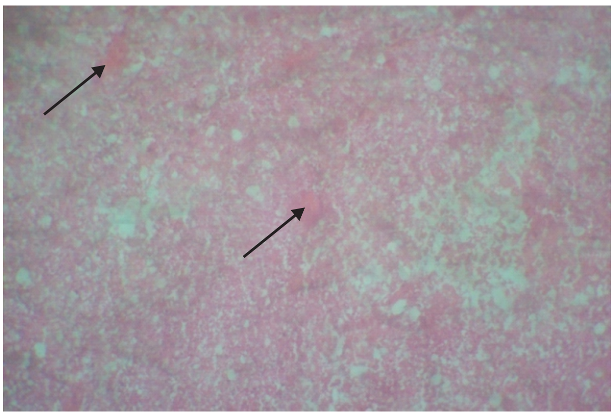

Plate 5: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 80% BSSM-based diet showing some foci of mild diffuse cytoplasmic vacuolations of hepatocytes and periportal congestion (arrows) (H and E; x40).

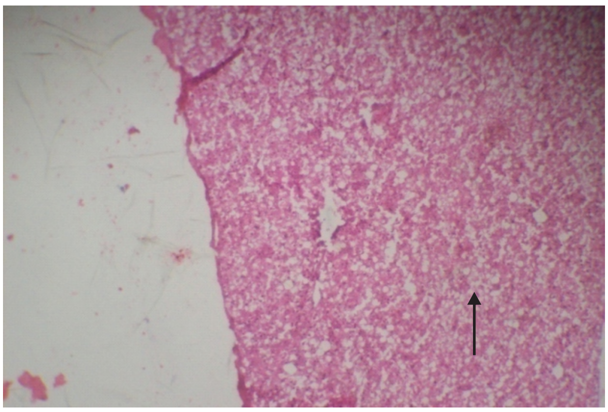

Plate 6: Photomicrograph of a section of the liver of *Clarias gariepinus* juveniles fed 100% BSSM-based diet showing severe diffuse cytoplasmic vacuolations (arrow) likely due to fatty infiltration of hepatocytes (H and E; x100).

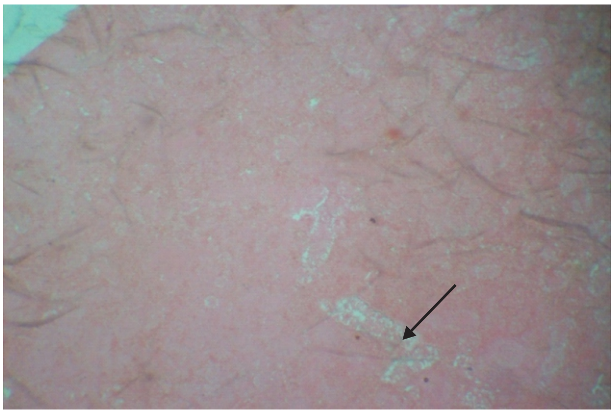

Plate 7: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 0% BSSM-based diet (control diet) showing no visible lesion in the renal tubules. Note the un-congested blood vessels (arrow) (H and E; x100).

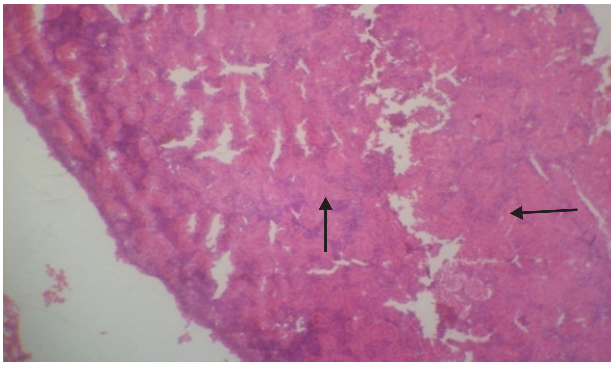

Plate 8: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 20% BSSM-based diet showing no visible lesion in the renal tubules (arrows) (H and E; x100).

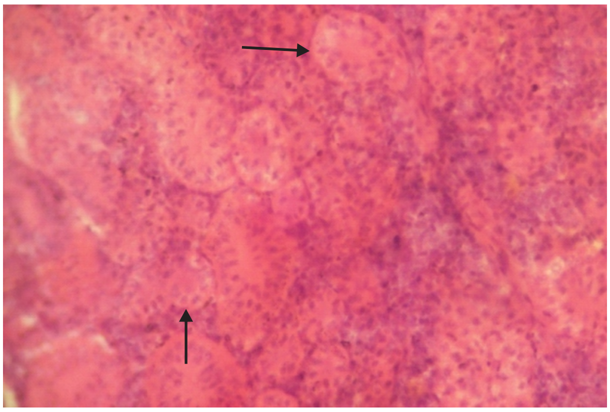

Plate 9: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 40% BSSM-based diet showing diffuse interstitial congestions as well as swelling and degeneration of the epithelia of the kidney tubules (arrows) (H and E; x100).

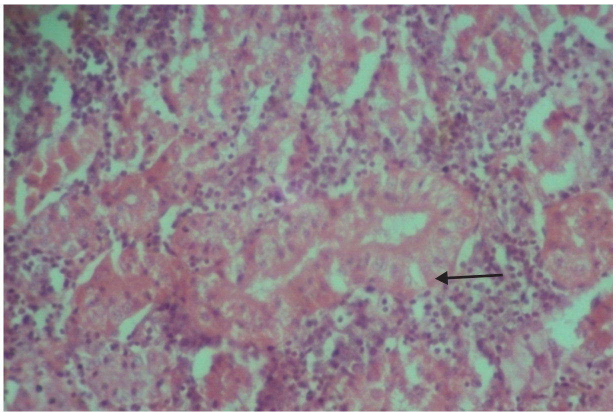

Plate 10: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 60% BSSM-based diet showing diffuse swelling and degeneration of the epithelia of the kidney tubules as well as necrosis; the affected degenerate tubular epithelia also contain vacuoles (arrow) (H and E; x100).

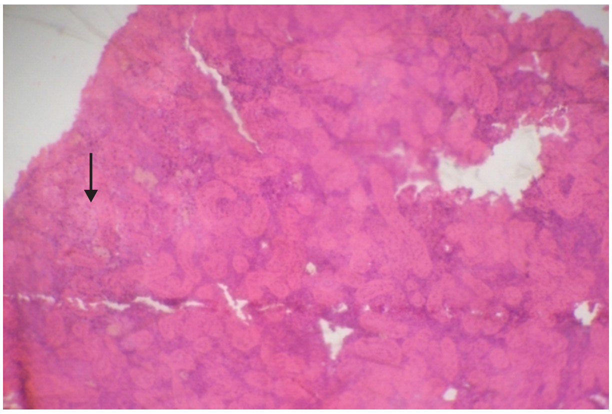

Plate 11: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 80% BSSM-based diet showing tubular epithelial degeneration (arrow) (H and E; x100).

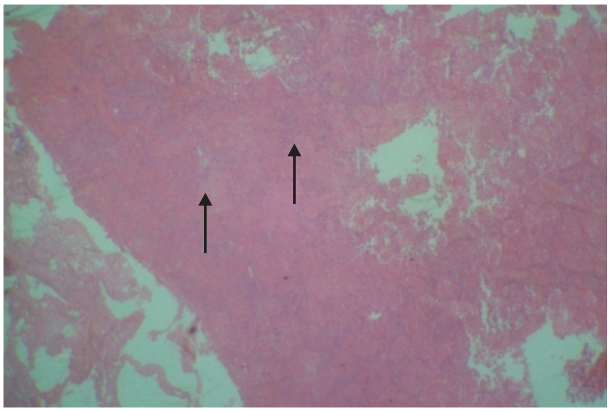

Plate 12: Photomicrograph of a section of the kidney of *Clarias gariepinus* juveniles fed 100% BSSM-based diet showing some foci of tubular epithelial degeneration (arrows) (H and E; x400).

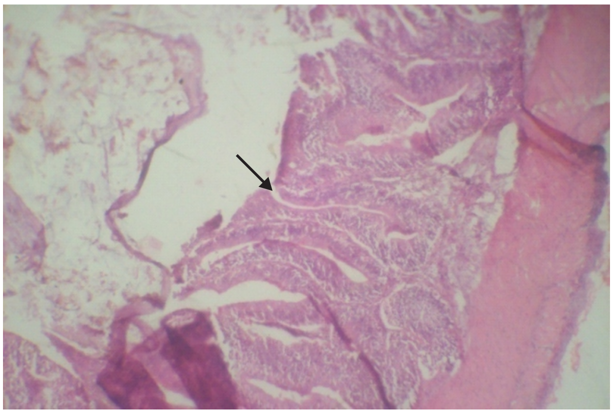

Plate 13: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 0% BSSM-based diet (control diet) showing no visible lesion (arrow) (H and E; x100).

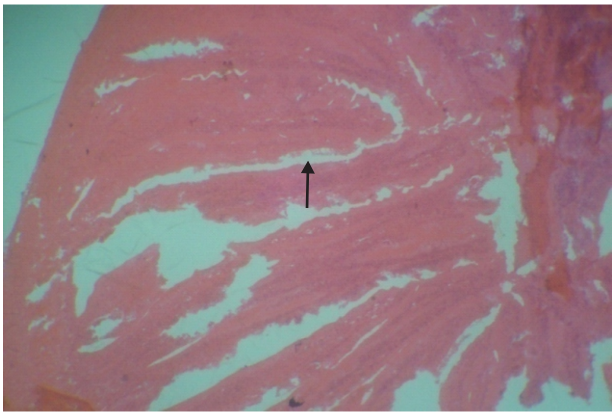

Plate 14: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 20% BSSM-based diet showing no visible lesion (arrow) (H and E; x100).

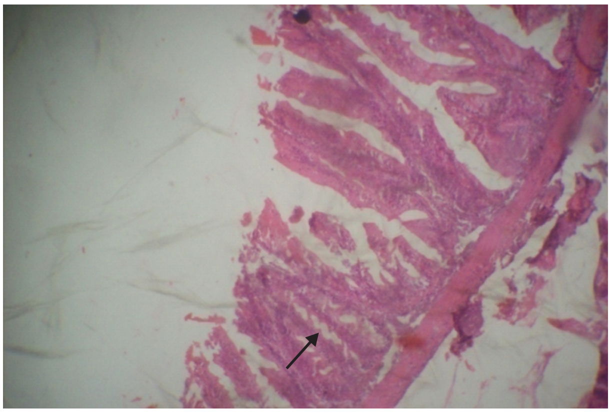

Plate 15: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 40% BSSM-based diet showing reduced sub-mucosal width and short villi (arrow) (H and E; x100).

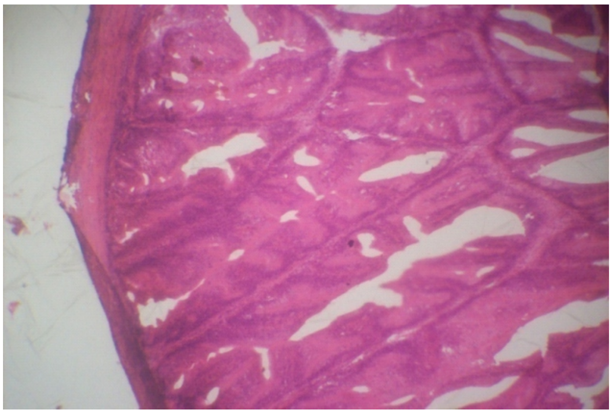

Plate 16: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 60% BSSM-based diet showing very long hyperplastic villi (arrows) (H and E; x100).

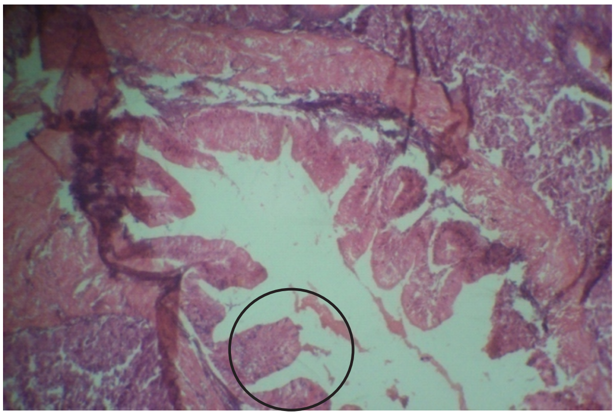

Plate 17: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 80% BSSM-based diet showing markedly reduced and stunted villi (circle) (H and E; x100).

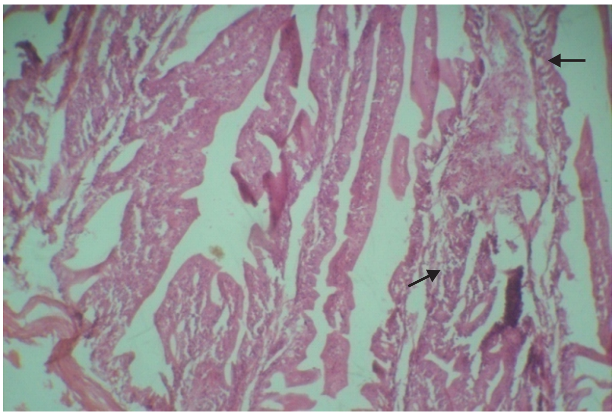

Plate 18: Photomicrograph of a section of the intestine of *Clarias gariepinus* juveniles fed 100% BSSM-based diet showing moderate sloughing off/erosion of the epithelia of villi (arrows), necrosis of enterocytes and presence of moderate amounts of inflammatory cells in *lamina propria* of the villi (H and E; x100).

DISCUSSION

Organosomatic indices *C. gariepinus* fed graded levels of BSSM diets

As the liver is a target organ for metabolism in the fish body, the liver index (HSI) is a useful biomarker to detect the hazardous effects of the environmental stressors (Pait and Nelson, 2003). 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). 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 HSI observed in the fish fed 0% BSSM-based diet (control diet) indicated normal liver growth resulting from dietary treatment without boiled sunflower seed meal at this level. It could be stated that the enhancement of HSI may reflect reduced body weight. On the contrary, Adejinmi (2000) noted that enlargement of organs such as liver, kidney and heart has been associated with dietary factors especially if diets contain toxins, anti-nutrients or heavy metals.

Barse *et al.* (2006) also reported elevated 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. The progressively reduced HSI values recorded for the fish fed 20% to 100% BSSM-based diets could be linked with reduced appetite which was probably associated with the presence of anti-nutrients in the boiled 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.

The significantly reduced value of kidney-somatic index (KSI) recorded for fish at 100% BSSM inclusion could be linked with the reduction of the total body weight as a result of reduced appetite that was likely induced by inherent anti-nutrients. The non-significant variation in the values of intestinosomatic index (ISI) suggested that the weight of the intestine and total body weight were not correspondingly affected by the increasing inclusion level of boiled sunflower seed meal in the diets. This result disagrees with that of Abdel-Hameid (2007) who reported that reduced ISI values for *O. aureus* juveniles could be associated with the reduction of the total body weight as a result of reduced appetite caused by phenol intoxication.

Histopathological observations on *C. gariepinus* fed graded levels of BSSM diets

Examination of the liver, kidney and intestine was done because of their physiological importance during absorption and metabolism of chemicals (Roberts, 1989). Evaluation of histological structure of digestive organs in fish fed new dietary ingredients generates valuable information about their digestive capacity and the potential health effects of such diets (Diaz *et al.*, 2006). Incorporating different inclusion levels of boiled sunflower seed meal (BSSM) in the diets of *C. gariepinus* juveniles in this study caused varying degrees of histopathological changes in their liver cells (hepatocytes) such as mild and severe diffuse vacuolations of the hepatocytes, vacuolar degeneration, some loci of extensive fatty infiltration, extensive hepatic degeneration and periportal venous congestion. These observations 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. Olasunkanmi (2015) also observed that *C. gariepinus* fed processed velvet beans showed both mild diffuse and marked widespread vacuolation of the hepatocytes. Vacuolated hepatocytes are usually accumulated with glycogen and have little or no regenerative ability (Nayak *et al.*, 1996) and the excessive vacuolation of the hepatocytes would result in abnormal functioning of the liver cells, for example, immobilization of fat, which could consequently result in fatty infiltration of the hepatic parenchyma (Adeyemo, 2005).

The result of the present study closely supports the finding of Hlophe and Moyo (2014) who observed that *C. gariepinus* fed high moringa leaf meal inclusion levels (>50%) showed an increase in the number of degraded irregularly shaped hepatocytes, small dark pyknotic nuclei, poor fatty deposition and isolated necrosis. Despite similar protein and energy levels in the experimental diets, liver histology showed that fish fed higher BSSM inclusion levels had necrotic signs associated with poor nutritional status (Tusche *et al.*, 2012). The malnutrition signs observed in *C. gariepinus* fed higher levels of BSSM 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 explains the nutritional necrosis observed in the hepatocytes.

In the present study, 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. In this situation, anti-nutritional substances present in sunflower seed meal must have been responsible for the observed histopathological changes in the liver sections.

Utilisation of BSSM-based diets by *C. gariepinus* also resulted in some histological changes in its kidney which included some loci of tubular epithelial degeneration, diffuse interstitial congestions and tubular degeneration as well as necrosis. Olasunkanmi (2015) also observed a marked congestion in the kidneys of *C. gariepinus* juveniles fed diets containing higher inclusion levels of processed velvet beans and associated such changes with ingestion of a high percentage of velvet bean meals which imposed stress on the organ above its physiological capacity. Benjamin (2009) earlier reported that congestion of the kidney tubules is the first stage in the development of kidney disease.

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). The presence of tubule disruption and necrosis in the kidney of fish fed higher BSSM inclusions indicates that the kidney suffered some damage which could be attributed to the presence of anti-nutritional substances in the BSSM.

The observed sections of the intestines of *C. gariepinus* juveniles fed boiled sunflower seed meal-based diets indicated mild histopathological changes such as reduced submucosal width, slightly reduced and stunted villi as well as moderate erosion of the epithelia of villi. This result agrees with that of Hlophe and Moyo (2014) who observed that the intestine histology of *C. gariepinus* fed diets containing higher moringa leaf meal inclusion levels (>50%) showed significantly shorter villi. The decrease in villi height resulted in reduced surface area for nutrient absorption (Da Silva *et al.*, 2012). 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 during nutrient absorption (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). Necrosis and mucosal degeneration may affect the permeability and absorption of substances across the stomach and intestinal walls. This may suggest that *C. gariepinus*, being an omnivore, is more capable of utilising plant diets than carnivorous fish.

CONCLUSION

Histological examinations of the thin sections of the liver, kidney and intestine of *C. gariepinus* fed graded levels of BSSM-based diets revealed changes which varied from mild to severe lesions and few anatomical alterations, particularly at the higher levels of BSSM incorporation. Histological analysis indicated that *C. gariepinus* was more stressed as the level of inclusion of boiled sunflower seed meal in the diets increased. Anti-nutritional factors (tannin, oxalate and phytate) were most probably responsible for the poor performance of the BSSM-based diets. Further studies should be conducted on the specific role of each anti-nutrient during absorption and utilisation of nutrients as well as more effective methods of processing that will significantly reduce the levels of anti-nutritional components in sunflower seeds as a suitable alternative fish feed ingredient.

ACKNOWLEDGEMENTS
The author wishes to acknowledge Messrs. I. C. Mpama, O. I. Tunji, J. U. Augustine, Ambrose Nwagbara and Miss Janet Oni as well as Drs. O. O. Aina and John Ogunsola of the Department of Veterinary Anatomy, University of Ibadan, for their thorough technical assistance in tissue processing and slide photomicrographic preparation and interpretation.

REFERENCES

Abdel-Hameid, N.A.H. 2007. Physiological and Histopathological Alterations Induced by Phenol Exposure in *Oreochromis aureus* Juveniles.*Turkish Journal of Fisheries and Aquatic Sciences* 7:131-138.

Abdel-Moneim, A.M., Al-Kahtani, M.A. and Elmenshawy, O.M. 2012. Histopathological biomarkers in gills and liver of *Oreochromis niloticus* from polluted wetland environments of Saudi Arabia. *Chemosphere* 88 (8):1028-1035.

Adejinmi, O.O. 2000. The chemical composition and nutritional potential of soldierfly (*Hermetia elucens*) larvae in poultry rations.PhD. Thesis.Department of Animal Science. University of Ibadan. 292 pp.

Adesina, S.A., Falaye, A.E., Olusola, S.E. and Ajani, E.K. 2013. Growth performance and nutrient utilization of *Clarias gariepinus* juveniles fed graded levels of boiled sunflower (*Helianthus annuus* L) seed meal-based diets. *Wudpecker Journal of Agricultural Research* 2 (12): 342-351.

Adeyemo, O.K. 2005. Haematological and histopathological effects of cassava mill effluent in *Clarias gariepinus*. *African Journal of Biomedical Research* 8: 179-183.

Akerman, G., Amcoff. P., Tjarnlund, U., Fogelberg, K., Torrissen, O. and Balk, L. 2003. Paraquat and menadione exposure of rainbow trout (*Oncorhynchus mykiss*). Studies of effects on the penthose phosphate shunt and thiamine levels in liver and kidney. *Chemical Biology International* 142: 269-283.

Akhilesh, K.Y., Prem, P.S., Joykrushna, J., Pradeep, S., Shipra,C. and Rajesh, D. 2014. Impact of dietary fats on histological alterations in the kidney tissue of Asian catfish, *Clarias batrachus* (Linnaeus, 1758). *International Journal of Fisheries and Aquatic Studies* 1(3): 147-151.

Ali, G., Nurcan, U., Ayhan,Ö., Belda, E., Sezin,D. and Göktuğ, G. 2014. Effects of some physicochemical properities of water on histopathological and haematological picture of fish inhabiting Emet Stream. *International Journal of Fisheries and Aquatic Studies* 2 (1): 65-71.

Ali, M.Z. 2001. Dietary protein and energy interactions in African catfish *Clarias gariepinus* (Burchell, 1822).PhD. Thesis. Department of Aquaculture. University of Aquaculture, Stirling, United Kingdom. 274pp.

Bancroft, J.D. and Cook, H.C. 1994. Manual of Histological Techniques and Their Diagnostic Application. Churchill Livingstone, London. 305pp.

Barse, A.V., Chakrabarti, T., Ghosh, T.K., Pal, A.K. and Jadhao, S.B. 2006. One-tenth dose of LC_{50} of 4-*tert*butylphenol causes endocrine disruption and metabolic changes in *Cyprinus carpio*. *Pesticide Biochemistry and Physiol*ogy 86 (3): 172-179.

Benjamin, H. 2009. Diseases of the liver, gall bladder, kidneys and pancreas. Accessed online from www: herbdatanz.com/liver gall bladder kidney pancreas harry-benjamin nd.htm on 21/05/2011.

Caballero, M.J., Izquierdo, M.S., Kjørsvik, E., Fernández, A.J. and Rosenlund, G. 2004. Histological alterations in the liver of sea bream, *Sparus aurata* L., caused by short or long-term feeding with vegetable oils. Recovery of normal morphology after feeding fish oil as the sole lipid source. *Journal of Fish Diseases* 27: 531–541. doi: 10.1111/j.1365-2761.2004.00572.x.

Chaudhuri A., Mukherjee S. and Homechaudhuri S. 2012. Diet composition and digestive enzymes activity in carnivorous fishes inhabiting mudflats of Indian Sundarban estuaries. *Turkish Journal of Fisheries and Aquatic Sciences* 12: 265-275. doi: 10.4194/1303- 2712-v12_2_11.

Coyle, S., Durborow, D.R.M. and Tidwell, J.H.

2004. Anaesthetics in Aquaculture. SRAC Publication, Texas, pp: 6.

Da Silva, M.R., Natali, M.R.M. and Hahn, N.S. 2012. Histology of the digestive tract of *Satanoperca pappaterra* (*Osteichthyes, Cichlidae*). *Acta Scientiarum Biological Sciences* 34: 319-326. doi: 10.4025/actascibiolsci.v34i3.8956.

Diaz, A.O., Escalante, A.H., Garcìa, A.M. and Goldemberg, A. L. 2006.Histology and histochemistry of the pharyngeal cavity and oesophagus of the Silverside *Odontesthes bonariensis* (Cuvier and Valenciennes). *Anatomia Histolologia Embryologia* 35: 42–46.

Duncan, D.B. 1955. Multiple range and multiple F tests. *Biometrics* 11: 1 – 42.

FAO. 2011. Demand and supply of feed ingredients for farmed fish and crustaceans: trends and prospects. http://www.fao.org/docrep/015/ba0002e/ba0002e.pdf (accessed January 22, 2014).

Figueiredo-Fernandes, A., Fontaínhas-Fernandes, A., Peixoto, F., Rocha,E., Reis-Henriques, M.A. 2006. Effect of paraquat on oxidative stress enzymes in tilapia *Oreochromis niloticus* at two levels of temperature. *Pesticide Biochemistry and Physiology* 85: 97-103.

Garza de Yta, A., Davis D. A., Rouse D. B., Ghanawi J. and Saoud I. P. 2012. Evaluation of practical diets containing various terrestrial protein sources on survival and growth parameters of redclaw crayfish *Cherax quadricarinatus*. *Aquaculture Research* 43 (1): 84 - 90.

Gernhofer, M., Pawet, M., Schramm, M., Müller, E. And Triebskorn, R. 2001. Ultrastructural biomarkers as tools to characterize the health status of fish in contaminated streams. *Journal of Aquatic Ecosystem, Stress and Recovery* 8: 241-260.

Hlophe, S.N. and Moyo, N.A.G. 2014. Replacing Fishmeal with Kikuyu Grass and Moringa Leaves: Effects on Growth, Protein Digestibility, Histological and Haematological Parameters in *Clarias gariepinus*. *Turkish Journal of Fisheries and Aquatic Sciences* 14: 795-806.

Hoque, M.T., Yusoff, F.M., Law, A.T. and Syed, M. A. 1998. Effect of Hydrogen Sulphide on Liver-Somatic Index and Fulton Factor in *Mystus nemurus*. *Journal of Fish Biology* 52: 23-50.

Hughes, G.M. and Perry, S.F. 1976. Morphometric study of trout gills: A light microscopic method for the evaluation of pollutant action. *Journal of Experimental Biology* 63: 447-460.

Nayak, N.C., Sathar, S.A., Mughal, S., Duttagupta, S., Mathur, M. and Chopra, P. 1996. The nature and significance of liver cell vacuolation following hepatocellular injury - an analysis based on observations on rats rendered tolerant to hepatotoxic damage. *Virchows Archives* 428: 353-365.

Olasunkanmi, J.B. 2015. Histopathological changes in the organs of *Clarias gariepinus* fed processed velvet beans. *International Journal of Fisheries and Aquatic Studies* 2 (5): 128-132.

Olvera–Novoa, M. A., Olivera–Castillo, L. and Martinez-Palacios, C. A. 2002. Sunflower seed meal as a protein source in diets for *Tilapia rendalli* fingerlings. *Aquaculture Research* 33(3): 223-230.

Pait, A.S. and Nelson, J.O. 2003. Vitellogenesis in male *Fundulus heteroclitus* (Killifish) induced by selected estrogenic compounds. *Aquatic Toxicology* 64: 331-342.

Roberts, R.J. 1989. The Pathophysiology and Systematic Pathology of Teleosts. In: Fish Pathology, Roberts, R. J. (Ed.). 2nd Edn., Bailliere Tendall, London, UK., pp: 56-134.

Shalaka, S. and Pragna, P. 2013. Gonadosomatic and Hepatosomatic Indices of Freshwater Fish *Oreochromis mossambicus* in Response to a Plant Nutrient. *World Journal of Zoology* 8(1): 110-118.

Siddhuraju, P. and Becker, K. 2001. Preliminary nutritional evaluation of mucuna seed meal (*Mucuna pruriens* var. *utilis*) in common carp (*Cyprinus carpio* L.): An assessment by growth performance and feed utilization. *Aquaculture* 196:105-123.

Sintayehu, A., Mathies, E., Meyer-Burgdorff, K.-H., Rosenow, H. and Gunther, K.-D. 1996. Apparent digestibilities and growth experiments with tilapia (*Oreochromis* *niloticus*) fed soybean, cottonseed meal and sunflower seed meal. *Journal of Applied Ichthyology* 12: 125-130. doi: 10.1111/ j.1439-0426.1996.tb00075.x.

Takashima, F. and Hibya, T. 1995. An atlas of fish histology: normal and pathological features. Edn 2, Tokyo, Kodansha.

Thophon, S., Kruatrachue, M., Upathan, E.S., Pokethitiyook, P., Sahaphong, S. and Jarikhuan, S. 2003. Histopathological alterations of white seabass, *Lates calcarifer*,in acute and sub-chronic cadmium exposure. *Environmental Pollution* 121:307-320.

Tusche, K., Arning, S., Wuertz, S., Susenbeth, A. and Schulz, C. 2012. Wheat gluten and potato protein concentrate - Promising protein sources for organic farming of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 349: 120-125. doi:10.1016/j.aquaculture. 2012.03.009.

Uwachukwu, S.N., Shoyinka, V.O. and Obioha, F.C. 2003. Chronic toxicity of raw lyon's bean (*Mucuna cochinchinensis*) in broilers [toxicidad cronica del frijol lyon (Mucuna cochinchinensis) pollos de engorda]. *Tropical and Subtropical Agroecosystems* 2: 23-30.

Yang, X. and Baumann, P.C. 2006. Biliary PAH metabolites and the hepatosomatic index of brown bullheads from Lake Erie tributaries. *Ecological Indicators* 6: 567-574.