Some aspects of histology of liver and intestine of *Cyprinus carpio* fingerlings fed raw and processed *Mucuna pruriens* diets

^{*1}Kakwi, D. G., ²Awolumate, S., ³Audu, B. S., ¹Oshibanjo, D. O., ¹Oyedapo, F. A.,
¹Mohammed, M. Y. and ⁴Ositimihin, K. M.

¹Animal Production Department, University of Jos, Jos.

²Department of Animal Science and Fisheries, Faculty of Agricultural Sciences,
National Open University of Nigeria.

³Department of Zoology, University of Jos, Jos.

⁴Department of Fisheries, Lagos State University, Lagos State Nigeria

Corresponding author: oshibanjoo@unijos.edu.ng; +2348055438564

Abstract

Mucuna contains anti-nutritional factors, anticoagulants, anti-inflammatory factors and L-dopa, a potentially neurotoxic agent. These anti-nutrients are endogenous toxic substances that are part of the normal feedstuff and affect its nutritional quality. The concentrations of these anti-nutritional factors can be reduced or completely eliminated if the seeds are properly processed. The impact of anti-nutritional factors and other toxic compounds in *mucuna* on fish have not been documented. Investigations on the dietary effects of raw and processed *Mucuna pruriens* seed diets on the histopathology of the liver and intestine of *C. carpio* fingerlings were carried out. The raw and the processed *mucuna* seed meals in two replacement levels of 20% and 50% as substitution of soybean meal in treatment diets were labelled Raw, MCD1 (20%) and MCD2 (50%), respectively, while a reference diet with no *mucuna* served as the control diet (CD). The diets were fed to *C. carpio* fingerlings (average weight, 15.40g) for 56 days. Water quality in the culture of *C. carpio* was measured using standard procedure. Histopathological changes in the liver and intestine sections of fish were observed consistently and variable in the treatment groups. Water quality parameters were measured for the period of evaluation. Significant difference was not observed for all the water quality parameters measured. The water temperature varied between 22.90°C to 22.96°C similar to the room temperature. The pH ranged between 7.02 and 7.07 while dissolved oxygen ranged from 6.24 to 6.34 ppm. The liver of the fish fed raw *mucuna* diets showed vacuolation and severe necrosis of hepatocytes in the liver parenchyma and periportal areas. The histological analysis of the intestine of *C. carpio* fingerlings fed raw *Mucuna* diet also showed severe atrophy in the musculature of the intestinal villi of the fish which was mild in the fish fed MCD1 and control diets. The MCD1 was considered the most appropriate for dietary inclusion of *Mucuna pruriens* seeds in the feeding of *C. carpio* fingerlings.

Keywords: *Mucuna pruriens* Seed, Liver, Intestine, Histopathology

Certains aspects de l'histologie du foie et de l'intestin des alevins '*Cyprinus carpio*' nourris de régimes *Mucuna pruriens* crus et transformés

Résumé

Mucuna contient des facteurs antinutrit nutritionnels, des anticoagulants, des facteurs anti-inflammatoires et de la L-dopa, un agent potentiellement neurotoxique. Ces antinutriments sont des substances toxiques endogènes qui font partie de l'aliment normal et affectent sa qualité nutritionnelle. Les concentrations de ces facteurs anti-nutritionnels peuvent être réduites ou complètement éliminées si les graines sont correctement traitées. L'impact des

Some aspects of histology of liver and intestine of Cyprinus carpio fingerlings

facteurs anti-nutritionnels et d'autres composés toxiques dans la mucuna sur les poissons n'a pas été documenté. Des études sur les effets diététiques des régimes crus et transformés de graine de Pruriens de Mucuna sur l'histopatologie du foie et de l'intestin des a ongles de C. carpio ont été effectuées. Les repas crus et transformés de graine de mucuna dans deux niveaux de remplacement de 20% et 50% comme remplacement de la farine de soja dans les régimes de traitement ont été étiquetés crus, MCD1 (20%) et MCD2 (50%), respectivement, tandis qu'un régime de référence sans mucuna a servi de régime de contrôle (CD). Les régimes ont été donnés aux acolings C. carpio (poids moyen, 15,40 g) pendant 56 jours. La qualité de l'eau dans la culture de C. carpiowas a été mesurée à l'aide d'une procédure standard. Des changements histopathologiques dans les sections hépatiques et intestinales des poissons ont été observés uniformément et variables dans les groupes de traitement. Des paramètres de qualité de l'eau ont été mesurés pour la période d'évaluation. Aucune différence significative n'a été observée pour tous les paramètres de qualité de l'eau mesurés. La température de l'eau variait entre 22,90 °C et 22,96 °C, semblable à la température ambiante. Le pH se situe entre 7,02 et 7,07 tandis que l'oxygène dissous varie de 6,24 à 6,34 ppm. Le foie des poissons alimentés régimes crus de mucuna a montré la vacoulation et la nécrose grave des hépatocytes dans le parenchyme de foie et les secteurs periportal. L'analyse histologique de l'intestin des ails de C. carpio alimentés au régime cru de Mucuna a également montré l'atrophie grave dans la musculature des villosités intestinales du poisson qui était douce dans les poissons alimentés MCD1 et les régimes de contrôle. Le MCD1 a été considéré comme le plus approprié pour l'inclusion diététique des graines pruriens de Mucuna dans l'alimentation des acolings de C. carpio.

Mots-clés: Mucuna pruriens, foie, Intestin, Histopatologie

Introduction

Aquaculture feed production is fast expanding with the current intensification towards meeting the growing food requirements of the world's population (Bostock *et al.*, 2010; FAO, 2014). Therefore, there is greater demand for higher quality feed and thus for increased knowledge of the fish dietary requirements for better growth. The presence of anti-nutritional factors in plant-based feed materials is known to prevent full expression of the high quality of plant protein in animal diets (Higgs *et al.*, 1982; Francis *et al.*, 2001). These anti-nutrients include fibre and other indigestible carbohydrates and phenolic compounds such as glucosinolates, phytates, protease inhibitors, non-starch polysaccharides (NSP), saponins, tannins, lectins, and gossypols (Francis *et al.*, 2002; Siddhuraju *et al.*, 2002). *Mucuna pruriens*, also known as velvet bean is a herbaceous legume

belonging to the Family *Fabaceae* and sub family *Papilionaceae* (Kay, 1979). *Mucuna*'s potential as a food and feed is affected, on the positive side, by its relatively good seed and forage yield and beneficial composition of nutrients and minerals (Bressani *et al.*, 2003). *Mucuna* also contains anti-nutritional factors (ANFs) such as tannin, haemagglutinin, cyanide and trypsin inhibitors (Ravindran and Ravindran, 1988 and Francis *et al.*, 2001), anticoagulants, anti-inflammatory factors and L-dopa, a potentially neurotoxic agent (Aguiyi *et al.*, 2003). These anti-nutrients are endogenous toxic substances that are part of the normal feedstuff and affect its nutritional quality (Ukachukwu, 2000; Bressani 2003). The impact of anti-nutritional factors and other toxic compounds in mucuna on fish have not been documented (Francis *et al.*, 2002). However, it has been observed that histopathological changes can be related to

growth of fish as assimilation of nutrients are affected as reported by Richardson *et al.* (1985); and Nogales *et al.* (2010). Also, Bozidar *et al.* (2011) observed the need for monitoring histological structure of fish liver and intestine as this contribute to better explanation of mechanism involved in weight gain, digestibility and food availability. Francis *et al.* (2001) reported different tolerance level to toxins by individual fish species and need to be considered before deciding on treatment procedures to reduce their levels. Tilapia fish species, for example, were found to be more tolerant than carp to the increased presence of antinutrients in their diets (Francis *et al.*, 2001). **The concentrations of these anti-nutritional factors can be reduced or completely eliminated if the seeds are properly processed (Ukachukwu, 2000; Siddhuraju *et al.*, 2002).**

In this study, the histopathological effects of dietary inclusion of raw and maize cob ash processed *Mucuna pruriens* seed meals on indices of liver and intestine of *C. carpio* fingerlings were investigated.

Materials and methods

Experimental site

This study and the feeding trial was conducted in concrete tanks in a private outdoor fish hatchery in Jos, Plateau State, Nigeria.

Experimental fish

About 500 fingerlings of Common carp (*C. carpio*) fish fingerlings of weight sizes between 15.10-15.20gm were selected from a stock (mixed sex full sibling) and kept in oxygenated concrete tank (2m x 5m x 1.5m) while being fed two times a day, on commercial pelleted diet (Vital Fish Feed, 25% Crude protein) for 10 days. The feeding was done daily at 10:00 hour daily.

Collection and processing of *M. pruriens* seeds into feedstuff

About 5kg sample of the speckled variety of

M. pruriens bean seeds was sorted out, cleaned of impurities, sun-dried and out of which 3kg was subjected to hydro-thermal processing method by boiling at 100-105°C in maize cob ash solution, while remaining 2kg was left raw.

Treatment of *M. pruriens* seeds with maize cob ash solution

About 300gm of ash was obtained from maize cobs burnt on a metal tray and put in plastic container with 1mm holes at the bottom. The ash was pressed down slightly to form a filter bed. Distilled water (1.5 l) was added slowly making sure that the ash layer was not disturbed. The filtrate, amounting to about 750ml was collected in a container. One hundred milliliters (100mL) of the filtrate was diluted to 500ml with distilled water to make a solution of 1:4 dilutions. The samples (100gm) of mucuna seeds were boiled in 1.5 litres of the maize cob ash solution in enamel pot for a duration of 60 minutes. The level of processing solution in the pot was maintained by the addition of boiling solution from a standby boiling pot and wooden spoon used to stir the content in order to minimize the risk of interference of metallic ions when metal spoon was used (Wanjekenje *et al.*, 2003). At the end of different boiling duration (60 minutes), the water was drained off using raffia basket after which the processed bean sample was washed with distilled water and dried in an open air under a shed for three days. The dried sample was then weighed, grinded and preserved in cellophane bag and kept in the refrigerator at 4°C for analysis.

Experimental diet composition and preparation

Materials used in the test diets formulation included mucuna seed meal, fishmeal, full-fat soybean meal, yellow maize flour, cassava flour (binder), vegetable oil and vitamin premix as shown in Table 1. All the materials apart from mucuna seeds were purchased locally from commercial

Some aspects of histology of liver and intestine of *Cyprinus carpio* fingerlings

livestock feed millers in the open market. Before the different diets were prepared, proximate analysis of the main ingredients (fishmeal, soyabean meal and maize flour) was carried out. Two sets of approximately isonitrogenous and isoenergetic experimental diets were prepared using the raw and Maize cob ash processed mucuna seeds as the main test ingredients. The details of test diet preparations were as given by Ufodike and Matty (1983). The ingredients were milled separately and sieved with laboratory 593µm mesh size sieve to obtain a homogenous particle size. Maize, soybean and mucuna seeds were grinded into powder individually and each was also sieved separately.

The required quantities of all the dry

ingredients were measured and thoroughly mixed together at an average room temperature of (45°C) in a plastic bowl. Vitamin premix was added and there was a thorough remixing again. Then vegetable oil (2mL) was added and the ingredients were again mixed, while 5ml of warm water was added and the ingredients finally mixed to form a paste and the moist mixture extruded through a 2-mm die manually operated extruder to form worm-like pellets. These pellets were then dried in the laboratory oven at a temperature of 60°C for ten hours. To each of the prepared diets, 1% chromic oxide was added as an external digestibility marker. The pellets were then stored in a cool, dry box, free from rodents.

Table 1: Composition of treatment diets fed to *Cyprinus carpio* fingerlings for 8 weeks

Ingredients (%DM)	Control (CD) 0%	Raw	MCD1 20%	MCD2 50%
Mucuna meal		49.97	9.98	24.99
Soya bean meal	49.97	-	39.99	24.99
Maize flour	35.03	35.03	35.03	35.03
Fish meal	10.00	10.00	10.00	10.00
Veg. Oil	3.00	3.00	3.00	3.00
Fish Oil	2.00	2.00	2.00	2.00
Vitalyle*	2.00	2.00	2.00	2.00
Cassava flour	8.00	8.00	8.00	8.00
Total	100.00	100.00	100.00	100.00
Protein (%)	35.00	32.24	32.16	31.16

*1kg of Vitalyte premix contains: Vitamin A (5,000,000 I.U), Vitamin D3. (1,000,000 I.U), Vitamin E (16,000mg), Vitamin K3 (800mg), Vitamin B1 (1,200mg), Vitamin B2 (22,000mg), Niacin (22,000mg), Calcium pantothenate (4,600mg), Vitamin B6 (2,000mg), Vitamin B12 (10mg), Folic acid (400mg), Biotin (32mg), Choline chloride (200,000mg), Manganese (48,000mg), Iron (40,000mg), Zinc (32,000mg), Copper (3,400mg), Iodine (600mg), Cabalt (120mg), Selenium (48mg)

MCD1 and MCD2: Processed *M. pruriens* was used for soybean protein at inclusion levels of 20% and 50% respectively.

Experimental design and layout

The four experimental diets were fed to the *C. carpio* fingerlings in a completely randomized design (CRD) experiment. Each treatment was replicated twice with 15 fish per replicate. All the fish were fed experimental diets twice a day in two equal parts between 08.00 – 09.00h and 15.00 – 16.00hr at a feeding rate of 5% body weight per day. The duration of the experiment was

eight weeks.

Water quality parameters

The standard methods employed in the determination of the various water physico-chemical parameters which include temperature, pH, total alkalinity, free carbon dioxide, dissolved oxygen and ammonia were those described by APHA/AWWA/WPCF (1985).

Histopathological examination of fish organs

The liver and digestive tract samples of *C. carpio* fingerlings were taken for determination of any significant alteration of organ by increased supplementation with raw and treated diets due to presence or reduction of antinutrients. The organs were fixed in 10% normal saline solution. They were prepared for histological presentation using the routine histological methods and haematoxylin-aosin staining techniques described by Culling (1974). The automatic duplex processor, Uniscope (standard and southern Model: C. 35H) was used to process the organs. Graded alcohol (70%, 90%, absolute I, II, III) and graded chloroform (I, II, III) were used in dehydrating and cleaning respectively. The organs were infiltrated and embedded in molten paraffin wax. The Cambridge Rock Microtome (Model: M 64) was used to section the organs to the desired thickness (0.2mm) and finally transferred into a clear slide. During the staining procedures, the sections were de-waxed in xylem and hydrated in graded alcohol (95%, 80%, 70% and 50%) respectively. The sections were stained in haematoxylin for 5 minutes, differential in 1% acid alcohol (hydrochloric acid and 70% alcohol), washed with tap water and counter-stained in 5% aqueous eosin for 5 minutes. The

section was thoroughly washed and dehydrated in graded alcohol and chloroform and further de-parafine in xylene. The section was fully mounted in Canada Balsam and carefully covered with slide cover slip. Photographs of the prepared sections were taken using a mounted photo-microscopic camera.

Statistical analysis

All experimental data obtained were subjected to one-way Analysis of Variance (ANOVA). Standard errors (SE) of the means for each replicate were calculated and the means were compared using Duncan's multiple-range test (Duncan, 1955) to determine significant differences ($P < 0.05$) among treatments for processing methods, proximate compositions of the raw and processed seeds and growth parameters. The PC software SPSS version 25 was used for the ANOVA analysis.

Results

The water quality parameters monitored during the experimental period were not significantly ($P > 0.05$) different as shown in Table 2. The water temperature varied between 22.90 to 22.96°C similar to the room temperature. The pH ranged between 7.02 and 7.07, while dissolved oxygen ranged from 6.24 to 6.34 ppm. Free Carbon dioxide (mg/l) ranged 2.14 – 3.32 while total alkalinity (mg/l) ranged 22.8 – 27 mg/l.

Table 2: Water quality parameters as affected by *M. pruriens* seed meal treated with maize cob ash solution

Parameters	Control	Raw	MCD1 (20%)	MCD2 (50%)	SEM
Temperature (°C)	22.90	22.92	22.94	22.96	0.63
Dissolved oxygen (mg/l)	6.34	6.30	6.26	6.24	0.13
Free Carbondioxide (mg/l)	2.14	2.88	3.18	3.32	0.23
Total alkalinity (mg/l)	22.80	24.80	26.00	27.00	1.34
pH	7.02	7.02	7.06	7.07	0.04
Ammonia (unionized NH ₃)	0.22	0.22	0.22	0.22	0.01

MCD1 and MCD2: Processed *M. pruriens* was used for soybean protein at inclusion levels of 20% and 50% respectively

SEM = Standard Error Mean

Some aspects of histology of liver and intestine of Cyprinus carpio fingerlings

The histopathological conditions of *C. carpio* fingerlings fed Raw and processed *M. pruriens* diets are presented as photomicrographs (Plates 1 to 8). Histopathological changes were observed consistently in the liver and intestine sections of fish fed diets containing the raw and processed mucuna seed meals. However, the degrees of these changes observed were variable in the treatment groups. The liver sections of *C. Carpio* fish fed control diets showed distinct hepatocytes (H) with no observable changes in the lobular architecture (Plates 1

and 2). Liver sections of fish fed 20% MCD1 processed Mucuna diets had no significant ($p < 0.05$) changes in the perivascular and periductula spaces in the portal areas (P) (Plates 3 and 4). However, feeding of 50% MCD2 processed Mucuna diets caused mild infiltration of polymorphonuclear leucocytes (PNL) and intravascular hemolysis (HL) in the portal vessels of the liver (Plates 5 and 6). The raw Mucuna diets caused severe vacuolation (v) and karyolysis (k) of the hepatocytes in centrolobular areas of the liver parenchyma of *C. Carpio* (Plates 7 and 8).

Plate 1: Liver Section of *C. carpio* Fed Control Diet X 100
H - Hemolysis

Plate 2: Liver Section of *C. carpio* Fed 20% MCD1 Processed *M. pruriens* Diet X100
H – Hemolysis P- perivascular and periductula spaces in the portal areas

Plate 3: Liver section of *C. carpio* Fed 50% MCD2 Processed *M. pruriens* Diet x 100
H – Hemolysis PNL- polymorphonuclear leucocytes
HL - intravascular hemolysis

Plate 4: Liver Section of *C. carpio* Fed Raw *M. pruriens* Diet
H – Hemolysis P- perivascular and periductula spaces in the portal areas V - vacuolation

Plate 5: Intestine Section of *C. carpio* Fed Control Diet
Mv – microvilli Snv – supranuclear vacuoles Le – lamina epithelialis
Lp – lamina propria Sc- stratum compactum Sg – stratum granulosum
Sr – stratum circulare Sl – stratum longitudinale Sm – serosa
Muc - mucosa layer Sub - submucosa layer

Some aspects of histology of liver and intestine of Cyprinus carpio fingerlings

Plate 6: Intestine Section of *C. carpio* Fed 20% MCD1 Processed *M. pruriens* Diet

Mv – microvilli Snv – supranuclear vacuoles Le – lamina epithelialis Lp – lamina propria Sc- stratum compactum Sg – stratum granulosum. Sr – stratum circulare Sl – stratum longitudinale Sm – serosa. Muc - mucosa layer Sub - submucosa

Plate 7: Intestine Section of *C. carpio* Fed 50% MCD2 Processed *M. pruriens* Diet

Mv – microvilli Snv – supranuclear vacuoles Le – lamina epithelialis. Lp – lamina propria Sc- stratum compactum Sg – stratum granulosum. Sr – stratum circulare Sl – stratum longitudinale Sm – serosa. Muc - mucosa layer Sub – submucosa

Plate 8: Intestine Section of *C. carpio* Fed Raw *M. pruriens* Diet. Mv – microvilli Snv – supranuclear vacuoles Le – lamina epithelialis. Lp – lamina propria Sc- stratum compactum Sg – stratum granulosum. Sr – stratum circulare Sl – stratum longitudinale Sm – serosa. Muc - mucosa layer Sub - submucosa

Discussion

The aquatic environment governs fish life; hence water quality should be suitable for fish culture. The successful aquaculture depends on the quality of different water parameters (Rahman, 1992). For a successful aquaculture venture, the dynamics and management of water quality in culture media must be taken into consideration (Davies and Ansa, 2007). Temperature as a water quality parameter is important as it directly affects the amount of oxygen that can be dissolved in water but also the growth of organisms and bacteria that can cause disease. Temperature also controls the rate at which food is transformed into energy which then in turn affects breathing, food intake, bodily growth and physiological behaviour. An increase in temperature will decrease the dissolved oxygen content in water which will in turn increase the fish metabolic rate and its demand for oxygen. The results obtained for this study is within the acceptable range which is in line with the report of Boyd (1981). Dissolved Oxygen (DO) is considered by most to be the most important water quality parameter to in aquaculture. Depending on the size and type of fish farm, DO levels can change very quickly indeed and can therefore endanger the lives of the fish in a matter of minutes or hours. Although fish can tolerate a drop in dissolved oxygen below 5-6 mg/L for short periods of time, it will lead to significant stress or indeed death if not increased to healthy levels. The results obtained for this study is within the acceptable range which is in line with the report of Boyd (1981). The recommended pH range for fish and vertebrates is between 7.0 and 8.0 as the average blood pH is around 7.4. With water passing through the fish gills and skin they are susceptible to changes in pH. Fish therefore become stressed if pH falls outside the pH5-10 range. pH does vary throughout the day and

depends on the oxygen demand in the water and photosynthesis. The CO₂ is also released overnight hence lowering pH by morning but rises to its peak in the afternoon when CO₂ consumption by algae (photosynthesis) is at its peak. Fish have a reduced capacity to carry oxygen if pH levels fall to critical levels but if the pH goes critically high, the portion of ammonia that is toxic also increases. The results obtained for this study is within the acceptable range which is in line with the report of Boyd (1981). Ammonia is the main waste product by fish and is regarded as the second most important water quality parameter after dissolved oxygen in intensive fish farming. Even in relatively small concentrations, ammonia can cause significant stress and damage to fish especially bacteriological infections. In higher concentrations where the nitrogen cycle is not in balance ammonia can wipe out whole fish stocks. The results obtained for this study is within the acceptable range which is in line with the report of Boyd (1981). For the histopathological assessment of fish organs, the liver of the fish fed raw Mucuna diets showed vacuolation and severe necrosis of hepatocytes in the liver parenchyma and periportal areas which are indications of pathological conditions. In line with the important function of the liver as an organ of detoxification which suffers most from toxic insults, this situation is a sign of direct involvement of cyanide and tannin which are found in raw Mucuna seed and as reported by Ukachukwu (2000). The degrees of these changes appeared to be reduced in the processed treatments especially with the inclusion level of 20% MCD1 processed Mucuna diet fed to the fish. Similar observations were made by Grabner and Hofer (1985) and Caballero *et al.* (2004) when they reported that various levels of protein in the diet can lead to histopathological changes in the alimentary tract and liver of rainbow trout

Some aspects of histology of liver and intestine of Cyprinus carpio fingerlings

(*Litopenacus vannamei*) and sea bream (*Spansa cerara*) respectively. Zakes *et al.* (2008) also reported that the most common nutritional changes in the liver include hepatocytes vacuolation and changes in morphometric parameters such as hepatocyte number, surface area and nuclei concentration. It was also reported that changes in metabolic activity, changes in liver parenchyma and necrosis are evident in liver as the organ of detoxification (Caballero *et al.*, 2004, Francis *et al.*, 2001).

The histological analysis of the intestine of *C. carpio* fingerlings fed raw mucuna diet also showed severe atrophy in the musculature of the intestinal villi of the fish, which was mild in the fish fed maize cob ash processed mucuna diets. Hendrix *et al.*, (1990) and Van der Ingh *et al.* (1991) reported some binding of soybean agglutinin and atrophy of the proximal and distal intestine in Atlantic salmon (*Salmo salar*) when fed soybean diets respectively. In this study, the observed lacerations could be attributed to the presence of some anti-nutrients (trypsin inhibitor and tannin) which was at high levels in the raw Mucuna seed diet (Kakwi and Audu (2016). In a similar study, Bozidar *et al.* (2011) reported that *C. carpio* reacted to the replacement of fish meal for soya bean meal which resulted to shortening of intestinal villi, loss of supra nuclear vacuolation of the enterocytes, widening of and infiltration of inflammatory cells in the lamila propria. Other antinutritional factors such as lectin have been implicated in histopathological changes in the digestive tract as reported by Osuigwe *et al.* (2006); Ogunji and Wirth, (2001). Tannin has also been reported to bind with protein of mucosa membranes and thus affecting protein digestibility (Onwuka, 1997; Akinmutimi and Okwu, 2006).

The non significance in the histopathological conditions of the fish fed

the processed MCD1 diet could be attributed low level of residual antinutritional factors that do not have observable effects on the organs assessed as reported by Francis *et al.* (2001). He also reported that the toxicity of some antinutritional factors in some legumes (such as lectins) depends on the animal species. Grant (1991) and Bond and Smith, (1989) also noted that some legumes produce seed extracts with anti-nutritional factors without any observable symptoms of toxicity but the seed could still be chronically toxic (Francis *et al.*, 2001). Liver and intestine are the most important organs for the digestion and absorption of nutrients from feed. Consequently, the observed histopathological changes can be related to growth of fish as assimilation of nutrients are affected as observed by Richardson *et al.* (1985) and Nogales *et al.* (2010). Thus the observed poor growth of fish fed raw Mucuna diets. Therefore, the need for monitoring histological structure of fish liver and intestine can contribute to better explanation of mechanism involved in weight gain, digestibility and food availability (Bozidar *et al.*, 2011).

Conclusion

In conclusion, the substitution of soybean meal with up to 20% maize cob ash processed mucuna seed meal in fish diet have no deleterious or severe effect on the liver and intestine of *Cyprinus carpio* fingerlings. This study thus provides information on histopathological effects of feed ingredients and some useful data base to feed millers and aquaculturists for the development of non-conventional feedstuff.

References

- Aguiyi, J. C., Ekwere, E. O., Umar, M. B. T., Shugaba, A. I., Ogbonna, A., Akweshi, E. A., Marinello, E. and Guerranti, R. 2003.

- Preliminary immunization of sheep using *Mucuna pruriens* seed protein against snake venom poisoning. *Nigerian Journal of Botany*, 16:49-55.
- Akinmutimi, A. H. and Okwu, N. D. 2006.** Effect of quantitative substitution of cooked *Mucuna utilis* seed meal for soybean meal in broiler finisher diet. *International Journal of Poultry Science* 5(5): 447-481.
- APHA, AWWA, WCPF (American Public Health Association, American Water Works Association and Water Control Pollution Federation) 1985.** Standard Methods for Examination of Water, 15th edn. Washington D.C. USA: APHA, 1976p.
- Bond, D. A. and Smith, D. B. 1989.** Possibilities for the reduction of antinutritional factors (ANFs) in grain legumes by breeding. In: Juisnam, T.F., Poel, B and I.E.Liener (Eds), *Recent Advances of Research in Antinutritional Factors in Legume Seeds: Animal Nutrition and Feed Technology, Analytical Methods*. Wageningen, (Netherlands), Pudoc, pp 285-296.
- Bostock, J. C. 2009.** Use of information technology in aqua-culture. In: Burnell, G and G. Allan (Eds), *New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management*. Oxford, (UK), Woodhead Publishing, 35:1064-1118.
- Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Handisyde, N., Telfer, T., Lorenzen, K., Little, D., Ross, L., Gatward, I. and Corner, R. 2010.** Aquaculture: Global Status and Trends. *Philosophical Transactions of the Royal Society*. 365, 2897- 2912.
- Bowen, S.H. 1987.** Dietary protein requirements of fishes: A reassessment. *Canadian Journal of Fisheries and Aquacultural Science*. 44, 1995-2001.
- Bozidar, S.R., Marko, B.S., Zoran Z.M and Vesna, D.P 2011.** Histological methods in the assessment of different feed effects on liver and intestine of fish. *Journal of Agricultural Science*, 56(1): 87-100.
- Boyd, C. E. 1982.** Water Quality Management for Pond Fish Culture. Department of Aquaculture and Fisheries Science, V.9 Elsevier
- Bressani, R., Lau, M. and Silvia-Vargas, M. 2003.** Protein cooking quality and residual content of dehydroxyphenylalanine and trypsin inhibitors of processed *Mucuna* beans (*Mucuna* spp). In: M.Eilita, J.Mureithi, R.Muinga, C.Sandoval, and N.Szabo (Eds), *Increasing Mucuna's Potential as a Food and Feed Crop. Proceedings of an International Workshop*. 23-26 September, 2002. Mombasa, Kenya. *Tropical and Sub-tropical Agro-Ecosystems*, 1(2-3):197-212
- Caballero, M.J. Izquierdo, M.S., Kprsulk, E., Fernandez, A.J. and Rosentund, G. 2004.** Histological alteration in the liver of sea bream *Spans acerara* by short or long-term feeding with vegetable oil. *Journal of Fish Disease* 27: 531-541.
- Culling, C.F.A. 1974.** Handbook of Histopathological and Histochemical Techniques. 3rd Edition, 726p.

- Davies, O. A. and E. Ansa. 2007.** Comparative Assessment of Water Quality Parameters of Freshwater Tidal Earthen Ponds and Stagnant Concrete Tanks for Fish Production in Port Harcourt, Nigeria. *Int. J. Sci. Nat.*, 1(1): 34
- Duncan D.B. 1955.** Multiple Range and Multiple F - Tests. *Biometrics*, 11:1-42.
- FAO (Food and Agriculture Organisation). 2014.** *Feed formulation*. Food and Agriculture Organization. Regional Aquaculture Annual Review. Accessed from <http://www.fao.org/fishery/nile-tilapia/feed-formulation/en>, January, 2014.
- Francis, G., Makkar, H.P.S. and Becker, K. 2001.** Antinutritional factors present in plant derived alternate feed ingredient and their effects in fish. *Aquaculture*. 199:197-227.
- Francis, G., Makkar, H.P.S. and Becker, K. 2002.** Products from little researched plants as aquaculture feed ingredients. <http://www.google.makkar.h.p.s>.html, 11th August, 2005, 13pp.
- Grabner, M. and Hofer, R. 1985.** The digestibility of the proteins of broad bean and soybean (*Glycine max*) under in-vitro conditions stimulating the alimentary tracts of rainbow trout (*Salmo gairdneri*) and Carp (*Cyprinus carpio*). *Aquaculture*, 48:111-122
- Grant, G. 1991.** Lectins. In: D'Mello, F.J.P. Duffus, C.M. Duffus, J.H. (Eds.), *Toxic substances in Crop plants*. The Royal society of chemistry, Thomas Graham House, Science Park, Cambridge CB4 4WF, Cambridge, pp. 49-67.
- Grant, G., More, L. J., McKenzie, N. H. and Pusztai, A. 1982.** The Effect of heating on the hemagglutinating activity and nutritional properties of bean (*Phaseolus vulgaris*) seeds. *Journal of the Science of Food and Agriculture*, 33: 1324-13.
- Hendriks, H.G.C.J.M., Van der Ingh, T.S.G.A.M., Kroghdahl, A., Olli, J. and Koninkx, J.F.J.G. 1990.** Binding of soybean agglutinin to small intestinal brush border membranes and brush border membrane enzyme activities in Atlantic salmon (*Salmo salar*). *Aquaculture*, 91: 163-170.
- Higgs, D.A., McBride, J.R., Markert, J. R., Dosanjh, B.S., Plotnikoff, D.M. and Clarke, C. W. 1982.** Evaluation of Tower and Candle rapeseed (*canola*) meal and Bronowski rapeseed protein concentrate as protein supplements in practical dry diets for juvenile Chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture*, 29: 1-31.
- Kakwi, D.G., and Audu, B.S. 2016.** Effect of Partial Replacement of Soyabean meal with *Mucuna pruriens* Meal in the diet of Common Carp (*Cyprinus carpio* Linnaeus, 1758) Fingerlings. *Nigerian Journal of Fisheries and Aquaculture* 4(2):48-60.
- Kay, D.E. 1979.** *Food legumes. Crop and Product Digest No 3*. Tropical Products Institute, London, UK, 435pp.
- Nogales-Merida, S., Tomas-Vidal, A., Matinez-Lorens, S. and Jover-Cerda, M. 2010.** Sunflower meal as a partial substitute in juvenile sharpnout sea bream (*Diplodus puntazzo*) diets: amino acid retention, gut

- and liver histology. *Aquaculture* 298:275-281.
- Ogunji, J.O. and Wirth, M. 2001.** Alternative protein sources as substitute for fish meal in the diet of young Tilapia, *Oreochromis niloticus* (L.). *Israeli Journal of Aquaculture- Bamidgeh* 53(1): 34-43.
- Onwuka, G. 1997.** Toxicology Studies of hemagglutinin in Soybean and *Mucuna cochinchinensis* and growth response of *Clarias gariepinus*. PhD Thesis, University of Nigeria, Nsukka, Nigeria, 216pp.
- Osuigwe, D.I., Obiekezie, A.I. and Onuoha, G.C. 2006.** Effects of jackbean seed meal on the intestinal mucosa of juvenile *Heterobranchius longifilis*. *African Journal of Biotechnology*, 5(13): 1294-1298.
- Rahman, M. S. 1992.** Water quality management in aquaculture. BRAC Prokashana, Bangladesh, 84 p
- Richardson, N.L., Higgs, D.A., Beames, R.M. and McBride, J.R. 1985.** Influence of dietary calcium, phosphorous, zinc and sodium phytate level on cataract incidence, growth and histopathology in juvenile Chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Nutrition*, 115: 553-567.
- Siddhuraju, P., Makkar, H.P.S. and Becker, K. 2002.** The effect of ionizing radiation on antinutritional factors and nutritional value of plant materials with reference to human and animal. Accessed on 5 – 9 – 2005 from <http://www.google.Siddhurajuhtml.pp9>.
- Ufodike, E.B.C. and Matty, A.J. 1983.** Growth response and nutrient digestibility in mirror carp (*Cyprinus carpio*) fed different levels of cassava and rice. *Aquaculture*, 31: 41-50
- Ukachukwu, S.N. 2000.** Chemical and nutritional evaluation of *Mucuna cochinchinensis* (Lyons bean) as an alternative protein ingredient in broiler diets. PhD Thesis, University of Nigeria, Nsukka, Nigeria. 96pp.
- Van der Ingh, T.S.G.M., Krogdahl, A., Olli, J.J., Hendriks, H.G.C.J.M. and Koninkx, J.F.J.G. 1991.** Effects of soybean- containing diets on the proximal and distal intestine in Atlantic salmon (*Salmo salar*): a morphological study. *Aquaculture*, 94: 297-305.
- Wanjekeche, E., Wakasa, V. and Mureithi, J.G. 2003.** Effect of alkali, acid and germination on nutritional composition and antinutritional factors of mucuna (*Mucuna pruriens*). In: *Increasing Mucuna's Potential as a Food and Feed Crop*. J. Mureithi, R. Muinga, C. Sandoval and N. Szabo (Eds). *Proceedings of an International Workshop, 23rd -26th September, 2002, Mombasa, Kenya. Tropical and Subtropical Agroecosystems*, 1(2-3): 183-192.
- Zakes, Z., Kowalska, A., Demska-Zakes, K., Jeney, G. and Jeney, Z. 2008.** Effect of two medicinal herbs (*Astragalus radix* and *Lonicera japonica*) on the growth performance and body composition of juvenile pike perch (*Sander lucioperca*). *Aquaculture Research* 39, 1149-1160.

Received: 12th November, 2020

Accepted: 5th February, 2021