

HISTOLOGY OF LIVER OF *Cyprinus carpio* FINGERLINGS FED RAW AND PROCESSED *Mucuna pruriens* DIETS

¹Kakwi, D.G., ²Audu, B.S., ³Awolumate, S, ⁴Ositimehin K.M, ⁵Haliru H. and ¹Oshibanjo D. O.

¹Animal Production Department, University of Jos, Jos State, Nigeria

²Department of Zoology, University of Jos, Jos State, Nigeria

³Department of Animal Science and Fisheries, Faculty of Agricultural Sciences, National Open University of Nigeria, Abuja, Nigeria

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

⁵National Veterinary Research Institute, Vom Jos Plateau State Nigeria

olusegunoshibanjo@gmail.com

08055438564

ABSTRACT

The effect of raw and processed *Mucuna pruriens* seed diets on histopathology of the liver 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 soya bean 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, 15.40 g) for 56 days. Histopathological changes in the liver sections of fish were observed consistently and variable in the treatment groups. 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. The MCD1 was considered the most appropriate for dietary inclusion of *Mucuna pruriens* seeds in the feeding of *C. carpio* fingerlings.

Key words: Raw *Mucuna pruriens* Seed, Liver, Histopathology, *Cyprinus carpio* and fingerlings

INTRODUCTION

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 most important antinutrient in *mucuna* is 3, 4 dihydroxy-L-phenylalanine (L-Dopa; Szabo, 2003). 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). Therefore, in this experiment 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

Treatment of *M. pruriens* Seeds with Maize Cob Ash (MCA) 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 750 ml was collected in a container. One hundred milliliters (100 ml) of the filtrate was diluted to 500 ml with distilled water to make a solution of 1:4 dilution. The samples (100 gm) of *mucuna* seeds were boiled in 1.5 litres of the maize cob ash solution in enamel pot for a duration of 60 minutes then 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 3 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 soya bean meal, yellow maize flour, cassava flour (binder), vegetable oil and vitamin premix as shown in Table 1.

Table 1: Composition of Treatment Diets Fed to *Ccprinus 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 (16,000 mg), Vitamin K3 (800 mg), Vitamin B1 (1,200 mg), Vitamin B2 (22,000 mg), Niacin (22,000 mg), Calcium pantothenate (4,600 mg), Vitamin B6 (2,000 mg), Vitamin B12 (10 mg), Folic acid (400 mg), Biotin (32 mg), Choline chloride (200,000 mg), Manganese (48,000 mg), Iron (40,000 mg), Zinc (32,000 mg), Copper (3,400 mg), Iodine (600 mg), Cobalt (120 mg), Selenium (48 mg)
MCD1 and MCD2: Processed *M. pruriens* was used for soybean protein at inclusion levels of 20% and 50% respectively.

Experimental Fish

500 fingerlings of Common carp (*C. carpio*) of weight sizes between 15.10-15.20g were selected from a stock (mixed sex full sibling) and kept in oxygenated concrete tank (2 m x 5 m x 1.5 m) while being fed two times a day, on commercial pelleted diet (Vital Fish Feed, 25% Crude protein) for 10 days. 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 (8) weeks (56 days).

Histopathological Examination of Fish Organs

The liver 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-eosin staining techniques described by Culling (1974).

RESULTS

The histopathological conditions of *C. carpio* fingerlings fed Raw and processed *M. pruriens* diets are presented as photomicrographs (Plates 1 to 4). 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 & 2). Liver sections of fish fed 20% MCD1 processed Mucuna diets has no changes in the perivascular and periductula spaces in the portal areas

(P). 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). 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 4).

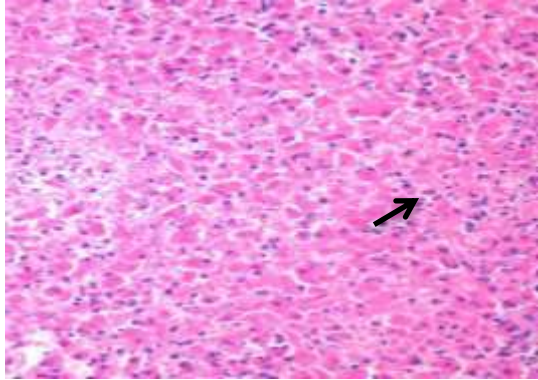


Plate 1: Liver Section of *C. carpio* Fed control

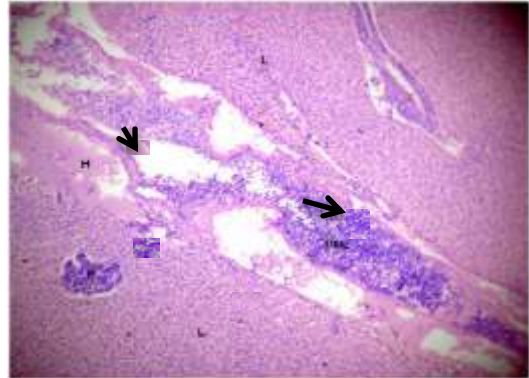


Plate 3: Liver section of *C. carpio* Fed 50% MCD2 Processed *M. pruriens* Diet

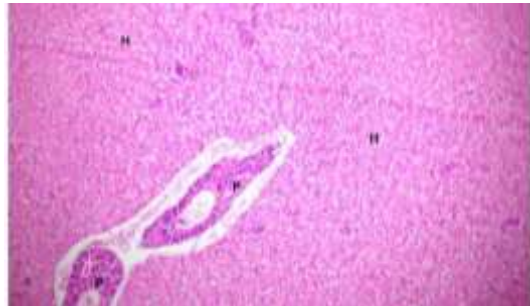


Plate 2: Liver Section of *C. carpio* Fed 20% MCD1 Processed *M. pruriens* Diet

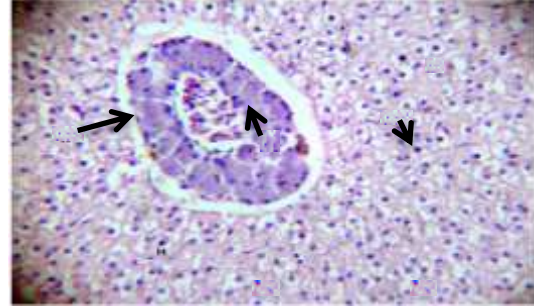


Plate 4: Liver Section of *C. carpio* Fed Raw *M. pruriens* Diet

DISCUSSION

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 (*Litopenacusrannaei*) 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 histopathological conditions of the fish fed the processed MCD1 diet could be attributed to low level of residual anti-nutritional 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 & 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). 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 (Bozidaret *et al.*, 2011).

CONCLUSION

In conclusion, the substitution of soya bean meal with upto 20% maize cob ash processed Mucuna seed meal in fish diet have no deleterious or severe effect on the liver and intestine of *C. 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

- 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.
- 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.
- 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.
- Duncan D.B. (1955). Multiple Range and Multiple F-Tests. *Biometrics*, 11:1-42.
- 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 digestability 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.
- 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.
- Nogales-Merida, S., Tomas-Vidal, A., Martinez-Lorens, S. and Jover-Cerda, M. (2010). Sunflower meal as a partial substitute in juvenile sharpnose sea bream (*Diplodus puntazzo*) diets: amino acid retention, gut and liver histology. *Aquaculture* 298:275-281.
- 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>.

- Ukachukwu, S.N. (2000). Chemical and nutritional evaluation of *Mucunacochinchinensis* (Lyons bean) as an alternative protein ingredient in broiler diets. PhD Thesis, University of Nigeria, Nsukka, Nigeria. 96pp.
- 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.