



Histological Changes in the Liver of Nile Tilapia (*Oreochromis niloticus*) Fed Watermelon (*Citrullus lanatus*) Seedmeal Diets

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ABSTRACT: The nutritive potential of watermelon (*Citrullus lanatus*) seedmeal as dietary protein source in the diet of Nile tilapia (*Oreochromis niloticus*) were evaluated in a 56 day feeding trial using histological changes in the liver as an index of assessment. 150 *Oreochromis niloticus* fingerlings (6.12±0.05g) were acclimatized for a week, weighed and allotted into five dietary treatments; containing 0, 15, 30, 45 and 60% *Citrullus lanatus* seedmeal replacement levels for soybean meal respectively. The diets were isonitrogenous and isolipidic. Each treatment was replicated three times with ten fish per replicate. Fish were fed 5% body weight on two equal proportions per day. The result from the study indicated that there was marked vacuolation of hepatocytes of the liver of the fish exposed to different dietary treatments after the experiment which is not dietary treatment related.

Keywords: Watermelon, Histopathology, Nile tilapia, Liver

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INTRODUCTION

Soybean meal (SBM) is currently the most commonly used plant protein source in fish feed (El-Sayed, 1999). It is known for its high protein content, high digestibility and relatively well balanced amino acid profile (Storebakken *et al.*; 2000). However, wider utilization and availability of this conventional source for fish feed is restricted by increasing demand for human consumption and by other animal feed industries (Siddhuraju and Becker, 2001). It is therefore required to focus on using less expensive and readily available plant protein sources to replace soybean meal without reducing the nutritional quality of the feed (Barros *et al.* 2002). Recent research works focus on the use of alternative

protein source feed ingredients; prominent among which are watermelon as protein source feed ingredients for *Clarias gariepinus* (Jimoh *et al.*; 2013 a and b); sunflower and sesame as protein source feed ingredients for *Clarias gariepinus* (Fagbenro *et al.*; 2010 a, b and c.); jackbean as protein source feed ingredients for *Oreochromis niloticus* (Fagbenro *et al.* 2007; Jimoh *et al.* 2010). Watermelon belongs to the family Curcubitaceae. It is a tropical, semi tropical and arid region crop of the world (Razavi and Milani, 2006). Its seeds have nutritional density comparable to other oilseed proteins including soybean and other conventional legumes (Mustapha and Alamin, 2012). Wani *et al.* (2011)

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reported that watermelon seedmeal contains adequate amount of nutritional protein that could be used as nutritional ingredients in food products.

Liver, being the centre of metabolic activities, its cells' architecture can serve as a reliable indicator of toxicity of feed ingredients which has potential in negatively affecting the growth of fish. Gargiulo *et al* (1998) reported that histology of fish species is important in the

understanding of the pathological changes related to nutritional sources. More so that there is paucity of information on the use of *Citrullus lanatus* seeds as dietary protein source of fish feed especially the effect it has on the histology of the liver. This study thus seek to investigate the histological changes in the liver of *Oreochromis niloticus* fed *Citrullus lanatus* seedmeal.

MATERIALS AND METHOD

Sources and Processing of Ingredients.

Sample of dried water melon seeds (1kg) were obtained in Bodija market, Ibadan, Oyo state. The water melon seeds were rinsed with water and boiled for 15 minutes after which they were sundried (29-31°C) for three days and then ground in a hammer mill and the oil therein was removed using the pressure generated from locally made screw press (cassava-presser type). The cakes therefore were analysed for their proximate composition (AOAC 1990). Fish meal, soybean meal and other feedstuffs obtained from commercial sources in Nigeria were separately milled and screened to fine particles size. Samples were taken in triplicates and analyzed for their proximate composition (AOAC, 1990).

Experimental Diets

The experimental diets were formulated (Table 2) using the nutrient composition of the protein feedstuffs (Table 1). The experimental diets

contained soybean meal which was replaced by cooked water melon seedmeal at the rate of 0, 15, 30, 45, and 60%. The diets were isolipidic and isonitrogenous containing 35% crude protein and 10% lipid with fish meal (72%), soybean meal (45%), fish oil, vitamin premix and starch serving as ingredients. Starch serves the purpose of binder and filler. The feedstuffs were ground and water was added to aid binding after which it was introduced into a pelleting and mixing machine to obtain a homogenous mass and then passed through a mincer to produce 2mm size pellet which was immediately sundried at 30 - 32°C. After drying for three days, the diet was kept in a cool place.

Experimental Fish and System

The experiment was conducted at the hatchery unit of the Federal College of Animal Health and Production Technology, Moor Plantation Ibadan. 150 *O.niloticus* fingerlings (6.12±0.05g) were obtained from Masopa fish farm, Ibadan, Oyo

Table 1: Proximate Composition (%) of the Protein Feed Ingredients

Parameter	Fish meal	Soybean Meal	**CLM
Moisture	9.75	10.70	9.69
Crude Protein	72.4	45.74	19.11
Crude Lipid	10.45	9.68	15.35
Crude Fibre	-	5.10	4.97
Ash	8.32	4.48	5.39
*NFE	-	30.00	45.49

*Nitrogen Free Extract

** *Citrullus lanatus* Meal

Table 2: Gross composition (g/100g) of experimental diets containing *Citrullus lanatus* seedmeal fed to *Oreochromis niloticus*

Ingredients	CTR	DT2	DT3	DT4	DT5
Fishmeal	19.44	19.44	19.44	19.44	19.44
Soybean Meal	33.333	28.33	23.33	18.33	13.33
Watermelon	-	11.77	23.55	35.22	47.09
Corn	10.00	10.00	10.00	10.00	10.00
Fish Premix	2.50	2.50	2.50	2.50	2.50
Fish Oil	2.50	2.50	2.50	2.50	2.50
Starch	32.33	25.46	18.68	11.91	5.13
Total	100.00	100.00	100.00	100.00	100.00

* Specification: each kg contains: Vitamin A, 4,000,000IU; Vitamin B, 800,000IU; Vitamin E, 16,000mg; Vitamin K, 800mg; Vitamin B₁, 600mg; Vitamin B₂, 2,000mg; Vitamin B₆, 1,600mg; Vitamin B₁₂, 8mg; Niacin, 16,000mg; Caplan, 4,000mg; Folic Acid, 400mg; Biotin, 40mg; Antioxidant 40,000mg; Chlorine chloride, 120,000mg; Manganese, 32,000mg; Iron 16,000mg; Zinc, 24,000mg; Copper 32,000mg; Iodine 320mg; Cobalt, 120mg; Selenium, 800mg manufactured by DSM Nutritional products Europe Limited, Basle, Switzerland.

state and transported live to the project site in an aerated bag. The tilapia fingerlings were acclimated for 7 days prior to the feeding trial while being fed on a commercial pelleted diet. 10 fingerlings were allotted into each of the fifteen 25 litre rectangular tanks containing 20 litres of water. Experimental diets were assigned randomly to the tanks with three replicates per treatment. Fish in each tank were fed 5% body weight per day in two equal proportions between 9.00–10.00am and 5.00–6.00 pm for 56 days.

Histological Examination of Test Organ

At the end of the experiment, three fish per treatment were sampled for histological analysis. The test organisms were killed with a blow on the head, using a mallet and were dissected to remove the liver. The organs were fixed in 10% formalin for three days after which the tissue was dehydrated in graded levels of 50%, 70%, 90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during

embedding. The tissues were then embedded in malted wax and sectioned into thin sections (5–7µm), by means of a rotatory microtome and each section was cleared by placing it in warm water (38°C), where it was picked with clean slide and oven-dried at 58°C for 30 minutes to melt the wax. The slide containing sectioned materials/tissue was cleared using xylene and graded levels of 50%, 70%, 90%, 95% and 100% alcohol for two minutes each and stained with Harris haematoxyllin-eosin (H&E) stain, Bancroft and Cook (1994). Other slides containing sectioned tissues were also stained with periodic acid Schiff's reagent (PAS) following the method of Hughes & Perry (1976). The stained slide was observed under a light microscope at varying X400 magnification, sections was examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus C35 AD4), a camera (Olympus C40 AB-4) and an automatic light exposure unit (Olympus PM CS5P).

RESULTS

Proximate Composition of Experimental Diets fed to *Oreochromis niloticus*

The proximate composition of experimental diets fed to *Oreochromis niloticus* is shown in table

3. It shows that there was no significant difference ($P > 0.05$) in moisture, protein, lipid, fibre, ash and Nitrogen Free Extract (NFE) showing that the various diets prepared were

Table 3: Proximate composition (g/100g dry matter) of experimental diets containing *Citrullus lanatus* seedmeal fed to *Oreochromis niloticus*

Parameters	CTR	DT2	DT3	DT4	DT5
Moisture	9.66±0.51	9.59±0.59	9.56±0.50	9.88±0.33	9.52±0.52
Crude Protein	35.22±0.05	35.14±0.16	35.23±0.33	35.222±0.06	35.17±0.23
Crude Lipid	10.16±0.09	10.15±0.06	10.08±0.03	10.04±0.27	10.19±0.13
Crude Fibre	4.37±0.36	4.17±0.08	4.12±0.03	4.15±0.05	4.13±0.05
Ash	5.15±0.20	4.90±0.28	4.66±0.50	5.12±0.37	5.09±0.16
NFE	35.43±0.53	36.0±0.51	36.34±0.86	35.57±0.57	33.90±0.61
Total	100	100	100	100	100

Figures in each row without superscript are not significantly different ($P>0.05$) from each other

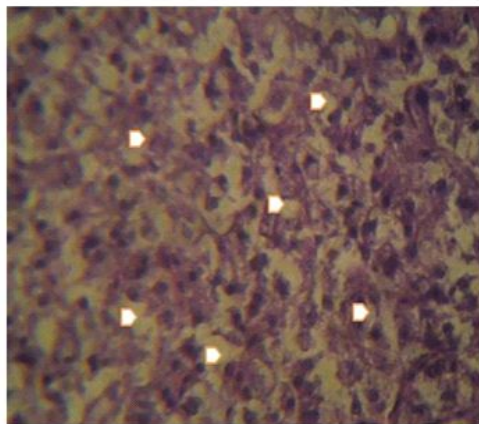


Plate 1: Photomicrograph showing liver of *O.niloticus* fed control Diets (CTR).
 Comment: Mild diffuse hepatic vacuolation (glycogen)
 Magnification: x400

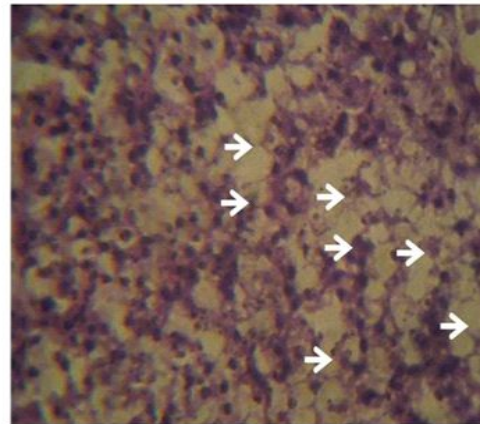


Plate 2: Photomicrograph showing liver of *O.niloticus* fed control Diets (CTR) Replicate.
 Comment: Severe diffuse hepatic vacuolation (glycogen)
 Magnification: x400

isonitrogenous, isocaloric and isolipidic. All the fish responded well to the dietary treatments given to them.

Plate 1 showed the photomicrograph of liver of *O. niloticus* fed diet CTR. There was mild diffuse hepatic vacuolation with its replicate having severe hepatic vacuolation (plate 2). In plate 3, there was no visible lesion seen in the photomicrograph of liver of *O. niloticus* fed diets DT2. The photomicrographs of liver of *O. niloticus* fed diet DT3 in plates 4 and 5 showed that there was diffuse hepatic vacuolation with melanomacrophage centres very prominent. Similar observation was recorded in plates 6, 7 and 9 for fish fed diet DT4 and DT5; although no visible lesion was observed in the liver of *O. niloticus* fed test diet DT5 (Plate 8). The histological changes were not dietary treatments related.

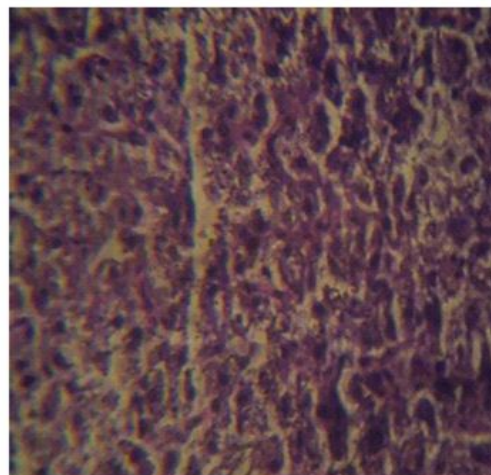


Plate 3: Photomicrograph showing liver of *O.niloticus* fed test Diets (DT2)
 Comment: No visible lesion seen
 Magnification: x400

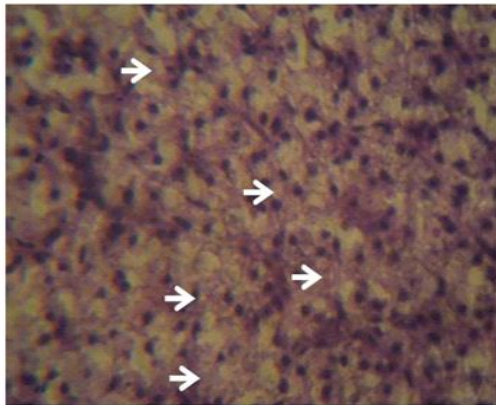


Plate 4: Photomicrograph showing liver of *O.niloticus* fed control Diets (DT3)
Comment: Severe diffuse hepatic vacuolation (glycogen)
Magnification: x400

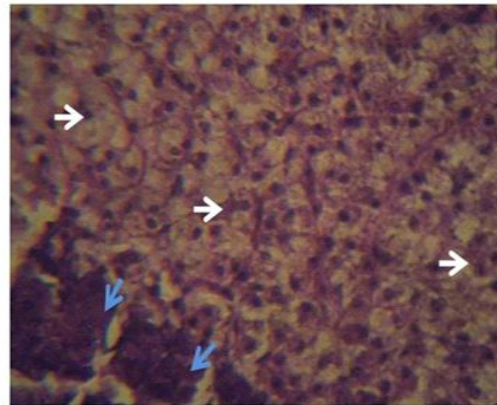


Plate 5: Photomicrograph showing liver of *O.niloticus* fed control Diets (DT3). Replicate
Comment: Severe diffuse hepatic vacuolation (glycogen-white arrows). The melanomicrophage centres are very prominent-blue arrows
Magnification: x400

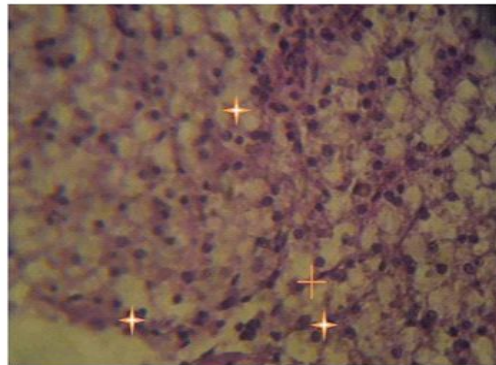


Plate 6: Photomicrograph showing liver of *O.niloticus* fed control Diets (DT4)
Comment: Severe diffuse hepatic vacuolation (glycogen) (stars)
Magnification: x400

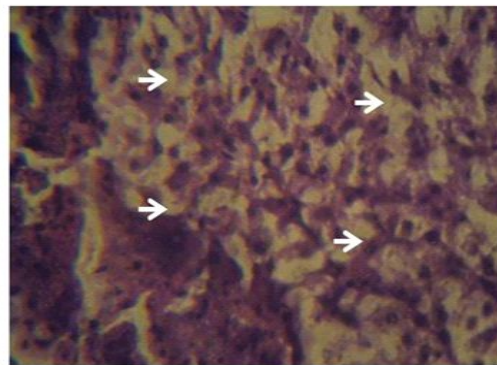


Plate 7: Photomicrograph showing liver of *O.niloticus* fed control Diets (DT4) -Replicate
Comment: Severe diffuse hepatic vacuolation (glycogen) (arrows)
Magnification: x400

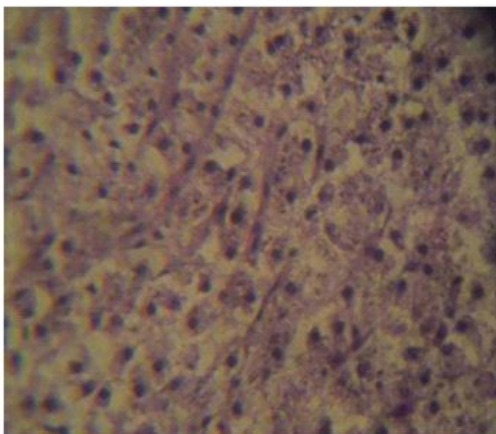


Plate 8: Photomicrograph showing liver of *O.niloticus* fed test Diets (DT5)
Comment: No visible lesion seen
Magnification: x400

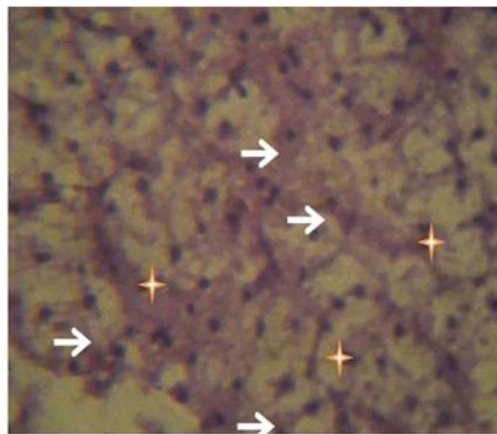


Plate 9: Photomicrograph showing liver of *O.niloticus* fed test Diets (DT5). Replicate
Comment: Severe diffuse hepatic vacuolation (glycogen) (stars); Hepatocytes appear necrotic (arrows)
Magnification: x400

DISCUSSION

The protein and lipid requirement of *Oreochromis niloticus* was met by the 35 and 10% provided in the experimental diets (Jauncey and Ross, 1982; Luquet 1991). Substitution of soybean with watermelon seedmeal in the diet of Nile tilapia in this study affected the histology of the liver by causing mild to severe vacuolation in the liver of all the fish exposed to different dietary treatments. This observation is in consonance with the observation of Meridah *et al.* (2010); Pereira *et al.* (2002) and Hansen *et al.* (2006). A marked vacuolation of hepatocytes among the different dietary recorded in this study was also reported by Olukunle (2011) and Jimoh (2012). Martin *et al.* (2007) and Valente *et al.* (2011). Gatta *et al.* (2011) explained that high vacuolation of the hepatocytes is physiological response to dietary lipid intake. Liver plays a central role in metabolism (Bernet *et al.*; 1999) helping in the regulation of metabolic activities of various nutrient substances in the body. Changes in liver

histological structure as recorded in this study may have plausibly been induced by the presence anti-nutrients in *Citrus lanatus*. This agrees with Merchand *et al.* (2008) who reported that large numbers of toxicants could cause histological alteration in the liver. Liver being the main organ of detoxification is a target organ of different xenobiotic substances (Cabot, 2000). Nero *et al.* (2005) reported that liver tissue damage may occur when it is constantly exposed to toxicants. The various degrees of vacuolation recorded in this study seem not to be dietary treatment related. Valente *et al.* (2011) made similar observation when Senegalese sole was fed diets containing mixtures of different plant proteins. The presence of numerous vacuolation in the liver as recorded in this study seems to be normal features of fish exposed to high dietary lipid intake. In conclusion, replacement of soybean meal by watermelon seedmeal can induce vacuolation and necrosis in hepatocytes.

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