Effect of Fish meal and Plant protein alternatives on the histological picture of different organs on Nile tilapia in Egypt.

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A B S T R A C T

The present study was designed to investigate the effect of utilization of Fish meal and plant protein alternatives of commercial protein supplements in diets of Nile tilapia in the terms of histological changes in liver, gills and Musculature tissue. A total of 42 apparently healthy Nile tilapia (*O.niloticus*) with an average weight of 21.24+1.03g, randomly divided into three comparable groups (14 fish each) based on type of diet protein supplement to be either fish meal (Gr. A), Soybean meal (Gr. B) or cotton seed meal (Gr. C). 52 days post feeding histological examination of liver showed hyperemic central vein and sinusoid with focal pigmentation in (Gr. A), Intra cytoplasmic round circumscribed fat vacuoles in (Gr. B) and Hyperemic central vein and sinusoids with focal extravasations of red cells surrounding the central veins in (Gr. C). Musculature tissue showed Degenerated muscle bundles in (Gr. A), intact muscle bundles in (Gr. B) and Hyper-cellularity in focal manner in Gr. C. while the gills showed Focal extravasation of red cells in the arch in (Gr. A), vascular lining epithelium with hyperplasia in the gill rakers in Gr. B and Hyperplasia and vacoulation the lining cells of the gill rakers in (Gr. C). In conclusion feeding Nile tilapia on soybean meal may be the better choice as protein supplement alternative due to its positive effect on the musculature tissue, no clinical signs beside it is much safer.

Keywords: Histology, liver, cottonseed meal, fishmeal, soybean meal, alternatives, Nile tilapia.

(http://www.bvmj.bu.edu.eg) **(BVMJ-28(2): 273-282, 2015)**

1. INTRODUCTION

Aquaculture is considered as important sources of production for meeting the worlds increasing demand for protein. Aquaculture is currently the fastest-growing animal production sector in the world, expanding at an average annual rate of about 8-11% since 1984 (FAO, 2006). There is a wide range of feed stuff to be used in the formulation of fish diets. Most important of these feed stuffs are energy and protein supplements. One of the major problems in fish industries in Egypt as well as in most development countries is the availability, cost, adulteration and bad treatments during manufacture of protein supplements. On the other hand due to adulteration, the nutritional name of protein supplement not express greatly on its chemical and physical specific features recommended by references as NRC (1993), since reflected in the terms of slow or low body growth, bad health that ended finally in the reduction the economical efficiency. The protein is the most expensive component in the diets of aquatic species. There are different commercial protein supplement in Egypt (native and exported). Fish meal, soybean meal and cotton seed meal are widely distributed. Fish meal has been used extensively as a chief of amino acids in fish diets. FAO, (2006).The benefits of good quality fish meal incorporated into fish diet: most commercial fish meal is made from small bony and oily fish that otherwise are not suitable for human consumption and

some is manufactured from by-products of sea food processing industries. Ninety percent of the non-food use portion was reduced to fishmeal and fish oil and 10% used as direct feed in fur bearing animals and aquaculture (FAO 2004). Alternatives to fishmeal in aquaculture feeds Tacon (2004) suggests the reduction in fishmeal and fish oil usage by the aquaculture sector will be due to economic/market pressures and the subsequent development of lower cost, more sustainable alternative feed components. Soybean meal is considered to be one of the most suitable candidates as a fish meal replacement (Dersjant-Li 2002). It has a favorable amino acid profile compared to other plant protein sources, is consistently available, and is cost effective (Watanabe 2002). Soybean meal, because of its high nutritional value, availability, palatability and low cost, is the most commonly used plant feed stuff in aquaculture diets, soybean meal is currently comprised up to 50% of the diet of tilapia. Replacement of soybean meal with less expensive plant protein would be beneficial in reducing feed costs. Replacement of fish meal and soybean meal with less expensive plant proteins would be beneficial in reducing feed cost. Soybean based products represent a major source of protein in diets for monogastric animals, and many have shown great potential as Fish meal replacements in diets for several fish species (Refstieet al., 1997; Refstieet al., 2000; Storebakken et al., 2000; Fagbenro and Davies, 2001; Schuchardt et al., 2008; Ali et al., 2008). Soybean is low in methionine can be used as a FM replacer. These sources are low in lysine (Gatlin et al., 2007). Cottonseed, *Gossypium hirsute* Linnaeus, is the third leading plant protein by weight (after soybean and rapeseed) used worldwide (Gatlin et al. 2007) and is available at relatively lower cost than animal proteins (Lovell 1989). According to National Cottonseed Production Association (NCPA) 450 Kgs of cottonseed meal can be extracted from a ton of cottonseed crushed. Cottonseed meal can be obtained in two methods from cottonseed; one is through mechanical and the other by solvent extraction. However, the meal extracted from the both crude protein must contain not less than 36 percent and the Proximate Composition of Cottonseed meal (NRC, 1993). Owing to its high protein value for human consumption (Alford et al. 1996) and animals, as well as low market price in comparison with other legumes and fishmeal, cottonseed meal (CSM) consequently has an immense potential for incorporation in high-protein aqua feeds (Gatlin et al. 2007). Nutritionally, cottonseed meal contains high levels of proteins (Forster and Cahloun 1995) and is very palatable to fish (Robinson and Li 1995).The amount of CSM that can be included in feeds depends on the fish species, developmental stages, dietary protein, available lysine (Martin 1990) and levels of anti-nutritional factors. The level of cottonseed meal inclusion in fish varies widely among fish species such as tilapia, *Sarotherodon mossambicus* (Jackson et al. 1982), *Oreochromis niloticus* (Ofojekwu and Ejike 1984; El-Sayed 1990; Rinchard et al. 2000; Mbahinzireki et al. 2001). Cotton seed meal, because of its availability, nutritive value, high protein content, palatability and low cost is a good alternative protein source to fish meal in fish diet. The amount of cotton seed meal that can be used in fish diets depend primarily on the levels of free gossypol and available lysine. The present study was designed to investigate the effect of utilization of Fish meal and the plant protein alternatives of different commercial diets on Nile tilapia in the terms of histological changes in liver, gills and muscles.

2. MATERIAL AND METHODS

2.1. The experimental fish;

The present study was conducted on a total number of 42 apparently healthy Nile tilapia (*O. niloticus*) with an average weight of 21.24+1.03g.They were brought from the central laboratory for Aquaculture Research

Hatchery, Abbassa, Abu Hammad, Sharkia, Egypt. Fish were transported by means of polyethylene bags containing oxygen to the Faculty of Veterinary Medicine, Moshtohor, department of Nutrition & Clinical Nutrition.

2.2. Aquaria:

Experiment was conducted in three equal groups reared in glass aquaria of 120 liters capacity. Each one was supplied with de-chlorinated tape water (water maintained for an overnight in large tanks before using in aquaria) with continuous aeration via an electric aerator connected with fine pipe 5mm in diameter stays in the bottom of each aquarium. Water was changed every week (Alabaster and Lioyd, 1980). The aquaria were cleaned every morning prior to feeding by siphoning the wastes which accumulation the bottom.

2.3. Diet preparation:

Three calculated iso-nitrogenous and iso-caloric diets were formulated according to nutrient requirements recommended by NRC (1993). The experimental diets consisted of three diets; A with fish meal, B with soybean meal and C with cotton seed meal. The three diets containing CP 32% and DE 3000Kcal| / Kg diet table (1).Diets were supplemented with mineral, vitamin mixture and corn oil and cod liver oil and then adding cold water until a stiff dough resulted then passed through a mincer with 2mm diameter and the resulting spaghetti like strings were dried in a forced conversion at dryer at 35°C after drying the diets were broken up and sieved into convent pellet sizes. To minimize loss of nutrients during storage, the diets were kept in polyethylene bags and stored in deep freezer.

2.4. Histological examination:

At the end of the feeding studies, three fish were selected from each aquarium. Fish were killed by cervical dislocation Specimens from liver, gills and muscles of three fish in different groups were collected at the end of experiment (52days) for histological examination. Histological studies were carried out by Drury and Wallington's method (1980) briefly: The specimens were fixed in formalin solution (10%) and serial dilution of alcohol (methyl, ethyl and absolute ethyl) to be dehydrated. Specimens were cleared in xylene and embedded in paraffin at 56oC in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 5.0u thickness by sledge microtom. The obtained tissue sections were collected on glass slides, deparaffinized and stained with Harris-hematoxylin and eosin (H&E) (Luna, 1968) as well as Prussin blue (Banchroft, et al., 1996) for histological examination through the light microscope.

3. RESULTS

The histological examination of Musculature tissue, liver, gills, are presented in Fig (1-8). The histological examination revealed alterations in the histological appearance in Musculature tissue of *O. niloticus* fed diets containing fish meal, cotton seed meal and as in Fig (1,3). On the other hand, the muscle bundles of fish fed diet contained soybean are intact as Fig (2). The liver of all experimental groups revealed histological alterations. Liver of *O. niloticus* fed diet containing fish meal showing Hyperemic central vein and sinusoid with focal pigmentation, Liver of *O. niloticus* fed diet containing SBM showing Intra cytoplasmic round circumscribed fat vacuoles and Liver of *O. niloticus* fed diet containing CSM showing Hyperemic central vein and sinusoids with focal extravasations of red cells surrounding the central veins Fig (4,5,6,7).

Gills of all experimental groups revealed histological alterations. Gills of *O. niloticus* fed diet containing fish meal showing: Vascular lining epithelium with hyperplasia in the gill rakers. Gills of *O. niloticus* fed diet containing SBM showing Vascular lining epithelium with hyperplasia in the Gill rakers of *O. niloticus* fed diet

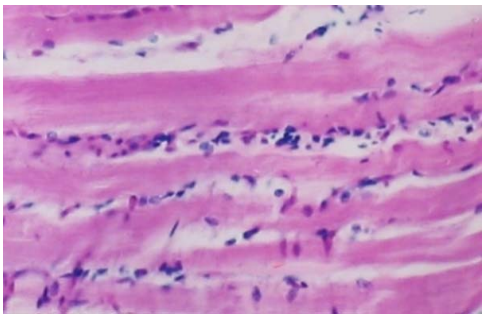

Fig (1): Musculature tissue of *O. niloticus* fed diet containing fish meal showing Degenerated muscle bundles (H&E x40).

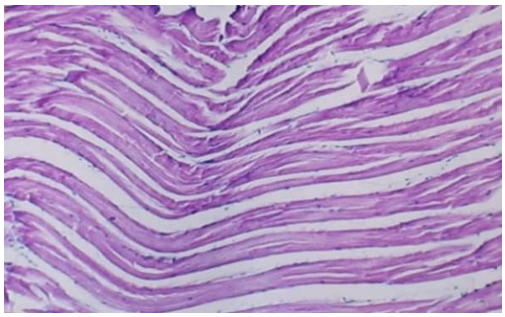

Fig (2): Musculature tissue of O. niloticus fed diet containing SBM showing Degenerated muscle bundles (H&E x40)

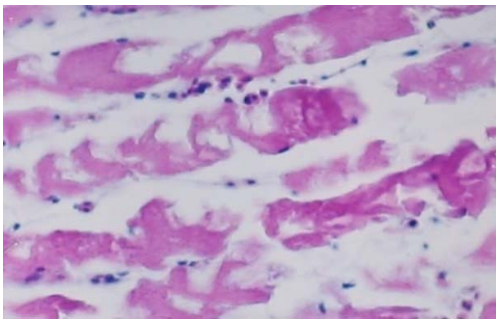

Fig (3): Musculature tissue of O. niloticus fed diet containing CSM showing Hyper-cellularity in focal manner (H&Ex40).

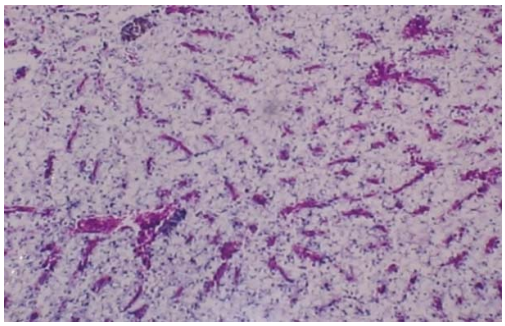

Fig (4): Liver of O. niloticus fed diet containing fish meal showing Hyperemic central vein and sinusoid with focal pigmentation (H&Ex40).

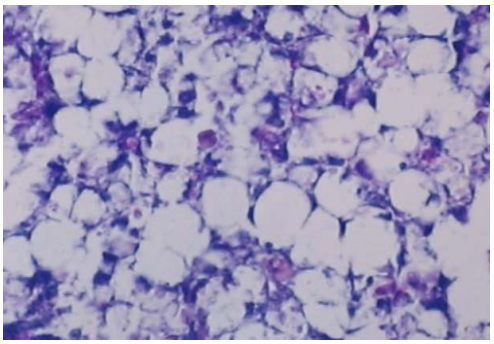

Fig (5): Liver of O. niloticus fed diet containing SBM showing Intra cytoplasmic round circum-scribed fat vacuoles Liver of O. niloticus fed diet containing CSM showing Hyperemic central vein and sinusoids with focal extravasations of red cells surrounding the central veins by higher magnification (H&Ex40).

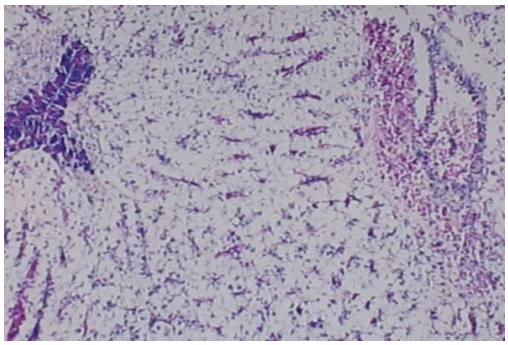

Fig (6): Liver of O. niloticusfed diet containing CSM showing Hyperemic central vein and sinusoids with focal extravasations of red cells surrounding the central veins (H&Ex40)

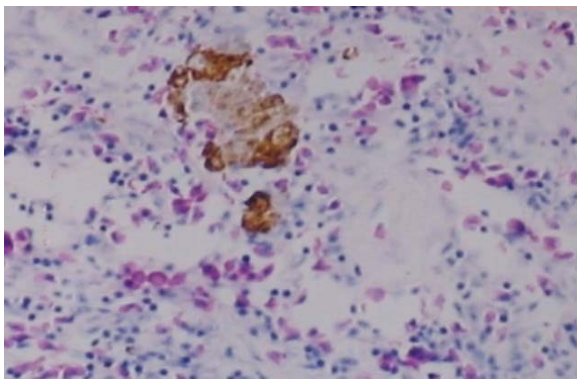

Fig (7): Liver of O. niloticus fed diet containing fish meal showing: Intra cytoplasmic round circum- scribed fat vacuoles (H&Ex40).

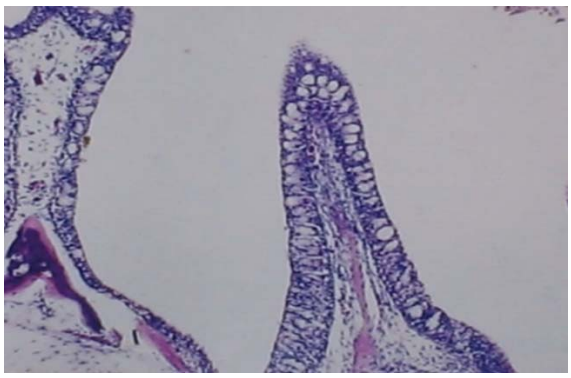

Fig (8): Gills of O. niloticusfed diet containingfish meal showing: Vascular lining epithelium with hyperplasia in the gill rakers (H&EX40).

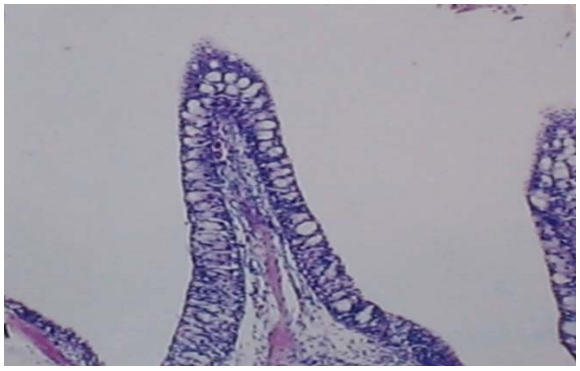

Fig (9): Gills of O. niloticus fed diet containing SBM showing Vascular lining epithelium with hyperplasia in the rakers. (H&E X40).

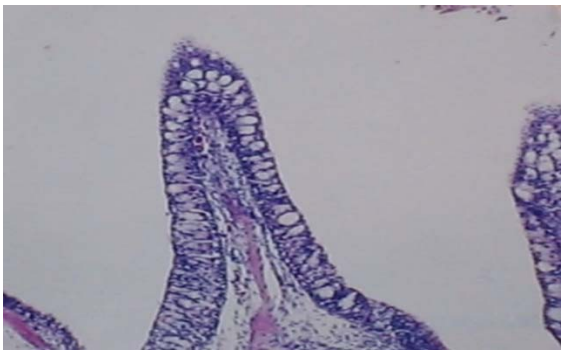

Fig (10): Gills of O. niloticus fed diet containing CSM showing Hyper-plasia and vaculation the lining cells of the rakers (H&E X40).

Table (1): The composition of experimental diets used for different experimental groups:

Feed ingredients	Units	Groups		
		A	B	C
Fish meal*	%	49.5	-	-
Soybean meal (44%)	%	-	50	-
Cotton seed meal	%	-	-	50
Wheat flour	%	10	10	9
Yellow corn	%	20	20	18
Fish oil	%	1.2	2.07	4.0
Corn oil	%	2	3	4.71
Vitamin mixture **	%	3	2	1.09
Mineral mixture***	%	4.5	4	2
Carboxy methyl cellulose	%	2.8	1.41	-
Casein	%	-	7.02	10.7
Cellulose	%	6.5	-	-
Chromic oxide	%	0.5	0.5	0.5
Total	%	100	100	100
Chemical composition :CP	%	40	40	40
DE K cal/ k gm diet		3000	3000	3000

* Commercial native fish meal used from 0-28 days and commercial exported fish meal from 28 to 52 days. The proximal analysis for CP of commercial native fish meal=27.9%. **Each 1 kg of the vitamin premix contains: Vit. A, 4,000,000 I U.; Vit k3, 400mg; VitB1, 400mg.; Vit B2, 1600mg; Vit., B6, 600mg; Pantothenic acid, 400mg;Vit. B12, 400mg; Vit.B12, 4mg; choline, 200g; Niacin, 8g; Biotin, 20mg; folic acid, 400mg and Vit. C, 200g. ***Each 1 kg of the Mineral premix contains: Calcium, 250gm; phosphorus, 180g; Zinc, 9g; Manganese, 8g;.Copper, 0.024g; Iron, 0.24g; Selenium, 0.8mg and Iodine, 2.4mg.

Table (2): Histological finding of *O. niloticus* fed different protein sources.

Organ	Group A	B	C
Musculature Tissue	Degenerated muscle bundles	Intact muscle bundles	Hyper-cellularity in focal manner
Liver	Hyperemic central vein and sinusoid with focal pigmentation	Intra cytoplasmic round circumscribed fat vacuoles by higher magnification	Hyperemic central vein and sinusoids with focal extravasations of red cells surrounding the central veins
Gills	Focal extravagation of red cells in the arch	Vascular lining epithelium with hyperplasia in the rakers	Hyperplasia and vaculation the lining cells of the rakers

containing CSM showing Hyperplasia and vacillation the lining cells of the gill rakers Fig (8, 9, 10)**.**

4. DISCUSSION

This experiment was carried out to investigate the histological picture of Musculature tissue, liver and gills of *O. niloticus* associated with feeding on diets containing commercial fish meal, soybean meal and cotton seed meal used in fish feed in Egypt. The histological examination of Musculature tissue of *O. niloticus* in both Gr A and Gr C fed diet containing fish meal and cotton seed meal showing Degenerated muscle bundles and Hyper-cellularity in focal manner. While Alexis, 1997 and showed that the muscles showed no histopathological changes. The authors also reported that plant based feeds significantly raises the protein and fat levels in the carcass composition of major carps. Regarding to Gr B fed on soy bean meal the histological findings revealed intact muscle bundles. In contrast, Van den Ingh et al., 1991; Baeverfjord and Krogdahl, 1996; Refstie et al., 2000; Krogdahl et al., 2003; Uran et al., 2008 and Knudsen et al., 2008 described some alterations in musculature tissue which may be responsible for the retardation of growth in response to SBM-based diets. The histological examination of *O. niloticus* liver: Gr A fed diet containing fish meal showed hyperemic central vein and sinusoid with focal pigmentation. Some experiments were carried out by (Caballero et al., 2003; Diaz et al., 2006) which showed alterations in liver of carp fed diets with fish meal (FM). Zoran Marković et al. (2012) also showed vacuolated hepatocytes, fatty changes, and focal fibroses were found on some liver samples regardless of feed and period of sampling (beginning or end of the experiment). The authors added that in all liver samples examined after 90 days of experiment signs of fatty degeneration of hepatocytes were found compared to the initial group. These alterations may be due to adulteration or bad quality of fish meal. Liver in Gr B fed diet containing Soya bean meal showed intra-cytoplasmic round circumscribed fat vacuoles. Also Fontagne et al., (1998; Power et al., 2000; Ostaszewskaet al., 2005) described vacuolated hepatocytes, fatty changes, and focal fibroses were observed in some liver samples. The authors explained that this may be due to the deficiency of soya bean meal in lysine and methionine. Also A reduction in nuclear size may be a sign of malnourishment reported by (Fontagne et al., 1998; Power et al., 2000; Ostaszewska et al., 2005). On other hand Figueiredo-Silva et al. (2005) reported that soybean oil replacing dietary fish oil up to 50 % had no significant effect on liver histology of the rainbow trout. While Gr C fed diet containing Cotton seed meal showing hyperemic central vein and sinusoids with

focal extravasations of red cells surrounding the central veins. Similar results on the *O. niloticus* reported by El-Kasheif, et al (2013) showed severe histological alterations in liver and spleen in fish fed on diet with high level of CSM (100% replacement of fish meal). While, results of the groups fed diet containing more than 50% CSM (50% replacement of fish meal) showed vacuolar degeneration of the hepatic cells. Also studies carried out by Evans et al. (2010) and Barros et al. (2000) stated that inclusion of gossypol in the diet had no statistically significant effect on liver glycogen deposition. While Herman (1970) observed liver necrosis in rainbow trout fed diets containing gossypol. This difference may be due to the difference in type of tested fish. Our results were supported by the findings reported by Takashima and Hibiya (1982); Roberts, (1989) and Poleksić et al., (2010) who stated that the most common changes observed in the liver are; hepatocytes vacuolization, fatty degeneration, changes in liver parenchyma and necrosis. The histological examination of gills *O. niloticus*: gills of all experimental groups revealed the same histological alterations in all groups (Gr A and Gr B) as presented in the results revealed vascular lining epithelium with hyperplasia in the gill rakers. While Gr C fed diet containing Cotton seed meal showed hyperplasia and vaculation the lining cells of the rakers. El-Kasheif, et al (2013) showed severe histological alterations in the gills, of *O. niloticus* fed diet containing more than 50% CSM, including edema of lamellar epithelial cells with breakdown of the pillar cells system. While Mustafa Yıldız et al (2013) showed no alterations of gill tissue samples of the rainbow trout fed different dietary treatments of Cottonseed meal. In conclusion, feeding Nile tilapia on soybean meal may be the best choice as protein supplement alternative due to its positive effect on the musculature tissue integrity, no clinical signs, beside it is much safer. Cottonseed meal economically viable as alternative plant protein but it may cause alternative changes diarrhea. Although fish meal is superior for achieving high growth rate it may accumulate harmful substances.

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