EFFECT OF DIETARY REPLACEMENT OF SOYBEAN MEAL PROTEIN WITH COTTON SEED MEAL PROTEIN ON BIOCHEMICAL AND HISTOLOGICAL FEATURES OF NILE TILAPIA MUSCLE

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SUMMARY

This experiment intended to assess the effect of cottonseed meal (CSM) as a partial and complete replacement of soybean meal on biochemical analysis, histological features, the carcass characteristics and meat quality traits of Nile tilapia (Oreochromis niloticus) after 22 weeks of feeding period. Fish with an average initial body weight of 46.4±1.8 g were stocked in 15 glass aquarium (90 L each) of 20 fish each. CSM was incorporated at levels of 0, 25, 50, 75, and 100% with soybean meal protein. Diets were fed to fish twice daily at a rate of 3% of body weight during 22 weeks the experimental period. The results of the present study revealed that groups of fish fed diet containing 50% CSM had significant highest final weight (189 g/fish), body weight gain percent (297.2%), carcass weight (96 g fish^{-1}), fillet weight (49.0 g fish^{-1}) and dressing % (50.5 %) compared with fish fed control and other diets. The lowest values of final body weight, body weight gain percent, carcass weight, fillet weight and dressing % were significantly obtained with fish fed diet containing 100% CSM. Biochemical analysis of muscles indicated that the maximum dry matter, crude protein crude lipid and ash contents were obtained with groups of fish fed on the diet containing 50% CSM, while the lowest values were obtained with groups of fish fed on the diet containing 100% CSM. Fish fed control diet and 50% CSM diet had significantly the highest values of water holding capacity (W.H.C.), 7.41 and 7.18, respectively, compared with others. The muscles showed histological alterations in fish included degeneration in muscle bundles accompanied with fat vacuole in muscle bundles. Splitting of muscle fibers and atrophy of muscle bundles were seen with increasing CSM concentration more than 50%. Therefore, these findings suggest that up to 50% of soybean meal protein can be replaced by cotton seed meal protein in Nile tilapia diets without any adverse effects on biochemical analysis, histological features, the carcass characteristics and meat quality traits of Nile tilapia.

Keywords: Nile tilapia, cotton seed meal, biochemical parameters, histology muscles

INTRODUCTION

Recently in Egypt, there is an increase in the production and consumption of freshwater fish reared in aquaculture systems, mainly the Nile tilapia, *Oreochromis niloticus*. The Nile tilapia, an important farmed fish produced in various parts of the world, very much sought by its low fat meat, shows a growing consumption in Egypt. Brown (1983) reported that tilapia are omnivorous fish which naturally feed on plankton, diatoms, small crustacea, higher plants, and decomposing vegetable matter. Historically, they have been utilized to recycle wastes into high quality fish flesh. They are capable of digesting high levels of carbohydrate in their diet (National Research Council, NRC, 1993), and effectively utilize alternative feed ingredients such as ricebran, cocoa, various flours, Soya, nut oil, and milling wastes (Brown, 1983).

Cottonseed meal has been tested in feeding numerous fish species including tilapia *Sarotherodon mossambicus* (Jackson *et al*., 1982), Nile tilapia, *Orechromis niloticus* (Rinchard *et al.*, 2000, Mbahinzireki *et al*., 2001 and El-Saidy and Amal, 2011) and channel catfish, *Ictalurus punctatus* (Robinson and Li, 1994 and Robinson and Tiersch, 1995). From nutritional point of view, cottonseed meal contains high levels of protein (Forster & Calhoun, 1995) and is very palatable to fish (Robinson and Li, 1993).

Traditionally, undecorticated cottonseed is crushed and screw-pressed, providing protein-rich crude oilcake that is characterized by high fiber and protein contents. In recent years, additional extraction procedures (fat, protein) deliver a dry cottonseed meal of potentially better quality (Lynn and Wedegaertner, 1986). Due to their high protein content of up to 40% (Ramachandran *et al*., 2007), cottonseed products - oilcake and meal - could be used as an inexpensive protein source in fish feeds. However, gossypol, a secondary metabolite stored in the pigment glands of cottonseed, is well-known for numerous antinutritional effects that limit its use as an alternative protein source (Rinchard *et al*., 2003; Yue and Zhou, 2008).

Thus, the replacement of fishmeal with less costly plant feedstuff must be emphasized. However, the presence of ant-nutritional factors and other active biological compounds limit the use of substitutes, and toxic effects have been reported in fish (Francis *et al.,* 2001; Lee *et al*., 2006).

The diet of the fish has a great influence on their general chemical composition, and particularly on their fatty acid composition (Henderson and Tocher, 1987). Fillet yield is considered as an important measurement for improving fish production efficiency (Flick *et al*., 1990). Many works were done to determine the effects of dietary protein levels on fillet yield and chemical composition of Nile tilapia (*Oreochromis niloticus*) and other fish species (Al-Hafedh, 1999; Li *et al.*, 2001 and Robinson *et al.,* 2007). A possible effect of the quality of the dietary protein source on the biochemical processes during early postmortem stages, with potential consequences on the shelf-life and quality characteristics of the final product was suggested by Parisi *et al.* (2004).

In tilapia, studies have been conducted to evaluate the effect of low-cost animal and plant protein sources on the fillet yield and meat quality. Therefore, the objective of this study was to investigate the possible different levels of cotton seed meals are used as a partial and complete replacement of soybean meal protein and their effects on biochemical analysis, the carcass characteristics and meat quality traits of Nile tilapia, Comprehensive histopathology of the major organ (muscle) was conducted for adverse gossypol impact.

MATERIALS AND METHODS

The present work was conducted at the fish research laboratory, Department of Poultry Production, Faculty of Agriculture, Minufiya University ,Egypt. The experiment intended to assess the effect of different levels of cotton seed meal as replacer to soybean protein on the carcass characteristics, meat quality traits biochemical constituent and histology of Nile tilapia (*Oreochromis niloticus*) muscle after 22 week feeding period.

Diet formulation:

Reared fish were fed on artificial experimental diets formulated to contain about 32% crude protein based on feedstuff values reported by National Research Council (NRC), 1993. The control diet 1(0 cotton seed meal) and four diets (2-5) with 25%, 50%, 75%, and 100% CSM in replacement to soybean meal protein, respectively, were prepared. Animal protein sources (FM) were supplemented in the diets as 3% of total crude protein. Other dietary ingredients included in the diet were soybean meal, wheat bran, soybean oil, fish oil, vitamin and minerals premixes. Each of the five diets was subjected to proximate analysis using standard methods (AOAC, 2000). The formulation and proximate composition of the dietary ingredients and experimental diets are presented in table (1)

Experimental system and animals:

About 350 Nile tilapia fish (*Oreochromis niloticus)* with an average initial weight of 46.4 g/fish collected from the Nile River at EL-Kanater EL-Khyria station during April, 2010. Fish were stocked in a concrete pond (40 m^2) for two weeks for adaptation before transportation to the fish research laboratory at Department of Poultry Production, Faculty of Agriculture, Minufiya University ,Egypt for experimental initiation. Fifteen glass aquaria each of 120 liter capacity and 60×40 × 50 cm, dimension were filled with 90 liter dechlorinated tap water. The water in each aquarium was provided with sufficient aeration by means of an electric air compressor. Throughout the experiment, each aquarium was partially cleaned daily and the water was completely changed once a week. At the start of the experiment, five experimental diets, namely from 1 to 5 were assigned each to triplicate groups (each group contained 20 fish in a random manner). Each group of fish was weighed at the beginning and every two weeks throughout the experimental period. Fish were fed six days a week at a rate of 3% of a body weight during the experimental period. The fish samples were collected from each aquarium before feeding at the end of the experiment (22 weeks).

Carcass characteristics:

At the end of the experiment fish was immediately weighed to obtain the final body weight (BW). Body weight gain % was calculated as follows: Final Wt- initial Wt/ initial Wt×100. Fins and viscera were removed. The body cavity was washed with tap water to remove any traces of blood. Fish was weighed again to calculate the dressing percentage. Fillet was separated and fillet weight (FW, with skin and ribs) in grams was recorded. Fillet yield was calculated according to Rutten, *et al.* (2004) as: F %= (FW/BW) X 100. Fillet samples were divided into two groups, the first group was used after harvest to determine the fillet chemical composition. The second group was stored at 4°C for 10 days to determine the effect of dietary treatments on fillet quality after this storage period. Fillet samples were minced using a meat mincer, and mixed for the chemical analysis.

Flesh quality traits:

Physical properties:

pH value was determined in the flesh using the method of Aitken, *et al*., (1962), Bechman pH meter. Water Holding Capacity (W.H.C.) of fish fillet samples was measured according to the method described by Wierbicki and Deatherage (1958). The sample of 0.3 g of fillet weight was placed under ashless filter paper (Whatman No. 41) and pressed for 10 minutes using 1 kg weight. Two zones were formed on the filter paper and measured using planimeter. The water holding capacity was calculated by subtracting the area of the initial zone from that of the outer zone. The data were expressed as cm^2.

Chemical composition:

Percentages of moisture, protein, fat, ash in fillet were determined according AOAC (2000) methods. The crude protein and fat in dry matter of experimental fish were determined using Kjedahl and Soxhlet apparatus. Water content was estimated by maintained the fish muscle at 105°C for 24h.

Histological study:

At the end of the experiment, 6 fish from each treatment, including the control and muscle samples were fixed in 10% buffered formalin for histopathological study. Samples were cosseted and processed using auto embedded (Shandon Excelsior, Thermo) examination for dehydration, clearing and wax impregnation. Processed fish tissues were embedded in paraffin wax and sectioned by microtome (Leica 2035 Biocut) set at 5µm thickness. Tissue sections were stained with haematoxylin and eosin and examined under a light microscope (Olympus BX51) to assess any histological changes in the muscle.

Statistical analysis:

Data were analyzed by analysis of variance (ANOVA) using the SAS ANOVA procedure (Statistical analysis system 1988). Duncan's multiple range test (Duncan 1955) was used to compare differences among individual means. Treatment effect were considered significant at $P \leq 0.05$. All percentage and ratio were transformed to arcsin values prior to analysis (Zar 1984).

RESULTS

Initial body weight, final body weight, body weight gain, carcass weight, fillet weight, dressing percentage and fillet percentage are shown in Table (2). Fish fed diet containing 50% CSM had significant ($p < 0.05$) the highest final body weight (189 g $fish^{-1}$), body weight gain % (297.2%), carcass weight (96 g $fish^{-1}$), fillet weight (49.0 g $fish^{-1}$) and dressing % (50.5 %) compared with fish fed control and other diets. The lowest ($p < 0.05$) values of final body weight, body weight gain percent, carcass weight, fillet weight and dressing % were significantly obtained with fish fed diet containing 100% CSM.

Physical properties:

Water holding capacity (W.H.C.) and pH values are presented in Table (3). Water holding capacity was identified as the ability of meat to hold its own or added water during the application of any force such as pressing, heating, etc. This phenomenon is one of the more important physical characteristics of meat and fish and mainly affects their texture, as well as is completely related to the proteins quality and quantity. Table 3 indicated that fish fed control diet and 50% CSM diet had the highest values of water holding capacity (W.H.C.), 7.41 and 7.10, respectively. The results indicated that pH values of Nile tilapia fillet ranged from 6.3 to 6.5. No significant differences were observed between the control diet and the other diets in pH records with different levels of CSM.

***Chemical composition*:**

Percentages of dry matter, protein, fat and ash contents of Nile tilapia fed the cotton seed meal experimental diets are presented in Table (4). There was a significant ($P<0.001$) effect of the tested diets on dry matter, crude protein, crude fat and crude ash contents of Nile tilapia muscle. Biochemical analysis of muscles indicated that the highest ($P<0.001$) values of dry matter, crude protein crude lipid and ash contents were obtained with groups of fish fed on the diet containing 50% CSM, while the lowest values were obtained with groups of fish fed on the diet containing 100% CSM. The highest significant crude protein value of 92.14% was obtained with groups of fish fed diet containing 50% CSM (Diet 3) compared with other diets. The lowest value of crude protein content (76.51) was obtained with groups of fish fed 100% CSM (Diet 5). The highest crude lipid content of 4.01 % was obtained with groups of fish fed diet containing 50% CSM compared with control and other diets.

Histological observation:

No recognizable changes were observed in the muscle of control group of fish (Fig. 1). The histological alterations in the muscle of

Nile tilapia fish fed diets containing 25 and 50% CSM diets 2 & 3 show fish muscle bundles splitting with edema appear (Fig.2 and 3). Muscles of fish fed diet 4 (75 % CSM) show that the fish muscle bundles containing vacuolar degeneration (fat vacuoles) and atrophy of muscle bundles were seen (Fig. 4). Muscles of fish fed diet 5 which contained 100% CSM show focal areas of necrosis with homogenous liquid appearance and the muscle tissue characterized by disappearance of striations, also an atrophy of nuclei of muscle fibers (Fig.5a and 5b).

DISCUSSION

The chemical composition of fish muscle varies greatly from one species to another and even among the individuals within the same species. Such variation depends on age, size, sex, environment and season (Huss, 1995); Silva and Chamul, 2000). In fact, the variation in the chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning (Sallam *et al.*, 2007).

An important intrinsic factor related to fish flesh is the very high post-mortem pH (>6.0). Most fish contain only very little carbohydrate (<0.5%) in the muscle tissue and only small amounts of lactic acid are produced post-mortem (Gram and Huss, 1996). In the present study, pH values of Nile tilapia fillet ranged from 6.3 to 6.5. Control diet had higher pH values (6.5) in the fillet compared to 75% and 100% CSM diets which had pH value of 6.3. No significant differences were observed between the control diet and the other diets. pH is related to the post-mortem evolution of the flesh and is influenced by the species, feeding, station of the year, etc.(Periago *et al*., 2005)

Water holding capacity was identified as the ability of meat to hold its own or added water during the application of any force such as pressing, heating, etc. Fish fed control and 50% CSM diets had significantly the highest values of W.H.C, values were 7.41 and 7.18, respectively. Regost *et al*. (2004) verified that Liquid holding capacity (LHC) was increased by dietary soybean oil. No significant differences were detected among 75 and 100% CSM diets. Sayed *et al*. (1999) indicated that body fat content of Nile tilapia was lower with increasing soybean meal protein. Results of the present study showed that total lipid contents ranged from 0.68 to 4.01 g 100 g^{-1} of tilapia fillet for experimental diets. The highest value of crude lipid content of 4.01 % was obtained with groups of fish fed diet containing 50% CSM diet 3, while the lowest value was obtained with groups of fish fed 100% CSM (diet 5). Total lipid contents was increased significantly ($P<0.05$) up to 50% CSM level then decreased with increasing CSM in the diets supplemented with 75 & 100% CSM (diets 4 &5). However, Clement and Lovell (1994) indicated that fat content of Nile tilapia fillet was lower than channel catfish while protein content was higher for tilapia. Meric *et al*. (2011) found that growth and feed efficiency was reduced when cottonseed meal was used as a replacement of soybean, suggesting the existence of antinutritional factors other than gossypol. This is further supported by the substantially reduced growth performance observed when glandless free cottonseed was used. Feed quality and composition may have contributed to this effect, (Cheng an Hardy, 2002) for rainbow trout fingerlings and Pham *et al.* (2007) for Japanese flounder. These findings contradicted the earlier report by Jackson *et al*. (1982) that tilapia grew well on CSM-based protein, even at 100% level of inclusion.

Winston (2008) indicated that the main oilseed protein sources soybean meal (SBM), Cottonseed meal (CSM) and Groundnut cake (GNC) could replace at least 50% of fish meal protein in the diet of *O. niloticus* fingerlings, without adversely affecting growth and feed efficiency which agree with the results obtained in the present study. Zheng *et al.* (2011) regarded that, supplementation and protein reduction had no effect on the viscerosomatic index of fish or moisture, lipid, and protein content of muscle samples.

Unfortunately, few studies have been concerned with histological effects of cottonseed meal (CSM) on muscle of *O. niloticus*, the effects detected in histological alteration of the muscle tissues are important in relation to the health of tilapia that depend on CSM as dietary protein source. The histological alterations in the muscles of studied fish included degeneration in muscle bundles accompanied with focal areas of necrosis and vacuolar degeneration, atrophy of muscle bundles, splitting of muscle fibers. In addition muscle tissue characterized by disappearance of striation was seen in groups of fish which fed diets containing more than 75% CSM level**.** This result emphasized high significant increase in water holding capacity result. In conclusion, the findings suggest that up to 50% of soybean meal protein can be replaced by cotton seed meal protein in Nile tilapia diets without any adverse effects on biochemical analysis, histological features, the carcass characteristics and meat quality traits of Nile tilapia.

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Table 1. Formulation and proximate analysis of the experimental diets (g kg^{-1} dry matter).

Experimental diets	1 Control (0 CSM)	2 (25%CSM)	3 (50%CSM)	4 (75%CSM)	5 (100%CSM)
Ingredients:					
Soybean meal	550	412.5	275.0	137.5	0.0
Cotton seed oil	0.0	137.5	275.0	412.5	550.0
Fish meal	50.0	50.0	50.0	50.0	50.0
Wheat bran	330.0	330.0	330.0	330.0	330.0
Fish oil	15.0	15.0	15.0	15.0	15.0
Soybean oil	15.0	15.0	15.0	15.0	15.0
Di-calcium phosphate	15.0	15.0	15.0	15.0	15.0
vitamin mixture[a]	12.0	12.0	12.0	12.0	12.0
Mineral mixture[b]	5.0	5.0	5.0	5.0	5.0
Lysine	0.0	1.2	2.4	3.6	4.8
Chromium oxide	0.5	0.5	0.5	0.5	0.5
Cellulose	7.5	6.3	5.1	3.9	2.7
Proximate composition[c]:					
Dry matter	891.2	887.1	887.5	890.8	889.6
crude protein	318.2	317.8	317.6	317.5	317.3
crude lipid	35.6	37.2	37.9	38.7	40.7
crude fibre	52.2	52.8	51.4	58.2	58.5
Ash	61.1	60.3	66.1	63.7	65.1
NFE[d]	424.1	419.0	414.5	412.7	408.0
Phosphorus	9.2	9.6	11.4	11.1	12.3
Free gossypol[e] (mg kg^{-1})	0.0	11.42	22.85	34.27	45.69
Met. Energy[f] (KJg $^{-1}$feed)	13.0	13.0	13.0	13.0	13.0

[a]Vitamin mixture (mg or IU if mentioned kg^{-1} diet): vit. A, 8000 I.U.; vit. D_3, 4000 I.U.; vit. E 50 I.U.; vit. K_3, 19 I.U.; vit. B_2, 25 mg; vit. B_3, 69 mg; nicotinic acid, 125 mg; thiamin, 10 mg; folic acid, 7 mg; biotin, 7 mg; vit. B_{12}, 75 mg; cholin, 400 mg and vit. C, 200 mg.

[b] Mineral mixture (mg kg^{-1} diet): FeSO4·7H2O, 40; ZnSO4·H2O, 150; MnSO4·H2O, 25; GuSO4·5H2O, 3; KI, 5; Na2SeO3, 0.09; CoSO4, 0.05.

[c] Values represent the mean of three sample replicates.

[d]Nitrogen-free extract =100 - (moisture+ crude lipid+ crude protein+ crude fiber+ ash).

[e]Free gossypol concentration in the diets was determined by high-performance liquid chromatography (Luo, *et al.*, 2006).

[f]Metabolizable energy was calculated based on the standard physiological values of 18.8 kJ g^{-1} protein, 13.8 kJ g^{-1} carbohydrate, and 33.5 kJ g^{-1} fat (Brett & Groves 1979).

Table 2. Averages initial body, final body, carcass, fillet, dressing % and fillet % of Nile tilapia *Oreochromis niloticus* fed diets of different levels of CSM. Values are mean[1] ± SD.

Diets	Initial Body Weight (g)	Final Body Weigh (g)	Body Weight Gain (%)	Carcass weight (g)	Fillet weight (g)	Dressing (%)	Fillet (%)
1 (0 CSM)	45.3±1.8	122.2±3.5^{c}	169.6±5.6^{c}	52.2±2.0^{c}	33.0±2.4^{c}	43.0±2.0^{b}	25.0±1.1
2 (25%CSM)	47.0±1.8	129.0 ±2.4^{b}	174.7±7.0^{bc}	54.2±1.7^{c}	31.0±2.2^{c}	43.2±2.0^{b}	26.0±1.3
3 (50%CSM)	47.7±2.2	189.0±1.8^{a}	297.2±17.8^{a}	96.0±1.5^{a}	49.0±1.7^{a}	50.5±1.5^{a}	27.0±1.1
4 (75%CSM)	46.0±0.9	131.0±1.7^{b}	185.1±11.4^{b}	64.0±1.5^{b}	39.0±2.5^{b}	45.0±2.8^{b}	25.0±1.8
5(100%CSM)	46.0±1.8	115.7±1.6^{d}	151.7±7.4^{d}	42.7±1.6^{d}	27.5±2.5^{d}	40.5±1.4^{c}	25.0±2.1

[1]Values in the same columns with different superscripts are significantly different ($P \leq 0.01$). Values represent the mean of three replicate groups.

Table 3. Water holding capacity (WHC) expressed as cm^2 and pH values of Nile tilapia (*Oreochromis niloticus*) muscles fed CSM diets. Values are mean[1] ± SD.

Diets	W.H.C	pH
1 (Control 0%)	7.41±0.01^{a}	6.5
2 (25% CSM)	6.19±0.02^{b}	6.4
3 (50% CSM)	7.18±0.14^{a}	6.4
4 (75% CSM)	5.79±0.20^{c}	6.3
5 (100% CSM)	5.72±0.05^{c}	6.3

[1]Values in the same columns with different superscripts are significantly different ($P \leq 0.01$). Values represent the mean of three replicate groups

Table 4. Percentages of dry matter, protein, fat and ash content of Nile tilapia (*Oreochromis niloticus*) muscle fed diets with different levels of cotton seed meal (CSM). Values are mean[1] ± SD

Diets	Dry matter	Crude protein	Crude lipid	Ash
1 (Control 0%)	22.75±0.59^{b}	89.88±1.03^{b}	1.46±0.30^{c}	5.29±0.28^{d}
٢ (25% CSM)	22.01±0.54^{c}	88.27±0.99^{b}	2.14±0.24^{b}	6.33±0.19^{b}
3 (50% CSM)	24.18±0.30^{a}	92.14±2.23^{a}	4.01±0.40^{a}	7.24±0.14 ^{a}
4 (75% CSM)	23.59±1.29^{ab}	81.37±1.14^{c}	1.31±0.07^{c}	6.65±0.34^{b}
5 (100% CSM)	21.01±0.90^{c}	76.51±1.05^{d}	0.68±0.20^{d}	5.66±0.40^{c}

[1]Values in the same columns with different superscripts are significantly different ($P \leq 0.01$). Values represent the mean of three replicate groups.

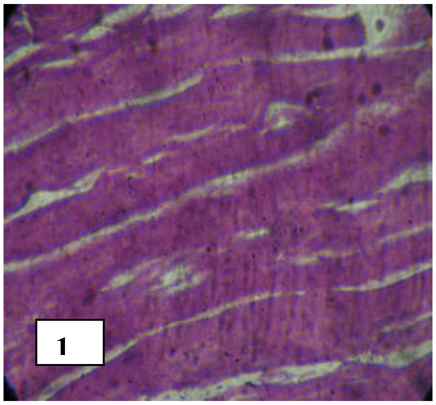

Figure 1. Muscle tissue of fish fed control diet1 showing striation of muscle tissues (X100).

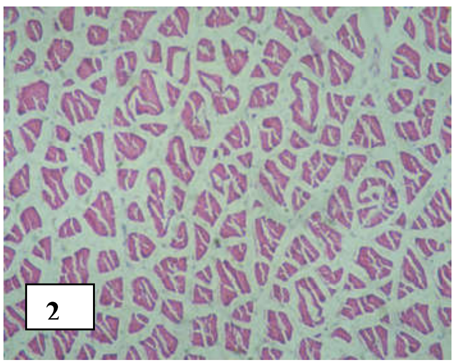

Figure 2.Muscle tissue of fish fed 25% CSM diet2 diet 5 showing edema and splitting muscle bundle (X100).

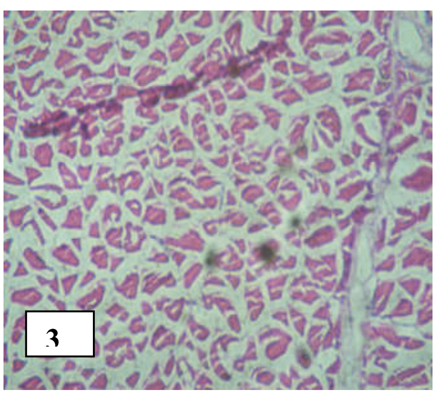

Figure 3. Muscle tissue of fish fed 50% CSM diet 3 showing atrophy and vacuolar degeneration of muscle bundles (X100).

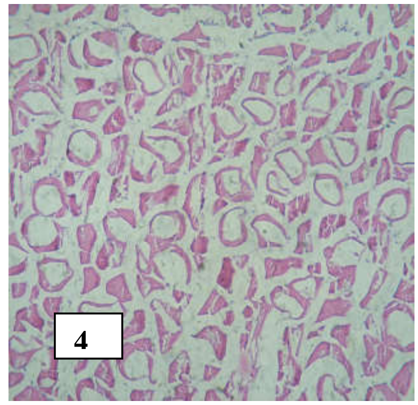

Figure 4. Muscle tissue of fish fed 75% CSM diet 4 showing splitting muscle tissue (X100).

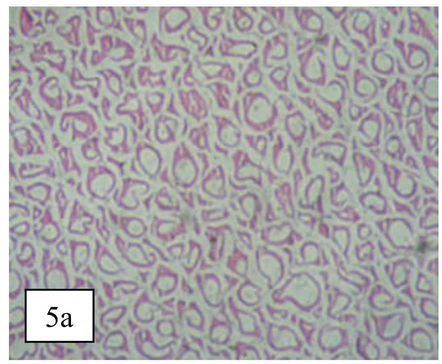

Figure 5a. Muscle tissue of fish fed 100% CSM showing vacuolar degeneration of muscle bundle (X100).

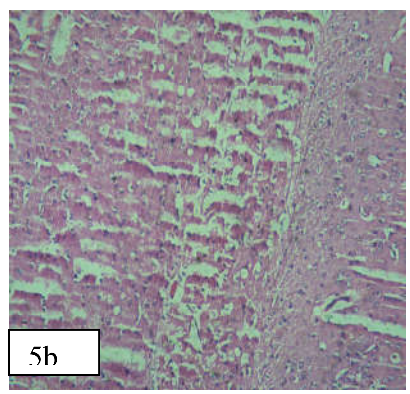

Figure 5b. Muscle tissue of fish fed100% CSM diet 5 showing disappearance of striation of muscle tissues (X100).

أثر إحلال بروتين كسب فول الصويا ببروتين كسب بذرة القطن فى علائق أسماك البلطى النيلى على مكونات اللحم البيوكيميائية والملامح الهستولوجية للعضلات

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أجريت هذة التجربة لدراسة تأثير اضافة بروتين كسب بذرة القطن كبديل جزئى أو كلى لبروتين كسب فول الصويا فى العلائق على المحتوى البيوكيميائى والمظاهر الهستولوجية وصفات الذبيحة وصفات جودة اللحم لأسماك البلطى النيلى المغذاة على العلائق التجريبية. أستخدم فى هذه التجربة أسماك البلطى النيلى متوسط وزن ابتدائى ٤٦.٤جم/سمكة والتى تم اسكانها فى ١٥ حوض زجاجى سعة الواحد منها ٩٠لتر مياة وبمعدل ٢٠ سمكة/ حوض لدراسة تأثير بروتين كسب بذرة القطن. وقد تم دراستة تحت مستويات أحلال مختلفة من العلائق وهى صفر (كنترول بدون كسب بذرة القطن) ,٢٥, ٥٠, ٧٥, ١٠٠% كبديل لبروتين كسب فول الصويا, وتم تغذية الأسماك بالعلائق مرتين يوميا بمعدل ٣% من وزن جسم السمكة يوميا ولمدة ٢٢ أسبوع.أوضحت الدراسة أن أعلى قيم لمتوسط وزن الجسم النهائى (١٨٩جم/سمكة) والزيادة النسبية فى وزن الجسم (٢٩٧.٢%) ووزن الذبيحة (٩٦جم/سمكة) ووزن العضلات (٤٩جم/سمكة) ونسبة التصافى (٥٠.٥%) كانت فى مجاميع الأسماك التى غذيت على العليقة المحتوية على٥٠% كسب بذرة القطن وذلك بالمقارنة بالعليقة الكنترول وباقى العلائق, بينما سجلت أقل النتائج فى مجاميع الأسماك التى غذيت على العليقة ١٠٠% كسب بذرة القطن . أوضح التحليل البيوكيميائى لعضلات الأسماك أن أعلى محتوى من المادة الجافة والبروتين الخام والدهن الخام والرماد كانت فى مجاميع الأسماك المغذاة على العليقة ٥٠% كسب بذرة القطن بينما سجلت مجاميع الأسماك التى غذيت على العليقة ١٠٠% كسب بذرة القطن أقل النتائج. وجد أن الأسماك المغذاة على العليقة كنترول , ٥٠% كسب بذرة القطن احتوت عضلات ألأسماك على أعلى قدرة للأحتفاظ بالماء حيث كانت ٧.٤١, ٧.٤٨ على الترتيب وذلك بالمقارنة بباقى العلائق .

وأوضحت الدراسة الهستولوجية لعضلات الأسماك وجود تغيرات هستولوجية تشمل تحلل فى الحزم العضلية مصاحب بوجود فجوات دهنية فى الحزم العضلية مع وجود تمزق فى الألياف العضلية أزداد بزيادة محتوى العليقة من كسب بذرة القطن لأكثر من ٥٠%. مما سبق نستنتج أنة يمكن أستبدال بروتين كسب فول الصويا ببروتين كسب بذرة القطن حتى ٥٠% بالعلائق دون تأثيرات عكسية على النمو والأداء والمحتوى البيوكيميائى والمظاهر الهستولوجية للعضلات وصفات الذبيحة وصفات جودة اللحم لأسماك البلطى النيلى.