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# EFFECT OF DIETARY SUBSTITUTION OF COD LIVER OIL BY VEGETABLE OILS ON GROWTH PERFORMANCE, BODY COMPOSITION, LIPID PEROXIDATION, LIVER AND MUSCLE HISTOPATHOLOGICAL STATE IN *Nile tilapia* (*Oreochromis niloticus*)

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**Abstract-** This study was carried out to evaluate the growth performance, body composition, lipid peroxidation, lipid profile, liver and dorsal muscle histpathological status of Juvenile *Nile tilapia* fed isonitrogenous (32%), isocaloric (3000 kcal/kg) diets containing different types of oils (cod liver oil (CLO), linseed oil (LO), olive oil (OO)) or a blend of equal proportions of CLO and vegetable oils (CLO: LO, CLO: OO) at 3 % of the diets for 60 days. Significant differences were observed in final body weight (BW), weight gain (WG), specific growth rate (SGR) and feed conversion ratio (FCR) due to feeding diets containing different types of oils. The diet containing 3% cod liver oil or linseed oil produced the best BW (81.69, 82.55), WG (34.53, 35.82), SGR (0.94, 0.93) and FCR (1.99, 1.94). Whereas, the diet containing a blend of CLO and LO (1:1) at 3% showed the poorest growth performance (BW, WG, SGR and FCR). Hepatosomatic index (HSI) was not significantly affected by feeding the different types of oils, meanwhile the viscerosomatic index (VSI) and the protein and ash percentages of whole fish body fed the diets containing CLO or LO were higher than other groups. The fish group fed the diet supplemented with OO had higher liver cholesterol and triglycerides levels than other experimental groups. The muscle cholesterol level was the highest in fish group fed the diet containing LO but had a lower level of triglycerides than other treatment groups. The type of lipid source significantly affected the lipid peroxidation of the liver and the fish flesh. The fish fed the diet containing 3% of OO presented the lowest level of the liver and the fish flesh lipid peroxidation (expressed as malondialdehyde (MDA) compared to another diets. Feeding of OO containing diet led to increase lipid deposition in the liver and the fish flesh. Liver histopathology of *Nile tilapia* fed 3% LO revealed mild lipid accumulation with central round nuclei. However, fish fed diet supplemented with a blend of equal proportions of CLO and OO and 3% OO showed intense steatosis with hepatocytes containing numerous lipid vacuoles and eccentric compressed or absent nuclei. Moreover, fish flesh of group fed diet containing 3% LO had only a focal area of fibrosis. In conclusion, dietary substitution of CLO with LO gave the best chemical, nutritional and histopathological results.

**Keywords-** *Nile tilapia*, lipids, performance, lipid peroxidation, histopathological changes

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## Introduction

It is accepted that the aquaculture is one of the fastest growing food production activities in the world [1]. Tilapia are considered the most important farmed fish in Egypt, since they represent about 52.8% of the total freshwater fish yield in Egypt [2]. The rapid growth rate of aquaculture leads to increase in the supply of feed sources [3]. Fish oil has been used at higher dietary lipid source as a general practice for its protein sparing effect, but the demand for fish oil by the aqua feed industry has been predicted to exceed the available resources within the next decade [4]. As a consequence, recently there has been an increasing interest in the investigation of sustainable alternative lipid sources. Several studies conducted on freshwater fish indicated that vegetable oils can successfully replace fish oil in the feed without affecting their survival and growth [5,6]. Tilapia, like other warm water fish, are more inclined to require greater amounts of n-6 fatty acids compared to n-3 fatty acids for maximal growth [7]. Different tilapia species however require approximately 1% of n-6 fatty acids in their diets [8]. Generally, n-3 fatty acids are not required by warm water fishes but for proper membrane structure and at least small quantity of these acids may be required [9]. Since vegetable oils contain high levels of n-6 and also huge quantities of n-3 fatty acids, they can be efficiently used in tilapia diets [10]. A review by Caballero, et al [11] found that little to no effect on performance was usually observed when certain plant-derived oils replaced up to 75% of the added dietary fish oil for most finfish species.

Dietary lipids play an important role of growth and development of fish. They are used as a source of energy and essential fatty acids

that are vital for normal growth, reproduction, metabolic functions and general wellbeing. Lipids are a part of the biomembrane structure, and they provide fat soluble vitamins (precursors for eicosanoids, hormones and vitamin E) and act as enzyme cofactors [11].

Lipid metabolism is mainly regulated by the liver including both the synthesis and degradation of fatty acids. Several enzymes are regulating these pathways show varying affinities for the different fatty acids available in the liver, and thus the imbalances in the dietary fatty acids could modify the function and morphology of this organ [12]. Liver steatosis (vacuolization) has been observed to be associated with nutritional imbalances, increase in dietary lipid, essential fatty acid deficiency and the use of vegetable oils in cultured fish [13-15]. Therefore, the objective of this research was to evaluate the effect of replacing cod liver oil with vegetable oils (linseed oil and olive oil) on growth performance, lipid profile, lipid peroxidation, chemical composition and histopathological changes.

## Materials and Methods

### Diet preparation

Five isonitrogenous (32% CP) isocaloric (3000 Kcal DE / Kg) diets were formulated [Table-1] to satisfy the nutritional requirements of *Nile tilapia* (*O. niloticus*) according to Teshima, et al [7]. Dietary ingredients used in the five diets were identical except the oil or the mixture of oils used as the lipid source, which was added at a level of 3% of the total diet. The dietary sources of lipid used were CLO, blend of equal proportions of CLO and LO (1:1), LO, blend of equal proportions of CLO and OO (1; 1), and OO. Diets were prepared in the form of water stable sinking pellet and stored in plastic bags in refrigerator during the time of use.

*Table 1- Ingredients (%) and proximate Composition of the experimental diets*

| Ingredients | Source of Supplementary Oil (3%) | | | | |
|---|---|---|---|---|---|
| | CLO | CLO:LO | LO | CLO:OO | OO |
| Yellow corn | 29 | 29 | 29 | 29 | 29 |
| Soybean meal | 20.5 | 20.5 | 20.5 | 20.5 | 20.5 |
| Fish meal | 26 | 26 | 26 | 26 | 26 |
| Corn gluten meal | 2 | 2 | 2 | 2 | 2 |
| Wheat bran | 9 | 9 | 9 | 9 | 9 |
| Egyptian clover meal | 7.2 | 7.2 | 7.2 | 7.2 | 7.2 |
| Cod liver oil | 3 | 1.5 | - | 1.5 | - |
| Linseed oil | - | 1.5 | 3 | - | - |
| Olive oil | - | - | - | 1.5 | 3 |
| gelatin | 2 | 2 | 2 | 2 | 2 |
| Vitamin & mineral premix* | 1 | 1 | 1 | 1 | 1 |
| salt | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |
| **Proximate Composition (Analyses)** | | | | | |
| Crude protein % | 31.78 | 31.57 | 31.9 | 31.88 | 31.96 |
| Ether Extract % | 7.15 | 7.18 | 7 | 7.17 | 7.18 |
| Ash % | 8.14 | 8.31 | 7.98 | 7.76 | 8 |

*Diet abbreviations, CLO: 3% cod liver oil; CLO: LO (1:1); LO: 3% linseed oil; CLO:OO (1:1)l; OO: 3% olive oil.*

**Trace minerals & vitamins premixes were prepared to cover the levels of the microminerals &vitamins for tilapia fish as recommended by (NRC, 1993).Vitamins premix (IU or mg/kg diet); vit. A 5000, Vit. D3 1000, vit. E 20, vit. k3 2, vit. B1 2, vit. B2 5, vit. B6 1.5, vit. B12 0.02, Pantothenic acid 10, Folic acid 1, Biotin 0.15, Niacid 30. Mineral mixture (mg/kg diet); Fe 40, Mn 80, Cu 4, Zn 50, I 0.5, Co 0.2 & Se 0.2.*

### Experimental Design

One hundred and fifty unsexed fish weighing approximately 46.9g were received and stocked in 10 glass aquaria (80 cm length, 35cm width & 40 cm height), 15 fish in each. Each diet was fed to the fish in duplicate aquariam at 3% body weight twice daily (9.00-10.00 h and 13.00-14.00 h) for 60 days. Fish were subjected to a photoperiod regimen of 12-13 h light and 12-11 h dark/ day and the temperature during the experimental period ranged from 24-27°C. Daily cleaning for each aquarium was carried out with partial replacement of water by previously stored (for 48 hours) dechlorinated tap water.

### Sample Collection

At the end of the experimental period (60 days), random fish samples (3 fish / aquarium) from all experiment groups were collected and minced for whole body approximate chemical analysis. Another 3 fish were allocated and sacrificed after being weighed to the nearest gram, eviscerated and the whole viscera were weighed. Livers were separated, weighed then fish were deboned and skinned for flesh analysis.

### Growth Parameter Measurements

The following equations were used to evaluate fish growth performance:

Weight gain (g) = Mean final weight (g) - Mean initial

Feed conversation ration (FCR) = Total dry weight of feed

Specific growth rate (SGR, %/day) = 100 x [(Ln (mean final body weight)-Ln (mean initial body weight)]/culture period (days) [16])

Protein efficiency ration (PER)=Wet weight gain/crude protein fed(g)

Hepatosomatic index (HSI) = (liver weight/body weight) x 100

Viscersomatic index (VSI) = (viscera weight/body weight) x 100

Carcass yield % = (carcass weight/body weight) x 100

### Chemical Analysis

The proximate analysis of diets, whole fish and fish flesh were performed according to the standard methods of AOAC (1985): moisture after drying in a hot air oven at 105°C until constant weight, crude protein (N x 6.25) by Kjeldhal method after acid digestion, ash content by incineration in a muffle furnace at 500°C for 18 h. Liver and fish flesh lipid were extracted according to Hayat, et al [17] then total lipids percentage of the fish flesh and the liver were determined gravimetrically [18]. Frozen samples of liver and fish flesh were collected and prepared for determination of malonaldehyde (MDA) by using vitro enzymatic colorimetric method using lipid peroxidation kit (Biodiognostic, Egypt) [19]. Lipid extract of the liver and the fish flesh were dried under N2 and then liver, and fish flesh triglyceride and cholesterol content were determined using vitro enzymatic colorimetric method using Triglycerides and cholesterol kits (Biodiogostic, Egypt) [20,21].

### Histopathological Examination

The remaining fractions of each sampled liver and samples from dorsal muscles were fixed in 10 % buffered formalin for histological examination. Subsequently, the samples were dehydrated in a graded ethanol series and then embedded in paraffin wax. All sections were cut at four µm and stained with haematoxylin and eosin (H&E). Liver sections were additionally stained with periodic acid-Schiff (PAS) and periodic acid-Schiff diastase (PASD) [22]. The PASD stain was necessary to discriminate the PAS positive reaction due to glycogen from other PAS positivity, such as ceroid pigment [23]. All slides were examined using light microscope. Hepatic

lesions were scored according to lipid accumulation and nuclear displacement from 0-3 scores (0, regular-shaped hepatocytes having centrally located round nuclei with no noticeable lipid accumulation in their cytoplasm; 1 mild lipid accumulation with many central round nuclei; 2 moderate lipid accumulation with many eccentric round nuclei and scattered central round nuclei; 3, severe lipid accumulation with diffuse eccentric compressed or absent nuclei).

### Statistical Analysis

The results were subjected to a one-way ANOVA to test the effect of feeding diets containing 3% CLO, LO or OO or a blend of CLO: LO (1:1) or CLO: OO (1:1) on fish growth performance, lipid profile, and chemical composition. Data were analyzed using statistical SPSS v20 (SPSS Inc., Chicago, IL, USA). Differences between means were compared using Duncan's multiple range test at significance of differences ($p < 0.05$) among dietary treatments.

### Results

At the end of the experiment (60 days period) all fish were survived and no mortalities were observed. The growth performance data of the fish groups fed the experimental diets supplemented with CLO and vegetables oils are presented in [Table-2]. Significance difference was observed in BW, WG, SGR and FCR. The diet containing a 3% of CLO or LO produced the best BW (81.69, 82.55), WG (34.53, 35.82), SGR (0.94, 0.93) and FCR (1.99, 1.94). Whereas, feeding the diet containing the blend of CLO and LO (1:1) showed the poorest values of growth performance (BW, WG, SGR and FCR). There was no significant difference of HSI among treatment groups, but the VSI of fish group fed diets containing CLO or LO were higher than other groups [Table-2].

*Table 2- Effect of substitution of cod liver oil by vegetable oil on growth performance of Nile tilapia*

| Items | Source of Supplementary Oil (3%) | | | | |
|---|---|---|---|---|---|
| | CO | CO:LO (1:1) | LO | CLO: OO (1:1) | OO |
| Initial weight (g)/fish | 47.16± 4.21 | 46.50± 3.58 | 46.93± 3.00 | 46.91± 3.35 | 47.00± 4.55 |
| Final weight (g)/fish | $81.69^{a}$± 4.15 | $68.98^{b}$± 3.69 | $82.55^{a}$± 3.66 | $74.32^{ab}$± 3.26 | $72.03^{ab}$± 4.66 |
| Body gain (g)/fish | $34.53^{a}$±0.25 | $22.48^{b}$± 2.3 | $35.62^{a}$± 1.38 | $27.41^{ab}$± 0.48 | $28.26^{ab}$± 1.7 |
| Feed Conversion Ratio | $1.99^{b}$±0.065 | $2.18^{ab}$± 0.071 | $1.94^{b}$±0.028 | $2.08^{ab}$±0.045 | $1.83^{b}$± 0.073 |
| Carcass yield % | $89.42^{a}$± 1.4 | $87.33^{a}$± 1.08 | $87.48^{a}$± 0.63 | $81.93^{b}$± 1.03 | $81.02^{b}$± 0.65 |
| Specific Growth rate % | $0.94^{a}$± 0.035 | $0.66^{b}$± 0.067 | $0.93^{a}$± 0.058 | $0.78^{ab}$± 0.037 | $0.75^{ab}$± 0.31 |
| PER | $1.08^{a}$±0.01 | $0.7^{c}$±0.01 | $1.10^{a}$±0.02 | $0.85^{b}$±0.02 | $0.87^{b}$±0.02 |
| HIS% | 1.97± 0.08 | 2.10± 0.17 | 2.07± 0.14 | 2.39± 0.30 | 1.88± 0.28 |
| VSI% | $3.56^{a}$± 0.33 | $2.49^{b}$± 0.08 | $3.19^{a}$± 0.18 | $2.67^{ab}$± 0.33 | $3.02^{ab}$± 0.66 |

$^{abc}$ *Means in the same row with the different superscript letter are significantly difference ($p<0.05$).*

### Chemical Analysis

Results of the proximate chemical analysis of the whole body and the flesh of the fish receiving the different dietary treatments are showed in [Table-3]. Results of the present study showed that there were no significant differences between moisture percentages of flesh or whole body of the fish fed the experimental diets supplemented with different oils. Also, no significant difference of the ash percentage of flesh of the fish groups fed diets containing different oils source was observed. However, the protein and ash percentages were highest in whole body of the fish group fed a blend of equal proportions of CLO and LO. There was a significant effect ($p<0.05$) of the diet on the lipid percentage of the muscle and the liver [Table-4].

*Table 3- Effect of source of oil supplementation on whole body and fillet proximate composition of Nile tilapia (on dry matter basis)*

| Items | Source of Supplementary Oil (3%) | | | | |
|---|---|---|---|---|---|
| | CLO | CLO: LO (1:1) | LO | CLO: OO (1:1) | OO |
| **Whole body composition** | | | | | |
| Moisture % | 72.13 ± 0.20 | 72.73 ± 0.25 | 72.72 ± 0.49 | 72.84 ± 0.25 | 72.98 ± 0.14 |
| Crude protein % | $56.75^{b}$ ± 1.00 | $60.50^{a}$ ± 1.01 | $57.36^{ba}$ ± 0.71 | $53.98^{b}$ ± 1.48 | $56.25^{b}$ ± 0.36 |
| Ash % | $21.66^{b}$ ± 0.33 | $26.00^{a}$ ± 1.15 | $23.66^{ba}$ ± 1.33 | $22.33^{b}$ ± 0.33 | $22.33^{b}$ ± 0.33 |
| **Fish Flesh** | | | | | |
| Moisture % | 73.73 ± 2.55 | 72.33 ± 2.66 | 76.68 ± 0.60 | 72.71 ± 1.37 | 75.67 ± 1.22 |
| Crude protein % | $66.58^{a}$ ± 1.22 | $64.97^{a}$ ± 0.92 | $61.86^{ba}$ ± 1.31 | $64.62^{a}$ ± 0.48 | $63.31^{a}$ ± 0.48 |
| Ash % | 6.14 ± 0.41 | 6.32 ± 0.51 | 5.93 ± 0.37 | 5.77 ± 0.25 | 6.47 ± 0.25 |

$^{abc}$ *Means in the same row with the different superscript letter are significantly difference($p<0.05$).*

*Table 4- Effect of different dietary lipid source on lipid profile of liver and muscle tissue of Nile tilapia.*

| Items | Source of Supplementary Oil (3%) | | | | |
|---|---|---|---|---|---|
| | CLO | CLO: LO (1:1) | LO | CLO: OO (1:1) | OO |
| **Liver** | | | | | |
| Cholesterol (mg/dl) | $15.81^{b}$ ± 1.10 | $18.43^{b}$ ± 1.71 | $17.32^{b}$ ± 2.01 | $19.54^{b}$ ± 1.81 | $28.00^{a}$ ± 2.61 |
| Triglycerides (mg/dl) | $21.56^{b}$ ± 0.91 | $19.43^{bc}$ ± 1.2 | $20.95^{bc}$ ± 0.91 | $24.90^{b}$ ± 0.61 | $41.29^{a}$ ± 1.21 |
| Lipid % | $1.35^{c}$ ± 0.05 | $2.56^{b}$ ± 0.50 | $2.88^{b}$ ± 0.24 | $4.80^{a}$ ± 0.10 | $4.63^{a}$ ± 0.50 |
| **Muscle** | | | | | |
| Cholesterol (mg/dl) | $43.12^{c}$ ± 1.33 | $41.50^{c}$ ± 1.56 | $57.08^{a}$ ± 1.53 | 48 0..$60^{b}$ ± 2.2 | $44.91^{cb}$ ± 0.97 |
| Triglycerides (mg/dl) | $72.19^{a}$ ± 4.15 | $70.29^{a}$ ± 4.46 | $55.13^{b}$ ± 2.32 | $54.14^{b}$ ± 0.19 | $73.76^{a}$ ± 4.66 |
| Lipid % | $0.86^{b}$ ± 0.08 | $0.84^{b}$ ± 0.12 | $0.58^{b}$ ± 0.08 | $0.70^{b}$ ± 0.043 | $1.22^{a}$ ± 0.11 |

$^{abc}$ *Means in the same row with the different superscript letter are significantly difference ($P<0.05$).*

The fish fed diet supplemented with OO had higher liver cholesterol and triglycerides levels than other experimental groups. While the muscle of the fish group fed the diet containing LO had higher level of cholesterol but had lower level of triglycerides than other treatment groups. The effect of substitution of CLO by vegetable oil on lipid peroxidation (MDA) is presented in [Table-5]. The lowest MDA concentration ($p<0.05$) of the liver and the muscle tissue were found in the fish fed diet containing OO.

*Table 5- Effect of substitution of cod liver oil by vegetable oil on muscle and liver peroxidation (MDA) of Nile tilapia*

| Source of Supplementary Oil | Liver lipid peroxidation (nmol MDA/g tissue) | Muscle lipid peroxidation (nmol MDA/g tissue) |
|---|---|---|
| 3% CLO | $26.72^{b} \pm 1.18$ | $4.48^{a} \pm 0.85$ |
| CO:LO (1:1) | $39.89^{a} \pm 0.66$ | $4.47^{a} \pm 1.06$ |
| 3% LO | $22.47^{c} \pm 2.14$ | $4.34^{a} \pm 0.90$ |
| CO: OO (1:1) | $15.27^{d} \pm 0.32$ | $4.16^{a} \pm 0.80$ |
| 3% OO | $10.10^{e} \pm 0.43$ | $2.22^{b} \pm 0.90$ |

$^{abcd}$ *means in the same column with the different superscript letter are significantly difference ($P<0.05$).*

### Microscopic Examination of the Liver and Muscle

The general architecture of the livers, the bile ducts and hepatopancreas showed no major abnormalities, with similar histological characteristics among all treatments. Histopathology of the fish liver sections from the different groups was shown in [Fig-1] - [Fig-5]. Dietary lipid seems to induce lipid accumulation in the liver. Different degrees of lipid accumulations in livers were detected.

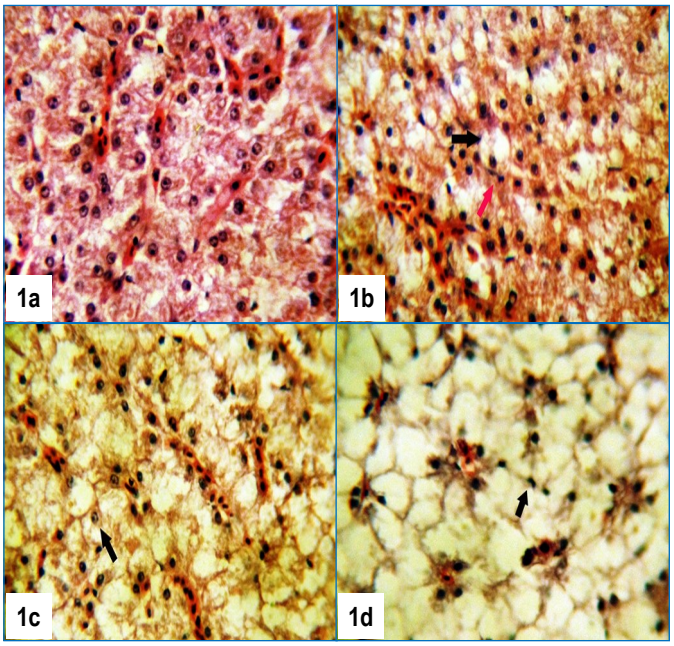


**Fig. 1- 1a-** Score 0; regular-shaped hepatocytes having centrally located nuclei with no noticeable lipid accumulation in their cytoplasm. Liver of fish fed 3% CLO. **1b-** Score 1; mild lipid accumulation in the cytoplasm of hepatocytes and many central round nuclei. The black arrow points to the swollen hepatocyte with large lipid vacuole and eccentric round nucleus, while the red arrow points to normal hepatocyte with central round nucleus. Liver of fish fed 3% LO. **1c-** Score 2; moderate lipid accumulation with eccentric round nucleus (black arrow); few central round nuclei are still seen. Liver of fish fed CLO: LO (1:1). **1d-** Score 3; severe lipid accumulation with few eccentric compressed nuclei (black arrow) and the majority of the nuclei were absent. Liver of fish fed 3%OO (HE, x 200).

Score 0 was given to livers from fish fed diet containing 3% CLO diet as they showed the best picture with regular-shaped hepatocytes having centrally located nuclei with no noticeable lipid accumulation in their cytoplasm [Fig-1a]. Livers from fish fed diet supplemented with 3% LO scored 1 as mild lipid accumulation in their cytoplasm and scattered irregularity in shape with many central round nuclei [Fig-1b] were seen. Livers from fish fed the diet containing a blend of CLO: LO (1:1 at 3% were scored 2 as the integrity of the hepatocytes was affected; swelling with higher degrees of vacuolization and multiple nuclear displacements were evident [Fig-1c]. Meanwhile, livers from fish fed a blend of CLO: OO (1:1) or 3% OO diets were scored 3 where apparent steatosis with intense lipid accumulation and diffuse eccentric compressed or absent nuclei were observed [Fig-1d].

Melanomacrophages (Mm) aggregates were observed in all livers samples randomly distributed in the parenchyma, but mostly close to hepatopancreas and portal veins. Ceroid pigment was found randomly distributed in hepatocytes of some sampled livers [Fig-2a]. Other histopathological findings were also recorded like rupture of portal vein, hemorrhage and congestion of hepatic blood vessels. Proliferation of the pancreatic acini (crowded pancreatic cells), absence of the acidophilic or basophilic portion of the pancreatic cells, bile stagnosis, shrunken and vacuolated pancreocytes were observed in some fish groups. Melanin was variably seen surrounding bile ducts. Periportal fibrosis with mononuclear cells (MNCs) and the presence of eosinophilic granulocytes (EGCs) in the pancreatic acini were also noticed. Some EGCs were degranulated as shown in [Fig-2], [Fig-3] and [Fig-4].

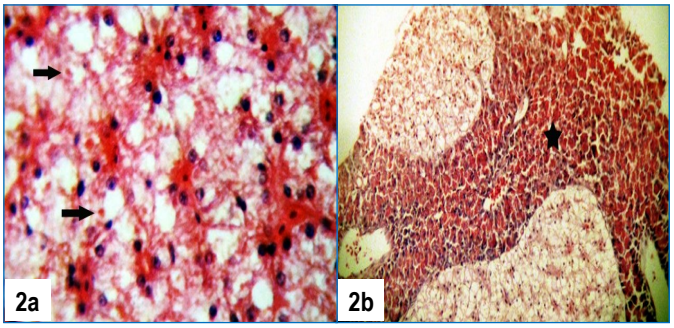


**Fig. 2-** Liver of *Nile tilapia* fed diet containing CLO: OO (1: 1). **2a-** Ceroid pigment in hepatocytes (arrows) (HE, x 200). **2b-** proliferation of pancreatic cells (asterisk). (HE, x 100)

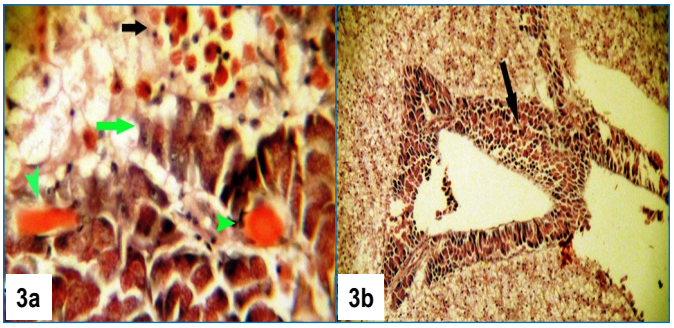


**Fig. 3-** Liver of *Nile tilapia* fed diet containing (3%) OO. **3a-** The absence of acidophilic portion of pancreocytes (green arrow) with bile stagnosis in side hepatopancreas (green arrowheads) and the presence of EGCs in portal region (black arrow) (HE, x 200). **3b-** shrinkage of pancreatic cells with infiltration of Mm (arrow) (HE, x 100).

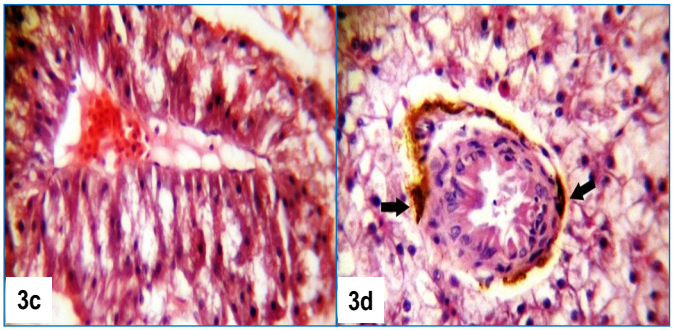


**Fig. 3-** Liver of Nile tilapia fed diet containing (3%) OO : **3c-** vacuolated pancreatic cells (HE, x 200). **3d-** melanin pigment is seen around bile duct (HE, x 200)

[Table-6] summarized the histopathological findings. According to PAS and PASD stains, fish fed 3% CLO or 3% LO containing diets showed higher glycogen deposits in their livers than other groups [Fig-5a], [Fig-5b]. Additionally, histopathological examination of dorsal muscle revealed normal appearance in 3% CLO diet group; mild hyaline degeneration in in muscle bundles of CLO: LO group; focal area of fibrosis in the LO diet group; hyaline degeneration with fibrosis in muscle bundles of the fish group fed a blend of CLO: OO and congestion, focal hemorrhage, few MNCs infiltration, edema among muscle bundles, hyaline degeneration in muscle bundles of the fish group fed 3% OO diet.

*Table 6- Summarized the histopathological findings*

| Histopathological findings/Groups No | 3% CLO | CLO:LO | 3% LO | CLO: OO | 3% OO |
|---|---|---|---|---|---|
| Lipid score | 0 | 2 | 1 | 3 | 3 |
| Ceroid pigment | - | + | + | ++ | - |
| Melanomacrophages | + | ++ | +/- | ++ | + |
| Rupture of portal vein | - | - | - | + | + |
| Congestion of hepatic BV | + | + | + | ++ | + |
| Melanin surrounding bile duct | - | - | - | ++ | ++ |
| Bile stagnosis | - | - | - | - | ++ |
| Absence of acidophilic portion | + | + | + | - | + |
| Proliferation of the pancreatic acini | - | - | - | + | - |
| Shrunken pancreocytes | - | - | - | + | ++ |
| Vacuolated pancreocytes | - | - | - | + | ++ |
| Periportal fibrosis with MNCs | - | + | + | ++ | + |
| The presence of EGCs in portal areas | + | + | +/- | ++ | +/- |
| Degranulation of EGCs | - | - | - | + | - |

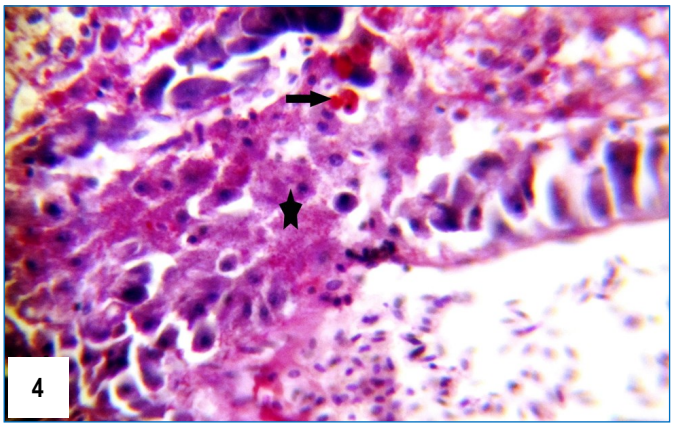


**Fig. 4-** Liver of *Nile tilapia* fed diet containing CLO: OO (1: 1). Hepatopancreas shows one EGC (black arrow) and many degranulated EGCs (star) (HE, x 200).

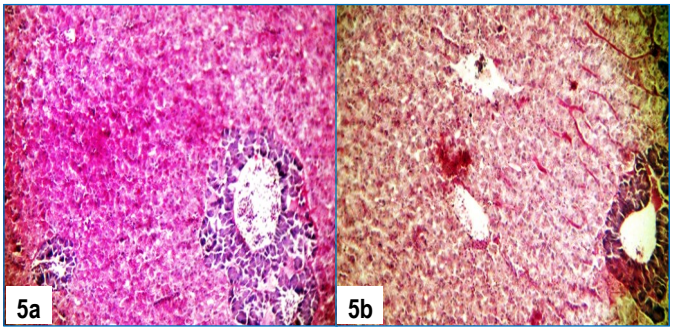


**Fig. 5-** Liver of fish fed diet containing (3%) LO. **5a-** The glycogen strongly stained in liver by PAS. **5b-** liver after diastase treatment shows weak staining indicating low glycogen content in liver (PAS, x 100).

## Discussion

The results of the present study indicated that the type of dietary supplemented oils significantly affected the growth performance parameters of *Nile tilapia*. The best growth rate, SGR and FCR and were observed in the groups fish fed the diet containing 3% CLO or LO at 3%. Many authors obtained conflicting results from their studies on the replacement of fish oil by vegetable oils. Shuichi, et al [24] found that 60% of fish oil could be replaced by rapeseed, linseed and olive oils without reduction of the growth rates in European sea bass *Dicentrarchus labrax*. With the same concept, Masiha, et al [25] observed that the highest body weight gain and feed efficiency of channel catfish fed diets containing CLO and LO. On the contrary, Kanazawa, et al [26] found no significant difference of body gain, specific growth rate, HSI and feed conversion ratio of rainbow trout fed diets containing either fish oil, flaxseed oil or a blend (1:1) of fish oil and flaxseed oil. The present data also showed that the lowest performance reported for the fish group fed the diet containing equal blend of CLO and LO [Table-2]. The blend of n-3 fatty acids (omega 3) of CLO and those of LO might have exceeded the biological tolerance limit of tilapia for n-3 fatty acids. High levels of n-3 PUFA (polyunsaturated fatty acids) have been reported to depress the growth of tilapia Huang, et al [27] Takeuchi, et al [28] and other researchers have showed that there was no enhancement in growth was obtained when 18:3n-3 (α linolenic acid) or n-3 HUFA (highly unsaturated fatty acids) was supplemented in tilapia diets [29].

The hepatosomatic index (HSI) values vary as a function of dietary protein, carbohydrates, and lipid level [30]. The viscerosoamtic index (VSI) is used to determine the rate of fat accumulated in all body of the fish. There was no significant difference in the HIS values obtained in the present study [Table-2]. Meanwhile, VSI of the fish fed diet supplemented with CLO or LO was higher than other groups. Ochang, et al [31] showed higher HSI of the rainbow trout fed diet had 100% fish oil than diet containing a blend of vegetable oils, but there were non significant differences of VSI among treatment groups. However, Hepher, et al [32] found that VSI of *Nile tilapia* fed diet containing fish oil was higher than VSI of other groups fed graded levels of palm oil and fish oil. The composition of fish showed no significant differences in moisture percentages of flesh or whole body of the fish and in ash percentage of fish flesh fed the experimental diets supplemented with different oils. However, the protein and ash percentages were highest in whole body of the fish group fed a blend of equal proportions of CLO and LO

According to Santiago, et al [33], endogenous and exogenous fac-

tors affect the body composition of fish. It should be noted that, the composition of the feed is the only factor, which could have influenced the difference chemical composition of fish, as other endogenous factors were maintained uniform during the study work. Moreover, Turchini, et al [10] stated that the data on the body composition of fish allows assessing the efficiency of transfer of nutrients from feed to fish and also helps to predict the overall nutritional status. Kanazawa, et al [26] observed that there was no significant difference in percentage of protein, ash, and moisture of whole body composition of rainbow trout fed diet supplemented with fish oil, flaxseed oil or equal proportions of fish oil and flaxseed oil. However, Turchini, et al [10] showed that the fish fed diet containing a blend of 75% of fish oil and 25% of vegetable oil at 6% of the diet had the highest crude protein among the other treatment groups. In this study, the total lipid percentage of the liver and the fish flesh was significantly affected by the different treatments as the diet supplemented with OO had higher lipid percentage in the liver and the flesh than other fed with other oils. In agreement with the present results other workers found differences in fillet and whole-body lipid levels of *Nile tilapia* depending on the source of dietary oil [34, 35]. With the same concept, Menoyo, et al [36] showed that the *Nile tilapia* fed diet containing 4% OO had the greatest lipid deposition in muscle accompanied by lower protein level compared to fish fed diets with fish and flaxseed oil. However, Ferreira, et al [37] reported that animal fed isocaloric diets and without changes in total lipid content usually do not differ in basic chemical composition. Ng, et al [38] observed that *Nile tilapia* fish fed diets with LO and FO had a lower percentage of total lipids in muscle compared with fish fed diet supplemented with olive, corn and soybean oil ($p < 0.05$). however, Phillips, et al [39] postulated that lipid deposition in the whole-body and muscle of African catfish was not significantly affected by different dietary lipid sources.

The fish group fed the diet containing 3% OO had higher levels of cholesterol and triglycerides in the liver samples than other experimental fish groups. While, the flesh of the fish group fed the diet containing LO had the highest level of cholesterol with a low level of triglycerides. However, Phillips, et al [39] found that the fish fed diet supplemented with FO had the highest total cholesterol and high-density lipoprotein and lower very-low-density lipoprotein concentrations ($p < 0.05$). On the other hand, Ochang, et al [31] observed that the diets of rainbow trout containing a blend of vegetable oils (55% rapseed oil, 30% palm oil, 15% linseed oil) induced a decrease in plasma cholesterol and low-density lipoprotein compared to diet containing a fish oil supplement. They attributed this to the type of fatty acids content of diet where in the diet containing blend of vegetable oils had high level of linolenic acid and oleic acid and also the presence of phytosterols which present in plant oisl and are known to decrease total cholesterol and LDL cholesterol in man [40,41].

The malonaldehyde (MDA), which is a secondary oxidation product during the breakdown of PUFAs, was used as an index compound for studying lipid peroxidation [42]. The lowest MDA concentrations ($p<0.05$) of the liver and the flesh were found in the fish fed diet containing 3% OO. This may be attributed to its high monounsaturated fatty acids content which mostly present in the form of oleic acid (18: 1n-9) which ranges from 70 to 80% of total fatty acids [43]. Moreover, Ali, et al [44] observed that the phenolics compounds of olive oil have antioxidant properties, higher than that of vitamin E, on lipids and DNA oxidation. In addition, a direct relationship was observed between lipid oxidation and the levels of PUFAs (polyunsaturated fatty acids) deposited in the tilapia fillets and liver. On the other hand, high levels of MDA in liver and flesh of the fish groups fed diets supplemented with CLO or LO is attributed to the richness of CLO and LO in long chain fatty acids and the flaxseed oil rich in alpha linolenic acid (18:3n-3) (53%) [7]. In addition, Lie, et al [45] reported that PUFAs are more easily oxidized than saturated fatty acids, and therefore, food products enhanced with the PUFAs n-3 are also more prone to lipid oxidation.

Results of histopathological examination in the current study demonstrated that different degrees of lipid accumulations in livers were detected indicating that the dietary lipid supplementation at 3% may induce lipid accumulation in the liver. Steatosis is classically described as a relatively mild liver alteration due to an excessive (or unbalanced) dietary intake of lipids which saturate the physiological capacity of the liver to handle, thus leading to lipid droplet (triglycerides; TG) accumulation. The synthesis and degradation of FA occurred mainly in the liver, and several enzymes regulating these pathways show varying affinities for the different fatty acids available in the organ [46]. Similarly, different degrees of lipid accumulations were detected in livers of catfish and common carp fed diets containing alternative lipid source: soyacid oil and yellow grease has been reported [47,48]. However, in sea bream, steatosis has been observed as a result of an EFA (essential fatty acids) deficiency [15], the use of artificial diets [49] and the inclusion of vegetable oils [14]. Our result indicated that, liver of fish groups supplemented with LO in the diet had lower vacuolization and higher glycogen content than in other groups. Dietary omega-3 and omega-6 polyunsaturated fatty acids (PUFA), present in fish oil, can regulate hepatic lipogenesis by reducing sterol-regulatory element-binding protein-1 in the liver [50]. Moreover, PUFA administration decreased fatty acids, such as oleate (C18:1 n-9), palmitate (C16:0) and palmitoleate (C16:1 n-7) [51]. LO is arguably one of the richest in polyunsaturated fatty acids (PUFA) and essential fatty acids (EFA), with 56.6% linolenic acid [$\omega$-3 (C18: 3 cis 9,12,15)] and 13.2% linoleic acid [$\omega$-6 (C18: 2 cis 9,12)], in addition to 17.8% monounsaturated fatty acid (MUFA) oleic acid [$\omega$-9 (C18: 1 cis 9)] [52]. Hepatic steatosis increased in CLO: LO, CLO: OO; that may be due to the imbalance in the dietary fatty acids. The latter could modify the functioning and morphology of liver. Also when dietary lipid or energy exceeds the capacity of the hepatic cells to oxidize fatty acids, the large synthesis and deposition of TG in vacuoles will result. Meanwhile, the increased hepatic steatosis in the fish group fed 3% OO supplemented diet was closely related to the higher levels of liver cholesterol and triglycerides. Hepatic steatosis due to excessive dietary fat contents is characterized by an increased collagen deposition, like other hepatic pathologies such as cirrhosis and fibrosis [53]. Similarly, fibrosis was absent in CLO supplemented group and variably detected around portal areas in other groups. Furthermore, EGC and MNCs were strongly present in CLO: OO (1:1) group; a marker of increased inflammatory state in their livers. Alternatively, they were weakly present in fish group fed LO supplemented diet. Fish oil found to increase arachidonic acid less than olive or butter-supplemented groups, because PUFA can confer anti-inflammatory effect [54]. Mm aggregates were observed in all livers samples. They seemed to be independent on type of diet. Coinciding with our results, Regost, et al [55] mentioned that the number, size and contents of Mm are highly variable, not only between species but also related to the health status of fish. LO significantly affected the organoleptic quality of flesh particularly odour, colour and texture [56]. Microscopically, muscle of fish group fed LO

had only a focal area of fibrosis that is very mild and expectedly nonspecific. The overall histopathological examination revealed the tendency of the different histopathological lesions to be concentrated in liver and muscle of fish fed a diet containing a blend of CLO: OO (1:1) or OO supplemented diets.

**Conclusion**

Supplementation of *Nile tilapia* diets with 3% LO was the best vegetable oil that can replace the CLO. Moreover, Inclusion of OO either at 3% in fish diet or mixed with CLO (1:1) at the same lvel did not improve any of the measured parameters and increased hepatic steatosis. However supplementation of the diet with OO reduced the lipid peroxidation of liver and fish flesh.

**Abbreviations**

CLO: Cod liver oil
LO: linseed oil
OO: olive oil
BW: body weight
WG: weight gain
SGR: specific growth rate
FCR: feed conversion ratio
HSI: Hepatosomatic index
VSI: viscerosomatic index
MDA: malondialdehyde
CP: crude protein
H&E: hematoxylin and eosin.
PAS: periodic acid-Schiff
PASD: periodic acid-Schiff diastase
Mm: melanomacrophages
MNCs: mononuclear cells
EGCs: eosinophilic granulocytes
FO: fish oil
PUFA: polyunsaturated fatty acids
HUFA: highly unsaturated fatty acids
TG: triglycerides
FA: fatty acids
EFA: essential fatty acids
LDL: low density lipoprotein
PER: protein efficiency ratio

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