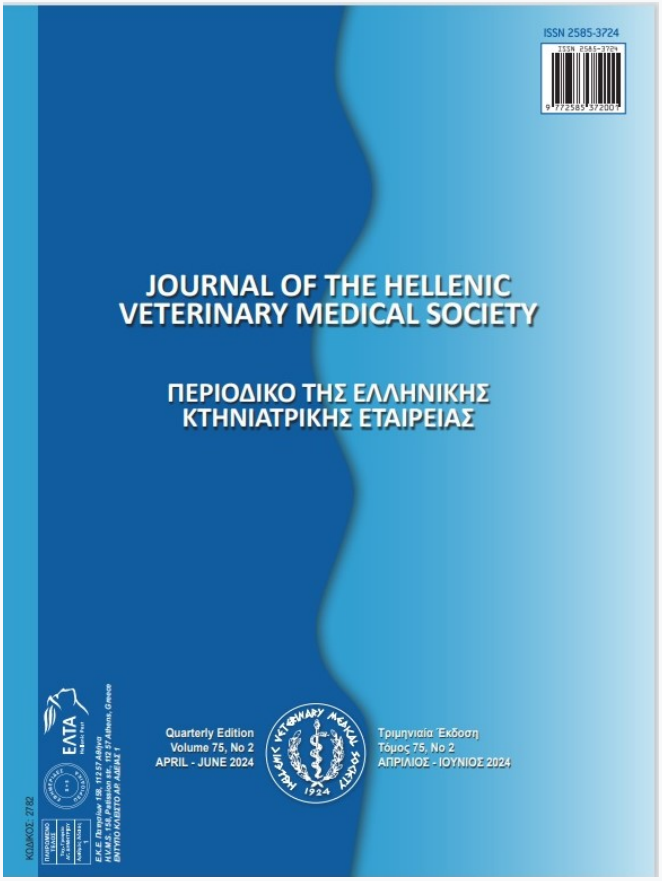
Journal of the Hellenic Veterinary Medical Society

Vol 75, No 2 (2024)



Canola oil and/or linseed oil improved growth performance, immune‐physiological and metabolic responses of Nile tilapia

AS Abdelhamid, AM Elnokrashy, NA Ebied, SH Al-Deriny, MF Abdelkader, NA Abozahra, RA Mohamed

doi: 10.12681/jhvms.34891

To cite this article:

Abdelhamid, A., Elnokrashy, A., Ebied, N., Al-Deriny, S., Abdelkader, M., Abozahra, N., & Mohamed, R. (2024). Canola oil and/or linseed oil improved growth performance, immune‐physiological and metabolic responses of Nile tilapia: plant oils improved tilapia health. *Journal of the Hellenic Veterinary Medical Society*, *75*(2), 7457–7470. https://doi.org/10.12681/jhvms.34891

J HELLENIC VET MED SOC 2024, 75 (2): 7457-7470
ΠΕΚΕ 2024, 75 (2): 7457-7470

Research article
Ερευνητικό άρθρο

Canola oil and/or linseed oil improved growth performance, immune-physiological and metabolic responses of Nile tilapia

A.S. Abdelhamid[1], A.M. Elnokrashy[2], N.A. Ebied[3], S.H. Al-Deriny[4], M.F. Abdelkader[5], N.A. Abozahra[6], R.A. Mohamed[7]*

[1]*Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafrelsheikh (P.O. 33516), Egypt*

[2]*Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafrelsheikh (P.O. 33516), Egypt*

[3]*Food Hygiene Unit, Animal Health Research Institute, Provisional Lab Kafr El-Sheikh, Agricultural Research Center (ARC), Egypt*

[4]*Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafrelsheikh (P.O. 33516), Egypt*

[5]*Food Hygiene Unit, Animal Health Research Institute, Provisional Lab Kafr El-Sheikh, Agricultural Research Center (ARC), Egypt*

[6]*Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafrelsheikh (P.O. 33516), Egypt*

[7]*Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafrelsheikh (P.O. 33516), Egypt*

Department of Fish health and Management, Sakha Aquaculture Research Unit, Central Laboratory for Aquaculture Research, A.R.C, Kafrelsheikh, Egypt

ABSTRACT: This study aimed to evaluate the synergistic effect of canola oil (CO) and linseed oil (LO) on growth performance, blood health, immune-oxidative status, and intestinal morphometry of Nile tilapia, *Oreochromis niloticus* fingerlings. Fish (n=270, 9.865±0.343g) were nearly similar initial body and randomly distributed in 18 hapas in triplicates at a rate of 15 fish per replicate for 90 days. Fish were divided into six groups: (1) fish received only basal diet (control group, CG), (2) basal diet containing 0.25% canola oil (CO 0.25%), (3) basal diet containing 0.5 % canola oil (CO 0.5%), (4) basal diet containing 1.0% canola oil (CO 1%), (5) basal diet containing 2% linseed oil (LO 2%), (6) basal diet containing a mixture of canola oil 0.1% and linseed oil 0.1% (CO1 x LO1%). The results showed that the fish received CO and/or LO had significantly ($p<0.05$) better growth performance, including final weight, weight gain, specific growth rate, and feed conversion ratio, than CG. The best results were found in fish that received CO/LO.

Corresponding Author:
Radi A. Mohamed, Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Kafr El-sheikh (P.O. 33516), Egypt
E-mail address: r.mohamed.vet@gmail.com

Date of initial submission: 08-07-2023
Date of acceptance: 04-12-2023

Hematological and biochemical profiles were conducted and showed that dietary CO and/or LO increased red blood cells, hemoglobin, white blood cells, total protein, globulin, cholesterol, triglycerides, and decreased lymphocytes and creatinine compared with CG ($p<0.05$) with the best findings in CO/LO. Antioxidant enzymes (increased catalase, superoxide dismutase, and reduced malonaldehyde), digestive enzymes (lipase and amylase), immune response (lysozyme and phagocytic index), and intestinal morphometry were improved when fish received CO and/or LO with the best results in CO/LO. To conclude, dietary incorporation of CO and/or LO improved growth performance, blood health, immune-oxidative status, and intestinal morphometry of Nile tilapia fingerlings. The best findings were reported in fish received CO/LO.

***Keywords*:** Nile tilapia; canola oil; linseed oil; growth performance; immune-physiological response.

INTRODUCTION

Aquaculture has a lot of promise to supply the growing demand for seafood and animal protein as an alternative to catch fisheries, accounting for approximately half of all fish consumed worldwide, with a high record production of 87.5 million tonnes in live weight in 2020 (FAO, 2022). Tilapia is the most farmed fish globally and Egypt is the African continent's top producer of cultured fish, with an average production of 1,591,900 tonnes in 2020 (FAO, 2022). Tilapia fish have their own qualities which enable their culture under different environmental conditions. These features include rapid growth, good acceptance of manufactured feed, improved feed efficiency, tolerance to a wide range of pH, salinity, and temperature, resistance to diseases, and stress (Elkaradawy et al., 2022).

Botanicals, phytobiotics, and phytogenies are terminology used to describe herbs, spices, and plant extracts (mostly essential oils). Phytobiotics are commonly employed in human traditional medicine due to their well-known pharmacological properties. Furthermore, phytobiotics have an important role in human nutrition as flavors and food preservatives with the potential to be used in livestock production (Sutili et al., 2018). Dietary lipids are a vital source of essential fatty acids which are needed for regular growth, general health, reproduction and biological functions (Bell, and Koppe, 2010). Vegetable oils are regarded as valuable lipid sources due to their low cost and widespread availability. Moreover, Orsavova et al., (2015) concluded that vegetables oils characterized by high concentrations of unsaturated fatty acids, monounsaturated fatty acids (MUFAs) have been shown to have several health benefits, including lowering low-density lipoprotein (LDL) cholesterol, slowing the progression of atherosclerosis by producing LDL that are highly resistant to oxidative modification, and lowering blood pressure. For instance, linseed oil (LO) is rich in polyunsaturated fatty acids (PUFAs) of n-3 series, and rapeseed oil (RO) is rich in monounsaturated fatty acids (MUFAs). The length of the carbon chain, degree of unsaturation, and concentration of fatty acids in lipid sources are the primary determinants influencing fish physiological and biochemical processes (Nakharuthai et al., 2020; Peng et al., 2016).

Rapeseed is known by many different names, including rape, oilseed rape colza, mustard, Rapa, and turnip rape, although it is increasingly being referred to as "canola". Canola oil (CO) has high-concentration of oleic acid (18:1n-9), followed by linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3), and characterized by constant availability, low price, superior edible, cooking characteristics, and not susceptible to oxidation (Wassef et al., 2016).

Linseed oil (LO), being one of the most common commercial vegetable oils that can be used to replace fish oils in fish diets. Linseed oils are rich in PUFAs especially α-linolenic acid (ALA) (18:3n-3) which can be converted to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), and linoleic acid (LA) to arachidonic acid (ARA) through the biosynthetic processes (Li, et al., 2016). Hence, the current study was aimed to evaluate the synergistic effect of CO and/or LO on growth performance, blood health, immune- oxidative status, and intestinal morphometry of Nile tilapia, *Oreochromis niloticus*, fingerlings.

MATERIALS AND METHODS

Fish husbandry experimental design, and diets preparation

The present study was conducted in concrete ponds (5x 3 x 1.5 m) in a private fish farm and hatchery (Kafr El-Sheikh governorate, Egypt). The Nile tilapia (*Oreochromis niloticus*) fingerlings used in the present study were obtained from the same place.

The fish was acclimated to the new environment for 2 weeks and fed the basal diet and the photoperiod was adjusted to 12 h light / 12 h dark. Fish (n=270, average weight= 9.865±0.343g) were nearly similar initial body and randomly distributed in triplicates at a rate of 15 fish per replicate. Each concrete pond was used to lodge 3 hapas (2 x 1 x 1.5 m) representing the 3 replicates. The fish were randomly divided into six experimental groups as follow:

The control group (CG): the fish received a basal diet (Table 1).

Canola oil 0.25 % (CO 0.25%) group: fish received basal diet contain 0.25% canola oil.

Canola oil 0.5% (CO 0.5%) group: fish received basal diet contain 0.5 % canola oil.

Canola oil 1% (CO 1%) group: fish received basal diet contain 1.0% canola oil.

Linseed oil 2% (LO 2%) group: fish received basal diet contain 2% linseed oil rich in polyunsaturated fatty acids (PUFA) (produced by Maasara Elnasr, Damanhur, Behera, Egypt.) as recommended by the manufacturer's instructions.

Canola oil 1% x linseed oil 1% (CO1 x LO1%) group: fish received basal diet contain a mixture of canola oil 0.1% and linseed oil 0.1%.

The tested feed additives (canola oil and linseed oil) were mixed with the experimental diet during feed manufacturing. The experimental diets were mixed, pelleted, and dried for 24 at room temperature, then stored at - 20 ˚C until used. Each hapa was supplied with air-stones as a source of aeration. About 30 % of the pond water volume was replaced every 2 days and the water column was maintained around 1 m. The water quality parameters were maintained around the normal levels of Nile tilapia (temperature: 25.67±1.32 °C, pH: 8.1±0.29, dissolved oxygen: 6.89±0.83 mg/ L, and total ammonia nitrogen: 0.081±0.007 mg/ L). Fish were fed diets (4 % of the total fish biomass) twice daily at 8:00 and 14:00 h for 90 days. Fish mortality was recorded daily, and dead fish were collected from each hapa.

Fish growth performance, feed utilization efficiency, and biometric indices

After a 90-day trial period, the sampled fish were collected in a clean polyethylene container and anaesthetized with clove oil (Merck, Germany, 50 µl/ litre water) in order to assess the performance of fish growth. Fish were weighed individually to obtain the final weight. The total length (L) of each fish was measured using a measuring board. Growth performance was determined and feed utilization was calculated as follows (Abozeid, et al.,2021): Weight gain (WG) = final body weight (W1) (g) - initial body weight (W0) (g); Specific growth rate (SGR % /day) =100× (lnW1−lnW0) / t; Feed conversion ratio (FCR) = feed intake (g)/ WG (g); Condition factor (K) =100 × (W1/ L^3); Hepato-somatic index (HSI)=100 ×(liver weight/

Table1. Ingredients and proximate chemical composition of control (basal) diet on dry matter bases

Ingredient	%	Chemical composition	%
Fish meal (60% CP)	3.2	Dry matter	90.0
Soybean meal	36.5	Crude protein	30.0
Corn gluten	8.0	Ether extract	6.02
Yellow corn	12.2	Crude fiber	4.95
Wheat middlings	22.5	Ash	5.1
Poultry byproducts meal	4.0	[2]NFE	53.93
Rice bran	8.0	Available phosphorus	0.4
Soy oil + rapeseed oil	2.0	Calcium	0.99
Mono-calcium phosphate	0.6	[3]Gross energy (MJ kg^1)	18.73
Common salt	0.5		
Calcium carbonate	0.5		
[1]Premix	2.0		

[1] Premix (mg kg^{-1}): vitamin A (3300 IU), vitamin D3 (410 IU), vitamin E (200 mg), vitamin B1 (133 mg), vitamin B2 (580 mg), vitamin B6 (410 mg), vitamin B12 (50 mg), biotin (9330 mg), choline chloride (4000 mg), vitamin C (500 mg), inositol (330 mg), para-amino benzoic acid (9330 mg), niacin (26.60 mg), pantothenic acid (2000 mg), manganese (325 mg), iron (200 mg), copper (25 mg), iodine, cobalt (5mg).
[2]NFE: nitrogen-free extract calculated as follows: NFE = 100 − (crude protein + ether extract + crude fiber + ash).
[3]GE: Gross energy calculated on the basis of 23.6, 39.4, and 17.2 k joule gross energy/g protein, ether extract, and NFE, respectively.

W1); Viscero-somatic index (VSI)= 100 ×(intestine weight/W1) and Survival rate (SR %) = (total number of fish at the end of the experiment /total number of fish at the start of the experiment) × 100. Where "t" is the experimental period (days).

Diet and whole-body chemical composition

Prior to starting the experiment, the diet's composition was analyzed. Five fish from each replication were randomly chosen at the end of the feeding trial to be used for measuring the whole-body chemical composition. The fish were then stored in the deep freezer at -40 °C until needed. According to the AOAC, (2007) procedures, the nutritional profile of test diets and the whole fish body composition (crude protein, crude fat, carbohydrates, moisture, and ash) were both calculated.

Blood sampling and serum separation

At the end of the experimental period, blood samples (9 fish/ group) were collected from the caudal vein in vacuum tubes. For blood serum collection, plain tubes without anticoagulants were used. The clotted blood was centrifuged at 300 rpm for 15 min at 4°C then the supernatant serum was aspirated and kept in plastic Eppendorf tubes at −40˚C. For hematological analysis, tubes containing an anticoagulant (Ethylenediaminetetraacetic acid, EDTA) was used (Elkadom et al. 2023).

Hematological analyses

Using hemocytometer and Natt-Herrick solution, the erythrocytes and leukocytes were counted according to the method described by Stoskopf (1993). Using the cyanmethemoglobin method Drabkin's solution, hemoglobin concentration was determined according to Stoskopf (1993). According to Dacie and Lewis (1991), the packed cell volume (PCV) was determined by the microhematocrit method. Thin blood films were prepared, air-dried, fixed with methanol for 3-5 minutes, stained with Gimsa stain for 8-10 minutes, and then allowed to dry in order to determine differential leukocytic count (DLC). The white blood cells were counted among one hundred blood smears according to Stoskopf (1993) and Thrall et al., (2004).

Serum biochemical analysis

Total proteins and albumins were determined according to the methods described by Doumas et al., (1981) and Dumas and Biggs (1972) respectively. Globulins content was calculated mathematically. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined using the colorimetric method at the wavelength 540 nm as described by Reitman and Frankel (1957). Serum creatinine was determined by the colorimetric method (Heinegård and Tiderström, 1973). Serum triglyceride and total cholesterol were analyzed according to the manufacturer's instructions of commercial clinical kit (GPO-PAP and CHOD-PAP) methods, respectively (Li et al., 2018).

Antioxidant capacity

The antioxidant activity was measured in 9 fish/ group. The activity of superoxide dismutase (SOD), catalase (CAT) and malonaldehyde (MDA) were measured using ELISA kits (Inova Biotechnology, China) at the wavelength 450 nm using the microplate ELISA reader (Kiron et al., 2011).

Digestive enzymes activity

The digestive enzyme activities in fish (9 fish/ group) serum were assayed using the diagnostic reagent kits according to the manufacturer's instructions (Cusabio Biotech Co. Ltd., Wuhan, Hubei, China). According to methods described by Abdel-Tawwab et al., (2018), the activities of digestive enzymes (lipase and amylase) were measured.

Immune response

According to Demers and Bayne's (1997) methodology, the lysozyme activity was measured by ELISA based on the ability of the enzyme to lyse the Gram-positive, lysozyme-sensitive bacteria *Micrococcus lysodeikticus* at a wavelength of 450 nm. Whole blood smears were made in accordance with the procedure described by Kawahara et al., (1991) for the measurement of phagocytic activity and phagocytic index. The phagocytic activity and index were calculated according to the following equations:

Phagocytic activity = macrophages containing yeast cells/total number of macrophages × 100

Phagocytic index = number of yeast cells phagocytized/number of phagocytic cells.

Liver and intestine histomorphology

Upon necropsy of the fish at the end of the 90 days experimental trial, liver and intestine samples were directly collected from 6 fish from each group, washed with ice-cold 1.15% KCl to remove any blood or undesirable materials, and then dried on filter paper

and preserved in 10% phosphate buffered formalin for at least 48 hours. The fixed specimens were processed by the conventional paraffin embedding technique, sectioned at 4-5 μm thick sections and finally stained with hematoxylin and eosin stain (H & E). By employing a light microscope (Lecia, DM750 P) with a camera-equipped section examination, the histological assessment was evaluated as previously described by Suvarna et al., (2018). The intestinal villi length was measured using Image-J analysis software, US National Institutes of Health, Bethesda, MD (http://rsb.info.nih.gov/ij/). In the calibrated images, the villus length was measured from submucosa to the villus tip at 5 locations per slide (Rahimi et al., 2019).

Statistical analysis

Data distribution normality was examined, and residual analysis was used to corroborate the normality. The processing of percentage data was preceded by an arcsine transformation before data analysis. Graph Pad Prism 7 software was used to statistically analyse the data (Graph Pad Prism v7.0, San Diego, CA, USA). Data were subjected to a one-way ANOVA and then Tukey's multiple comparison tests to identify the differences between the means that were statistically significant. Comparisons were made at a 5% probability level.

RESULTS

Growth performance and biometrics indices

As presented in Table 2, the growth performance of Nile tilapia in terms of final body weight (FBW), weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) showed significant difference ($P<0.05$) in fish fed diets supplemented with CO and/or LO as compared to the CG. However, hepato-somatic index (HSI), viscera-somatic index (VSI), final length (FL), condition factor (CF), and survival rate showed non-significant differences ($P>0.05$). Fish received diets supplemented with CO1xLO1%, CO1%, and LO2% increased significantly more than CG in FBW, WG, and SGR respectively except with LO2% in SGR *($P<0.05$)*. Fish received CO and/or LO diets showed significant improvement in FCR compared to the CG. The lowest FCR values were observed in fish received diets supplemented with CO1xLO1% followed by CO1% and LO2%.

Proximate chemical composition of fish body

The percentage of protein, lipid, ash, fiber, and nitrogen-free extract of the fish body showed a non-significance difference($P>0.05$) between all experimental groups, and high protein percent was reported in the CO1x LO1 % group (Table 3).

Table2. Growth performance and biometric indices (Mean ± SEM) of fish fed experimental diets for 90 days.

	CG	CO 0.25%	CO 0.5%	CO 1%	LO 2%	CO1xLO1%	*P*-value
IBW (g)	9.85±0.19	9.78±0.24	9.96±0.17	9.84±0.16	9.94±0.16	9.82±0.14	0.9673
FBW (g)	74.90±2.75^{b}	81.20±1.46^{ab}	82.60±1.81^{ab}	86.60±1.44^{a}	85.20±2.18^{a}	89.40±2.44^{a}	0.0010
WG (g)	65.05±2.82^{b}	71.42±1.49^{ab}	72.64±1.96^{ab}	76.76±1.51^{a}	75.26±2.07^{a}	79.58±2.46^{a}	0.0013
SGR (%/d)	3.38±0.07^{b}	3.53±0.05^{ab}	3.53±0.06^{ab}	3.63±0.05^{a}	3.58±0.03^{ab}	3.68±0.05^{a}	0.0141
FI (g)	94.28±3.37	99.79±3.27	98.54±3.08	102.0±2.35	99.95±2.89	101.20±4.03	0.6147
FCR	1.45±0.03^{c}	1.40±0.02^{bc}	1.36±0.01^{b}	1.33±0.01^{ab}	1.33±0.01^{ab}	1.27±0.02^{a}	<0.0001
FL (cm)	14.98±0.30	15.30±0.40	15.34±0.48	15.66±0.45	15.52±0.49	15.86±0.49	0.7791
CF (K)	2.22±0.04	2.27±0.01	2.29±0.03	2.26±0.03	2.28±0.03	2.24±0.03	0.6940
HSI (%)	1.69±0.07	1.59±0.06	1.58±0.08	1.60±0.06	1.61±0.07	1.70±0.09	0.7637
VSI (%)	4.22±0.23	4.12±0.08	4.43±0.09	4.35±0.17	4.18±0.15	4.27±0.08	0.6757
Survival %	100	100	100	100	100	100	--------

Means within the same row lack common superscripts are significantly different at $P<0.05$.
Initial body weight (IBW), Final body weight (FBW), weight gain (WG), specific growth rate (SGR), feed intake (FI), feed conversion ratio (FCR), final length (FL), condition factor (CF), Hepato-somatic index (HSI), viscera-somatic index (VSI)

Table3. Proximate chemical composition (Mean ± SEM) of fish (% on dry matter basis) fed experimental diets for 90 days

%	CG	CO 0.25%	CO 0.5%	CO 1%	LO 2%	CO1xLO1%	*P*-value
Protein	61.52±3.56	62.66±4.69	63.82±3.91	63.51±3.52	63.64±4.67	64.02±2.43	0.3821
Lipid	15.60±1.39	15.71±2.03	16.02±0.69	17.73±1.83	15.94±1.03	15.99±1.37	0.7924
Ash	16.87±2.02	16.53±1.38	14.68±1.29	14.42±1.04	16.06±0.72	15.83±0.91	0.8141
Fiber	4.79±0.14	3.23±0.06	3.45±0.13	2.45±0.05	2.91±0.16	2.61±0.08	0.1591
NFE	1.22±0.08	1.87±0.07	2.03±0.03	1.89±0.04	1.45±0.02	1.55±0.02	0.4517

Means within the same row lack common superscripts are significantly different at $P<0.05$.
Nitrogen-free extract (NFE)

Hematological profile

The results of hematological parameters are presented in Table 4. Red blood cells (RBCs), hemoglobin (Hb), White blood cells (WBCs), and lymphocytes showed a significant difference (P<0.05) in fish fed CO and/or LO diets compared to the CG. Higher significant values of RBCs and Hb were observed in fish received diets supplemented with CO1xLO1% followed by LO2% and CO1%. Moreover, WBCs were significantly higher in fish received LO2 % diet than CO1% and CO1xLO1% diets. Lymphocytes showed a significant decrease in fish received CO 0.5% diet followed by CO1xLO1%, LO2%, CO.25%, and CO1% respectively compared to the CG. while a non-significant difference was reported in PCV, neutrophils, and monocytes between all experimental groups (P>0.05).

Serum biochemical profile

The results in Table 5 revealed that total protein (TP), globulin, creatinine, cholesterol, and triglyceride were significantly different in fish received CO and/or LO diets compared to the CG (P<0.05). TP, globulin, cholesterol, and triglycerides showed a rise in fish fed CO and/or LO diets compared with the CG with the highest values of TP, globulin, and cholesterol were observed in CO1xLO1%, followed by CO 0.5%, then LO2%, while the highest triglycerides values were reported in CO1xLO1%, followed by LO2%, then CO 0.5%. Creatinine level was decreased in fish fed CO and/or LO diets except for the CO 0.25 % group in respect to the CG. However, values of albumin, ALT, and AST showed non-significant differences in respect to the CG (P<0.05).

Antioxidant parameters

Figure 1 presented the activity of CAT, SOD, and MDA in fish fed CO and/or LO diets whereas there was a significant difference (P<0.05) in comparison to the CG. The best findings of CAT activity with a significant increase were found in fish received LO2% diet followed by CO1xLO1% and CO1% (Figure 1A). In addition, fish received CO1xLO1% diet showed the highest increase in the SOD activity followed by LO2% diet (Figure 1B). Dietary supplementation of Nile tilapia with CO and/or LO significantly reduced MDA activity (P < 0.05) and the lowest level was reported in LO2% and CO1xLO1% groups and the highest level was reported in the CG (Figure 1C).

Digestive enzymes activities

Figure 2 showed the activity of amylase and lipase

Table 4. Hematological parameters (Mean ± SEM) of fish fed experimental diets for 90 days

	CG	CO 0.25%	CO 0.5%	CO 1%	LO 2%	CO1xLO1%	*P*-value
RBCs (x10^6/mm^3)	3.22±0.14^b	3.97±0.05^{ab}	4.24±0.07^a	4.23±0.16^a	4.31±0.23^a	4.50±0.11^a	0.0005
Hb (g/dL)	9.82±0.38^b	12.0±0.20^{ab}	12.87±0.23^a	12.95±0.43^a	13.04±0.67^a	13.57±0.34^a	0.0004
PCV (%)	38.50±1.44	38.50±0.29	41.50±0.29	41.00±1.16	42.00±2.31	40.00±1.16	0.5123
WBCs (x10^3/mm^3)	11.30±0.27^c	12.21±0.39^{bc}	13.05±0.23^b	13.78±0.02^b	15.15±0.71^a	13.44±0.23^b	0.0001
Lymphocytes (%)	13.50±2.87^a	9.50±1.29^b	8.50±2.29^b	10.50±1.27^b	9.50±1.24^b	9.50±1.27^b	0.0314
Neutrophils (%)	78.50±7.28	80.50±6.27	82.00±4.58	79.00±3.11	81.50±3.44	81.50±3.87	0.2750
Monocytes (%)	6.00±0.58	8.00±0.34	8.00±0.57	8.50±0.29	7.50±1.44	7.50±0.28	0.2537

Means within the same row lack common superscripts are significantly different at P<0.05.
Red blood cells (RBCs), hemoglobin (Hb), packed cell volume (PCV), white blood cells (WBCs)

Table 5. Serum biochemical parameters (Mean ± SEM) of fish fed experimental diets for 90 days

	CG	CO 0.25%	CO 0.5%	CO 1%	LO 2%	CO1xLO1%	*P*-value
TP (g/dL)	4.29±0.10^b	4.52±0.08^{ab}	4.92±0.05^{ab}	4.45±0.27^{ab}	4.67±0.24^{ab}	5.18±0.02^a	0.0161
Albumin (g/dL)	1.63±0.01	1.67±0.02	1.67±0.03	1.63±0.02	1.62±0.02	1.65±0.03	0.3358
Globulins (g/dL)	2.66±0.11^c	2.85±0.09^{bc}	3.25±0.02^a	2.83±0.29^{bc}	3.06±0.22^{ab}	3.53±0.01^a	0.0216
ALT (U/l)	33.98±2.43	29.74±2.05	30.70±0.32	27.57±0.41	27.97±1.20	29.34±1.04	0.0898
AST (U/l)	31.13±1.14	27.48±1.78	24.82±1.97	26.60±1.97	26.39±0.42	23.64±0.87	0.0535
Creatinin (mg/dL)	0.39±0.01^a	0.40±0.02^a	0.37±0.02^{ab}	0.33±0.01^b	0.32±0.01^b	0.33±0.02^b	0.0064
Cholesterol (g/dL)	84.34±3.34^b	92.57±4.31^{ab}	100.3±3.44^a	93.20±3.93^{ab}	99.08±4.60^a	101.1±3.59^a	0.0156
Triglyceride (mg/dL)	91.19±0.63^b	98.20±0.60^{ab}	99.57±0.31^{ab}	96.07±3.51^{ab}	106.1±3.42^a	108.10±5.2^a	0.0140

Means within the same row lack common superscripts are significantly different at P<0.05.
Total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST)

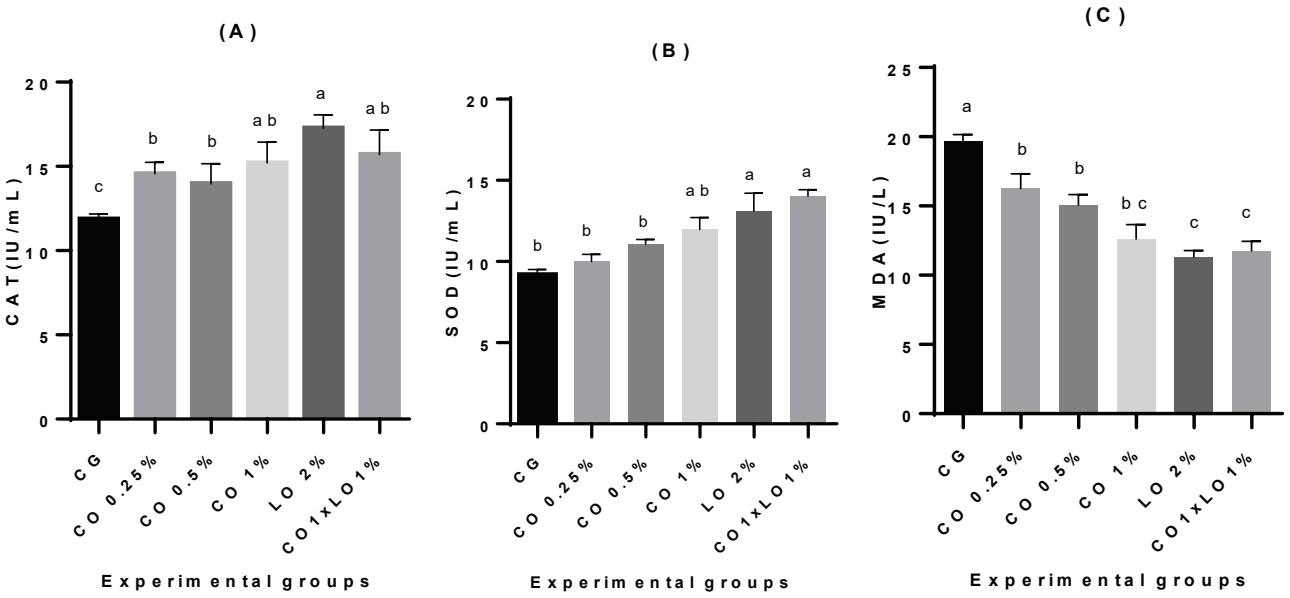


Figure 1. Oxidative parameters (A- CAT, B- SOD, and C- MDA) of fish fed experimental diets for 90 days. The columns (mean± SEM) with different letters are significantly different (P<0.05).

Catalase (CAT), superoxide dismutase (SOD), and malonaldehyde (MDA)

enzymes among experimental groups. The results showed a significant increase (P<0.05*)* in Nile tilapia fed CO and/or LO diets in comparison to CG. The highest levels of digestive enzymes were reported in CO1xLO1%, CO1%, and LO2%.

Immune response

The immune response of Nile tilapia fed different experimental diets are presented in figure 3. Significant differences were found between experimental and control diets in lysozyme activity, and phagocytic index (P<0.05). Lysozyme activity showed a significant increase in all experimental diets compared to the control one. Fish received CO1xLO1% diet showed more increase than other groups in lysozyme activity and phagocytic index. Meanwhile, the CG was the lowest in all measured immune response parameters.

Intestinal and hepatic histomorphometry

Figure 4 and Table 6 showed significant improvement in intestinal histomorphology (villi length, width, surface area, crypt depth, goblet cells) in fish fed CO and/or LO diets compared to control diet (P<0.05). The best findings of different parts of intes-

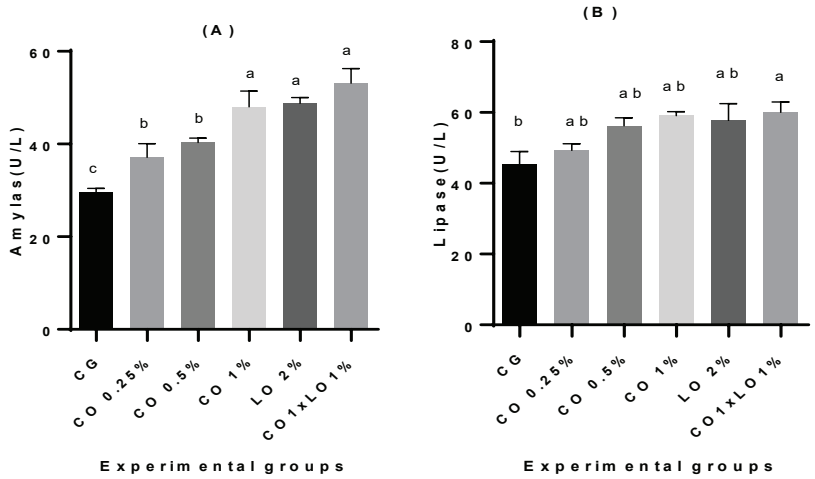


Figure 2. Digestive enzyme activity (A- amylase, B- lipase) of fish fed experimental diets for 90 days. The columns (mean± SEM) with different letters are significantly different (P<0.05).

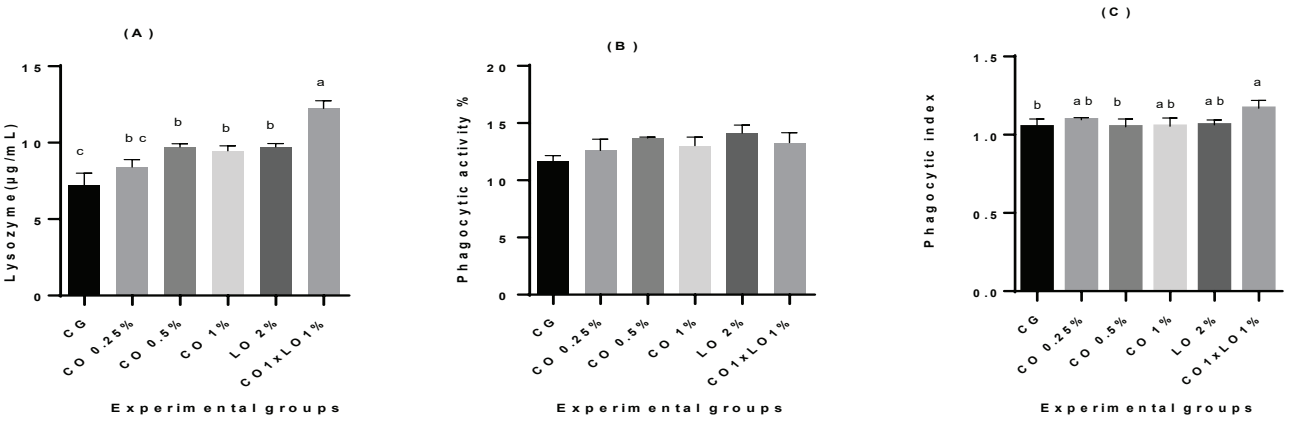


Figure 3. Immune response (A- lysozyme activity, B- Phagocytic activity, and C- Phagocytic index) of fish fed experimental diets for 90 days. The columns (mean± SEM) with different letters are significantly different (P<0.05).

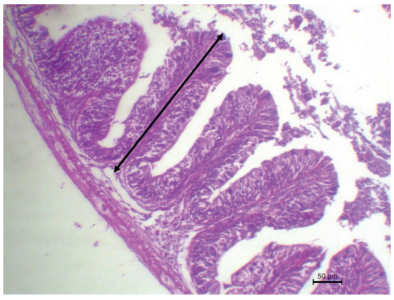
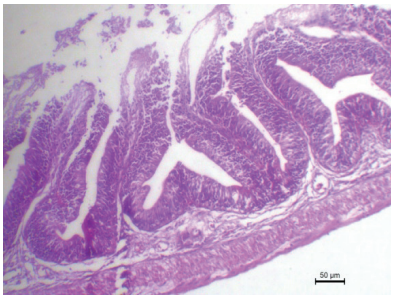
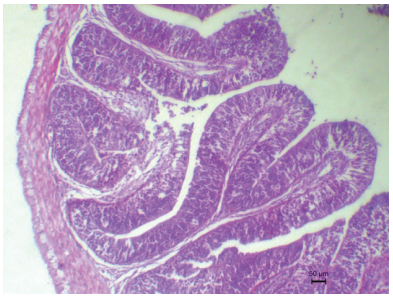
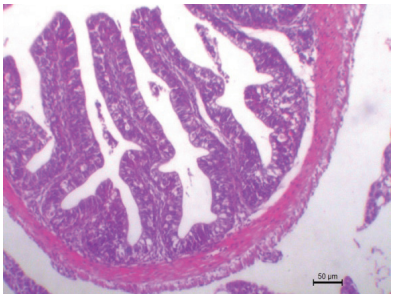
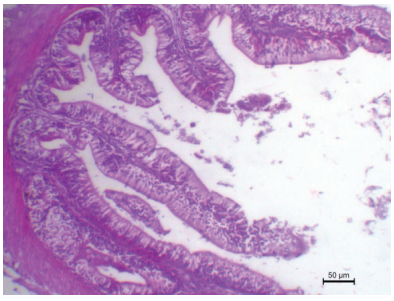
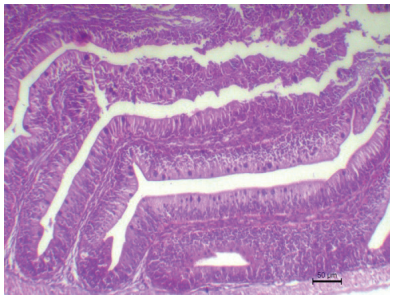
Experimental groups	**CG**	**CO 0.25%**
Photomicrograph of middle intestine		
Comment	Normal villi histology. Histomorphometry of intestinal villi length was measured from submucosa to the villus tip (arrow).	Normal villi histology with slight increase of villi length
Experimental groups	**CO 0.5%**	**CO 1%**
Photomicrograph of middle intestine		
Comment	Increase the villi length	Considerable increase of villi length
Experimental groups	**LO 2%**	**CO1xLO1%**
Photomicrograph of middle intestine		
Comment	Considerable increase of villi length	Marked increase of villi length

Figure 4. Haematoxylin-eosin-stained (H&E, X50) photomicrograph of the middle parts of intestine of fish fed experimental diets for 90 days

Table 6. Histo-morphometric analysis of middle intestine (Mean ± SEM) of fish fed experimental diets for 90 days

	CG	CO 0.25%	CO 0.5%	CO 1%	LO 2%	CO1xLO1%	*P*-value
Villi length(µm)	298.8±16.90^{d}	374.7±7.85^{cd}	425.2±14.34^{c}	520.2±24.13^{b}	564.1±12.21^{b}	798.4±24.72^{a}	0.0001
Villi width (µm)	149.4±8.45^{d}	187.4±3.93^{cd}	212.6±7.17^{c}	260.1±12.07^{b}	282.1±6.11^{b}	399.2±12.36^{a}	0.0001
Crypt depth (µm)	37.34±2.11^{d}	46.84±0.98^{cd}	53.15±1.79^{c}	65.03±3.02^{b}	70.52±1.53^{b}	99.80±3.09^{a}	0.0001
Villi surface area (mm^2)	0.018±0.004^{d}	0.048±0.002^{cd}	0.054±0.003^{c}	0.066±0.002^{b}	0.072±0.002^{b}	0.10±0.005^{a}	0.0001
Inter-villi space (µm)	99.8±3.09^{a}	70.52±1.53^{b}	53.15±1.79^{c}	51.03±3.15^{c}	46.84±2.98^{dc}	37.34±2.11^{d}	0.0041
Goblet cells/mm^2	18.67±1.06^{d}	23.42±0.49^{cd}	26.58±0.90^{c}	32.52±1.51^{b}	35.26±1.76^{b}	49.90±1.55^{a}	0.0001

Means within the same row lack common superscripts are significantly different at $P<0.05$.

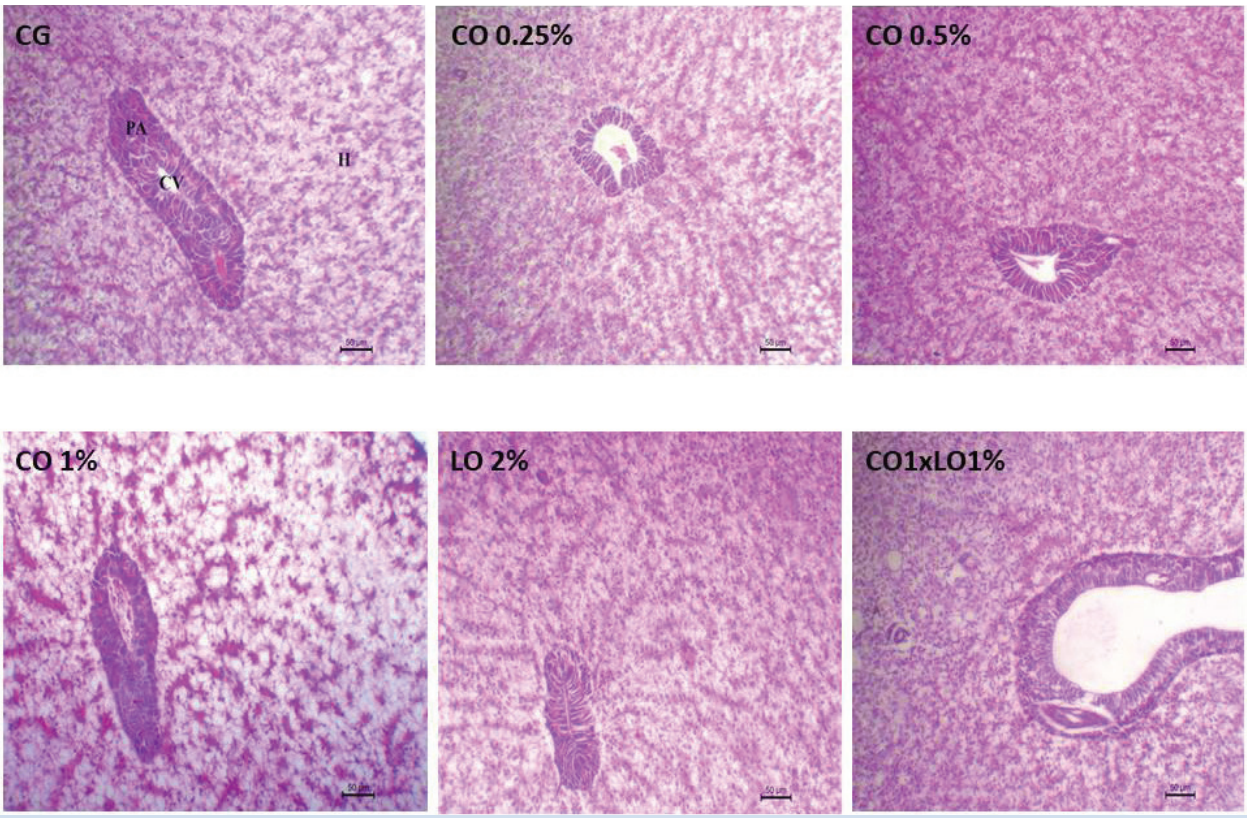


Figure 5. Haematoxylin-eosin-stained (H&E, X50, 200 µm) photomicrograph of the liver of fish fed experimental diets for 90 days. CG (control) showing normal histology featuring polygonal hepatocytes cords (H), and pancreatic acini (PA) around central vein (CV). Liver CO 0.25%, CO 0.5%, CO 1%, LO 2%, and CO1xLO1% show normal appearance of the hepatic histology.

tinal morphometry were found in CO1xLO1% group followed by LO2%, CO1%, CO 0.5% then CO 0,25% with a reduction in inter villi space respectively. The liver histology of fish received CO and/or LO diets showed a normal appearance (normal shaped vacuolated hepatocytes) (Figure 5).

DISCUSSION

Recently, it has become essential to search for alternative ingredients that maintain the welfare, health and immunity of the fish, while reducing production costs to maintain sustainable aquaculture production (FAO, 2020). Plant essential oils in last years had been used as feed additives due to its sustainable nutritional values and benefits in many aquatic species such as improving growth, immunity, general health condition, antioxidant capacity, and flavor and texture of fillet of fish (Turchini et al., 2009; Mahmoud & Miyashita, 2011; Sutili et al., 2018; Abdel-Rahim et al., 2023). Accordingly, the current study was aimed to assess synergistic effect of canola (rapeseed) oil, linseed oil on performance, blood health, immune-oxidative status, intestinal morphometry and liver histology of Nile tilapia *(Oreochromis niloticus)* fingerlings. Canola oil (CO) and linseed oil (LO) characterized by high levels of monounsaturated fatty acids (MUFAs) and n-3 polyunsaturated fatty acids (PUFAs) respectively (20:3n-6, 20:3n-3, 18:2n-6, 18:3n-3) (Turchini, et al., 2011; Francis et al., 2010; Dupont-Cyr et al., 2022). One of the primary variables that affect the physiolog-

ical and biochemical processes of fish are the length of the carbon chain, the degree of unsaturation, and the amount of fatty acids present in lipid sources (Peng et al., 2016; Cottrell et al., 2020). In general, fatty acids are essential for many biological processes in fish like reproduction, immune, and growth performances. For instance, most of the cellular membrane is made up of phospholipids, which also preserve the flexibility and structure of the membrane. Polyunsaturated fatty acids (PUFAs) with 20 carbons (n-3 and n-6) are the source of eicosanoids. Eicosanoids are bioactive signaling lipids that control immune response and inflammation, and they are made up of prostanoids, thromboxanes, prostacyclins, and leukotrienes (De Pablo & Álvarez De Cienfuegos, 2000; Tocher, 2003; Nakharuthai et al., 2020; García-Meilán et al., 2023).

In the current study, Nile tilapia fingerlings fed diets supplemented with CO (with different concentrations) and/or LO showed improvement in growth performance and feed utilization, expressed by markedly increase in final body weight, weight gain, specific growth rate with a better feed conversion ratio compared to the control group, and the best findings were found in fish received diets with CO1xLO1% followed by CO1% and LO2% with non-significant difference in viscero-somatic index (VSI) and hepato-somatic index (HSI) between all experimental groups. Similarly, these results were reported by Elkaradawy, et al., (2022) who stated that Nile tilapia received a diet supplemented by LO 2% showed a significant improvement in growth performance parameters. Also, in other study reported by Nakharuthai et al., (2020) who used different vegetables lipid sources on the feed of Nile tilapia (palm oil, soybean oil, and linseed oil) and there were no significant differences in all groups in weight gain and feed efficiency with a high result observed in linseed oil. In addition, Rossetto et al., (2021) stated that substitution of soybean oil with linseed oil improved the growth performance of Nile tilapia. Furthermore, Taylor et al., (2013) reported that replacement of 50% fish oil with canola oil does not affect the growth performance of Nile tilapia, similar results were found in Zhou & Yue, (2010) who replaced soybean meal and fish oil meal with canola meal respectively without affecting growth performance of Nile tilapia. The results of the current study were consistent with previous studies that proved that using linseed oil and canola oil improved growth performance of Nile tilapia (Peng et al., 2016), Atlantic salmon (*Salmo salar L.*) (Rosenlund et al., 2001), Murray cod (*Maccullochella peelii peelii*) (Francis et al., 2010), as well as using of canola oil in gilthead sea bream feed (*Sparus aurata L.)*(Fountoulaki et al., 2009), rainbow trout (Turchini et al., 2013). Those results indicate that PUFAs supplied from CO and/or LO met the demands of fish for physiological competence and normal growth. Canola oil is rich in 18:2n-6, 18:3n-3 fatty acids and MUFAs which gives a better ratio between both of them which reflects on a good tilapia growth performance. Additionally, 18:1(n-9) in a high concentrated CO diet is regarded as a desirable substrate to produce energy. Likewise, LO is rich by n-3 fatty acids which are a source for linolenic acid (18:3n-3), eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3) which are important for fish health (Wall et al., 2010, National Research Council, 2011, Peng et al., 2016: Huang et al., 2022) Consequently, the diet with CO and LO in the present study showed a synergistic effects induced the best findings in growth performance and feed utilisation of tilapia.

The present study has demonstrated that the inclusion of CO and/or LO in the diets of Nile tilapia fingerlings did not induce any significant changes in their proximate chemical composition, including protein, lipid, and ash. These findings are consistent with those of previous studies, such as Peng et al. (2016), who used rapeseed oil and linseed oil in Nile tilapia feed and observed no variations in moisture, lipid, protein, and ash of the fish. Furthermore, Francis et al. (2010) found similar results when canola oil and linseed oil were used in Murray cod feed. Additional studies by Teoh et al. (2011), Rossetto et al. (2021), and Elkaradawy et al. (2022) documented that using LO in Nile tilapia diets have also reported similar findings. Similarly, Guan et al. (2023), Taylor et al. (2013), and Fountoulaki et al. (2009) observed that the inclusion of canola oil did not significantly affect the chemical composition of Tilapia, Rainbow trout, and Gilthead seabream, respectively. The use of CO and/or LO as a combination feed additive for fish and fish consumers was shown to be safe.

Hematological parameters are important indicators for the overall health and nutritional status of fish. The current study showed that there was a significant difference with an increase in RBCs, Hb, WBCs in Nile tilapia received diets with CO and /or LO except for CO 0.25% than the control group. Besides, a significant decrease in lymphocytes was reported in experimental fish groups that received CO and /or LO supplemented diet than control group. However, PCV,

neutrophils, and monocytes showed non-significant differences between different experimental groups. The increase of RBCs, Hb, WBCs in fish received diet supplemented with canola oil may be due to the better utilization of protein from the feed (Taşbozan et al. 2015). Zhou & Yue, (2010) reported that replacement of soybean meal by canola meal (with different concentrations) improved WBCs, Hb, and PCV significantly on juvenile hybrid tilapia with no change in RBCs count which remained within the normal range between different groups. While Taylor et al., (2012) stated that dietary supplemented LO for twelve weeks in Nile tilapia had no effect on RBCs and WBCs numbers, PCV, and Hb. Likewise, Nakharuthai et al., (2020) demonstrated that LO had no significant impacts on RBCs count, Hb, and PCV of adult Nile tilapia when compared to palm oil and soybean oil. These different results in some blood parameters may be attributed to different concentrations of vegetable oils, feeding duration, and life stages of Nile tilapia. There are not enough studies on the effect of CO and LO on hematological parameters, even though dietary lipids play a crucial role in membrane composition of RBCs (Dougherty, et al., 1987).

Serum biochemical parameters of the fish group received CO1xLO1% diet in the present study showed best findings and improved significantly followed by other fish groups received LO2% or CO (with different concentrations) compared to the control group with no significant difference in liver enzymes (ALT and AST). Total protein, globulin, and albumin were increased in fish fed diets with CO1xLO1% followed by CO 0.5% then LO2% diets. A decrease was observed in ALT with CO1%, LO2%, and CO1xLO1% groups respectively. While AST level decreased in dietary CO1xLO1% then CO 0.5% then LO2%. Furthermore, creatinine decreased in fish received LO2% then CO 0.5% then CO1&LO1% diets. Significant increases in cholesterol and triglycerides levels were found in CO1xLO1% group followed by LO2% and CO 0.5% alternatively. Similarly, Peng et al., (2016) stated that ALT and AST levels were decreased when total substitution of fish oil with rapeseed oil on Nile tilapia diets with no negative effect of replacing fish oil by LO on ALT and AST activities. Also, 25 % substitute of fish oil by LO showed non-significant difference in ALT and AST level in the diet of Tilapia (Li, et al., 2016). Protein content in canola oil varies between 32% and 45%. According to the essential amino acid index, the quality of protein of canola meal is comparable to that of herring meal and superior to that of soybean meal and accordingly the serum total protein of fish received canola meal is high (Soltan, 2005) and canola oil may increase the utilization of protein from the feed (Taşbozan et al. 2015). In addition, serum total protein in the Nile tilapia fed LO supplemented diet was higher than other sources of dietary lipids due to high content of n-3 PUFAs in LO (Taylor et al., 2012). In contrast, Elkaradawy, et al., (2022) reported that AST and ALT were increased while cholesterol and triglycerides were decreased in fish fed diet supplemented with LO than control group, moreover TP, globulin, albumin, and creatinine showed non-significant difference. Therefore, using CO and/or LO as feed additives has hepatoprotective effects and improve liver and kidney functions of Nile tilapia.

Catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA) are important enzymes as indicators for cellular immunity and antioxidant processes in fish (Elkadom et al. 2023; García-Meilán et al., 2023). Lipid peroxidation appears to be a prominent consequence of oxidative stress, and excessive peroxide concentrations may damage membrane function and inhibit endogenous antioxidant enzyme activation (Peng et al., 2016). The present study revealed a significant increase in CAT and SOD and a decrease in MDA activities in fish received diets supplemented with CO and/or LO. The best findings of CAT and MDA were found in LO2% group while SOD in CO1xLO1% followed by LO2% groups. Similar results were demonstrated by Li, et al., (2016) who found that substitution of 50% of fish oil by LO in Nile tilapia feed increased SOD and decreased MDA activities significantly. Furthermore, Peng et al., (2016) stated that LO diets didn`t differ from fish oil diets on activities of SOD and MDA, even more LO diets resulted in greater enzyme activity in serum and reduced MDA levels in the liver. Linseed oil has high n-3 fatty acids which may be able to moderate antioxidant enzyme inhibition and reduce peroxidation of lipids through the reduction of oxidants, such as free radicals, which target lipids with carbon-carbon double bonds, particularly polyunsaturated fatty acids (PUFAs). In contrast, Elkaradawy, et al., (2022) stated that there was no significant difference between fish received LO and control diets in CAT, SOD, and MDA activities. As far as, canola oil in the current study had a positive effect on CAT, MDA activities with no effect on SOD, these results didn`t coincide with results reported by Peng et al., (2016) who stated that rapeseed oil (RO) increased MDA level and

decreased SOD level than fish oil in diets of Nile tilapia for 56 days, and this maybe attributes to high proportion of 18:2(n-6) and 18:3(n-3) in RO which would promote accumulation and oxidation of hepatic lipid and increase reactive species production, and consistently lead to oxidative stress. Also, Mohammadi et al., (2020) stated that liver tissue MDA content, CAT, and MDA activity were not affected by processed canola meal diets in juvenile Nile tilapia. Moreover, García-Meilán et al. (2023) stated that feeding sea bream on diets supplemented with LO and CO induced an increase in CAT and SOD while reduced MDA level. The inconsistent results could be attributed to differences in oil sources, extraction, diet formulation, fish size, feeding duration and dietary fat content. So, the relationship between canola oil and antioxidant activities is still unknown and needs more investigation.

Activities of lipid metabolism enzymes are affected by dietary lipids (Peng et al., 2016). So, measurement activity of digestive enzymes is critical for comparing nutrient absorption efficiency across different feed sources (Elkaradawy, et al., 2022). The current study reported that a significant increase in amylase and lipase activities in fish received CO and/or LO diets with the best findings in CO1xLO1% group. Similar results of lipase activity were reported by Peng et al., (2016) when used RO or LO instead of fish oil in Nile tilapia feed and indicated that lipase is essential for lipid metabolism and transport. On the other side, Mohammadi et al., (2020) demonstrated that different concentrations of dietary processed canola meal didn't affect digestive enzyme activity of juvenile Nile tilapia including lipase and amylase. Also, Elkaradawy, et al., (2022) stated that dietary LO had no effect on lipase and amylase activity on Nile tilapia fed for 60 days. Moreover, substitution of fish oil with LO did not affect amylase and lipase activity in diets of Chinese mitten crab (*Eriocheir sinensis*) (Wei, et al., 2018).

Lysozyme activity, phagocytic activity, and index are indicators used to assess the immunological response of fish. The innate immune system is a key of the defense system in fish, especially humoral innate components. Lysozyme is one of the humoral-related parameters (Wang et al., 2022). The n-3 PUFAs have been shown to have immunomodulatory properties by altering the eicosanoids profiles generated and reducing proinflammatory cytokines levels through both lipid-mediator-related and nonlipid-mediator-related processes (Wall et al., 2010). The current study presented that the fish received experimental diets were significantly increased in lysozyme activity and phagocytic index than control diet with the best result being observed in CO1xLO1% group. Similar result of lysozyme activity was reported by Nakharuthai et al., (2020) who found that linseed oil increased lysozyme significantly than soybean oil in diets fed to Nile tilapia for 90 days. Mohammadi et al., (2020) revealed that inclusion of processed canola meal instead of fish meal by 37.5% increased lysozyme activity in juvenile Nile tilapia for 36 days. While Taylor et al., (2012) demonstrated a non-significant difference in lysozyme level in Nile tilapia fed LO7% diet from other diets containing corn oil, beef tallow, and fish oil. Linseed and canola oils have a positive effect on response of innate immunity and that may be owing to beneficial action of n-3 and n-6 PUFAs on cellular membrane of many immune cells which consequently stimulate production of humoral innate components (Gutiérrez, et al., 2019).

Height of intestinal villi and number of goblet cells is considered the main feature of many factors that affect the health of the intestine which consistently affect nutrient digestion and absorption. Also, the intestine contains mucosa-associated lymphoid tissue (MALT), with goblet cells as an active component. Goblet cells are intestinal epithelial cells that release mucus and play an important role in innate defense, through prevention of pathogenic microbe invasion to intestinal wall (Mohammadi et al., 2020; Ma et al., 2018; Mohamed et al., 2021). The present study showed a significant increase in villi length, width, crypt depth and goblet cells number with a significant decrease in inter-villi space in fish received diets supplemented with CO and/or LO, and the best results were reported in CO1xLO1% group. These results were accomplished with normal appearance of hepatic cells that showed normal histology featuring polygonal hepatocytes cords, and pancreatic acini around the central vein. Similar results stated by Mohammadi et al., (2020) who replaced fish oil with processed canola oil (PCM) till 25 % in the diet of Nile tilapia and showed a positive effect on intestine, while increasing PCM content to 37.5 and 50% proved to had adverse effect on intestinal histology (decreases in villi length, goblet cell goblet, and increasing thickness of villi). In addition, Wassef et al., (2016) reported that incorporation of dietary canola oil till 50 % instead of fish oil did not make any alterations in morphology of liver and intestine of European seabass, but

70 % replacement of fish oil with CO made liver and intestine morphological alterations due to high levels of CO stimulate lipid accumulation in hepatocyte and increase cellular vacuolization in the enterocyte of distal intestine. As for LO, it improves health of the intestine through increasing intestinal folds height, goblet cells, and microflora (Ma et al., 2018). Also, replacement of fish oil with vegetables oils (rapeseed or linseed oils) did not make any alterations on intestinal histomorphology of European sea bass (Castro et al., 2015; Mourente et al., 2005), Gilthead Sea bream (Fountoulaki et al., 2009), Rainbow trout (Yildiz, et al.,2013). Interestingly, the improvement of intestine histomorphology may improve feed absorption and subsequently improve the growth performance of the fish received diets supplemented with LO and/or CO.

CONCLUSION

Canola oil and/or linseed oil dietary supplementation to Nile tilapia fingerlings improved its growth performance, blood health, immune-oxidative status, and intestinal morphometry. The fish received CO and LO enriched diet were reported to have the best findings and CO or LO proved to have a positive effect as feed additives for farmed Nile tilapia.

ACKNOWLEDGMENT

The authors would like to thank the Department of Aquaculture, Faculty of Aquatic and Fisheries Sciences, Kafrelsheikh University, Egypt, for providing facilities to carry out this experiment.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abdel-Rahim M.M., Elhetawy A.I., Mansour A.T., Mohamed R.A., Lotfy A.M., Sallam A.E., Shahin S.A. (2023). Effect of long-term dietary supplementation with lavender, Lavandula angustifolia, oil on European seabass growth performance, innate immunity, antioxidant status, and organ histomorphometry. Aquac. Inter.,1-19.

Abdel-Tawwab M., Samir F., Abd El-Naby A.S., Monier M.N. (2018). Antioxidative and immunostimulatory effect of dietary cinnamon nanoparticles on the performance of Nile tilapia, *Oreochromis niloticus* (L.) and its susceptibility to hypoxia stress and *Aeromonas hydrophila* infection. Fish shellfish immunol., 74: 19-2.

Abozeid A.M., Abdel-Rahim M. M., Abouelenien F., Elkaradawy A, M. R. A. (2021). *Quillaja saponaria* and/or *Yucca schidigera* ameliorate water quality, growth performance, blood health, intestine and gills histomorphology of Nile tilapia, *Oreochromis niloticus*. Aquac. Res., 53: 1-15.

AOAC. (2007). Official methods of analysis of the Association of Official Analytical Chemists, Washington, DC.

Bell J.G. and Koppe W. (2010). Lipids in aquafeeds. Fish oil replacement and alternative lipid sources in aquaculture feeds , pp.21-59.

Castro C. O., Castro C., Couto A., Serra C. R., & Fernandes R. (2015). Effects of fish oil replacement by vegetable oil blend on digestive enzymes and tissue histomorphology of European sea bass (*Dicentrarchus labrax*) juveniles. Fish Physiol Biochem., 41: 1-15.

Council N. R. (2011). Nutrient requirements of fish and shrimp. National Academies Press, Washington.

Cottrell R.S., Blanchard J.L., Halpern B.S., Metian M., Froehlich H.E. (2020). Global adoption of novel aquaculture feeds could substantially reduce forage fish demand by 2030. Nat. Food, 1(5), 301-308.

Dacie J. V., Lewis S. (1991). Practical haematology, ELBS with Churchill Livingston. Churchil Livingstone.

De Pablo M.A., Álvarez De Cienfuegos G. (2000). Modulatory effects of dietary lipids on immune system functions. Immunol. Cell Biol., 78: 31-39.

Demers N.E., Bayne C.J. (1997). The immediate effects of stress on hormones and plasma lysozyme in rainbow trout. Dev Comp Immunol., 21(4): 363-373.

Dougherty, R.M.; Galli, C.; Ferro-Luzzi, A., Iacono, J. M. (1987). Lipid and phospholipid fatty acid composition of plasma, red blood cells, and platelets and how they are affected by dietary lipids: A study of normal subjects from Italy, Finland, and the USA. Am. J. Clin. Nutr., 45: 443-455.

Doumas B.T., Bayse D.D., Carter R.J., Peters T. S. R. (1981). candidate reference method for determination of total protein in serum. I. Development and validation. Clin.Chemi., 27(10): 1642-1650.

Dumas B.T., Biggs H. G. (1972). Standard Methods of Clinical Chemistry. Ed., Academic Press, New York.

Dupont-Cyr B.A., Le François N.R., Christen F., Desrosiers V., Savoie A., Vandenberg G.W., Dufresne F., Blier, P.U. (2022). Linseed oil as a substitute for fish oil in the diet of Arctic charr (*Salvelinus alpinus*), brook charr (*S. fontinalis*) and their reciprocal hybrids. Aquac. Rep., 22, 100949.

Elkadom E.M., Abd El-Kader M.F., Bakr B.A., Abozeid A.M., Mohamed, R.A. (2023). Impacts of various single and mixed colors of monochromatic LED light on growth, behavior, immune-physiological parameters, and liver and brain histology of Nile tilapia fingerlings. *Aquaculture*, *577*, 740007.

Elkaradawy A., Abdel-Rahim M.M., R. A. M. (2022). *Quillaja saponaria* and / or linseed oil improved growth performance, water quality , welfare profile and immune- oxidative status of Nile tilapia , *Oreochromis niloticus* fingerlings. Aquac. Res., 53: 576-589.

FAO (2020). *The State of Food and Agriculture* 2020; FAO: Rome, Italy.

FAO Yearbook. Fishery and Aquaculture Statistics. (2022). The state of world fisheries and aquaculture production 2020, table number 1 (p.3) and table number 7 (p. 30).

Fountoulaki E., Vasilaki A., Hurtado R., Grigorakis K., Karacostas I., Nengas I., Rigos G., Kotzamanis Y., Venou B., & Alexis M. N. (2009). Fish oil substitution by vegetable oils in commercial diets for gilthead sea bream (*Sparus aurata* L.); effects on growth performance, flesh quality and fillet fatty acid profile Recovery of fatty acid profiles by a fish oil finishing diet under fluctuating water temperatures. Aquaculture, 289: 317-326.

Francis D. S., Turchini G. M., Jones P. L., & De Silva S. S. (2006). Effects of dietary oil source on growth and fillet fatty acid composition of Murray cod, *Maccullochella peelii peelii*. Aquaculture, 253: 547-556.

García-Meilán I., Fontanillas R., Gutiérrez J., Capilla E., Navarro I., Gallardo, Á. (2023). Effects of Dietary Vegetable Oil Mixtures including

Soybean Oil on Intestinal Oxidative Stress in Gilthead Sea Bream (*Sparus aurata*). Animals, 13(6),1069.

Gutiérrez S., Svahn S.L., Johansson, M. (2019). Effects of Omega-3 Fatty Acids on Immune Cells. Int. J. Mol. Sci., 20: 5028.

Heinegård D, T. G. (1973). Determination of serum creatinine by a direct colorimetric method. Clinica chimica acta, 43(3): 305-310.

Huang X., Chen F., Guan J., Xu, C., & Li Y. (2022). Beneficial effects of re-feeding high α -linolenic acid diets on the muscle quality, cold temperature and disease resistance of tilapia. Fish Shellfish Immunol., 126: 303-310.

Kawahara E., Ueda T. and Nomura S. (1991). In vitro phagocytic activity of white-spotted char blood cells after injection with *Aeromonas salmonicida* extracellular products. Fish Pathology, 26(4): pp.213-214.

Kiron V., Thawonsuwan J., Panigrahi A., Scharsack J.P., Satoh S. (2011). Antioxidant and immune defences of rainbow trout (Oncorhynchus *mykiss*) offered plant oils differing in fatty acid profiles from early stages. Aquac Nutr., 17: 130 - 140.

Li F. J., Lin X., Lin S. M., Chen W. Y., & Guan Y. (2016). Effects of dietary fish oil substitution with linseed oil on growth, muscle fatty acid and metabolism of tilapia (*Oreochromis niloticus*). Aquac Nutr., 22(3): 499-508.

Stoskopf M. (1993). Anatomy and physiology of sharks. Fish Medicine. Stoskopf, MK (Ed), WB Saunders Co., Philadelphia, Pennsylvania: 751.

Ma Q., Li L., Le J., Lu D., Qiao F., Zhang M., & Du Z. (2018). Dietary microencapsulated oil improves immune function and intestinal health in Nile tilapia fed with high-fat diet. Aquaculture, 496: 19-29.

Mahmoud B. S. M., & Miyashita K. (2010). Essential oils: natural antimicrobials for fish preservation. Mississippi, USA, Academic Press, 217-225 pp. ref.67

Mohammadi M., Imani A., Farhangi M., Gharaei A., & Hafezieh M. (2020). Replacement of fishmeal with processed canola meal in diets for juvenile Nile tilapia (Oreochromis *niloticus*): Growth performance , mucosal innate immunity , hepatic oxidative status , liver and intestine histology. Aquaculture, 518: 734824.

Mohamed R.A., Yousef Y.M., El-Tras W.F., Khalafallaa M.M. (2021). Dietary essential oil extract from sweet orange (Citrus sinensis) and bitter lemon (Citrus limon) peels improved Nile tilapia performance and health status. Aquac. Res., 52(4), 1463-1479.

Mourente G., Good J. E., & Bell J. G. (2005). Partial substitution of fish oil with rapeseed, linseed and olive oils in diets for European sea bass (*Dicentrarchus labrax* L .): effects on flesh fatty acid composition , plasma prostaglandins E 2 and F 2 a , immune function and effectiveness of a fish. Aquac Nutr. 11: 25-40.

Nakharuthai C., Rodrigues P. M., Schrama D., Kumkhong S., & Boonanuntanasarn S. (2020). Effects of Different Dietary Vegetable Lipid Sources on Health Status in Nile Tilapia (*Oreochromis niloticus*): Haematological Indices, Immune Response Parameters and Plasma Proteome. Animals, 10: 1-19.

Orsavova J., Misurcova L., Vavra Ambrozova J., Vicha R. and Mlcek J. (2015). Fatty acids composition of vegetable oils and its contribution to dietary energy intake and dependence of cardiovascular mortality on dietary intake of fatty acids. Int J Mol Sci., 16(6): pp.12871-12890.

Peng X., Li F., & Lin S. (2016). Effects of total replacement of fish oil on growth performance, lipid metabolism and antioxidant capacity in tilapia (*Oreochromis niloticus*). Aquac. Inter., 24: 145-156.

Rahimi S., Kathariou S., Fletcher O., Grimes J. L. J. P. s. (2019). Effect of a direct-fed microbial and prebiotic on performance and intestinal histomorophology of turkey poults challenged with Salmonella and Campylobacter.Poult sci., 98: 6572-6578.

Reitman S., Frankel S. (1957). A Colorimetric Method for the Determination of Serum Glutamic Oxalacetic and Glutamic Pyruvic Transaminases. Am J Clin Pathol., 28(1), 56-63.

Rosenlund G., Obach A., Sandberg M. G., Standal H., & Tveit K. (2001). Effect of alternative lipid sources on long-term growth performance and quality of Atlantic salmon (*Salmo salar* L .). Aquac. Res., 32: 323-328.

Rossetto V., Karla B., Tsujii M., Dena L., Barriviera L., Paola F., Paulovski A., Antônio J., Miranda G., Tolentino M., Wilson M., & Furuy M. (2021). Substitution of soybean oil with linseed oil on growth performance , fatty acid profile and texture attributes in large Nile tilapia , O*reochromis niloticus* reared under cold suboptimal temperature. Aquac. Res., 53: 5136-5145.

Soltan M. A. (2005). Potential of using raw and processed canola seed meal as an alternative fish meal protein source in diets for nile tilapia (*Oreochromis niloticus*). Egypt J Nutr Feed, 8(1): 1111-1128.

Sutili F. J., Iii D. M. G., Heinzmann, B. M., & Baldisserotto, B. (2018). Plant essential oils as fish diet additives: benefits on fish health and stability in feed. Rev. Aquac., 10: 716-726.

Suvarna K.S., Layton C., Bancroft J. D. (2018). Bancroft's theory and practice of histological techniques E-Book. Elsevier Heal Sc.

Taşbozan O., Gökçe M.A., Erbaş C., Özcan, F. (2015). Effect of Different Concentrations of Canola Oil in Diets on Body Chemical Composition and Growth Performance of Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1758). Pakistan J. Zool, 47(6),1761-1769.

Taylor P., Mulligan B., & Trushenski J. (2013). Use of Standard or Modified Plant-Derived Lipids as Alternatives to Fish Oil in Feeds for Juvenile Nile Tilapia. J Aquat Food Prod Technol., 22(1): 47-57.

Taylor P., Yildirim-aksoy M., Lim C., Davis D. A., Shelby R., Phillip H., & Davis D. A. (2012). Influence of Dietary Lipid Sources on the Growth Performance, Immune Response and Resistance of Nile Tilapia , *Oreochromis niloticus* , to *Streptococcus iniae* Challenge. J Appl Aquac., 19(2): 37-41.

Thrall M, Baker D, L. E. (2004). Veterinary Haematology and Clinical Chemistry Lippincott Williams and Wilkins. Philadelphia, USA.

Tocher D. R. (2003). Tocher, D.R. Metabolism and Functions of Lipids and Fatty Acids in Teleost. Rev. Fish Sci. 2003, 11: 107- 184.

Turchini G. M., Moretti V. M., Hermon K., Caprino F., Busetto M. L., Bellagamba F., Rankin T., Keast, R. S. J., & Francis D. S. (2013). Monola oil versus canola oil as a fish oil replacer in rainbow trout feeds: Effects on growth , fatty acid metabolism and final eating quality. Food Chem., 141(2): 1335-1344.

Turchini G. M., Torstensen B. E., & Ng W. (2009). Fish oil replacement in finfish nutrition. Rev. Aquac., 1: 10-57.

Turchini G.M., Francis D.S., Senadheera S.P.S.D., Thanuthong T. & De Silva S.S. (2011) Fish oil replacement with different vegetable oils in Murray cod: evidence of an 'omega-3 sparing effect' by other dietary fatty acids. Aquaculture, 315: 250-259.

Wall R., Ross R. P., Fitzgerald G. F., & Stanton C. (2010). Fatty acids from fish: the anti-inflammatory potential of long-chain omega-3 fatty acids. Nutr. Rev., 68(5): 280-289.

Wang B., Thompson K. D., Bondad-reantaso M. G., Surachetpong W., & Tattiyapong P. (2022). Strategies to enhance tilapia immunity to improve their health in aquaculture. Rev. Aquac. 10: 1-16.

Wassef E. A., Wahbi O. M., Saqr, E. M., & Saleh N. E. (2016). Response of European seabass (*Dicentrarchus labrax*) to canola oil diets: effect on growth performance, fish health and liver and intestine histomorphology. Aquac. Inter., 24(4): 1073-1088.

Wei B., Yang Z., Cheng Y., Wang J., & Zhou J. (2018). Effects of the complete replacement of fish oil with linseed oil on growth, fatty acid composition, and protein expression in the Chinese mitten crab (*Eriocheir sinensis*). Proteome Sci., 16(1): 6.

Yildiz M, Eroldogan OT, Engin K, Gulcubuk A, B. M. (2013). Effects of dietary cottonseed and/or canola oil inclusion on the growth performance, FA composition and organ histology of the juvenile rainbow trout, *Oncorhynchus mykiss*. Turkish J Fish and Aquatic Sci., 13: 453-464.

Zhou Q., & Yue Y. (2010b). Effect of replacing soybean meal with canola meal on growth, feed utilization and haematological indices of juvenile hybrid tilapia , *Oreochromis niloticus* x *Oreochromis aureus*. Aquac. Res., 41: 982-990.