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Can Dietary Hazelnut Oil Inclusion Alleviate the Negative Effects of Oxidized Fish Oil in Rainbow Trout (*Oncorhynchus mykiss*) Juveniles?

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Abstract

The aim of the present study was to check if dietary hazelnut oil alleviates the negative effects of oxidized fish oil in rainbow trout juveniles. Dietary fresh and oxidized fish oils were totally or partially (40 and 60%) replaced in a 9-week feeding trial. Growth performance and feed efficiency were significantly influenced by oxidized fish oil ($P<0.05$), but not by hazelnut oil inclusion and their interaction. Fish fed fresh fish oil containing-diets had better growth and feed efficiency than those fed oxidized fish oil-containing diets. Protein and lipid digestibility decreased with the increase in both dietary oxidized fish oil and hazelnut oil. Fatty acid profile in whole-body lipids was significantly influenced by the experimental diets ($P<0.05$). Polyunsaturated fatty acids (PUFA) showed a significant decrease with both dietary hazelnut and oxidized fish oils inclusion level. Dietary hazelnut oil resulted in a significant increase in hepatic vitamin E and a significant decrease in lipid peroxidation level ($P<0.05$). Our overall results suggested that dietary oxidized lipids caused growth depletion, lowered nutrient digestibility, lipid peroxidation, and decreased eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic (DHA; 22:6n-3) levels in rainbow trout. However, dietary hazelnut oil inclusion alleviated the negative effects of oxidized oil, sparing n-3 long-chain PUFAs such as EPA and DHA, and increasing hepatic α-tocopherol levels which lowered lipid peroxidation despite no significant impact on growth.

Introduction

Due to the rapid increase in the world population, food security has become the main concern worldwide. The global demand for fish is expected to increase significantly in the next decades as the world population is estimated to reach 10 billion in 2050 (FAO, 2022). The expected increase in fish products is likely to be secured by aquaculture which has been the fastest-growing food production sector for the last 4 decades, providing more than half of the world's fish for human consumption now (Cai & Zhou, 2019). The rapid growth of aquaculture resulted in an increasing requirement for the raw materials used in aquafeeds (Tacon et al., 2011).

Traditionally, fish oil has been used as the main lipid resource in feeds for carnivorous fish species for a long time. Fish oil is very rich in n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) such as eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), which have very important physiological roles in fish and human health. On the other hand, LC-PUFAs make fish oil highly vulnerable to lipid peroxidation in fish tissues, resulting in toxic oxidation products such as aldehydes, ketones or fatty acid alkoxy radicals, which can damage cellular integrity (Janssens et al., 2000).

In manufactured aquafeeds, LC-PUFAs of fish oil are readily prone to autoxidation in the presence of atmospheric oxygen, high temperature, humidity, metal ions and light when preparation and storage of feeds are not properly done. In this case, instead of being useful, LC-PUFAs of fish oil can turn into an issue for fish (Hsieh

& Kinsella, 1989). Oxidation products such as hydrocarbons, alcohols, aldehydes, ketones, volatile organic acids and epoxy compounds negatively influence the nutritional quality of the diet, resulting in impaired growth and health status in fish (Chen et al., 2019). Previous studies showed that oxidized dietary fish oil caused growth depletion in several fish species including rainbow trout, *Oncorhynchus mykiss* (Fontagné-Dicharry et al., 2014), *Rhynchocypris lagowski* Dybowski (Chen et al., 2019), hybrid grouper (♀ *Epinephelus fuscoguttatus* × ♂ *Epinephelus lanceolatus*) (Long et al., 2021), genetically improved farmed tilapia, *Oreochromis niloticus* (Yu et al., 2020a), orange-spotted grouper, *Epinephelus coioides* (Liu et al., 2017) and channel catfish, *Ictalurus punctatus* (Liang et al., 2019). Negative effects of dietary oxidized fish oil in fish were also reported as lipid peroxidation, oxidative stress (Yang et al. 2015; Peng et al. 2009), liver degeneration (Chen et al., 2012), lowered nutrient digestibility (Yong et al., 2022), and depletion in vitamin E (Gao et al., 2013; Chen et al., 2012).

Vitamin E, as an effective lipid-soluble antioxidant, reduces lipid peroxidation in aquafeed with oxidized oil (Peng et al., 2009) and protects PUFAs against oxidation in fish tissues (Hamre, 2011). Hazelnut oil plays an important role in human nutrition due to its unique nutritional quality, including its high amount of vitamin E content (Terruzi et al., 2018). It was also successfully used in fish diets (Atasever et al., 2014; Tasbozan et al., 2015; Katsika et al., 2021). In the present study, therefore, we aimed to investigate the possible impact of dietary hazelnut oil inclusion on growth, digestibility, lipid peroxidation, antioxidant response, fatty acid profile and liver histology in rainbow trout fed oxidized fish oil.

Materials and Methods

Diets and Experimental Design

Seven isonitrogenous and isolipidic (48% protein and 20% lipid) experimental diets were formulated (Table 1). Chromic oxide as an indigestible marker was added to the diets at 1% to investigate nutrient digestibility. As lipid resources, fresh fish oil (peroxide value 5.5 meq/kg), oxidized fish oil (peroxide value 135.5 meq/kg) and fresh hazelnut oil (peroxide value 3.4 meq/kg) were used either solely (FO, OxFO and HO diets, respectively) or in combination where both fresh fish oil and oxidized fish oil were replaced with hazelnut oil at 40 and 60% (HO40 and HO60, and OxHO40 and OxHO60 diets, respectively). Fatty acid profiles of the experimental diets are presented in Table 2. Excluding the HO diet, a 2 × 3 factorial design was applied to investigate the effect of different inclusion levels of oxidized fish oil and hazelnut oil on the target parameters. Fresh fish oil was exposed to vigorous aeration at 70°C for 12 hours in order to get oxidized fish

Table 1. Formulation and chemical composition of experimental diets

Ingredients (%)	HO	FO	HO40	HO60	OxFO	OxFO40	OxFO60
Fishmeal	14.58	14.58	14.58	14.58	14.58	14.58	14.58
Poultry meal	34.03	34.03	34.03	34.03	34.03	34.03	34.03
Soy protein concentrate	12.26	12.26	12.26	12.26	12.26	12.26	12.26
Blood meal	3.65	3.65	3.65	3.65	3.65	3.65	3.65
Pre-gelatinized starch	17.60	17.60	17.60	17.60	17.60	17.60	17.60
Carboxymethyl cellulose	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Vitamin mixture[a]	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Mineral mixture[b]	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Fresh fish oil	0.00	15.78	9.47	6.31	0.00	0.00	0.00
Hazelnut oil	15.78	0.00	6.31	9.47	0.00	6.31	9.47
Oxidized fish oil	0.00	0.00	0.00	0.00	15.78	9.47	6.31
Choline chloride	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Chromic oxide	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Chemical composition							
Protein (%)	47.8	48.2	48.3	47.5	47.3	47.5	47.6
Lipid (%)	20.0	19.9	19.6	19.5	19.6	20.0	20.2
Ash (%)	10.3	10.9	10.4	11.2	10.2	11.3	11.0
Moisture (%)	6.9	5.5	5.2	6.9	6.9	6.1	6.8
α-tocopherol (mg/kg)	19.9	6.1	9.9	15.5	2.2	8.8	12.7
Peroxide value of lipids (meq/kg)	3.4	5.5	5.2	3.6	135.5	92.7	61.1

[a]Trouw Nutrition Premix (Ankara, Turkey), composition per g of the vitamin mixture: vitamin A, 2645.50 IU; vitamin D_3, 220.46 IU; Vitamin B_{12}, 13 µg; riboflavin, 13.23 mg; niacin, 61.73 mg; D-pantothenic acid, 22.05 mg; menadione, 1.32 mg; folic acid, 1.76 mg; pyridoxine, 4.42 mg; thiamin, 7.95 mg; D-biotin, 0.31 mg.

[b]Bernhart Tomarelli salt mixture (ICN Pharmaceuticals, Costa Mesa, CA), composition (g/100g): calcium carbonate, 2.1; calcium phosphate dibasic, 73.5; citric acid, 0.227; cupric citrate, 0.046; ferric citrate (16 to 17% Fe), 0.558; magnesium oxide, 2.5; manganese citrate, 0.835; potassium iodide, 0.001; potassium phosphate dibasic, 8.1; potassium oxide, 6.8; sodium chloride, 3.06; sodium phosphate, 2.14; and zinc citrate, 0.133. Five milligrams of Se in the form of sodium selenite was added per kilogram of the salt mixture.

oil (Gao et al., 2012b). Peroxide value was measured at 3-hour intervals, using the method Ja 8–87 of the American Oil Chemists Society, heating and aeration were stopped when the desired peroxide value (135.5 meq/kg) was reached at the end of 12 hours. The diets were prepared by cold-pelleting (2.0 mm diameter) after 35–40 mL of distilled water was added into 100 g mixture of ingredients, dried in an oven at 35°C for 2 days to decrease moisture below 10%, crushed into suitable particle sizes (0.4–2.0 mm), and stored at -20°C until use.

Fish and Feeding Experiment

Feeding experiment was carried out with juvenile rainbow trout with approximately 1.1 g initial mean weight at the Aquaculture Experimental Research Center of Atatürk University, Erzurum, Turkey. Fish were distributed in 35-L rectangular acrylic aquariums, 30 fish per each in triplicates, in a semi-closed recirculating water system equipped with biological filtration and constant oxygenation. Fish were fed 3 times a day (9.00 AM, 1.00 PM and 5.30 PM) at the rate of 5% body weight daily for 9 weeks. Temperature and dissolved oxygen were 13±1°C and 7.5±0.5 mg/L, respectively during the feeding trial. Fish were weighed every 15 days to check their growth performance and re-adjust the feeding rate. Collection of feces for the determination of digestibility was carried out daily during weeks 6–8. After the last feeding, aquariums were siphoned to remove the uneaten feed. Feces were collected by siphoning before the first feeding in the next morning and stored at −20°C until analyzed. Feces were freeze dried before the analyses.

At the termination of the feeding trial, five fish per aquarium were sampled and immediately frozen in liquid nitrogen, and stored at -80°C for whole-body lipid and fatty acid analysis. Another eighteen fish from each aquarium were sacrificed by an overdose of clove oil, followed by dissection. The livers were separated, immediately frozen in liquid nitrogen and stored at -80°C for α-tocopherol, antioxidant enzymes activities, reduced glutathione and lipid peroxidation analyses. The procedures and methods used in the current study followed the ethical guidelines of Atatürk University Animal Care and Use Committee under project number PRJ2015/382.

Growth Performance

At the termination of the feeding trial, growth performance was evaluated with the parameters presented below:

Table 2. Fatty acid profile (% of total detected) of experimental diets

	Experimental diets						
Fatty acids	HO	FO	HO40	HO60	OxFO	OxFO40	OxFO60
14:0	6.8	6.1	8.4	7.5	5.4	10.3	8.1
14:1	ND	0.2	1.1	ND	0.2	ND	ND
15:0	ND	0.8	0.5	0.3	0.8	0.4	0.3
16:0	9.1	19.8	14.4	12.4	20.4	14.6	12.5
16:1	1.0	4.8	3.0	2.3	5.1	3.1	2.3
17:0	ND	0.6	0.4	ND	0.6	ND	ND
18:0	3.4	4.9	4.0	3.8	4.9	4.1	3.8
18:1n-9	61.4	24.3	38.3	47.2	24.9	38.6	47.1
18:2n-6	15.0	9.0	10.7	12.3	9.4	11.2	12.6
18:3n-3	0.7	2.0	1.5	1.0	2.0	1.3	1.1
18:4n-3	ND	1.4	0.8	0.6	1.2	0.7	0.6
20:0	0.2	0.7	0.5	0.3	0.8	0.5	0.5
20:1n-9	ND	0.2	0.2	ND	0.2	1.0	ND
20:2n-6	ND	0.3	1.1	0.1	0.3	0.2	ND
20:3n-3	ND	ND	0.2	0.2	0.5	0.2	0.1
20:4n-6	0.4	0.9	0.6	0.5	0.9	0.6	0.5
20:4n-3	ND	0.0	0.2	1.0	0.3	0.2	0.8
20:5n-3	0.6	8.0	4.6	3.2	7.7	4.6	3.0
22:0	ND	0.8	0.7	1.0	0.2	0.2	0.7
22:4n-6	ND	0.6	0.3	0.3	0.5	0.2	0.2
22:5n-3	ND	0.8	0.4	0.3	0.7	0.6	0.4
22:6n-3	1.3	13.4	7.9	5.4	12.5	7.3	5.1
24:1n-9	ND	0.5	0.2	0.2	0.4	0.2	0.3
∑SFA	19.4	33.7	28.9	25.3	33.1	30.1	25.9
∑MUFA	62.5	30.0	42.9	49.7	30.7	42.9	49.7
∑PUFA	18.1	36.3	28.2	25.0	36.2	27.0	24.4
∑n3	2.7	25.5	15.6	11.8	25.1	14.8	11.1
∑n6	15.4	10.8	12.7	13.3	11.1	12.2	13.3
n3/n6	0.2	2.4	1.2	0.9	2.3	1.2	0.8

ND: not detected

% Weight gain (%WG) = [(final weight – initial weight) / initial weight] × 100

Specific growth rate (SGR) = [(ln final weight – ln initial weight) × 100] / time (days)

Feed conversion ratio (FCR) = feed intake (g) / weight gain (g)

Digestibility

Chromic oxide contents of the seven experimental diets and feces from each experimental unit (21 tanks) were separately analyzed as described earlier (Kimura & Miller, 1957). Calculations of apparent digestibility coefficients (ADC) for dry matter, protein and lipids were performed using a standard formula:

ADC = 100 – [100 (Cr_2O_3 in diet) ÷ (Cr_2O_3 in feces) × ((% nutrient in feces) ÷ (% nutrient in feed))]

Chemical Analyses

Whole body, liver and feed lipids were extracted according to Folch et al. (1957) method. The fatty acid methyl esters (FAME) were prepared according to the Metcalfe & Schmitz (1961) method and analyzed by gas chromatography (Agilent 6890 N, USA), using CP-Sil 88 100 × 0.25 (0.2) column (Agilent, USA) as described by Arslan et al. (2009). The injector and detector temperatures were set at 270 and 300°C, respectively. The initial temperature of the oven was 175°C for 26 min, increased to 205°C by increments of 2°C/min, and subsequently held at 205°C for 24 min. Fatty acids were then identified by comparing their retention times to those of a standard mix of fatty acids (Supelco 37 Component FAME Mix) (Sigma-Aldrich, Germany).

Dietary proximate analyses were done according to standard procedures described by the Association of Officiating Analytical Chemists (AOAC, 2006). In order to determine the dry matter, the moisture content of samples was detected by drying them to constant weight in an oven at 105°C for 6 h. Dry matter was subsequently calculated by subtracting the moisture content from 100. Crude protein content (N × 6.25) was determined by using a Kjeldahl system. Ash content was detected by incinerating the samples in porcelain crucibles in a Muffle Furnace at 550°C for 12 h.

Dietary and liver vitamin E (α-tocopherol) was analyzed in HPLC (Agilent, USA) equipped with a C18 (4.6 × 150 mm, 5 µm) column and fluorescent detector (G1321A FLD) based on Cort et al. (1983) method. The amount of reduced glutathione (GSH) in the liver was detected according to Sedlak et al. (1968) at 412 nm after incubating with 5,5'-dithio-bis (2-nitrobenzoic acid) (DTNB) at 37°C for 30 minutes. Liver superoxide dismutase (SOD) activity was determined by xanthine-xanthine oxidase inhibition as a result of O_2 reduction at 560 nm (Sun et al., 1988). Hepatic lipid peroxidation level was determined by measuring the amount of malondialdehyde (MDA) in the spectrophotometer at a wavelength of 532 nm according to the method described by Ohkawa et al. (1979).

Liver Histology

Liver tissues were fixed in a buffered 10% formalin solution followed by a rutin alcohol and xylene procedure. Tissues embedded in paraffin were then cut into 5 µm thick sections by using a microtome (Leica, Germany). The sections were stained in Mayer's hematoxylin and eosin, and examined by light microscopy (Presnell & Schreibman, 1997).

Data Analysis

Data were expressed as means±SD. The normality of the data and homogeneity of variance were checked using Levene's test. Excluding HO-fed fish, data were subjected to a two-way analysis of variance (ANOVA) and subsequent comparison of means by Duncan's multiple range test when investigating the potential effect of oxidation and hazelnut oil inclusion levels on target parameters. A one-way ANOVA was applied to data from each dietary treatment when comparing with negative control (HO). Percentage data were arcsin transformed prior to statistical analysis. Differences were considered statistically significant at $P \leq 0.05$.

Results

Growth Performance

Growth performance and feed efficiency were significantly influenced by oxidized fish oil ($P<0.05$), but not by the hazelnut oil inclusion and their interaction (Table 3). When HO fed group was taken into account, dietary treatments significantly influenced growth performance and feed efficiency as well ($P<0.05$). Fish fed the diets containing fresh fish oil (FO, HO40 and HO60) had significantly higher final weight, % weight gain and SGR, and lower FCR than those fed the diets containing oxidized fish oil (OxFO, OxHO40 and OxHO60). Fish fed the HO diet, where hazelnut oil was the only dietary lipid resource, had significantly lower growth performance than those fed fresh fish oil-containing diets, while FCR was similar with both fresh and oxidized fish oil-containing diets.

Digestibility

Apparent protein and lipid digestibilities were significantly influenced ($P<0.05$) by hazelnut inclusion level, fish oil oxidation, and their interaction, while dry matter was significantly affected ($P<0.05$) by fish oil oxidation and the interaction (Table 4). Dietary fish oil oxidation caused a decrease in the ADC of nutrients. On the other hand, fish fed diets with only fresh fish oil had

significantly higher ADC than those fed diet with only hazelnut oil. The increase in dietary hazelnut oil level decreased ADC values in fish fed fresh fish oil containing diets (HO40 and HO60), while ADC values increased in those fed oxidized fish oil containing diets (OxFO40 and OxFO60) under the same circumstance.

α-tocopherol

Hepatic vitamin E level was significantly affected by dietary hazelnut inclusion level and fish oil oxidation (P<0.05) (Figure 1). The level of α-tocopherol increased with an increasing amount of dietary hazelnut oil, while it decreased with fish oil oxidation. When comparing all experimental groups, the highest hepatic vitamin E was detected in fish fed the HO diet while those fed the OxFO diet had the lowest value.

Lipid Peroxidation and Antioxidant Response

Liver lipid peroxidation level (MDA level) was significantly influenced by the dietary hazelnut inclusion (P<0.05); however, it was not influenced by oxidation and interaction. The level of MDA clearly tended to decrease with the amount of dietary hazelnut oil inclusion and increased with fish oil oxidation, which was opposite to the tendency of hepatic α-tocopherol (Figure 1). The amount of GSH and the activity of SOD were not significantly influenced by the dietary treatments under any circumstances (Figure 2).

Lipids and Fatty Acids

Whole-body lipid content was not influenced by the dietary treatments, averaging 10.6±1.4%, while the dietary fatty acid profile was reflected by the fish fed corresponding diets. Fatty acid compositions of fish fed hazelnut oil-containing diets were dominated by oleic acid (OA; 18:1n-9) while those fed fish oil-containing diets were characterized by the high amounts of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3). Furthermore, total saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), PUFA and n3/n6 ratio was significantly influenced by hazelnut oil inclusion, while the amount of total MUFA and PUFA as well as n3/n6 ratio were significantly affected by oxidation, hazelnut oil inclusion and their interactions (P<0.05) (Table 5). Most of the individual fatty acids were significantly influenced by hazelnut inclusion while EPA and DHA were the only fatty acids, which were significantly influenced by all factors: hazelnut oils inclusion, oxidation and their interaction. DHA levels decreased with fish oil oxidation and hazelnut oil inclusion while oxidized fish oil did not influence OA levels (Figure 3).

Liver Histology

Fish fed oxidized fish oil had severe degenerations and necrosis in the liver; however, hazelnut oil inclusion seemed to have an alleviative effect. Fish fed the OxHO60 diet rarely had the aforementioned hepatic disorders (Figure 4).

Discussion

Fish oil is a very valuable dietary lipid source for fish as it contains physiologically important LC-PUFAs such as EPA and DHA, but this superiority makes it vulnerable to oxidation at the same time. Dietary oxidized fish oil

Table 3. Growth performance in rainbow trout fed diets with different levels of fresh fish oil, oxidized fish oil and hazelnut oil for 9 weeks. Data are expressed as mean±SD. Data with different superscripts in a row are significantly different (P<0.05)

Parameters	Experimental diets							*2-way ANOVA*		
	HO	FO	HO40	HO60	OxFO	OxFO40	OxFO60	Oxidation	Hazelnut oil	Interaction
Initial weight (g)	1.1±0.04	1.1±0.02	1.1±0.05	1.1±0.04	1.1±0.03	1.1±0.02	1.1±0.01	NS	NS	NS
Final weight (g)	12.4±0.3^{b}	14.4±0.5^{a}	14.3±0.8^{a}	15.7±1.4^{a}	12.0±0.6^{b}	12.4±0.6^{b}	12.6±0.9^{b}	S	NS	NS
Weight gain (%)[1]	1028±33^{b}	1195±52^{a}	1187±17^{a}	1313±121^{a}	1015±42^{b}	1050±102^{b}	1021±103^{b}	S	NS	NS
SGR[2]	3.8±0.0^{b}	4.1±0.1^{a}	4.1±0.0^{a}	4.2±0.1^{a}	3.8±0.1^{b}	3.9±0.1^{b}	3.8±0.1^{b}	S	NS	NS
FCR[3]	1.4±0.1^{ab}	1.3±0.1^{b}	1.3±0.0^{b}	1.2±0.0^{b}	1.6±0.1^{a}	1.5±0.1^{a}	1.6±0.1^{a}	S	NS	NS
Survival (%)	97.8±1.9	97.8±1.9	100.00±0.00	99.7±0.5	96.7±0.0	96.5±1.9	98.9±1.9	NS	NS	NS

[1]Weight gain (%) = [(final weight – initial weight) × 100] / initial weight
[2]Spesific growth rate (SG) = [(ln final weight – ln initial weight) × 100] / duration (day)
[3]Feed conversion ratio (FCR) = feed consumed (g) / weight gained (g)
S: significant (P<0.05); NS: not significant (P>0.05)

Table 4. Apparent digestibility coefficient (%) in rainbow trout fed diets with different levels of fresh fish oil, oxidized fish oil and hazelnut oil for 9 weeks. Data are expressed as mean±SD. Data with different superscripts in a row are significantly different (P<0.05)

Parameters	Experimental diets							*2-way ANOVA*		
	HO	FO	HO40	HO60	OxFO	OxFO40	OxFO60	Oxidation	Hazelnut oil	Interaction
Dry matter	76.3±0.8^{c}	80.8±0.7^{a}	79.8±0.5^{ab}	78.6±0.3^{b}	74.3±0.8^{d}	75.3±1.1^{cd}	75.6±1.0^{c}	S	NS	S
Protein	82.7±0.4^{d}	88.5±0.5^{a}	86.1±1.6^{b}	83.9±1.0^{cd}	84.3±0.4^{c}	82.7±0.5^{d}	83.3±0.7^{cd}	S	S	S
Lipid	89.0±0.7^{cd}	93.1±0.1^{a}	91.5±0.5^{b}	89.8±0.3^{c}	89.7±1.0^{c}	88.6±0.6^{d}	89.2±0.6^{cd}	S	S	S

S: significant (P<0.05); NS: not significant (P>0.05)

was reported to cause several unfavorable consequences in aquatic animals (Chen et al., 2019). In the present study, fish fed oxidized fish oil-containing diets had decreased growth performance and feed efficiency in comparison to those fed fresh fish oil-containing diets. Similar results were also reported in several fish species such as Japanese sea bass, *Lateolabrax japonicus* (Gao et al., 2012a), rainbow trout (Fontagné-Dicharry et al., 2014), *Rhynchocypris lagowski* Dybowski (Chen et al., 2019; Yu et al., 2020b), hybrid grouper (Long et al., 2021), genetically improved farmed tilapia (Yu et al., 2020a), orange-spotted grouper (Liu et al., 2017), *Labeo rohita* (Fatima et al., 2018), and channel catfish (Liang et al., 2019). Decreased growth caused by oxidized fish oil was mostly related to induced oxidative stress, reduced feed intake due to the low palatability, and decreased digestibility of some macronutrients (Hasanpour et al., 2019). Vitamin E, as a strong antioxidant, was tested against the negative effect of dietary oxidized fish oil in earlier studies. Fish such as *Labeo rohita* and seabream (*Sparus aurata*), which were exposed to dietary oxidized fish oil, exhibited improved growth performance when they had vitamin E supplementation in their diets (Gao et al., 2012a; Fatima et al., 2018). In the present study, dietary vitamin E levels increased with the increasing amount of hazelnut oil inclusion; however, no significant difference was observed in growth based on hazelnut oil inclusion level. Unlike our results, an increasing tendency in growth with graded levels of dietary palm oil, rich in vitamin E, was observed in seabream fed oxidized fish oil-containing diets (Gao et al., 2012b). On the other

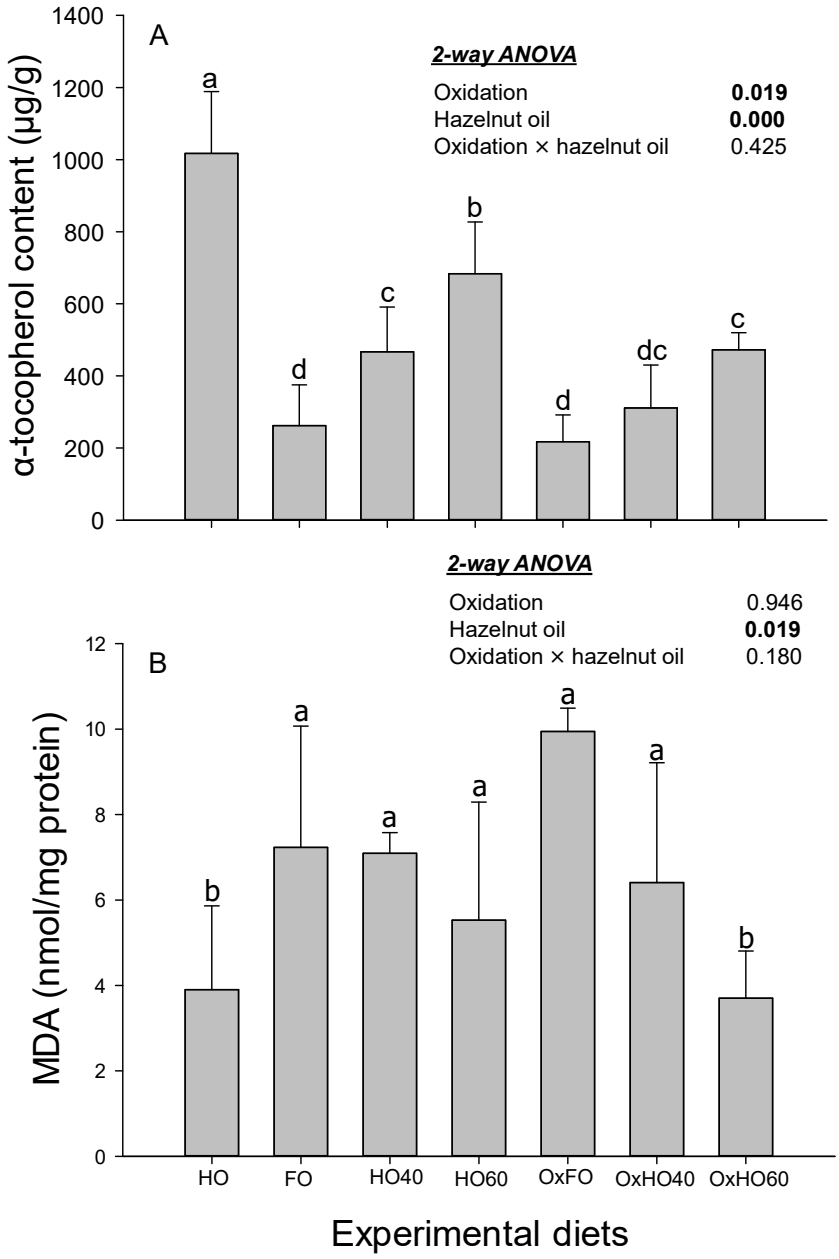

Figure 1. Liver α-tocopherol (A) and lipid peroxidation (MDA) (B) level in rainbow trout fed diets with different levels of fresh fish oil, oxidized fish oil and hazelnut oil for 9 weeks. Data are expressed as mean±SD. Data with different letters are significantly different (P<0.05).

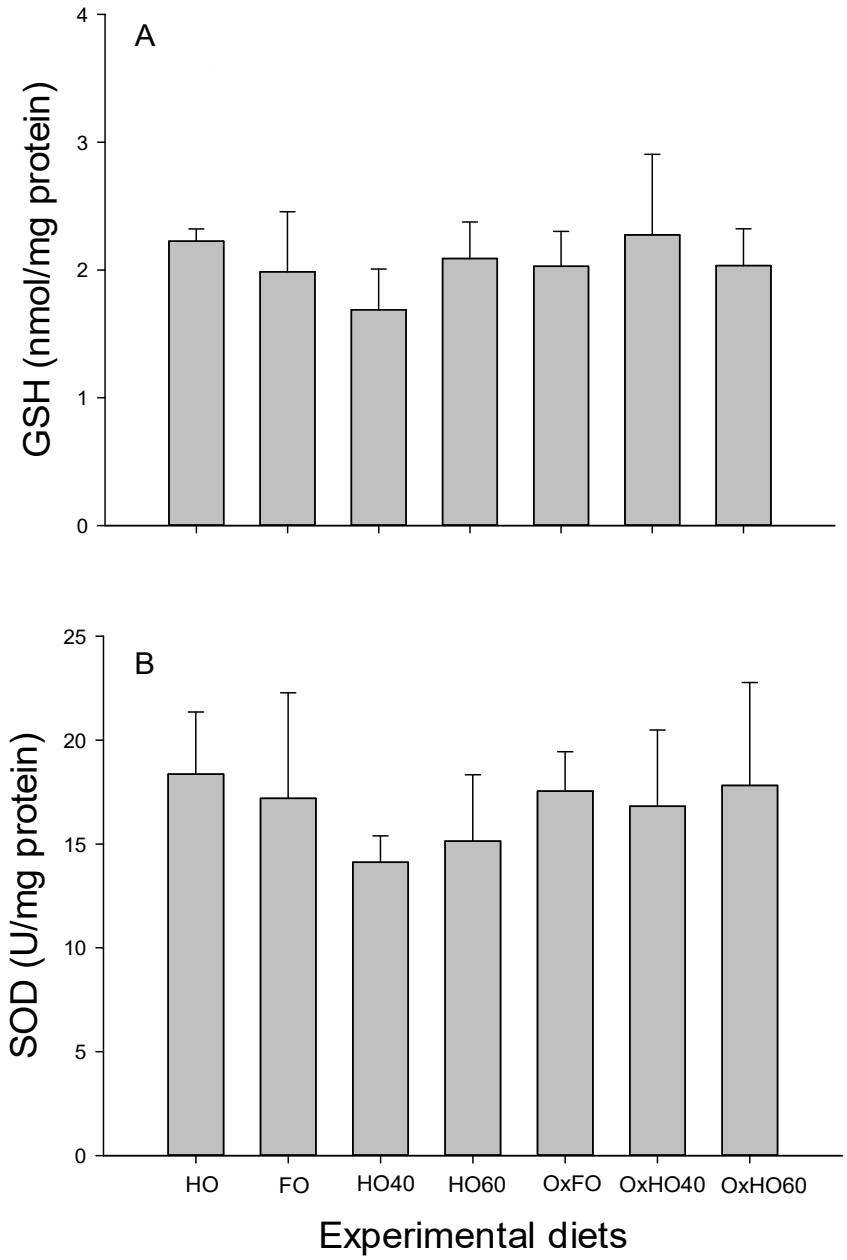

Figure 2. The level of reduced glutathione (GSH) (A) and the activity of superoxide dismutase (SOD) (B) in liver of rainbow trout fed diets with different levels of fresh fish oil, oxidized fish oil and hazelnut oil for 9 weeks. Data are expressed as mean±SD.

hand, we detected growth depletion in fish fed diet with hazelnut oil solely. Similar results were reported in brown trout, *Salmo trutta* (Arslan et al., 2012) and meagre, *Argyrosomus regius* (Katsika et al., 2021), referring to the deficiencies of essential fatty acids such as linoleic acid (18:2n-6) and linolenic acid (18:3n-3), and EPA and DHA, respectively.

We observed a decreasing tendency in the ADC values with the increase in both dietary oxidized fish oil and hazelnut oil. However, the decrease caused by the dietary lipid oxidation was more severe in comparison to the dietary hazelnut oil inclusion. Similar to our results, the ADC values of dry matter, protein and lipids were lower in hybrid grouper fed diet with fresh palm oil in comparison to those fed diets with oxidized palm oil when the peroxide value of the diet reached 36.1 meq/kg diet (Yong et al., 2022). Decreased digestibility of the nutrients in fish fed oxidized oil-containing diets was considered the consequence of oxidative stress, leading to intestinal tissue disruption which can cause toxicity (Janssens et al., 2000) and reduction in digestion and absorption of nutrients (Yong et al., 2022).

In the present study, hepatic vitamin E content increased with an increasing amount of hazelnut oil inclusion; however, this increment was lower in fish fed oxidized fish oil-containing diets in comparison to those fed diets with fresh fish oil. Similarly, liver vitamin E levels positively correlated with dietary vitamin E supplementation in *Labeo rohita* (Fatima et al., 2018) and red sea bream, *Pagrus major* (Gao et al., 2012b), exhibiting lower accumulation in the oxidized oil-fed fish. Depleted amounts of vitamin E in fish fed oxidized

Table 5. Fatty acid composition of whole body total lipids in rainbow trout juveniles fed diets with different levels of fresh fish oil, oxidized fish oil and hazelnut oil for 9 weeks. Data are expressed as mean±SD. Data with different superscripts in a row are significantly different (P<0.05)

	Experimental diets							*2-way ANOVA*		
Fatty acids	HO	FO	HO40	HO60	OxFO	OxFO40	OxFO60	Oxidation	Hazelnut oil	Interaction
14:0	6.0±0.3^{b}	4.0±0.1^{c}	2.5±0.1^{d}	5.9±3.4^{b}	3.9±0.1^{c}	2.6±0.1^{d}	7.8±0.5^{a}	NS	S	NS
14:1	ND	0.2±0.0^{a}	0.1±0.1^{b}	0.1±0.0^{b}	0.1±0.1^{b}	0.1±0.0^{b}	0.0±0.0	NS	S	NS
15:0	0.1±0.0^{d}	0.4±0.3^{b}	0.4±0.0^{b}	0.3±0.0^{c}	0.6±0.0^{a}	0.4±0.0^{b}	0.3±0.0^{c}	NS	NS	NS
16:0	10.2±0.1^{d}	16.4±0.3^{a}	14.3±0.5^{b}	12.7±0.6^{c}	16.7±0.4^{a}	14.7±0.3^{b}	12.7±0.2^{c}	NS	S	NS
16:1	1.4±0.1^{d}	5.0±0.0^{a}	3.3±0.0^{b}	2.6±0.2^{c}	5.0±0.1^{a}	3.5±0.1^{b}	2.5±0.1^{c}	NS	S	NS
17:0	0.1±0.0^{d}	0.4±0.0^{a}	0.3±0.0^{b}	0.2±0.0^{c}	0.4±0.0^{a}	0.3±0.0^{b}	0.3±0.0^{b}	NS	S	NS
18:0	3.5±0.1^{d}	4.0±0.0^{b}	4.0±0.1^{b}	3.7±0.3^{c}	4.3±0.1^{a}	3.9±0.1^{b}	3.7±0.1^{c}	NS	S	NS
18:1n-9	56.1±0.8^{a}	26.8±0.5^{d}	41.3±0.1^{c}	46.5±1.6^{b}	29.0±0.6^{d}	42.4±0.6^{c}	46.0±0.2^{b}	NS	S	NS
18:2n-6	12.1±0.4^{a}	10.4±0.1^{b}	11.8±0.2^{a}	11.8±0.7^{a}	10.7±0.3	12.3±0.3^{a}	12.0±0.1^{a}	NS	S	NS
18:3n-3	1.7±0.1^{c}	2.4±0.3^{a}	2.0±0.1^{b}	1.4±0.9^{d}	2.2±0.0^{a}	2.1±0.1^{b}	1.8±0.1^{c}	NS	S	NS
18:4n-3	0.5±0.2	1.1±0.2	0.7±0.1	0.9±0.8	1.0±0.2	0.4±0.1	0.5±0.2	NS	NS	NS
20:0	ND	0.3±0.1^{a}	0.2±0.1^{b}	0.1±0.1^{c}	0.3±0.1^{a}	0.1±0.1^{c}	0.1±0.1^{c}	NS	S	NS
20:1n-9	0.8±0.0^{a}	0.2±0.1^{c}	0.2±0.0^{c}	0.3±0.0^{b}	0.2±0.1^{c}	0.2±0.2^{c}	0.3±0.0^{b}	NS	NS	NS
20:2n-6	0.6±0.1^{a}	0.5±0.0^{b}	0.7±0.1^{a}	0.7±0.1^{a}	0.5±0.0^{b}	0.7±0.1^{a}	0.6±0.1^{a}	NS	S	NS
20:3n-3	0.0±0.0	0.2±0.2^{b}	0.2±0.0^{b}	0.0±0.1	0.3±0.0^{a}	0.1±0.1^{b}	0.0±0.0	NS	S	NS
20:4n-6	1.0±0.0	1.2±0.0^{a}	0.9±0.0^{b}	0.8±0.0^{c}	1.2±0.0^{a}	0.9±0.0^{b}	0.8±0.0^{c}	NS	S	NS
20:4n-3	ND	0.6±0.0^{b}	0.4±0.0^{c}	0.3±0.1^{cd}	0.5±0.1^{b}	0.2±0.2^{d}	0.1±0.1^{e}	S	S	NS
20:5n-3	0.5±0.0^{g}	5.9±0.1^{a}	3.0±0.0^{c}	1.8±0.1^{e}	5.3±0.0^{b}	2.8±0.1^{d}	1.6±0.0^{f}	S	S	S
22:0	0.9±0.0^{a}	0.3±0.0^{c}	0.4±0.0^{b}	0.5±0.0^{b}	0.4±0.0^{b}	0.3±0.2^{c}	0.4±0.0^{b}	NS	NS	NS
22:4n-6	0.5±0.0^{a}	0.5±0.0^{a}	0.4±0.0^{b}	0.3±0.0^{c}	0.5±0.0^{a}	0.3±0.1^{c}	0.2±0.1^{d}	NS	S	NS
22:5n-3	0.1±0.1^{g}	1.4±0.1^{a}	0.8±0.0^{c}	0.5±0.1^{e}	1.2±0.0^{b}	0.6±0.1^{d}	0.4±0.0^{f}	S	S	NS
22:6n-3	3.6±0.1^{g}	17.7±0.3^{a}	11.8±0.3^{c}	8.6±0.2^{e}	15.6±0.2^{b}	11.1±0.1^{d}	7.8±0.1^{f}	S	S	S
24:1n-9	0.2±0.0	0.1±0.1	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	0.2±0.0	NS	NS	NS
∑SFA	20.9±0.4^{c}	25.8±0.9^{a}	22.2±0.7^{b}	23.3±2.6^{b}	26.5±0.7^{a}	22.3±0.5^{b}	25.3±0.2^{a}	NS	S	NS
∑MUFA	58.5±0.8^{a}	32.3±0.5^{g}	45.2±0.2^{e}	49.6±1.8^{b}	34.5±0.6^{f}	46.3±0.5^{d}	49.0±0.3^{c}	S	S	S
∑PUFA	20.6±0.6^{g}	41.9±0.4^{a}	32.7±0.7^{c}	27.1±0.9^{e}	39.0±0.1^{b}	31.5±0.3^{d}	25.7±0.1^{f}	S	S	S
∑n3	6.3±0.2^{g}	29.2±0.2^{a}	18.9±0.4^{c}	13.6±0.3^{e}	26.1±0.2^{b}	17.3±0.4^{d}	12.1±0.1^{f}	S	S	S
∑n6	14.3±0.5^{a}	12.7±0.2^{b}	13.8±0.3^{a}	13.5±0.8^{a}	12.9±0.3^{b}	14.2±0.4^{a}	13.6±0.2^{a}	NS	S	NS
n3/n6	0.4±0.0^{g}	2.3±0.0^{a}	1.4±0.0^{c}	1.0±0.1^{d}	2.0±0.1^{b}	1.2±0.1^{d}	0.9±0.0^{e}	S	S	S

S: significant (P<0.05); NS: not significant (P>0.05); ND: not detected

lipids were also observed previously (Baker and Davies, 1997; Peng et al., 2009; Fontagné-Dicharry et al., 2014). It is evident that vitamin E is utilized more in the case of oxidative stress/lipid peroxidation, which is induced by oxidized fish oil in this particular case. Confirming this, hepatic lipid peroxidation or MDA level decreased with increasing α-tocopherol amount in our study. Previous reports also confirmed that dietary oxidized oil-related lipid peroxidation level was lowered by dietary vitamin E supplementation (Tocher et al., 2003; Fatima et al., 2018; Gao et al., 2012a; Gao et al., 2012b).

In the present study, dietary treatments did not change the amount of reduced GSH and the activity of SOD, which are often used as indices for the oxidative response. Similar to our results, GSH level did not change in largemouth bass (*Micropterus salmoides*) fed the oxidized fish oil-based diets while SOD activity increased with the increasing degree of oxidation (Yin et al., 2019). However, Liang et al. (2019) reported that dietary oxidized lipids caused depletion in GSH which was consumed first as an endogenous reducing substance, while the activity of SOD fluctuated depending on the inclusion level of dietary oxidized lipids, stimulated at a low inclusion level but suppressed by the high amount of dietary oxidized lipids. Similarly, the activity of antioxidant enzymes, including SOD in hybrid grouper (Long et al., 2022) and the level of GSH in Wuchang bream, *Megalobrama amblycephala* (Song et al., 2019) decreased by dietary oxidized lipids. In general, antioxidant enzymes have been reported to be stimulated by low levels of oxidative stress then the enzyme system is damaged when the degree of oxidation exceeds the tolerance limit of the organism (Long et al., 2022). On the other hand, the enzymatic antioxidant defense response against the dietary oxidized lipids exhibited variations depending on the species and the degree of oxidation (Chen et al., 2019). Studies also reported that the existence of vitamin E decreased the activities of antioxidant enzymes (Arslan et al., 2016), taking the scavenger duty over against oxidative radicals (Mourente et al., 2002; Fatima et al., 2018).

Dietary fatty acid profiles were reflected by fish fed corresponding diets. The amount of OA increased with the increasing amount of dietary hazelnut oil whereas EPA and DHA amounts elevated with the increase in dietary fish oil. However, fish fed the oxidized fish oil-containing diets exhibited significantly reduced amounts of n-3 PUFAs including physiologically important fatty acids such as EPA and DHA. Similar to our findings, EPA and DHA significantly decreased in rainbow trout (Fontagné-Dicharry et al., 2014), Atlantic cod, *Gadus morhua* (Zhong et al., 2008) and African catfish, *Clarias gariepinus* (Baker and Davies, 1997) fed dietary oxidized lipids. This can be considered an expected consequence, as LC-PUFAs are known to be the most susceptible fatty acids to lipid peroxidation (Tocher, 2003). On the other hand, increasing dietary vitamin E levels significantly increased the amount of EPA and DHA in our study. Similarly, dietary vitamin E supplementation spared EPA

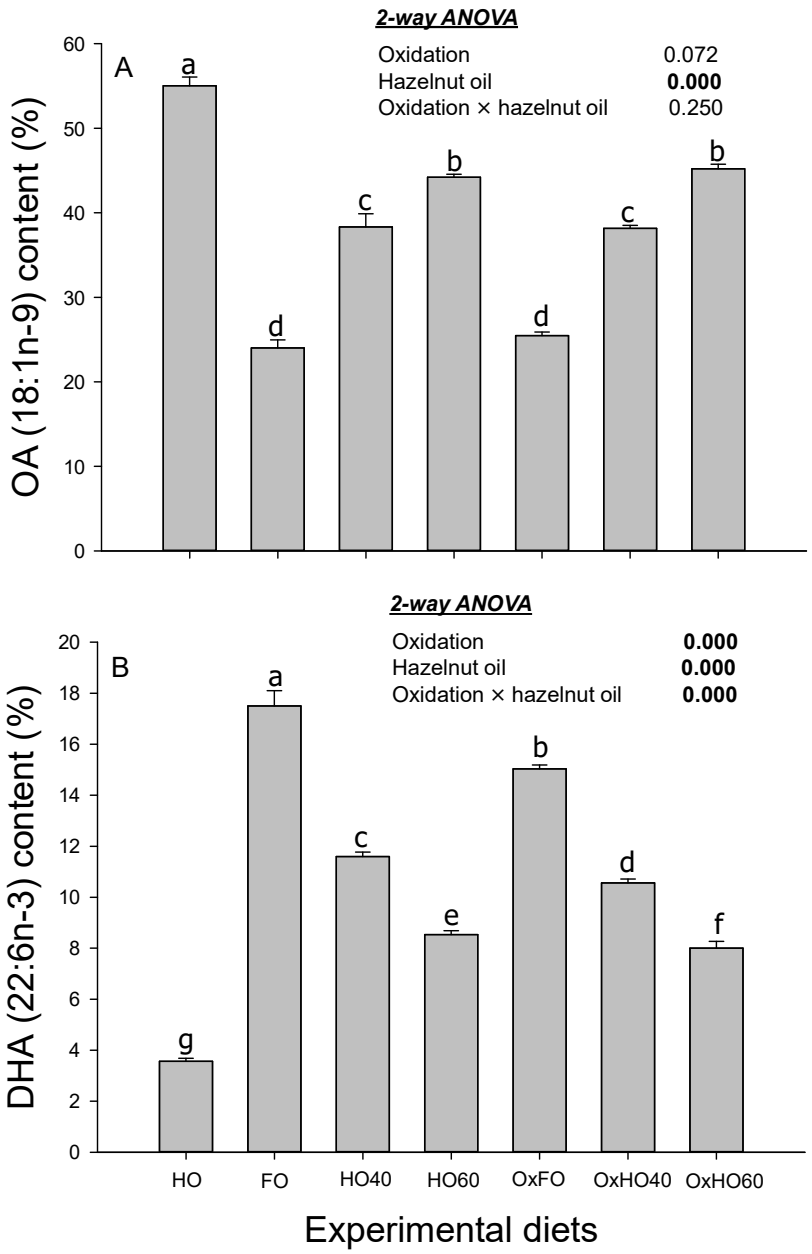

Figure 3. Oleic acid (18:1n-9) (A) and docosahexaenoic acid (22:6n-3) (B) level in whole body total lipids of rainbow trout fed diets with different levels of fresh fish oil, oxidized fish oil and hazelnut oil for 9 weeks. Data are expressed as mean±SD. Data with different letters are significantly different (P<0.05).

and DHA level in red seabream (Gao et al., 2012b), African catfish (Baker and Davies, 1997) and Atlantic cod (Zhong et al., 2007), proving the antioxidant capacity of α-tocopherol.

Previous studies reported liver degenerations such as hepatocytes with lipid vacuoles filled and nuclear migration in largemouth bass (Chen et al., 2012), high amount of lipid droplets in hybrid grouper (Long et al., 2021), and cytoplasmic vacuolation, cellular hypertrophy, nuclear migration and indistinguishable cellular outlines in genetically improved farmed tilapia (Yu et al., 2020a) fed oxidized lipids. Similarly, we observed severe hepatic degenerations and necrosis in fish fed the OxFO diet where the lipid resource was oxidized fish oil solely. However, hazelnut oil inclusion at 60% (OxHO60 diet) seemed to alleviate the negative effect of dietary oxidized fish oil considerably.

Conclusions

In conclusion, dietary oxidized lipids caused growth depletion, lowered nutrient digestibility, lipid peroxidation, decreased amount of EPA and DHA, and liver degeneration in rainbow trout. Nevertheless, dietary hazelnut oil inclusion alleviated the negative effects of oxidized oil, increasing hepatic α-tocopherol which lowered lipid peroxidation and spared n-3 LC-PUFAs such as EPA and DHA despite no significant impact on growth.

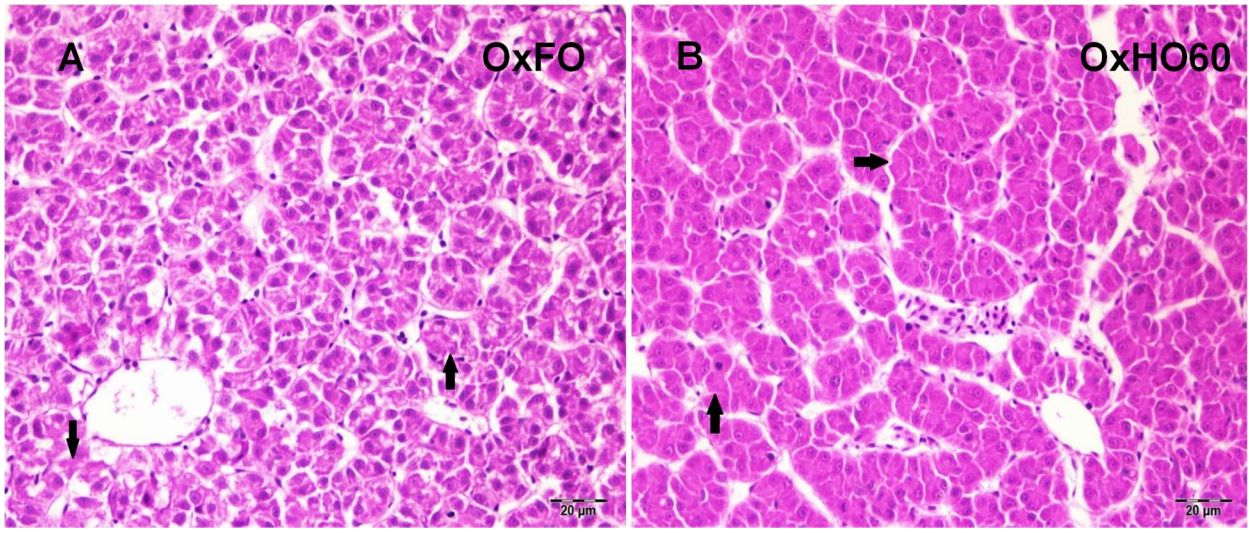

Figure 4. Liver cross-sections in rainbow trout fed the OxFO (A) and OxHO60 diets (B) for 9 weeks.

Ethical Statement

The procedures and methods used in the current study followed the ethical guidelines of Atatürk University Animal Care and Use Committee under project number PRJ2015/382.

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Author Contribution

Seyda Tacer-Tanas: Writing - original draft, Investigation, Formal analysis, Visualization. **Murat Arslan:** Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing - review & editing, Visualization, Validation, Formal analysis.

Conflict of Interest

All authors have no conflicts of interest to declare.

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