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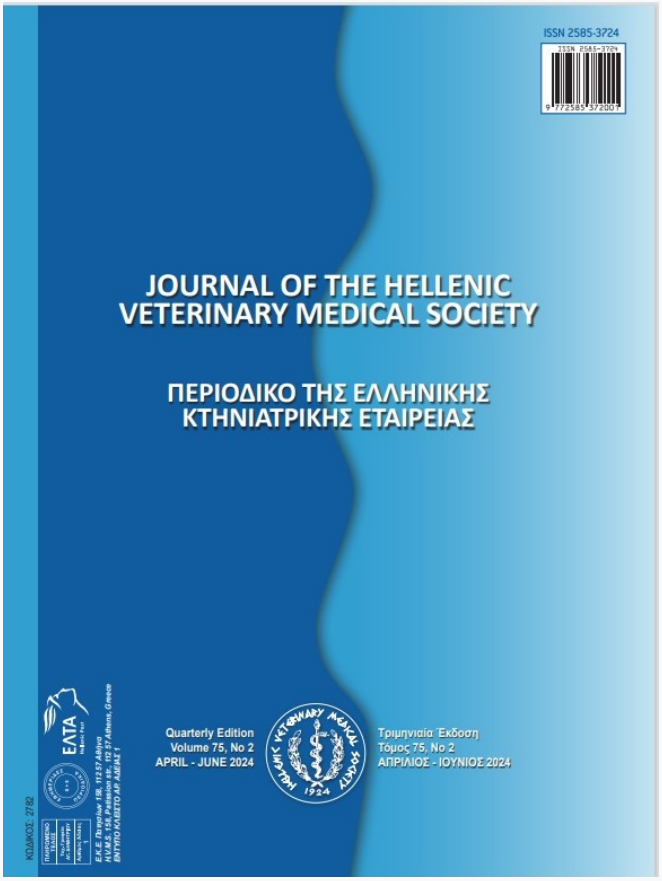

Impact of oregano (Origanum vulgare) supplementation on antioxidant status, and related gene expression in Black Sea salmon, Salmo labrax

OT Özel, ZD Düzgüneş, SE Gürkan, E Çakmak

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Research article
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Impact of oregano (*Origanum vulgare*) supplementation on antioxidant status, and related gene expression in Black Sea salmon, *Salmo labrax*

O.T. Özel[1,*], Z.D. Düzgüneş[2], S.E. Gürkan[3], E. Çakmak[1]

[1]*Central Fisheries Research Institute, Department of Aquaculture, Trabzon, Türkiye*

[2] *Central Fisheries Research Institute, Department of Breeding and Genetics, Trabzon, Türkiye*

[3]*Çanakkale Onsekiz Mart University, Faculty of Science, Department of Biology, Çanakkale, Türkiye*

ABSTRACT: The aim of this study was to to evaluate the effect of diets containing 0, 50, 100, 200, and 400 mg kg^{-1} oregano (Origanum vulgare) essential oil on antioxidant enzymes including glutathione proxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) and expression levels of their-relevant genes in liver and muscle tissues of Black Sea salmon (Salmo labrax) juvenile. This study was conducted in the freshwater recirculating aquaculture system (RAS). Fish with average initial weights of 3.52±0.01g were distributed randomly in triplicate to 50 liter fiberglass tanks, and 45 fish were placed in each experimental tank. Fish were fed 3% level of live weight for 90 days. The addition of oregano essential oil had no significant impacts on CAT in liver and GPx in muscle ($p>0.05$), but increased SOD activity was observed in muscle. The increased GPx gene expression were observed in both muscle and liver tissues. The addition of oregano essential oil had no significant impacts on the mRNA expression of SOD in muscle, nevertheless increased SOD genes expression were determined in liver. In terms of the mRNA expression of CAT, control and 200 mg kg^{-1} concentration were similar to each other and had the highest level. Present study suggests that the addition of oregano essential oil can enhanced the antioxidant enzyme activities, and related gene expression of Black Sea salmon (Salmo labrax) juvenile.

Keywords: aquaculture, herbal additive, muscle, liver, mRNA

Corresponding Author:
Özel O.T., Central Fisheries Research Institute, Department of Aquaculture, Trabzon, Türkiye, Tel: +90 462 341 10 53; Fax: +90 462 341 11 52
Email: osmantolgaozel@gmail.com

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INTRODUCTION

In recent years, researchs have focused on the use of biological additives such as enzymes, probiotics, proteins, and medicinal plants to increase fish rearing in the shortest possible time with minimum side effects. To this end, medicinal herbs have been in demanded as a dietary supplement in both aquaculture and in many studies (Asadi et al., 2018). Essential oils derived from herbs (plants) have antioxidant activity due to their radical removaling, intestinal flora modification, digestibility and nutrient absorption enhancing properties, and improving growth performance and immune response, and thus have been used as biological additives in aquaculture feeds in recent years (Cagol et al., 2020). Oregano (*Origanum vulgare*) is among of the most important medicinal and aromatic plant species distributed around the Mediterranean area (Beltran et al., 2020). Its essential oil has a high phenolic content which its main components are carvacrol and thymol (Zhang et al., 2019). Plant extracts used in aquaculture can reduce the cost of culture and be more environmentally friendly, as they are more biodegradable than chemical drugs and play a role in defense against a wide variety of pathogens (Sun et al., 2018). Oregano is used as dietary additive in the feeding of farm animals such as lambs, pigs, rabbits, broilers, rats, mice and boar for the expectation of showing antioxidant, anti-stress, antimicrobial, protective and immunostimulatory activities (Beltran et al. 2020). In studies conducted on shrimp, Nile tilapia, Channel catfish, Common carp and gilthead seabream, the dietary oregano essential oil supplementation was determined the improved growth performance, muscle protein content, feed utilization, survival rates and disease resistance (Abdel-Latif and Khalil, 2014).

Organisms develop antioxidant systems consisting of proteins including antioxidant enzymes to protect against oxidative stress (Azambuja et al., 2011). Antioxidants play a vital role in maintaining optimal health and well-being by providing a line of defense against damaging unstable molecules known as free radicals(Abd El-Naby et al., 2017). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) enzymes are important parts of antioxidant defense systems (Ning et al., 2020) and can preserve cells against oxidative damage by inhibiting ROS production (Abd El-Naby et al., 2017). Of these enzymes, SOD and CAT decompose O_2 and H_2O_2, respectively. GPx, one of glutathione-dependent enzymes, detoxifies both H_2O_2 and organic hydroperoxides (Azambuja et al., 2011). Antioxidant enzymes are accepted as an important indicator in understanding the oxidative cell damage (Abdel-Tawwab et al., 2018a), and take on important functions by neutralizing free radicals and antagonizing oxidative stress. The decrease in the levels of these enzymes in tissues is accepted as an indicator of oxidative stress and cellular damage (Abdelkhalek et al., 2020). If antioxidant enzymes cannot eliminate the excess of free radicals in time, this situation leads to a decrease in cellular antioxidant activity and restriction of enzyme activity by affecting CAT synthesis. (Baldissera et al., 2017b).

Fish are likely to be exposed to stress under aquaculture conditions. This situation may negatively affect some physiological processes as well as growth. This negative effect can be reduced with some abiotic factors such as dietary treatments. Improvement in the antioxidant defence system has a positive effect on fish welfare. Antioxidant enzymes, which have important roles in the metabolisms of fish, are significantly affected by dietart treatments. In particular, natural feed additives added to the feeds can improve culture performance by positive affecting the metabolic activities as well as the physiological properties of the fish. Considering such information, this study was aimed to determine effect on the antioxidant defense system of Black Sea salmon of oregano (O*riganum vulgare*) essential oil.

MATERIAL AND METHODS

Fish and experimental procedure

In this work, seventh filial generation of Black Sea salmon (*Salmo labrax*) with initial weights of 3.52±0.01g were used. The present work carried out at the freshwater recirculating aquaculture system (RAS) at the Trabzon Central Fisheries Research Institute. Experimental fish were placed randomly in experimental tanks into 5 treatment groups, including 45 fish in each tank. Fish were fed 3% of body weight four times daily for 90 days. Every experiment was carried out in triplicate. The experiment was carried out in fiberglass tanks (39x39-33x33 cm) and the water was changed 22 times a day. Experimental tanks were cleaned by siphoning daily. The water supplied to the experimental tanks was passed through 100, 25, 10 and, 5 μ filters from system, respectively. Experimental fish were exposed to artificial photoperiod regime with 12 hours of darkness and 12 hours of light. Water temperature, oxygen, pH, and mortality were recorded daily. The ammonia level was measured weekly.

Essential oil and feeds

Origanum vulgare oil was procurced from Talya Herbal Products operating in Antalya province, and analysed using the Agilent GC/MS system (7890B-5977B model) having an HP-Innowax column (60 m x 0.25 mm x 0.25 µm). Essential oils are insoluble in aqueous solutions, liquid, and having yellow appearances. Specific gravity was 0.935 g/cm3, refractive index was 1.49071 in 25ºC. Physical and chemical specifications of the essential oils conform to the industrial standards. First, oregano essential oils in different levels (50, 100, 200 or 400 mg/kg) were added to the fish oil, and it was mixed as homogenous. This mixture was then permeated into the extruded feeds by vacuum coating. Oregano oil was added to experimental diets at levels of 50 (O50), 100 (O100), 200 (O200) or 400 (O400) mg/kg (Table 1).

Table 1. Composition of the experimental feed (%)

Ingredients	%
Fish meal[1]	31
Soybean meal	20
Wheat gluten	6
Pea protein concentrate	12
Sunflower seed meal	7
Wheat flour	12.5
Fish oil[2]	11
Vitamin mix[3]	0.22
Mineral mix[4]	0.16
Vit C	0.12
Nutrient analyses	
Crude protein	46.20
Crude lipid	14.97
Crude Ash	9.38
Moisture	6.14

[1] Contained 65.37% protein and 10.7% lipid, and derived European sprat (*Sprattus sprattus)* and Atlantic herring (*Clupea harengus*).
[2] Derived from European anchovy (*Engraulis encrasicolus*).
[3]Supplied the following: inositol 300 mg, biotin (Vit B7) 200 mg, tocopherol (Vit E) 200 mg, calcium pantothenate (Vit B5) 50 mg, riboflavin (Vit B2) 30 mg, pyridoxine (Vit B6) 20 mg, thiamine (Vit B1) 20 mg, menadione (Vit K3) 12 mg, niacin (Vit B3) 6 mg, retinol (Vit A) 0.6 mg, folic acid (Vit B9) 0.5 mg, cholecalciferol (Vit D3) 0.05 mg, cobalamin (Vit B12) 0.05 mg.
[4]Supplied the following: ferric sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) 50 mg, manganese (II) oxide (MnO) 50 mg, zinc oxide (ZnO) 50 mg, copper sulfate pentahydrate ($CuO_4S \cdot 5H_2O$) 10 mg, calcium iodate (Ca_2IO_6) 0.8 mg, cobalt carbonate hexahydrate ($CoCO_3 \cdot 6H_2O$) 0.15 mg, sodium selenite (Na_2SeO_3) 0.15 mg.

Ethic statement

The sampling was performed in accordance with European Union Directive (2010/63/EU) (European Commision, 2010) and ARRIVE ethical guidelines (Kilkenny et al., 2010). Besides, procedures were performed with the approval (coded as ETIK-2017/1) of the Ethical Committee of Animal Experiments of Central Fisheries Research Institute in Trabzon province of Türkiye.

Analytical methods

To determined the activity of antioxidant enzymes in the muscle and liver, 6 fish were selected from each experimental groups, and then lightly anesthetized with 50 ppm benzocaine. The tissues were gently removed from the fish, placed in ethanol 70% and stored at -80°C. Tissue samples were homogenized with phosphate buffer (0.05 M, pH:7.5, 1:5 w/w) to obtain cytosolic fractions. The homogenate was centrifuged 2 times for 20 minutes at 10000 g at 4 °C and kept at -45 °C. The amount of protein in each homogenate was determined by the method of Bradford (1976) and the enzyme-specific activities measured spectrophotometrically were calculated as mU/mg protein^{-1}. SOD enzyme activity was measured according to the method of Flöhe and Ötting (1984). SOD activity is determined by the color formation resulting from the reduction of the superoxide radical to Nitro Blue tetrazolium (NBT). This reduction is completed by the formation of blue colored formazone, which gives maximum absorbance at 550 nm. Catalase activity was measured using Clairborne (1985) based on the decomposition of H_2O_2. Samples were measured every 15 seconds at 240 nm for a total of 90 seconds. It was noted that when any decrease in absorbance was observed, it was directly proportional to the CAT enzyme activity. GPx activity was determined by the method of a decrease in absorbance as long as GR degradation continued (Wendel, 1980). Although this decrease in absorbance is directly proportional to the GPx activity in the environment, the measurements were recorded at 340 nm wavelength.

The tissues were taken from 3 fish with each groups to determined antioxidant-related gene expression, and placed in cryo tubes (1.5 ml) into RNA later stabilization reagent solution. Then, tissue samples were stored at -80°C until RNA extraction. The muscle and liver samples of each tiral group were collected. Total RNA was isolated after treatment from muscle and liver tissues with RNeasy Lipid Tissue Mini Kit (Qiagen cat. no. 74804). The concentration and purity of RNA was quantified based on the ratio of absorbance at 260 nm to that at 280 nm using a nanodrop spec-

trophotometer (Thermo Fisher), and its quality was tested by electrophoresis in 1% agarose gel stained with ethidium bromide. RNA samples were stored at -80°C for cDNA synthesis. Extracted RNA samples were reverse transcribed into cDNA using the ThermoScript™ RT-PCR System for FirstStrand cDNA Synthesis Kit (Invitrogen) in accordance with the manufacturer's protocol. Synthesized cDNA samples were stored at -20°C for Real-time q-PCR. Expression of mRNA for SOD, CAT and GPX in muscle and liver was analysed using a Roche LightCycler 480 II instrument. Forward (F) and reverse (R) primer were as follows: for superoxide dismutase, F 5'-ccacgtccatgcctttgg-3', R 5'-tcagctgctgcagtcacgtt-3', for catalase, F 5'-ccgaccgtccgtaaatgcta-3', R 5'-gcttttcagataggctcttcatgtaa-3', for glutathione peroxidase, F 5'-gattcgttccaaacttcctgcta-3', R 5'-gctcccagaacagcctgttg-3', for β-Actin (Housekeping gene), F 5'-ccaaagccaacagggagaa-3', R 5'-agggacaacactgcctggat-3 (Olsvik et al., 2005). Each reaction, with a total volume of 20 µl, contained 6 µl ddH_2O, 10 µl QiagenTag PCR master mix, 1 µl qPCR Master Mix with SYBR Green (GoldBio), 1 µl forward primer, 1 µl reverse primer and 1 µl cDNA. The thermal program included 1 min at 94°C, and followed 10 s at 95°C and at 60°C for 45 s at 40 cycles. The mRNA levels in expression of antioxidant-related genes were analysed using the efficiency (e)(−ΔCt) method. The relative expression of each gene was calculated using the formula $E=10^{(-1/slope)}$ (Pfaffl, 2001).

Data analysis

All data were subjected to statistical analysis using one-way ANOVA using the Statistical Package for Social Science (SPSS for Windows Version 21). Duncan's multiple range test was used to determine spesific differences among the experimental groups. The results of the data obtained were presented as means ± SE. The levels of statistical significance were determined as P<0.05.

RESULTS

The main compounds in oregano essential oil added to experimental diets at concentrations higher than 1% are: carvacrol (68.3%), ρ-cymene (9.2%), thymol (5.2%), γ-terpinene (4.3), β-caryophyllene (2.1%), myrcene (1.4%) and caryophyllene oxide (1.0%).

Water temperature (15.10±0.98 °C), oxygen (8.78±0.21 mg/l), pH (7.43±0.18) and mortality were recorded daily. The ammonia level (0.05±0.05 mg/l) was measured weekly.

Antioxidant status

Supplemental effects of dietary oregano essential oil on antioxidant activity (SOD, CAT, and GPx) in muscle and liver tissues of fish were demonstrated in Table 2 and 3. CAT activity in muscle of fish samples that fed O200 was lower than whole experimental groups while other essential oil supplementations were not different from control (P>0.05). In terms of SOD activity in muscle, O50 and O400 were similar to each other and had the highest levels compared to other experimental treatments. Although, GPx activity in muscle and CAT activity in liver were not affected by the dietary oregano essential oil treatments, supplementation of O100 and O200 significantly increased SOD activity in liver compared with the control group (P<0.05). Additionally, supplementation of oregano essential oil increased the activity of SOD in muscle was increased in O50 and O400 and the activity of CAT in muscle was decreased in O200 in a linear and quadratic manner compared to other experimental groups.

Gene expression

The expression levels of SOD, CAT, and GPx genes were evaluated in in muscle and liver of experimental fish using the qRT-PCT technique (Figure 1). The expression levels of GPx in muscle and liver were significantly enhanced (P<0.05) in whole essen-

Table 2. Effect of dietary supplementation of oregano essential oil on muscle antioxidant status of Black Sea salmon (n=6)

Item[2]	Treatment[1]					Statistical analyses		
	Control	O50	O100	O200	O400	ANOVA	Polynomail contrasts	
						P	P_{linear}	$P_{quadratic}$
GPx	2.23±0.45	2.28±0.20	2.60±0.29	3.03±0.31	1.88±0.12	0.105	0.057	0.137
SOD	19.68±4.48^{c}	73.32±12.68^{a}	53.72±11.11^{ab}	31.65±6.63^{bc}	62.49±11.75^{a}	0.004	0.003	0.002
CAT	27.50±2.57^{a}	32.67±1.41^{a}	31.00±2.37^{a}	8.00±1.37^{b}	28.00±1.88^{a}	0.000	0.000	0.000

^{a,b,c}Mean values (mean ± standard error) with different superscript letters in a row are significantly different (95% confidence intervals).

[1]O50: 50 mg/kg, O100: 100 mg/kg, O200: 200 mg/kg and O400: 400 mg/kg oregano essential oil.

[2]GPx: Glutathione proxidase; SOD: Superoxide dismutase; CAT: Catalase

Table 3. Effect of dietary supplementation of oregano essential oil on liver antioxidant status of Black Sea salmon (n=6)

Item[2]	Treatment[1]					Statistical analyses		
	Control	O50	O100	O200	O400	ANOVA	Polynomail contrasts	
						P	P_{linear}	$P_{quadratic}$
GPx	8.66±0.81[ab]	10.47±0.69[a]	5.99±1.32[b]	7.71±0.93[ab]	6.36±1.15[b]	0.027	0.077	0.035
SOD	5.02±0.82[b]	7.40±0.52[b]	53.72±17.96[a]	38.05±11.76[a]	27.69±6.19[ab]	0.009	0.024	0.074
CAT	84.61±7.09	94.33±6.92	67.87±11.19	100.77±8.26	82.77±7.11	0.087	0.047	0.020

[a,b]Mean values (mean ± standard error) with different superscript letters in a row are significantly different (95% confidence intervals).
[1]O50: 50 mg/kg, O100: 100 mg/kg, O200: 200 mg/kg and 0400: 400 mg/kg oregano essential oil.
[2]GPx: Glutathione proxidase; SOD: Superoxide dismutase; CAT: Catalase

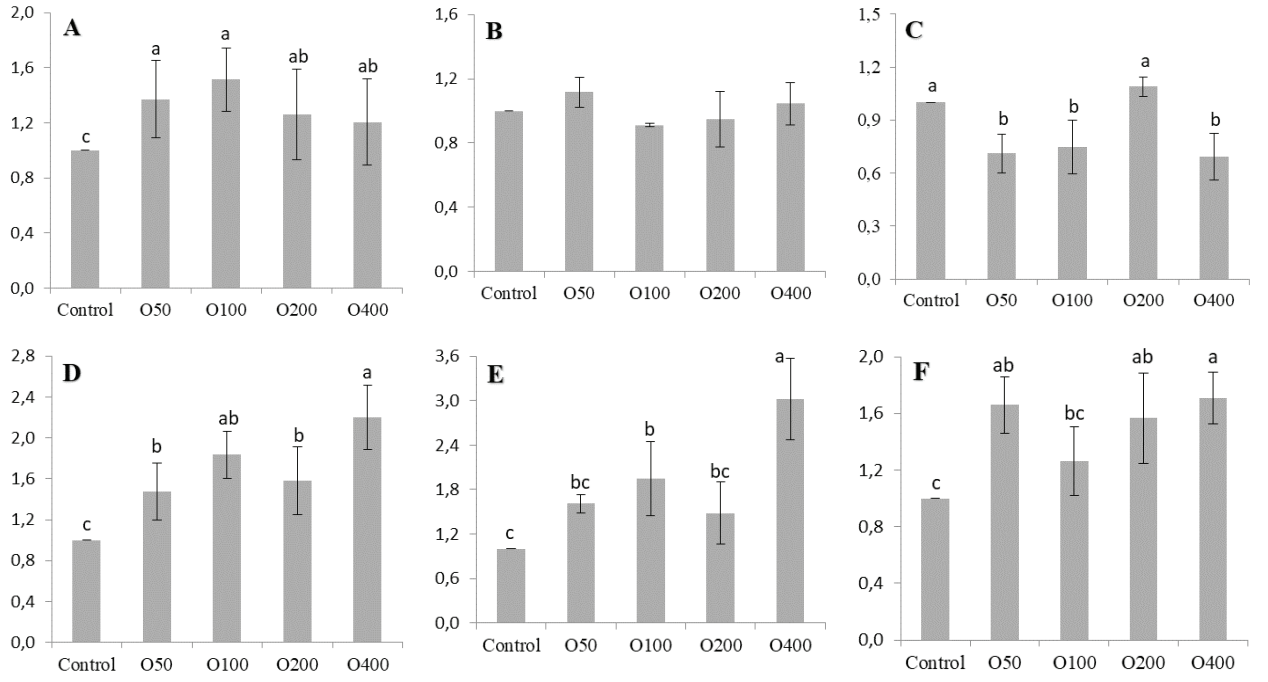

Figure 1. Effect of dietary oregano essential oil on the relative antioxidant enzymes expression of muscle and liver tissues of Black Sea salmon. O50: 50 mg/kg, O100: 100 mg/kg, O200: 200 mg/kg and 0400: 400 mg/kg oregano essential oil. (A): glutathione peroxidase (GPx), (B): superoxide dismutase (SOD) and (C): catalase (CAT) in muscle, (D): glutathione peroxidase (GPx), (E): superoxide dismutase (SOD) and (F): catalase (CAT) in liver. Mean values (mean±standard error) with different superscript letters (a, b, c) in column are significantly different (95% confidence intervals).

tial oil groups compared with that in control group. In muscle, the highest value was observed in O50 and O100 groups. This was followed O200 and O400 groups. In liver, the highest value was observed in O400 and O100 groups. This was followed O200 and O50 groups, respectively. There were no significant differences in the expression level of SOD gene in muscle among the experimental treatments. A significant increase was obtained in the expression level of SOD gene in liver of fish, with the highest values being observed in supplementation with O100 and O400 groups as compared to control group. However, the SOD level in muscle was not affected by any dietary oregano essential oil supplementation. In terms of the mRNA expression of catalase gene, while muscle tissue was higher in O50, O200 and O400 groups than in the control groups, liver tissue was higher in control and O200 groups.

DISCUSSION

Origanum vulgare is one of the herbal species with the highest antioxidant content, and is added to animal feed as dietary additive to prevent lipid peroxidation of feed and oxidative stress (Beltran et al., 2020). Its antioxidative capacity is attributed in their essential oil including thymol, carvacrol, γ -terpinene, and p-cymene (Khafaga et al., 2020). A similar trend was observed in *Zataria multiflora* and *Thymus vulgaris*, which are high of thymol and carvacrol concentrations (Mirghaed et al., 2020). Thymol and carvacrol are ca-

pable to modulate immune and antioxidant systems of fish species (Mirghaeda et al., 2018). Antioxidant compounds can be synthesized by fish and plants to prevent oxidative damage caused by increased ROS production during tissue reoxygenation (Saccol et al. 2013). Excessively ROS production in fish can cause oxidative stress. This adversely affects the health of the fish by causing oxidative damage. The presence of SOD and CAT eliminates the adversely effects of excessive ROS production (Abdel-Latif et al. 2020). In the presence of a factor that increases exceed ROS production (cypermethrin etc.), the addition of *Origanum vulgare* essential oil improves serum SOD and CAT activity, clearing excess ROS production, thus helping to prevent oxidative damage (Khafaga et al., 2020). While *Origanum majorana*, which belongs to the same family as *Origanum vulgare*, increased plasma SOD activity in common carp, not change CAT activity (Yousefi et al., 2020). Besides, Nano-oregano essential oil improved serum GPx activity in the rainbow trout, *Oncorhynchus mykiss* (Dokou et al. 2023). A similar result was observed in our study in SOD and GPx levels in muscle and CAT levels in liver.

SOD and CAT antioxidant enzymes are considered as the first line of defense against free radicals (Zeppenfeld et al., 2016). Blend of cinnamaldehyde, thymol, carvacrol significantly increased serum SOD activity of tilapia under hypoxia stress (Ning et al., 2020). A previous study, Abdel-Latif et al. (2020) reported that dietary supplementation of oregano (*Origanum vulgare*) essential oil significantly increased SOD activity in liver of common carp (*Cyprinus carpio*). An additional study, Saccol et al. (2013) found that a significant increase in muscle SOD in silver catfish in dietary supplementation *Lippia alba*. The increase in SOD activity in fish may maintain the superoxide anion level (Mohammed et al., 2020), reduce MDA level (Yousefi et al. 2020), and protect the cells against lipid peroxidation and free radicals (Asadi et al. 2018). In our study, oregano essential oil added to Black Sea salmon diets showed an antioxidant effect by significantly increasing the SOD activity in muscle and liver. Antioxidant property of oregano essential oil are believed to be due to its ability to scavenge free radicals, chelate transition metal ions, and decompose peroxides (Zhang et al., 2019). Lopez et al. (2020) reported that the increasing effect of *Citrus* × *latifolia* essential oil (0.5 and 1 ml/kg) added to fish feed on SOD activity shows that it has good capacity to scavenge the anion superoxide and can improve the primary antioxidant defense mechanism system. *Origanum majorana*, which belongs to the same family as *Origanum vulgare*, did not change plasma CAT activity in common carp (Yousefi et al., 2020). However, dietary *Zataria multiflora* essential oil stimulated plasma and hepatic CAT activity of rainbow trout (*Oncorhynchus mykiss*) (Mirghaed et al., 2020). Similarly, dietary supplementation with carvacrol or thymol to diet incerased the serum CAT activity of rainbow trout (Giannenas et al. 2012). In our study, it decreased significantly in the O200 group although CAT activity in muscle did not change in the other treatment groups. Similarly, Khafaga et al. (2020) reported that there was no difference in serum CAT in common carp (*Cyprinus carpio*) fed 1 % *Origanum vulgare* essential oil diet. Whereas a significant decrease CAT was observed in those fed 0.5 %. This indicates that CAT's capacity to scavenge hydrogen peroxide produced in response to oxidative stress decreases, thus contributing to oxidative damage, as reported by Baldissera et al. (2017a). Decreased CAT activity in the cell leave defenseless the immune system and damages the liver (Baldissera et al., 2017b). Additionally, Our result in liver CAT activity is in accordance with those reported by Zheng et al. (2009) who found that there was no difference in serum CAT activity of channnel catfish fed with carvacrol or thymol supplemeted feeds. No significant effects were observed in liver GPx activity of gilthead seabream (*Sparus aurata*) fed dietary *Origanum vulgare* (Beltran et al. 2020), silver catfish in dietary supplementation of *Lippia alba* (Saccol et al., 2013) and *Colossoma macropomum* that fed *Mentha piperita* essential oil (Ribeiro et al. 2018). Similarly, there was no difference in hepatic and muscular GPx in tambaqui (*Colossoma macropomum*) juvenile fed *Citrus* × *latifolia* essential oil diet (Lopez et al., 2020). In our study, supplementation of oregano essential oil to Black sea salmon had no influence on the GPx activity both in muscle and liver.

Antioxidant enzyme related gene expression analyses may have positive effects on the antioxidant defense system. At the molecular level, however, gene expression data alone may not be sufficient. Thus, the results obtained by evaluating the antioxidant enzyme activities need to be confirmed (Safari et al., 2017). In our study, in order to reveal the effects of oregano essential oil on tissue antioxidant enzyme levels in Black sea salmon, it was aimed to determine the gene expression levels as well as the activities of antioxidant enzymes in tissues. In according to our results, oregano essential oil added to Black Sea salmon diets

showed significantly increase the mRNA expression of CAT, SOD and GPX genes activity in liver. Increased SOD and CAT in fish may be related to the role of essential oil added to the diet in regulating antioxidant enzyme activity (Mohammed et al. 2020). In previous studies, Mousavi et al. (2020) found that a significant improvement in the expression of intestine CAT and GPx genes in rainbow trout fed dietary supplementation of grape (*Vitis vinifera*) seed extract at level of 10 g kg^{-1} was observed. Mohammed et al. (2020) found that SOD and CAT enzymes and their-related gene expression were significantly increased in feeding with sweet orange (*Citrus sinensis*) or lemon (*Citrus limon*) essential oils supplemented diets in Nile tilapia. We also found that dietary administration of oregano essential oil has a improvement effect on the expression of GPx in muscle of Black Sea salmon, whereas there was no difference in muscle SOD. In contrast, O50, O100 and O400 diets reduced CAT expression. The antioxidant activity of tissues and organs are different. This differences can arise from difference in rates of free radical generation, in susceptibility to oxidative damage and in antioxidant capacities of the tissues (Saccol et al. 2013). Hassaan et al. (2019) reported that relative expression level of the SOD and CAT genes mRNA was significantly increased with dietary incorporation of *Silybum marianum* seeds. Also, our results regarding liver CAT expression are in accordance with those reported by He et al. (2017) who found that the expression of CAT gene in the hepatopancreas of Pacific White shrimp (*Litopenaeus vannamei*) was significantly increased with a blend of organic acids and essential oils (thymol, 1.7%; vanillin, 1.0%) supplemented feed. Moreover, we found that Black Sea salmon fed oregano essential oils at levels of 50, 100, and 400 mg kg^{-1} showed an reduce of muscle CAT gene mRNA level. A similar trend was observed by Jafarinejad et al. (2020) who found that a significant reduce in the expression level of liver CAT gene of common carp juvenile fed with dietary ginger (*Zingiber officinale*) powder supplemented feds at levels of 2% and 5%.

CONCLUSIONS

Our results indicate that dietary oregano essential oil significantly changed antioxidant enzyme activity and related gene expression in Black Sea salmon. The addition of oregano essential oil at 50, 100 and 400 mg kg^{-1} for muscle and 100 and 200 mg kg^{-1} for liver was increased only SOD activity among antioxidant enzymes. Whereas, muscle CAT activity at 200 mg kg^{-1} and liver GPx activity at 100 mg kg^{-1} were considerably decreased. Besides, dietary supplementation of oregano essential oil improved the gene expression levels of GPx, as well as, the those of CAT and SOD to some extent also.

As a conclusion, oregano essential oil can be used effectively in Black Sea salmon diets for improving the antioxidant enzyme activity, and antioxidant-related gene expression. However, in order to better understand the possible effects of oregano essential oil on the antioxidant status of Black Sea salmon, further studies are needed by also being monitor their response to some stressors.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Research data will be made available on request.

DECLARATION OF ETHICAL ISSUES

The experimental protocols were conducted in accordance with the approval of the experimental animals ethics committee of the Trabzon Central Fisheries Research Institute (protocol No.: ETIK-2017/1).

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