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The effects of Fennel (*Foeniculum vulgare*) Essential Oils on Growth Performance and Digestive Physiological Traits in Black Sea Salmon (*Salmo labrax* PALLAS 1814) Juveniles

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ABSTRACT
The present study was carried out in the freshwater recirculating aquaculture system to determine the effects of the fennel (*Foeniculum vulgare*) essential oil on Black Sea salmon (*Salmo labrax*). Fish were distributed randomly to 50 L experimental tanks, and 45 fish were in each tank. The experiment was triplicate in each group, and the results were averaged. Five diets of equal isonitrogenous and isocaloric content with 50, 100, 200, and 400 mg kg^{-1} of fennel essential oil were prepared. Fish were fed with diets at the rate of 3% of live body weight four times daily by hand for 90 days. The results revealed that dietary supplementation with fennel essential oil did not have any significant effect on the growth performance. Supplementation with 200 mg fennel kg^{-1} increased lipase activity in contrast with control group. Supplementation with 400 mg kg^{-1} fennel showed similar results with the control group in terms of pepsin activity. The intestinal villi length of fish fed 200 mg kg^{-1} fennel was higher than the control group. The thickness of muscularis in group fed with 50, 100 and 200 mg kg^{-1} fennel was similar to each other and higher than the control group. Lactic acid bacteria were reduced by fennel essential oil supplementation. Results showed that fennel essential oil can be used in diets of Black Sea salmon without the growth performance.

Keywords: *Salmo labrax*, Feed additive, Essential oil, Nutrition

1. Introduction

Black Sea salmon aquaculture started in 2007, and today has been reached the level of 2311 tons/year in Turkey (TÜİK 2021). Currently, Black Sea salmon widely cultivated in the Southeastern Black Sea, has quite suitable cultural characteristics for global aquaculture (Çakmak et al. 2013). The importance of aquaculture is quite high in meeting animal protein needs which tends to increase constantly in human nutrition. Increasing demand and consumer preferences to seafood has prompted researchers to carry out new studies to increase species diversity along with the amount of production in aquaculture. As well as the rainbow trout and the Atlantic salmon, the Black Sea salmon have been expected to play an important role in the future in meeting the global demands which are in an ever increasing trend.

Numerous feed additives used to improve growth performance in fish, are chemical substances such as hormones and antibiotics that have undesirable side effects (Khalafalla 2009). However, using natural supplements in aquaculture have begun to gained importance due to the adverse effects of antibiotics and synthetic antioxidants on human health (Bilal et al. 2008). The natural and harmless compounds have the potential to be used in aquaculture as an alternative to antibiotics (Navarrete et al. 2010). World Health Organization encourages medicinal herbs to substitute or minimize the use of chemicals (Tonsy et al. 2011). The increasing concern about the use of antibiotics in animal production is the search for aromatic herbal and essential oil alternatives, which have a stimulating effect on both microbial activity and animal digestion (Kim et al. 2011). It has been stated that the plant extracts could be used as alternative additives as a result of both in vivo and in vitro studies (Bilal et al. 2008). Essential oils and their extracts derived from medicinal and aromatic plants increase the absorption of nutrition and the benefit of fishmeal by having a stimulant effect on the enzyme activities of pancreas and bile. They also increase intracellular absorption, accelerate the renewing of epithelium cells in the small intestine, regulate the intestinal microflora and prevent the habitation of

disease-causing bacteria into the digestive system. Therefore, these oils provide both improvements in growth performance and strengthening the immune system (Khalafalla 2009; Tonsy et al. 2011). Essential oils are recognized as safe according to the Food and Drug Administration (Snuossi et al. 2016). The essential oil derived from herbs and species are an alternative feed additive, and have antimicrobial, antioxidant and antifungal properties (Çabuk et al. 2014). Fennel (*Foeniculum vulgare*) essential oil is a biennial medicinal and aromatic plant belongs to Apiaceae (Umbelliferae) family (Hassaan & Soltan, 2016), and also has antioxidant, anticancer, antibacterial, antifungal properties, antidiabetic (Sotoudeh & Yeganeh 2016), hepatoprotective effects and antioxidant activities (Hassaan & Soltan 2016).

The goal in the aquaculture sector is to achieve maximum profit with minimum expense under optimum conditions. The use of products involving natural compounds which has short term effects are preferable in aquaculture by both producers and consumers. In order to commercialize the use of such products in aquaculture, it is important to investigate these medicinal-aromatic plants in all aspects. However, studies carried out on essential oils in fish nutrition are limited. In this study, it was aimed to investigate the effect of fennel essential oil on the growth performance and digestive physiology of Black Sea salmon, which is an endemic for Turkey and an increasing demand for farming.

2. Material and Methods

2.1. Fish, maintenance and feeding experiment

The study was performed at the freshwater recirculating aquaculture system at the Central Fisheries Research Institute. Fifth filial generation (F5) of Black Sea salmon (*Salmo labrax*) with average initial weights of 3.52±0.01g were used in the study. Every experiment was carried out in triplicate. Treatments were performed in 15 experimental tanks. The 675 fish (135 individuals per group) were randomly distributed into 50 L tanks at a density of 45 fish per experimental tank. Fish were fed by hand 3% of body weight four times daily. Experiments were carried out in square tanks with refreshing used water 22 times daily. Water temperature (15.10±0.98 °C), oxygen (8.78±0.21 mg/L), pH (7.43±0.18) and mortality were recorded daily. Ammonia (0.05±0.05 mg/L) was measured weekly.

2.2. Diet formulation

Fennel oil was supplied from Talya Herbal Products which are extracted from cultured *Foeniculum vulgare* provided from in the Mediterranean region of Turkey. The analyses were carried out by Anadolu University with the Agilent GC/MS system (7890B-5977B model) having an HP-Innowax column (60 m x 0.25 mm x 0.25 µm). In the analysis, carrier gas was selected as helium (0.7 mL/min), injection temperature was set as 250 ºC, ion source temperature was set as 230 ºC, and 70 eV electron was used for ionization. Ultimately, obtained results were evaluated with Wiley 9-Nist 11 Mass Spectral Database in Anadolu University. The volatile components of fennel essential oil were shown in Table 1. Experiment diets were prepared to contain fennel essential oils at levels of 50, 100, 200 and 400 mg kg^{-1}. All raw materials except fish oil and fennel essential oil were homogeneously blended with grinder. The mixture was extruded at 70 °C. Then the fennel oils were first included into the fish oil and then were penetrated into the extruded baits by vacuum coating. Similarly control diet without fennel oil was prepared by being penetrate into fish oil extruded post. Extruded feeds were cut 2.0 mm pellet size. Five diets were formulated, involving control. The control diet did not contain fennel oil. Fish meal was a mixture of European sprat (*Sprattus sprattus)* and Atlantic herring (*Clupea harengus*) meals containing 65.37% of crude protein and 10.7% of crude lipid, whereas fish oil was derived from European anchovy (*Engraulis encrasicolus*) the most used oil source for feed ingredients. Ingredients and nutrient compositions of diets were shown in Table 2.

Table 1- Volatile components of fennel essential oil*

Compound	*%*
(E)-Anethole	72.6
Limonene	6.3
Anisaldehyde	4.4
Methyl chavicol	3.7
(E,E)-2,4-Decadienal	3.2
(E)-2-Heptenal	2.3
(E,Z)-2,4-Decadienal	2.2
Carvone	1.4
α-Fenchone	1.3
(E)-2-Decenal	1.0
Anisketone	0.9
α-Pinene	0.7

*: The most abundant chemical compounds of essential oils were listed according to amounts that were found higher than 0.5%.

2.3. Fish performance

Before weight measurements, fish were starved for 1 day, then lightly anesthetized with 50 ppm benzocaine. The growth performance was calculated with equations shown below.

$$\text{Specific growth rate (SGR)}\ \% = 100 \times [(\ln \text{Final weight} - \ln \text{Initial weight})/\text{days}]$$
$$\text{Weight gain (WG)}\ g = (\text{Final weight - Initial weight})$$
$$\text{Feed conversion ratio (FCR)} = (\text{Feed intake}/\text{Weight gain})$$
$$\text{Survival rate (SR)}\ \% = 100 \times (\text{Final number of fish}\ /\text{Initial number of fish})$$ (Hoseinifar et al. 2014).

Table 2- Formulation and chemical parameters of the basal diet (%)

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

[1]: 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. **[2]:**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.

2.4. Digestive enzyme assays

Midgut samples were taken in 45th minutes after feeding and were kept at -80 °C until analyses. Tissue samples brought to Çanakkale Onsekiz Mart University, Faculty of Arts and Science, Biology Department, Water Ecology Laboratory in the cold chain. It was necessary to prepare homogenate from the digestive tract to be used and to obtain cytosolic fractions to analyse the digestive enzymes. The tissues were weighed and homogenized in a 1:5 ratio with homogenization buffer (0.05 phosphate buffer pH 7.4). The specific activity of each enzyme evaluated in the study was measured spectrophotometrically. Obtained values were proportioned to the protein value in homogenate and interpreted in terms of mU / mg.protein^{-1}. For this, Bradford (1976) method was used to calculate the amount of protein.

For the measurement of trypsin enzyme activity, Tseng et al. (1982), the analysis method used in their study and Na-Benzoyl-DL-arginine-p-nitroanilide (BAPNA) was used as substrate. Enzyme activities of the samples were measured in a spectrophotometer at 253 nm wavelength for 5 minutes. Measurement of pepsin enzyme activity was performed using a revised version of the analysis method used by Worthington (1982) by Infante & Cahu (1994). Besides, bovine hemoglobin was used as a substrate. Samples were measured at a wavelength of 280 nm for 5 minutes. Monitoring the α-amylase enzyme activity depended on the study conducted by Bieth & Metais (1968) which they used soluble starch as a substrate. Samples were measured at 540 nm wavelength for 5 minutes. To measure lipase enzyme activity, α -naphthyl caprylate was used as the analysis method and substrate used in the study conducted by Versaw et al. (1989). Spectrophotometric measurement at 490 nm wavelength for 10 minutes.

2.5. Histomorphological studies

Intestinal sampling was sampled from the beginning part of the middle intestine, which is final point of section attached to the intestine of pyloric caeca. Tissue samples were taken from 6 fish with each group cut into 1.0 cm pieces and placed into 10% formalin for further processing. Then, tissue samples were carried to Kırsehir Ahi Evran University, Faculty of Agriculture, Zootechni Department to tissue processing. Tissues were placed into tissue cassettes for dehydration process and were embedded in paraffin blocks, then subsequently cut 5-µ thickness and placed on a slide. A tissue sample of each intestine was prepared and stained with hematoxylin and eosin solution by using the standard paraffin-embedding procedure. After the embedding process,

the muscularis layer, villi length and villi width were photographed with ZEISS Primostar HD Light microscope and evaluated by using an image processing and analysis system.

2.6. Enumeration of intestinal microbiota

A total of 25 fish (5 fish from each diet group and control) were used for bacterial examination. The intestinal tract of fish was aseptically removed in the Fish Health laboratory of Trabzon Central Fisheries Research Institute. A gram of digestive content was homogenized with 9 ml of 0.1% peptone-water containing 0.9% NaCl using the stomacher apparatus (BagMixer CC, Interscience). Five-fold serial dilutions of content were prepared and streaked on de Man, Rogosa and Sharpe (MRS, Merck) for the count of the lactic acid bacteria (LAB). LAB was allowed to incubate at 30 °C for 48h in anaerobic jars (Merck), (Harrigan and McCance 1976). The dilution (100µl) was also streaked on Coliform Agar (CES, Merck) and incubated for 24 h at 35 °C for the count of the total aerobic mesophilic bacteria (TAMB) and *Escherichia coli* (Ture et al. 2018). At the end of incubation, the total number of LAB, *E. coli* and TAMB were calculated by counting the colony-forming units.

2.7. Statistical analyses

Data are presented as means with standard errors. The data were statistically analysed by one-way ANOVA. Duncan's multiple range test was performed for the significance of differences of means among groups. The intestine microbiota data were log10 transformed, then analysed. The result was considered significant at $p<0.05$. Data analysis performed using SPSS 21.0.

3. Results

3.1. Growth and feed utilization

The results obtained from measurements at the end of the experiment are shown in Table 3. No differences were observed in growth performance between the control diet and fennel oil levels ($p>0.05$). Fish exposed to the 100 mg kg^{-1} fennel oil group reached a final weight of 31.26 g, but not higher statistically from the other group. Besides insignificance results among the groups, all trials had high survival rates at the end of the experiment.

Table 3- Growth parameters of Black Sea salmon fed with fennel oil supplemented diets

Performance	*Levels of fennel (Foeniculum vulgare) oil (mg kg^{-1})*					*P values*
	0	*50*	*100*	*200*	*400*	
FW	29.83±1.11	30.46±0.69	31.26±1.06	29.72±0.25	30.62±0.45	0.646
FI	23.30±0.72	23.71±0.55	24.18±0.44	23.43±0.45	23.91±0.22	0.730
WG	26.31±1.11	26.95±0.69	27.74±1.05	26.20±0.25	27.10±0.45	0.644
FCR	1.07±0.04	1.00±0.05	0.97±0.04	1.08±0.04	1.00±0.04	0.486
SGR	2.30±0.04	2.32±0.03	2.35±0.04	2.29±0.01	2.33±0.01	0.600
SR	91.85±2.67	97.04±1.48	91.11±2.22	96.30±0.74	94.82±0.74	0.133

No difference between means ($P>0.05$), values are given as means with standard errors. FW (g): Final weight, FI (g): Feed intake, FCR: Feed conversion ratio, WG (g): Weight gain, SGR (%): Specific growth rate, SR (%): Survival rate.

3.2. Enzyme status

Table 4 illustrates the results obtained from the digestion enzyme activities. Supplementation with fennel essential oil affected on pepsin and lipase enzyme activities. α- amylase activity was similar among trial groups, which was higher in fish fed 50 mg kg^{-1} fennel oil versus the control. The same observation was done for the trypsin activity, which was higher in fish fed 200 mg kg^{-1} fennel oil versus the control although there was no statistical difference among groups. Pepsin enzyme activity decreased lightly in Black Sea salmon fed with 400 mg kg^{-1} fennel oil, but it was in those fed with 50 and 200 mg kg^{-1} fennel oil that the lowest rate was determined.

Table 4- The activity of digestion enzymes of Black Sea salmon fed with fennel oil supplemented diets, U mg^{-1}

Enzymes	*Levels of fennel (Foeniculum vulgare) oil (mg kg^{-1})*					*P values*
	0	*50*	*100*	*200*	*400*	
Pepsin	$69.95±7.29^{a}$	$15.83±2.91^{b}$	$27.24±8.90^{b}$	$16.40±2.65^{b}$	$58.13±2.67^{a}$	0.000
Trypsin	34.52±5.93	30.99±0.61	35.48±3.15	44.17±5.51	22.39±3.81	0.052
Amylase	1.46±0.46	7.08±2.90	5.21±1.45	6.69±2.50	2.05±0.63	0.177
Lipase	$0.02±0.00^{bc}$	$0.04±0.00^{ab}$	$0.02±0.01^{bc}$	$0.06±0.02^{a}$	$0.01±0.00^{c}$	0.013

Means with different superscript letters in a row are significantly different at $P<0.05$, values are given as means with standard errors.

3.3. Intestine histomorphology

The results are summarized in Table 5 and shown in Figure 1. The essential oil groups except for 400 mg kg^{-1} fennel oil decreased intestinal villi length. In addition to, the intestinal villi length of the 200 mg kg^{-1} fennel oil was higher than that of the control one ($P<0.05$). Although there was no statistical difference among them in terms of villi length, it was higher in fish fed 50 and 100 mg kg^{-1} fennel oil compared to control. Moreover, adding fennel essential oil to the diet increased the muscularis layer. Muscularis layer was found to be higher for fish fed all other fennel oils except for 400 mg kg^{-1} fennel oil compared to fish fed control diet. However, supplementation with fennel essential oil did not effect on villi width.

Table 5- Intestine histomorphology of Black Sea salmon fed with fennel oil supplemented diets, µm

Histomorphology	*Levels of fennel (Foeniculum vulgare) oil (mg kg^{-1})*					*P values*
	0	*50*	*100*	*200*	*400*	
VL	223.80±5.35^{bc}	252.58±16.30^{ab}	255.57±16.36^{ab}	281.43±10.01^{a}	191.17±7.66^{c}	0.000
VW	65.15±2.88	69.67±4.65	78.72±7.26	75.78±5.65	82.14±4.32	0.145
Muscularis	44.53±1.82^{b}	57.48±4.55^{a}	56.61±2.77^{a}	57.71±2.72^{a}	51.87±1.70^{ab}	0.008
VL/VW	3.58±0.25^{a}	3.85±0.33^{a}	3.87±0.56^{a}	4.03±0.34^{a}	2.46±0.21^{b}	0.020

Means with different superscript letters in a row are significantly different at $p<0.05$, values are given as means with standard errors. VL: Villi length, VW: Villi width

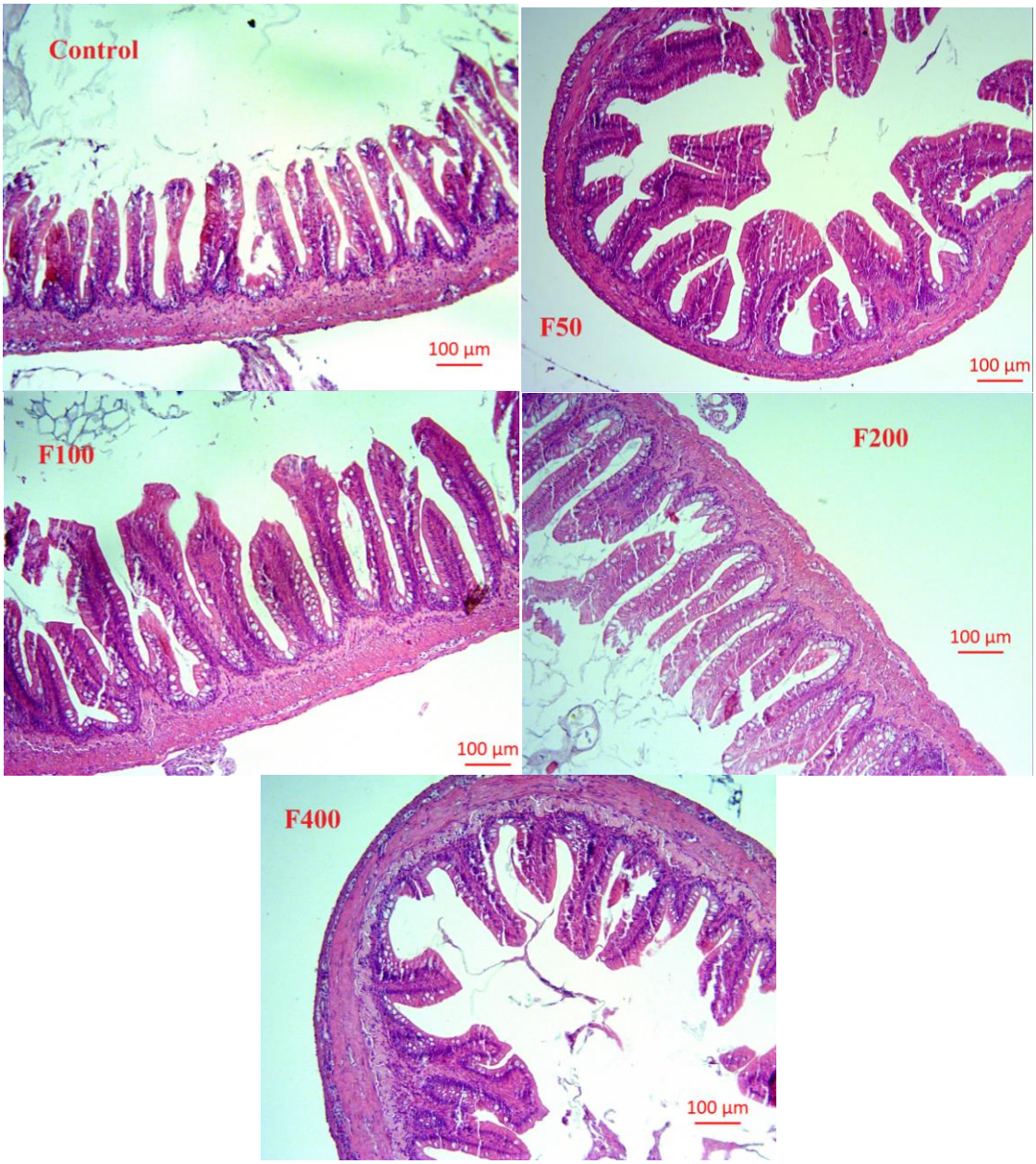

Figure 1- Intestine histology of Black Sea trout fed with fennel oil supplemented diets (4x, H&E)

3.4. Intestinal microbiota

Administration of fennel essential oil to the diet led to significant differences among the groups in terms of intestine microbiota. Feeding with a diet containing different levels of fennel essential oil decreased significantly the amount of lactic acid bacteria. The control group showed higher lactic acid bacteria than all other fennel oils. Conversely, the 200 and 400 mg kg^{-1} fennel oils showed a higher *E. coli* and coliform than the other groups. The lowest *E.coli* and coliform counts were found in fish fed 100 mg kg^{-1} fennel oil among groups. However, the lowest lactic acid bacteria were found in fish fed 400 mg kg^{-1} fennel oil (Table 6).

Table 6- Intestine microbiota of Black Sea salmon fed with fennel oil supplemented diets, CFU log/g

Microbiota	*Levels of fennel (Foeniculum vulgare) oil (mg kg^{-1})*					*P*
	0	*50*	*100*	*200*	*400*	*values*
E.Coli	5.46±0.16^{b}	4.90±0.35^{bc}	4.38±0.52^{c}	6.95±0.22^{a}	6.97±0.18^{a}	0.000
Coliform	12.47±0.15^{b}	12.07±0.30^{b}	11.36±0.55^{b}	13.86±0.39^{a}	13.96±0.45^{a}	0.003
LAB	11.05±0.05^{a}	5.29±0.10^{c}	5.98±0.09^{b}	5.28±0.15^{c}	4.58±0.18^{d}	0.000

Means with different superscript letters in a row are significantly different at P<0.05, values are given as means with standard errors.

4. Discussions

In our study, all the experimental diets were willingly consumed by fish during the trial. This has shown that fennel oil was attractive for Black Sea salmon. Abo-State et al. (2017) stated that phytogenic feed additives increase the flavour and palatability of feed. Additionally, Seden et al. (2009) stated that increased feed intake is due to high demand for nutrients or increased appetite. Phytogenic feed additives have been recognized in aquaculture as natural eco-friendly growth promoters in recent years (Abd El-Naby et al. 2019). In the present study, no effect of dietary fennel essential oil at levels 50, 100, 200 and 400 mg kg^{-1} was observed on the fish performance (P>0.05). The findings of the present study in terms of final weight, weight gain and specific growth rate are in accordance with those reported by Cruz Villeda (2013). Hassan & Soltan (2016) found that dietary supplementation of fennel oil at the level of 1 ml kg^{-1} increased the growth performance of Nile tilapia fry. Besides, Sotoudeh & Yeganeh (2016) found that feeding with dietary fennel oils (75, 100, 125 and 150 mg kg^{-1}) of convict cichlid (*Cichlasoma nigrofasciatum*) did not make a difference on growth performance (final weight, weight gain and specific growth rate) except FCR. However, our results with fennel oils were similar to the results of Mahdavi et al. (2014) found that feeding with fennel essential oil at levels of 100, 200, 400 and 600 mg kg^{-1} did not find significant differences in the growth performance of the Caspian kutum (*Rutilus frisii kutum*) fry and those of Abo-State et al. (2017) found that feeding with phytogenic feed additive containing oregano oil at levels of 1 g kg^{-1} did not find significant differences in the growth performance of the Nile tilapia (*Oreochromis niloticus*) fingerling. Being obtained different results on growth performance in the studies conducted on essential oils may change depending on essential oils or their composition and levels.

The activity of the digestive enzymes of fish is an important indicator in understanding the digestive physiology and revealing the digestion and absorption capacity of the nutrients taken (Wei et al. 2010). Digestion of nutrients begins with the activities of digestive enzymes in the stomach and continues in the intestine by trypsin, chymotrypsin, amylase and lipase enzymes secreted by the pancreas (Mohamed et al. 2018). The profiles or activity levels of digestive enzymes can be affected by size, age, origin, temperature, season and food (Hani et al. 2017). Medicinal herbs or their derivatives increase digestive enzyme secretions (Amhamed et al. 2018). In a previous study, Mohamed et al. (2018) found that trypsin, amylase and lipase activities in common carp (*Cyprinus carpio*) fed diets with 0.1%, 0.5% or 1% *Apium graveolens* extract at a level of 0.1%, 0.5% or 1% increased significantly. An additional study, De Souza et al. (2019) reported that, amylase enzyme activity in Nile tilapia increased at the level of 1 ml kg^{-1} *Ocimum basilicum* essential oil, but did not change at levels of 0.25, 5 or 2 mL kg^{-1}. The findings obtained from our study demonstrated that feeding with fennel essential oil affected activity of pepsin and lipase enzymes, but not trypsin, amylase. The highest lipase enzyme activity was determined in 200 mg kg^{-1} fennel essential oil. Moreover, our results with the amylase enzyme activity of Black sea salmon fed with fennel essential oil is in accordance with those reported by Magouz et al. (2021). Also, our results obtained on trypsin and amylase activities are similar to those reported by Heidarieh et al. (2012). The pepsin activities in the carnivorous is higher as compared to herbivorous fish. Unlike, the amylase enzyme in carnivorous fish is moderate level due to low or lack of carbohydrate intake in natural environment, and lowest than herbivorous (Natalia et al, 2004). According to Mohamed et al. (2018), trypsin activity in common carp (*Cyprinus carpio*) fed with garlic (*Allium sativum*) powder was not higher than amylase and lipase. Our study showed that trypsin enzyme activity was higher than amylase and lipase in all the trial groups. A similar result was seen in those obtained with the administration of *Apium graveolens* extract in Japanese seabass, *Lateolabrax japonicus* (Xu et al. 2019).

The small intestine is the primary site for absorption of nutrients, and thus playing a vital role in fish growth (Abdel-Latif et al. 2020). Intestinal villi play an important role in the digestion and absorption of nutrients exposed to digestive enzymes (Munglue 2016). In fish, the healthy intestinal tract can improve growth performance and gut health. In general, high intestinal villi play an important role in the feed efficiency and absorption of nutrients (Alagawany et al. 2020). The findings of our study about the intestinal morphology demonstrated that the addition of fennel essential oil to the diet increased the thickness of the

muscularis, but had no effect on intestinal villi width. Besides, intestinal villi length was enhanced in levels in except for 400 mg kg^{-1} fennel essential oil. Valladao et al. (2019) found that, in Nile tilapia, supplementation with thyme essential oil did not alter the intestinal morphology including villi height and width. According to Munglue et al. (2019), intestinal villi length and width in catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fed with the diet containing rice paddy herb (*Limnophila aromatica*) extract were significantly higher than those of the control. Similarly, Addam et al. (2018) found that villi length in the anterior intestine significantly enhanced in Nile tilapia fed *Lippia origanoides* essential oil diet. In fish, intestinal villi height is related to the digestive and absorptive functions of the intestines (Munglue & Dasri 2015). In our study, the increased villi length may cause improve absorption of nutrients as stated by Alagawany et al. (2020), the increase of the intestinal villi height and width may increase the surface area for absorption. Also, in terms of intestinal villi width, our results are harmonious with those of reported by Addam et al. (2018). In Black sea salmon, muscularis layer decreases gradually from the beginning of the anterior intestine to the end of the posterior intestine (Özel et al. 2019). One of the functions of this structure contribute to the mixing of feed with digestive enzymes (Mumford et al. 2007). In the posterior intestine, also, enhanced muscularis thickness assists defecation and moisture reabsorption (Munglue 2016). Our results with fennel essential oil were similar to those reported by Munglue (2016), dietary supplementation of lotus (*Nelumbo nucifera*) stamen extract enhanced muscularis thickness in anterior and posterior intestines in the Catfish (*Clarias gariepinus*).

In fish, the intestinal microbiota is influenced by genetic, nutritional, microbiological and environmental factors. Among these factors, the diet one of the main factors responsible for changes in bacterial diversity of the digestive tract (Sutili et al. 2017). Diet ingredients are important for the composition and activity of gut microbiota in fish (Yusuf et al. 2017). According to Giannenas et al. (2012), the inclusion of phytogenic substances to the diet can affect the intestine populations of trout. However, little is known about the antimicrobial effect of essential oils in fish (Cruz Villeda 2013). Findings of our study showed that feeding with dietary supplemented fennel essential oil had a significant effect on intestinal microbial activity including *E.coli*, coliform and lactic acid bacteria. According to Yusuf et al. (2017), the inclusion of 4 g kg^{-1} fumaric to the diet in juvenile tilapia (*Oreochromis niloticus*) increased the lactobacillus count and decreased the fecal coliform. In our study, suppressive effect on lactic acid bacteria of diets with fennel essential oil can be explained according to Alagawany et al. (2020), the antimicrobial effect of essential oils is depended to the effective and phenolic substrates obtained from some herbs. Lactic acid bacteria are not among the dominant bacterial communities due to found in low abundance in fish intestine, but can have positive effects on fish health and disease resistance due to can inhibit the growth of pathogenic bacteria (Hoseinifar et al. 2019). Findings of our study shown that fennel essential oil had a decreasing effect on intestinal lactic acid bacteria in the Black Sea salmon. Besides, diets supplemented with 200 and 400 mg kg^{-1} fennel oil increased *E.coli* and coliform counts. This result may be due to antimicrobial, antifungal and antibacterial activities of fennel essential oil as stated by Sotoudeh & Yeganeh (2016).

5. Conclusions

Results of our study showed that feeding the juvenile Black Sea salmon with fennel essential oil had not an effect on growth performance including feed intake, final weight, weight gain, feed conversion ratio and specific growth rate. Dietary supplementation of fennel oil affected the enzymes except for trypsin and amylase. Feeding with 200 mg kg^{-1} fennel oil increased lipase activity. Moreover, feeding with 50, 100 and 200 mg kg^{-1} fennel essential oil may have the potential to increase the surface area required for digestion by increasing intestinal villi length. Dietary supplemented fennel essential oil showed antimicrobial properties by decreasing lactic acid bacteria count. Similarly, 50 and 100 mg kg^{-1} fennel oil had the same effect on *E.coli* and coliform counts. In aquaculture, the number of studies on the possible effects of essential oils on the digestive physiology of fish is quite limited. Detailed studies are required to better understand of the mechanism of action of these oils in fish.

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Data Availability Statement

Research data are not shared. All the related data has been given with the article.

Animal Welfare Statement

The authors confirm that the ethical policies of the journal have been adhered to and the appropriate ethical review committee approval has been received from the Animal Ethics Committee of Central Fisheries Research Institute, Turkey (application number ETIK-2017/1). The authors confirm that they have followed EU standards for the protection of animals used for scientific purposes and feed legislation.

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