Tropical Animal Health and Production (2022) 54:390
https://doi.org/10.1007/s11250-022-03396-0

REGULAR ARTICLES

Influence of laurel (*Laurus nobilis*) essential oil on gut function of Black Sea salmon (*Salmo labrax*) juveniles

Osman Tolga Özel[1] · Ekrem Cem Çankiriligil[2] · Selin Ertürk-Gürkan[3] · Isa Coskun[4] · Mustafa Türe[5]

Received: 7 February 2022 / Accepted: 9 November 2022 / Published online: 21 November 2022
© The Author(s), under exclusive licence to Springer Nature B.V. 2022

Abstract
The present work investigated the effects of dietary incorporation of laurel (*Laurus nobilis*) essential oil on the zootechnical performance and digestive physiology of juvenile Black Sea salmon (*Salmo labrax*). In this trial, 15 fiberglass tanks (39 × 39 cm square and 33 cm high) were used. Forty-five fish (3.52 ± 0.01 g) were placed randomly per tank. Fish were fed for 90 days with the diet containing 50, 100, 200, or 400 mg kg^{-1} laurel (*Laurus nobilis*) essential oils, respectively. The work was performed in the recirculating aquaculture system (RAS) operating with freshwater. Fish were manually fed 3% level of live weight during the experiment period. Final weight (FW), weight gain (WG), feed conversion rate (FCR), and specific growth rate (SGR) were not affected by laurel essential oil supplementation. Dietary laurel essential oil (50 mg kg^{-1}) affected positively the surface area of fish intestinal villus that required for digestion. Both villus height and villus width were affected positively in fish fed with 50 mg laurel essential oil kg^{-1}. While incorporation with 100 mg laurel essential oil kg^{-1} increased the total α-amylase enzyme, 50 mg laurel kg^{-1} increased lipase enzyme. Moreover, 50 mg laurel essential oil kg^{-1} increased lactic acid bacteria (LAB) count in fish. Besides, 50 mg laurel essential oil kg^{-1} reduced the number of total coliform and *E. coli*.

Keywords Trout · *Salmo trutta labrax* · Herbal · Feeding · Growth · Digestion physiology

Introduction

In fish, the digestion process begins in the stomach, and it proceeds throughout the whole intestine (Caruso et al. 2009). Various types of digestive enzymes are produced in the stomach, pancreas, and intestine, and participate in the digestion of feed. The digestive enzymes, for instance pepsin, trypsin, amylase, and lipase for fish, have an important role in the gastrointestinal physiology of fish species, and digestion and absorption capacity of nutrients (Napora-Rutkowski et al. 2009; Silva and Anderson 1995), along with many microbial ecosystems localized in the digestive tract (Vaughn et al. 2005). In fish, intestinal villus is a good indicator of the surface area that is needed in the absorption, and this can be increased with villus development. The muscularis layer helps to the mixing of digestion enzymes and ingested feed (Mumford et al. 2007; Özel et al. 2019). The intestinal villus contributes to the absorption of nutrients, thereby helps to the transferring of absorbed nutrients into the lymph or blood (Karabulut 1991), thanks to its possession of absorptive cells (Balbi and Ciarletta 2013). Besides, in fish feeding studies, enzymological, histomorphological, and microbiological structures should be evaluated to better understand the physiologic mechanisms of digestion (Özel et al. 2019), especially on the new culture lines. Microbiota also play a crucial role in animal health (Pérez et al. 2019). Lactic acid bacteria (LAB) are an important factor of the gut microbiota of a healthy fish, and they are associated with the nutritional and environmental factors that affect the digestive tract (Ringø and Gatesoupe 1998). Similarly,

✉ Osman Tolga Özel
osmantolga.ozel@tarimorman.gov.tr

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

[2] Department of Fisheries, Sheep Breeding Research Institute, Bandırma, Türkiye

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

[4] Department of Animal Science, Faculty of Agriculture, Ahi Evran University, Kırşehir, Türkiye

[5] Department of Fisheries Health, Central Fisheries Research Institute, Trabzon, Türkiye

coliform bacteria, as well as *Escherichia coli*, are found in the fish intestine in different counts according to environmental conditions (Cahill 1990). Although a great majority of the coliform strains are not accepted as pathogen microorganisms, they can be defined as opportunistic pathogens that caused infections when the immune system is weakened (Feng et al. 2002).

Today, medical plants and their derivatives are efficiently used as dietary supplements to improve growth and immunity in fish and other vertebrates (Saad et al. 2015; Fattepur et al. 2018; Zharif et al. 2018; Nurin and Halim 2019; Othman et al. 2019; Khan et al. 2020; Shukri et al. 2020; Khojastehfard et al. 2021; Chehreh et al. 2021). These supplements are accepted to be potential growth-promoting alternatives for animal production as well as safe feed additives and alternatives to antibiotics (Ahmadifar et al. 2011). They can enhance feed consumption and flavor, secrete digestive enzymes, and increase gastric and intestinal activity, and also can have antimicrobial, antiviral, antiparasitic, antifungal, immunomodulating, antioxidant, and anti-inflammatory activities (Giannenas et al. 2016), and also contribute to increasing nutrient absorption and improving performance parameters in animals by changing gut morphometry and gut microbiota, increasing digestive enzymes, and improving nutritional digestibility (Ghafarifarsani et al. 2022a, b, c; Heluy et al. 2020; Yousefi et al. 2022). The laurel plant is an evergreen shrub or small tree that can grow up to 8–10 m high and 9 m wide (Giannenas et al. 2016). Laurel or bay laurel (*Laurus nobilis*) is a medicinal plant as a member of the Lauraceae family with distribution throughout the Mediterranean region, Europe, and Asia Minor (Özcan and Chalchat 2005). Laurel essential oils are stated to have antimicrobial activity against foodborne spoilage and pathogenic bacteria and fungi, and antiviral and antibiofilm activities. The main active component of *Laurus nobilis* essential oils is 1.8-cineole (Snuossi et al. 2016).

The intestine physiology is an important indicator in understanding the nutritional status of fish. In order to understand the physiological mechanisms of this structure more clearly, it should be supported with nutritional studies and should be followed with enzymological, histomorphological, and microbiological monitoring (Özel et al. 2019). The present work aimed to determine the usage of herbal essential oil–supplemented diets on the Black Sea salmon (*Salmo labrax* Pallas, 1814) to improve growth characteristics and digestive physiology. Black Sea trout is an endemic and anadromous fish species of the Eastern Black Sea (Aydin and Yandi 2002). The first cultivation studies aiming at conservation of the species led to intensive culture operations. Nowadays, species' culture is an essential part of the aquaculture sector of the region (Çakmak et al. 2019; Kasapoglu et al. 2020). Even with this species' high production, feeding studies conducted on this species are limited. Especially in salmonid culture, some parameters such as feed conversion efficiency, survival rate, and growth performance have crucial importance. Especially in novel culture lines, these parameters can be effected negatively due to stress caused by coping with domestication process and adaptation to the culture environment. It is reported that essential oils obtained from medicinal and aromatic plants have a positive effect on growth by providing effective use of feeds with their antimicrobial activity and digestive stimulating properties. With these benefits, the usage of herbal essential oils in the novel culture lines just as Black Sea salmon is essential. Besides, essential oil–supplemented diets for the Black Sea salmon are unique for this study. Considering such information, effect of laurel oil (*Laurus nobilis*) on the growth-related parameters, digestive enzymes, intestinal histomorphology, and gut microbiota were evaluated in this study.

Materials and methods

Diet preparation

The laurel essential oil used in the experimental study was given free of charge from Talya Herbal Products which was obtained from cultured *Laurus nobilis* produced in the Mediterranean region of Turkey. The volatile constituents of the laurel essential oil were determined by the method of Ergun et al. (2016) with GC–MS by using Sigma-Aldrich standards at 80 ℃ and are presented in Table 1. During the preparation of the experimental feeds, all raw materials except fish oil and laurel essential oil were homogeneously blended with a grinder. The mixture was extruded at 70 °C. Then, the laurel

Table 1 The volatile constituents of laurel essential oil

Compound	%
1,8-Cineol	56.1
γ-Terpinyl acetate	8.2
α-Pinen	4.9
Sabinen	4.9
β-Pinen	3.9
Terpinen-4-ol	3.7
ρ-Simen	2.8
α-Terpinene	1.8
Limonene	1.5
Linalool	1.5
Methyl eugenol	1.0
(Z)-Osimenol	0.6
Pinocarvone	0.5

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

oils were first included into the fish oil and then were penetrated into the extruded baits by vacuum coating. Extruded feeds were cut into 2.0-mm pellet size. Experiment diets were prepared to contain laurel essential oils at doses of 50 (L50), 100 (L100), 200 (L200), and 400 (L400) mg kg^{-1}. European anchovy (*Engraulis encrasicolus*) was the main source of fish oil. Fish meal was a blend of Atlantic herring (*Clupea harengus*) and European sprat (*Sprattus sprattus*) meals. Formulation and proximate composition of diets are demonstrated in Table 2.

Fish and rearing conditions

Fifth filial generation of Black Sea salmon (3.52 ± 0.01 g) obtained by culture activities in Trabzon Central Fisheries Research Institute (SUMAE) was used. The work was designed as three triplicates (45 for each). This experimental work was carried out in recirculating aquaculture system (RAS), and lasted for 90 days. Experimental materials were distributed randomly in 50 m^3 (39 × 39 cm size and 33 cm high) fiberglass square tanks into 5 treatment groups, including 45 fish in each experiment tank. Fish were fed by hand with 3% of body weight four times daily. Water temperature, oxygen, and pH were respectively measured as 15.10 ± 0.98 °C, 8.78 ± 0.21 mg/L, and 7.43 ± 0.18 daily. Ammonia measured weekly was saved as 0.05 ± 0.05 mg/L. During the experimental period, water changing in tanks was 22 times daily. Samplings were performed in accordance with ARRIVE ethical guidelines (Kilkenny et al. 2010) and European Union Directive (2010/63/EU) (European Commission 2010).

Growth parameters

Before weight measurements, fish were starved for 24 h, slightly anesthetized with benzocaine (50 ppm). At the end of the experiment, the final body weight was measured to calculate the growth-related parameters including weight gain (WG) (Rampelotto et al. 2018), specific growth rate (SGR), feed conversion ratio (FCR), hepatosomatic index (HSI) (Tibaldi et al. 2006), and survival rate (SR) (Ebrahimi and Ouraji 2012) using the equations shown below.

$$\text{WG g} = (\text{Final weight} - \text{Initial weight})$$

$$SGR\% = 100 \times \left[(\ln\ Final\ body\ weight - \ln\ Initial\ body\ weight) \div days\right]$$

$$\text{FCR} = (\text{Feed intake} \div \text{Weight gain})$$

$$\text{HSI\%} = 100 \times (\text{Liver weight} \div \text{body weight})$$

Table 2 Ingredient composition and proximate analysis of the basal diet

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
Chemical parameters	
Crude protein	46.20
Crude lipid	14.97
Crude ash	9.38
Moisture	6.14

[1]Fish meal contained 65% of crude protein and 8.5% of crude lipid

[2]Fish oil was European anchovy (*Engraulis encrasicolus*) oil

[3]Supplied the following: inositol 300 mg, biotin (Vit B_7) 200 mg, tocopherol (Vit E) 200 mg, calcium pantothenate (Vit B_5) 50 mg, riboflavin (Vit B_2) 30 mg, pyridoxine (Vit B_6) 20 mg, thiamine (Vit B_1) 20 mg, menadione (Vit K_3) 12 mg, niacin (Vit B_3) 6 mg, retinol (Vit A) 0.6 mg, folic acid (Vit B_9) 0.5 mg, cholecalciferol (Vit D_3) 0.05 mg, cobalamin (Vit B_{12}) 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

$$\text{SR\%} = 100 \times (\text{Final number of fish} \div \text{Initial number of fish})$$

Digestive enzyme activity analysis

Sampling was carried out at the 45th min when enzyme levels were highest for Black Sea salmon (Özel and Ertürk-Gürkan 2019). The digestive tract (midgut section) samples were transferred to COMU Biology Department in favorable conditions for the enzyme analysis. The tissues were stored at − 45 °C until analysis. For enzyme analysis, primarily the homogenization of the tissues was achieved. For this, homogenization solution (0.05 M phosphate buffer) and liquid nitrogen were used. Enzyme activities and total protein amounts of the tissues were determined spectrophotometrically. The amount of protein was determined based on the method of Bradford (1976). In the measurement of trypsin enzyme activity, the method is based on Bieth and Metais (1968). Accordingly, trypsin levels of the samples were measured at 253 nm wavelength for 5 min. The method reported by Worthington (1982) and then edited by Infante and Cahu (1994) was used to determine the level of pepsin. Measurements were recorded at 280 nm for 5 min. α-Amylase enzyme levels were determined based on the analysis method reported by Tseng et al. (1982). The measurement wavelength was 540 nm. The method reported by Versaw et al. (1989) was used for the measurement of lipase enzyme levels. The measurement time was 10 min and the wavelength was 490 nm.

Intestinal histomorphology

Intestine sampling was taken from the beginning section of the middle intestine that had the highest villus development (Özel et al. 2019). Tissue samples were taken from 6 fish with each group for analysis at the end of the experimental work. Intestine tissues were cut into 1.0-cm pieces, cleaned using distilled water, and placed in a 100-mL plastic container containing 10% formaldehyde. Subsequently, tissues were fixed for 1 day at + 4 °C. Fixed tissues were embedded in paraffin blocks by transferring into tissue cassettes, and then placed on slides by cutting 5-µ thickness. Processed tissue samples were subjected to the standard paraffin-embedding procedure, and followed staining procedure with H&E stains after fixation. As the final process, intestinal villus height, intestinal villus width, and muscularis thickness were photographed under a ZEISS microscope Primostar HD Light, and measured by using an image processing and analysis system.

Intestinal microbiota analysis

A total of 25 fish (5 fish from each diet group and control) were used for examination of bacteria including the total aerobic mesophilic bacteria (TAMB), lactic acid bacteria (LAB), and *Escherichia coli*. The intestinal tract of fish was removed aseptically. Feces sample of 1 g was aseptically taken from the whole intestine section, and was made homogeneous in 9 mL of 0.1% sterile distilled water with 0.9% NaCl solution using the stomacher bag. After homogenization, six-fold serial dilutions were made for the next processes. LAB count was determined according to Harrigan and McCance (1976). For this, prepared dilutions were spread on de Man Rogosa and Sharpe Agar (MRS, Merck) for the determination of the number of LAB, and incubated at 30 °C for 50 h using anaerobic jars (Merck). The number of the TAMB and *E. coli* was determined in accordance with Ture et al. (2018). For this, 100 µL dilution was spread on Coliform Agar (CES, Merck) and incubated overnight at 35 °C for the determination of number of the TAMB and *E. coli.* After incubation, processed samples were counted the colony-forming units, and intestinal *E. coli*, LAB, and TAMB and were calculated.

Data analysis

The data analysis was performed using SPSS 21, and was shown as the means with standard error. Data analysis was performed with the one-way analysis of variance. Statistical differences were determined by Duncan's multiple comparison test. Intestinal microbiota were analyzed after transforming logarithm to base 10. Statistical differences were considered to be significant at a level of $p < 0.05$.

Results

Zootechnical performance

During the 90-day trial, all the trial diets were well consumed by fish. No significant differences among all dietary treatments in final weight (FW), weight gain (WG), feed conversion rate (FCR), specific growth rate (SGR), feed intake (FI), hepatosomatic index (HSI), and survival rate (SR) were observed. The growth-related parameters such as WG, FW, SGR, FCR, and FI improved as the doses of laurel oil in the diet increased, but this was not significant statistically. After 90 days of feeding, the FCR was founded to be about 1 in all the trial groups without significant difference among groups (Table 3).

Enzyme activity

In Table 4, the digestion enzyme activities of all experimental groups are presented. No significant difference between experimental groups was found in the trypsin enzyme activity. Black Sea salmon fed with 400 mg kg^{-1} laurel oil had a high trypsin enzyme activity, but not statistically different from the other groups. However, amylase and lipase enzyme activities were significantly higher in Black Sea salmon fed 50 and 100 mg kg^{-1} laurel oils compared to the control, respectively. Although no significant difference among the two was found, pepsin enzyme activity was higher in fish fed 400 mg fennel oil kg^{-1} versus control.

Intestine histomorphology

Table 5 and Fig. 1 illustrate the histomorphologic results of fish fed with the trial diets. At the end of the trial, intestinal histomorphology was significantly affected by the dietary treatment. Intestinal villus height was significantly increased in the middle intestine of Black Sea salmon fed 50 or 100 mg laurel kg^{-1} compared to the control group. The same observations were done for the muscularis layer. The intestinal villus width was significantly increased in those fed diet 50 mg laurel oil kg^{-1} compared to fish fed control.

Intestinal microbiota

As shown in Table 6, the intestinal microbiota for instance coliform, *E. coli*, and lactic acid bacteria were significantly affected by dietary laurel essential oils. Lactic acid bacteria were significantly increased in 50 mg kg^{-1} laurel oil when we compared with other diets, including control. The essential oils except for 50 mg kg^{-1} laurel oil were found to be statistically similar to each other. The highest levels of *E. coli* were found in 400 mg kg^{-1} laurel oil, and the lowest in 50 mg kg^{-1} laurel oil. As the doses of laurel essential oil in the experimental diet increased, *E. coli* in the intestine increased, and lactic acid bacteria decreased.

Discussion

Growth performance

It is understood in the literature that limited studies investigated the effect of dietary laurel essential oil incorporation in fish nutrition studies. In studies conducted on different essential oils, they found that some essential oils have an increasing effect on growth while others do not have any effect. The findings of our study about the growth experiment showed that laurel essential oil can be used in diets of Black Sea salmon, without negative effects on growth performance. Similar to our study, Dedi et al. (2016) found that supplementation with cinnamon (*Cinnamomum burmannii*) leaf

Table 3 Growth parameters of Black Sea salmon fed with laurel oil–supplemented diets

Parameters	Control	Laurel essential oil, mg kg^{-1}				ANOVA *p* value
		50	100	200	400	
IW	3.52 ± 0.01	3.52 ± 0.00	3.52 ± 0.00	3.53 ± 0.00	3.52 ± 0.00	0.351
FW	29.83 ± 1.11	30.28 ± 0.55	30.45 ± 0.06	31.22 ± 0.58	31.90 ± 0.19	0.211
FI	23.30 ± 0.72	23.56 ± 0.13	23.81 ± 0.22	24.10 ± 0.30	24.34 ± 0.77	0.621
FCR	1.07 ± 0.04	1.02 ± 0.02	1.01 ± 0.01	0.98 ± 0.01	0.97 ± 0.04	0.430
WG	26.31 ± 1.11	26.76 ± 0.56	26.93 ± 0.05	27.70 ± 0.58	28.38 ± 0.19	0.211
SGR	2.30 ± 0.04	2.31 ± 0.02	2.32 ± 0.00	2.35 ± 0.02	2.37 ± 0.01	0.205
HIS	1.92 ± 0.18	1.57 ± 0.10	1.57 ± 0.29	1.48 ± 0.23	1.47 ± 0.23	0.597
SR	91.85 ± 2.67	93.33 ± 1.28	88.89 ± 1.28	91.85 ± 2.67	91.85 ± 3.23	0.757

No difference between means ($p > 0.05$); values are given as mean ± SE. *FW* (g), final weight; *FI* (g), feed intake; *FCR*, feed conversion rate; *WG* (g), weight gain; *SGR* (%), specific growth rate; *SR* (%), survival rate

Table 4 The activity of digestion enzymes of Black Sea salmon fed with laurel oil–supplemented diets

Enzymes (U mg^{-1} protein)	Control	Laurel essential oil, mg kg^{-1}				ANOVA *p* value
		50	100	200	400	
Pepsin	69.95 ± 7.29^{a}	39.16 ± 1.40^{b}	10.44 ± 1.58^{b}	33.05 ± 18.92^{b}	94.60 ± 5.27^{a}	0.001
Trypsin	34.52 ± 5.93	24.09 ± 4.38	33.75 ± 1.50	29.74 ± 4.43	42.42 ± 4.73	0.130
α-Amylase	1.46 ± 0.46^{c}	1.91 ± 1.04^{bc}	9.45 ± 1.42^{a}	1.27 ± 0.54^{c}	4.83 ± 1.21^{b}	0.01
Lipase	0.02 ± 0.00^{c}	0.08 ± 0.01^{a}	0.02 ± 0.00^{c}	0.03 ± 0.01^{c}	0.05 ± 0.01^{b}	0.00

Means with different superscript letters in a row are significantly different at $p < 0.05$; values are given as mean ± SE

Table 5 Intestine morphology of Black Sea salmon fed with laurel oil–supplemented diets

Intestine (μm)	Control	Laurel essential oil, mg kg^{-1}				*p* value
		50	100	200	400	
VL	223.80 ± 5.35^{b}	309.17 ± 14.79^{a}	311.92 ± 11.52^{a}	246.95 ± 9.99^{b}	215.52 ± 12.73^{b}	0.000
VW	65.15 ± 2.88^{b}	81.28 ± 4.92^{a}	61.75 ± 3.56^{b}	70.34 ± 4.65^{ab}	59.63 ± 2.91^{b}	0.001
ML	44.53 ± 1.82^{c}	64.09 ± 5.52^{b}	76.78 ± 3.23^{a}	51.95 ± 2.24^{c}	51.82 ± 2.04^{c}	0.000
VL/VW	3.58 ± 0.25^{b}	3.99 ± 0.30^{b}	5.29 ± 0.35^{a}	3.73 ± 0.27^{b}	3.83 ± 0.39^{b}	0.002

Means with different superscript letters in a row are significantly different at $p < 0.05$; values are given as mean ± SE. *VL*, villi length; *VW*, villi width; *ML*, muscularis

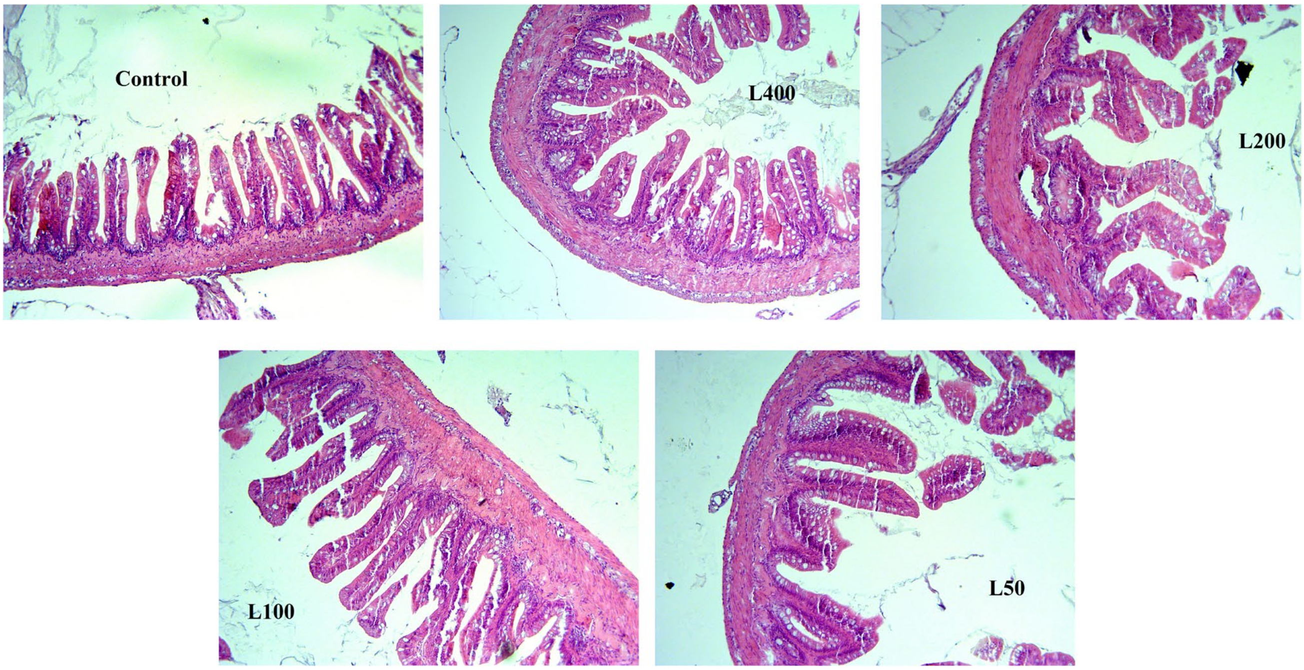

Fig. 1 Intestine histology of Black Sea salmon fed with laurel oil–supplemented diets (4×, H&E)

Table 6 Intestine microbiota of Black Sea salmon fed with laurel oil–supplemented diets

Microbiota (CFU, log/g)	Control	Laurel essential oil, mg kg^{-1}				ANOVA *p* value
		50	100	200	400	
E. coli	5.46±0.16^{d}	4.75±0.20^{e}	5.99±0.16^{c}	7.59±0.10^{b}	8.07±0.07^{a}	0.000
Coliform	12.47±0.15^{a}	9.81±0.32^{c}	11.35±0.05^{b}	12.23±0.29^{a}	10.46±0.30^{c}	0.000
Lactic acid	11.05±0.05^{b}	13.03±0.08^{a}	6.81±0.32^{c}	6.76±0.41^{c}	6.18±0.18^{c}	0.000

Means with different superscript letters in a row are significantly different at $p<0.05$; values are given as mean±SE

had no effect on final weight and growth rate of carp (*Cyprinus carpio*). However, there are also studies with different results about essential oils. Marques et al. (2016) stated that the essential oil combination supplemented with laurel is a potential growth promoter for broilers. In a previous work, Zaki et al. (2016) reported that feeding with alpinia meal (*Alpinia galanga*) had an improved effect on the final weight, weight gain, specific growth rate, and feed conversion ratio in the Nile tilapia (*Oreochromis niloticus*) fingerlings. In an additional work, Giannenas et al. (2016) reported that laurel oil (*Laurus nobilis*) improved the weight and feed conversion ratio in broiler chickens from the monogastric species. However, Bulbul et al. (2015) found that there was no difference in final body weights, body weight gain, feed intake, and feed conversion ratio in supplementation of laurel essential oil in quail diets. Results obtained with essential oils may vary depending on animal species, essential oils, and their doses. Also, our study showed that growth parameters such as feed intake, final weight, weight gain, feed conversion ratio, and specific growth rate were in parallel with the improvement in the doses of laurel essential oil, although there was a statistical difference. A similar result was seen in those announced by de Souza et al. (2019) who determined the effects of *Ocimum basilicum* essential oil in Nile tilapia juvenile.

Enzyme activity

Digestion starts in the stomach and continues in the intestine with digestive enzymes, for instance trypsin, chymotrypsin, amylase, and lipase, secreted from the pancreas (Zahran et al. 2014). Digestion performance generally depends on the type and function of the digestive enzymes (Farhoudi et al. 2012). The digestive enzyme activities of fish are influenced by factors such as diet, feeding habits, fish species, fish age, growth stage, pH, temperature, and digestive system structure (Amhamed et al. 2018). Laurel leaves may increase concentration of digestive enzymes including chymotrypsin lipase, amylase, and trypsin, and improve digestion and absorption capacity of ingested feed (Ali and Al-Shuhaib 2021). Similarly, laurel (*Laurus nobilis*) extract may improve digestive enzymes such as protease and amylase in common carp (*Cyprinus carpio* L.) (Al-Harbi and Al-Faragi 2019). Our results showed that digestive enzyme activity in the midgut section was significantly affected by dietary treatments. Gabriel et al. (2017) found that variations in digestive enzymes activities of GIFT tilapia (*Oreochromis niloticus*) fed aloe vera powder extract were observed. Similar results were obtained in our study, in which the activity of enzymes of amylase, lipase, and pepsin varied depending on different levels of oregano, laurel, and fennel essential oils. In a previous work, Xu et al. (2019) reported that, when compared to control, the levels of lipase and trypsin in Japanese seabass (*Lateolabrax japonicus*) increased at the level of 10 g kg^{-1} garlic (*Allium sativum*) powder, but did not change at doses of 15, 20, or 25 g kg^{-1}. In an additional work, Shabana et al. (2019) reported that levels of lipase and amylase improved in the *Catla catla* fed with different levels (2–10 g kg^{-1}) of *Citrus sinensis* peel extract. Our work showed that feeding with the laurel oil 400 mg kg^{-1} increased pepsin activity while feeding with the laurel oil 50 mg kg^{-1} increased lipase activity along with decreased pepsin activity. Similar results were also obtained in the activities of trypsin and amylase enzymes. Trypsin activity is higher compared to the activities of amylase and lipase in all days of the experiment. Unlike our study, Hani et al. (2018) found that the amylase enzyme activity in the intestine of Stickleback (*Gasterosteus aculeatus*) was higher than trypsin enzyme. Al-Saraji and Nasir (2013) stated that the activity of digestive enzymes in the fish varies depending on different factors, for instance the fish age, pH, and temperature, diet composition and feeding habit of the fish, the nutritional value of the feed, food digestion, nutrient absorption, and metabolic factors. Also, the results of our study with lipase enzyme were similar to the result determined by Amhamed et al. (2018) who found that the activity of lipase enzyme in the juvenile common carp (*Cyprinus carpio*) fed the *Chenopodium album* increased in comparison to that of the control or was not different from the control. Moreover, in our study, the effect of laurel essential oil on the activity of amylase enzyme is compatible with those announced by de Souza et al. (2019).

Intestine histomorphology

The intestinal villus height plays an important role in the digestive and absorption functions of fish (Munglue and Dasri 2015). The height villus in the fish intestine improves intestinal health and efficiency of nutrient absorption. In this way, it enhances fish performance by making intestinal tracts healthier (Abdel-Latif et al. 2020). Feeding of fish with immunostimulants enhances the intestinal villi length (Heidarieh et al. 2013). There are limited studies explaining the effect on fish gut histomorphology of laurel essential oil in fish nutrition. Khattak et al. (2014) found that feeding with natural blend of essential oil at a level of 300 mg kg^{-1} supplemented laurel improved villi width and surface area in broiler. El (2014) reported that herbal essential oil mixture supplemented with laurel increased intestinal villi length and tunica muscularis thickness in Nile tilapia juvenile. A similar result was also obtained in broilers fed blend of essential oil–supplemented laurel (Bozkurt et al. 2012). In the study of Salem and Abdel-Ghany (2018), intestinal villus height in the anterior section of Nile tilapia progressively increased as the orange peel level (0, 1, 2, and 4 g kg^{-1}) added to the diets increased. In an additional work, Munglue et al. (2019) reported that intestinal villus height, villus width, and muscularis thickness in the middle part of hybrid catfish (*Clarias macrocephalus* × *C. gariepinus*) fed with different levels (1, 3, and 5%) of Lasia extract were significantly increased. The findings of our study demonstrated that feeding with laurel essential oil had a significant effect on the intestinal morphology of Black Sea salmon. In our study, while 50 mg kg^{-1} and 100 mg kg^{-1} laurel essential oil increased intestinal villi length and muscularis layer, 50 mg kg^{-1} laurel essential oil increased intestinal villus height. Our result obtained with laurel essential oil supplementation in terms of intestinal villus height is harmonious with that reported by Yusuf et al. (2017). Ferreira et al. (2016) found that the increased villus height and width in fish fed with oregano essential oil may be due to the antimicrobial property of this product. According to Snuossi et al. (2016), laurel essential oils have antimicrobial activity. The increased villus height in our study can be explained by the decrease in the number of *E. coli* in particular. Besides, the increased villus height and villus width obtained at the 50 mg kg^{-1} laurel essential oil can be explained by the decrease in the number of *E. coli* and coliform and the increase in the lactic acid bacteria. Ariyadi and Harimurti (2010) found that, in a broiler, supplementation of lactic acid bacteria had a positive effect on intestinal mucosa profile. Similarly, Aliakbarpour et al. (2012) found that the addition of lactic acid bacteria–based probiotic in the broiler diets significantly increased the number of goblet cells and villus height.

Intestinal microbiota

Fish intestinal microbiota exhibit an important symbiotic relationship in nutritional provisioning, metabolic homeostasis, and immune defense (Egerton et al. 2018). Lactic acid bacteria can stimulate host digestive system development, digestive function, mucosal tolerance, and immune response, and enhance disease resistance. Thus, lactic acid bacteria are considered suitable microorganism (Ringø et al. 2018). In terms of cecal microbiota, the differences among different animal species may observed. Irawan et al. (2021) reported that in broiler chicken fed with essential oil, while the population of *Lactobacillus* is increased, the *E. coli* population is decreased, which increases feed efficiency. However, Odeh et al. (2020) reported that *Lactobacillus* count was significantly decreased in feeding with laurel in rats. In a previous study, Yusuf et al. (2017) found that turmeric (*Curcuma longa*) powder had an enhancing effect on *Lactobacillus* in the intestine of Nile tilapia (*Oreochromis niloticus*). In an additional study, Hoseinifar et al. (2019) found that galactooligosaccharide addition as a prebiotic in diets of Caspian roach and Caspian white fish fingerlings has significantly enhanced intestinal lactic acid bacteria and can be considered an effective prebiotic. A similar observation was seen in our results obtained regarding improved lactic acid bacteria in fish fed with the experimental diet with 50 mg laurel oil kg^{-1}. According to Rawling et al. (2009), some of lactic acid bacteria have shown positive effects on fish health and growth as probiotics. Similarly, Ringø et al. (2018) stated that lactic acid bacteria are considered the most promising bacteria species as probiotic in aquaculture. In addition, findings of our study show that *E. coli* and coliform counts in the intestine significantly decreased in feeding with diet containing 50 mg kg^{-1} laurel oil. Similar to our study, Berendika et al. (2022) found that the inclusion of laurel (*Laurus nobilis* L.) extract at level of 100 mg kg^{-1} to rat diet increased the number of *Lactobacillus* by about two times. Similarly, Yusuf et al. (2017) reported that fumaric supplementation at level of 4 g kg^{-1} to diet increased total *Lactobacillus* count and decreased fecal coliform. In our study, the increase in lactic acid bacteria and the decrease in *E. coli* and coliform showed that 50 mg kg^{-1} laurel oil had a probiotic effect on Black Sea salmon. Besides, all diets except 50 mg kg^{-1} laurel oil had a decreasing effect on lactic acid bacteria compared to the control group. This result is compatible with those reported by Giannenas et al. (2012), who obtained a lower *Lactobacillus* population in rainbow trout (*Oncorhynchus mykiss*) fed with dietary thymol supplementation.

Conclusion

The results of our study show that feeding the juvenile Black Sea salmon with laurel essential oil did not affect the growth performance. The feed intake, final weight, weight gain, feed conversion ratio, and specific growth rate increased in parallel with the increase in the doses of laurel essential oil, but were not found to be statistically significant. Dietary supplementation of 100 mg kg^{-1} laurel oil and 50 mg kg^{-1} laurel oil had an increasing effect on amylase and lipase, respectively. Besides, 50 mg kg^{-1} laurel oil may have the potential to show prebiotic or probiotic effects due to improvement effect on the intestine lactic acid bacteria, and also 50 or 100 mg kg^{-1} laurel oil may have the potential to increase the surface area required for digestion by increasing intestinal villus development in terms of both villi length and villi width. As a result, supplemented 50 and 100 mg kg^{-1} laurel oil in the Black Sea salmon diet can be recommended as a natural feed additive.

Acknowledgements This study was conducted as a part of the research project named "Investigation of Possibilities of Using Some Phytobiotic Containing Diets in Black Sea Trout Nutrition." Part of intestinal microbiota was presented as oral presentations at IV International Agriculture Congress, 05–08 July 2018, Nevşehir.

Author contribution Osman Tolga Özel: methodology, experimental plan, feed formulations, experiments and feeding, analysis, writing, and reviewing. Ekrem Cem Çankiriligil: experiments and feeding. İsa Coşkun: histomorphologic examination. Selin Ertürk-Gürkan: enzyme analysis. Mustafa Türe: microbiota detection.

Funding The General Directorate of Agricultural Research and Policies (TAGEM) supported this research (project number: TAGEM/HAYSUD/2017/A11/P-01/3) financially.

Data availability We declare that there is no shared data related to this manuscript.

Code availability Not applicable.

Declarations

Ethical approval The procedures applied in this study were performed with the approval (coded as ETIK-2017/1) of the Ethical Committee of Animal Experiments of SUMAE.

Consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

Abdel-Latif, H.M.R., Abdel-Tawwab, M., Khafaga, A.F. and Dawood, M.A.O. 2020. Dietary oregano essential oil improved the growth performance via enhancing the intestinal morphometry and hepato-renal functions of common carp (*Cyprinus carpio L.*) fingerlings. Aquaculture 526, 735432. https://doi.org/10.1016/j.aquaculture.2020.735432.

Ahmadifar, E., Falahatkar, B. and Akrami, R. 2011. Effects of dietary thymol-carvacrol on growth performance, hematological parameters and tissue composition of juvenile rainbow trout, *Oncorhynchus mykiss*. Journal of Applied Ichthyology, 27, 1057-1060. https://doi.org/10.1111/j.1439-0426.2011.01763.x.

Al-Harbi, S. W. and Al-Faragi, J. K. 2019. Efficacy of bay laurel Laurus nobilis as feed additives on growth performance and digestive enzymes of common carp (*Cyprinus carpio* L.). Biochemical and Cellular Archives. 19 (1), 2039–2043.

Ali, N. A. and Al-Shuhaib, M. B. S. 2021. Highly effective dietary inclusion of laurel (*Laurus nobilis*) leaves on productive traits of broiler chickens. Acta Scientiarum. Animal Sciences, v. 43, e52198. https://doi.org/10.4025/actascianimsci.v43i1.52198.

Aliakbarpour, H.R., Chamani, M., Rahimi, G., Sadeghi, A.A. and Qujeq, D. 2012. The Bacillus subtilis and lactic acid bacteria probiotics influences intestinal mucin gene expression, histomorphology and growth performance in broilers. Asian-Aust. J. Anim. Sci. Vol. 25, No. 9 : 1285 - 1293.

Al-Saraji, A.Y.J. and Nasir, N.A.N. 2013. Effect of different dietary proteins and fats on the digestive enzymes activities in the common carp fingerlings (*Cyprinus carpio L.*) reared in floating cages. Mesopot. J. Mar. Sci., 28(2): 121 – 130.

Amhamed, I.D., Mohamed, G.A., Almabrok, A.A., Altief, T.A.S and Bilen, S. 2018. Efficacy of dietary Chenopodium album extract on some health parameters, digestive enzymes and growth performance in juvenile *Cyprinus carpio*. Alinteri Journal of Agriculture Science, 33, 165-176. https://doi.org/10.28955/alinterizbd.412455.

Ariyadi, B. and Harimurti, S. 2010. The effects of single lactic acid bacteria probiotic supplementation on intestinal mucosa profile and immune response in broilers. The 5th International Seminar on Tropical Animal Production Community Empowerment and Tropical Animal Industry October 19–22, 2010, Yogyakarta, Indonesia. 314–319.

Aydin, H. and Yandi, I. 2002. The general status of spawning areas of Black-sea trout in the East Blacksea regions (*Salmo trutta labrax* Pallas1811). Ege Journal of Fisheries and Aquatic Sciences, 19, 501–506.

Balbi, V. and Ciarletta, P. 2013. Morpho-elasticity of intestinal villi. Journal of Royal Society Interface, 10, 1-12. https://doi.org/10.1098/rsif.2013.0109

Bieth, J. and Metais, P. 1968. The simultaneous presence of trypsin and trypsin inhibitors in some pathological effusions. Clinica Chimica Acta, 22, 639-642.

Bozkurt, M., Küçükyilmaz, K., Çatli, A. U., Özyildiz, Z., Çinar, M., Çabuk, M. and Çoven, F. 2012. Influences of an essential oil mixture supplementation to corn versus wheat-based practical diets on growth, organ size, intestinal morphology and immune response of male and female broilers, Italian Journal of Animal Science, 11:3, e54, https://doi.org/10.4081/ijas.2012.e54.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry, 72, 248-254.

Berendika, M., Drozdek, S.D., Odeh, D., Orsolic, N., Dragicevic, P., Sokolovic, M., Garofulic, I.E., Dikic, D. and Jurcevic, I.L. 2022. Beneficial effects of laurel (*Laurus nobilis* L.) and myrtle (*Myrtus communis* L.) extract on rat health. Molecules 27, 581. https://doi.org/10.3390/molecules27020581.

Bulbul, T., Ozdemir, V. and Bulbul, A. 2015. Use of sage (*Salvia triloba* L.) and laurel (*Laurus nobilis* L.) oils in quail diets. Eurasian J Vet Sci. 31 (2), 95–101. https://doi.org/10.15312/EurasianJVetSci.2015210080.

Cahill, M.M. 1990. Bacterial flora of fishes: A review. Microbial Ecology, 19, 21-41.

Çakmak, E., Firidin, Ş., Düzgüneş, Z.D. and Parlak, R. (2019). The Age-Dependent Reproductive Performance of 4th Generation Black Sea Salmon (*Salmo labrax* Pallas, 1814) Females. Turkish Journal of Fisheries and Aquatic Sciences, 19(6), 496-502. https://doi.org/10.4194/1303-2712-v19_6_05

Caruso, G., Denaro, M.G. and Genovese, L. 2009. Digestive enzymes in some teleost species of interest for Mediterranean aquaculture. The Open Fish Science Journal, 2, 74-86.

Chehreh, R., Sayehmiri, K., shohani, M., Vahidnia, S. and Tavan, H. 2021. Comparing the effect of herbal drugs and non-steroidal anti-inflammatory drugs on primary dysmenorrhea in Iran: a Systematic Review and Meta-Analysis. Journal of Chemical Health Risks, 11(1), 41-53. https://doi.org/10.22034/jchr.2020.1882587.1061.

European Commission, 2010. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The Official Journal of the European Union, 276, 1–79.

De Souza, E.M., de Souza, R.C., Melo, J.F.B., da Costa, M.M., de Souza, A.M. and Copatti, C.E. 2019. Evaluation of the effects of *Ocimum basilicum* essential oil in Nile tilapia diet: growth, biochemical, intestinal enzymes, haematology, lysozyme and antimicrobial challenges. Aquaculture 504, 7–12. https://doi.org/10.1016/j.aquaculture.2019.01.052.

Dedi, J., Hutama, A.A., Nurhayati, T., Wasjan, and Vinasyiam A. 2016. Growth performance and flesh quality of common carp, *Cyprinus carpio* feeding on the diet supplemented with cinnamon (*Cinnamomum burmannii*) leaf. AACL Bioflux, 9, 937-943. http://www.bioflux.com.ro/docs/2016.937-943.pdf.

Ebrahimi, C. and Ouraji, H. 2012. Growth performance and body composition of kutum fingerlings, Rutilus frisii kutum (*Kamenskii 1901*), in response to dietary protein levels, Turkish Journal of Zoology, 36, 551-558, https://doi.org/10.3906/zoo-1008-139.

Egerton, S., Culloty, S., Whooley, J., Stanton, C. and Ross, R.P. 2018. The Gut Microbiota of Marine Fish. Front. Microbiol. 9:873. https://doi.org/10.3389/fmicb.2018.00873

El, M., R. 2014.Supplementation of herbal essential oil mixture to diet of Nile tilapia and evaluating growth performance, health status and intestinal histology. Zag. Vet. J. Vol.42 No.1 pp 1-10.

Ergun, M., Ergun, N. and Ozbay, N. 2016. Analysis of volatile constituents of *Sideritis pisidica* Boiss. & Heldr. by head space gas chromatography mass spectrometry (HS-GC/MS). Zeitschrift fur Arznei- und Gewurzpflanzen, 21 (2), 68–72.

Farhoudi, A., Abedian Kenari, A.M., Nazari, R.M. and Makhdoomi, Ch. 2012. Changes of digestive enzymes activity in common carp (*Cyprinus carpio*) during larval ontogeny. Iranian Journal of Fisheries Sciences, 12, 320-334.

Fattepur, S., Nilugal, K. C., Darshan, T. T., Bacayo, M. F. D. C., Asmani, F., Abdullah, I., Yusuf, E. and Goudanavar, P. 2018. Toxicological and pharmacological activity of ethanolic extracts of *Catharanthus roseus* in expermental animals. International Journal of Medical Toxicology & Legal Medicine, 21(3and4), 141–144.

Feng, P., Weagant, S.D., Grant, M.A. and Burkhardt, W. 2002. Chapter 4: Enumeration of Escherichia coli and the Coliform bacteria. In: Food and Drug Administration (FDA), Bacteriological Analytical Manual Online, 8th Edition, Silver Spring, Berlin, 1998.

Ferreira, P.M.F., Caldas, D.W., Salaro, A.L., Sartori, S.S.R., Oliveira, J.M., Cardoso, A.J.S. and Zuanon, J.A.S. 2016. Intestinal and liver morphometry of the yellow tail tetra (*Astyanax altiparanae*) fed with oregano oil. Annals of the Brazilian Academy of Sciences, 88, 911-922. https://doi.org/10.1590/0001-3765201620150202.

Gabriel, N.N., Qiang, J., Ma, X.Y., Xu, P. and Nakwaya, D.N. 2017. Effects of dietary Aloe vera crude extracts on digestive enzyme activities and muscle proximate composition of GIFT tilapia juveniles. South African Journal of Animal Science, 47, 904-913. https://doi.org/10.4314/sajas.v47i6.18.

Ghafarifarsani, H., Hoseinifar, S. H., Javahery, S., Yazici, M. and Van Doan, H. 2022a. Growth performance, biochemical parameters, and digestive enzymes in common carp (*Cyprinus carpio*) fed experimental diets supplemented with vitamin C, thyme essential oil, and quercetin. Italian Journal of Animal Science, 21(1), 291-302.

Ghafarifarsani, H., Hoseinifar, S. H., Javahery, S. and Van Doan, H. 2022b. Effects of dietary vitamin C, thyme essential oil, and quercetin on the immunological and antioxidant status of common carp (*Cyprinus carpio*). Aquaculture, 553, 738053.

Ghafarifarsani, H., Hoseinifar, S. H., Aftabgard, M. and Van Doan, H. 2022c. The improving role of savory (*Satureja hortensis*) essential oil for Caspian roach (*Rutilus caspicus*) fry: Growth, haematological, immunological, and antioxidant parameters and resistance to salinity stress. Aquaculture, 548, 737653.

Giannenas, I., Triantafillou, E., Stavrakakis, S, Margaroni, M., Mavridis, S., Steiner, T. and Karagouni, E. 2012. Assessment of dietary supplementation with carvacrol or thymol containing feed additives on performance, intestinal microbiota and antioxidant status of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 350-353, 26-32. https://doi.org/10.1016/j.aquaculture.2012.04.027.

Giannenas, I., Tzora, A., Sarakatsianos, I., Karamoutsios, A., Skoufos, S., Papaioannou, N., Anastasiou, I. and Skoufos, I. 2016. The effectiveness of the use of oregano and laurel essential oils in chicken feeding. Ann. Anim. Sci., Vol. 16, No. 3, 779–796, https://doi.org/10.1515/aoas-2015-0099.

Hani, Y.M.İ., Marchand, A., Turies, C., Kerambrun, E., Palluel, O., Bado-Nilles, A., Beaudouin, R., Porcher, J.M., Geffard, A. and Dedourge-Geffard, O. 2018. Digestive enzymes and gut morphometric parameters of threespine stickleback (*Gasterosteus aculeatus*): Influence of body size and temperature. PLoS ONE, 13, e0194932. https://doi.org/10.1371/journal.pone.0194932.

Harrigan, W.F. and McCance, M.E. 1976. Laboratory Methods in Food and Dairy Microbiology. London, UK: Academic Press Inc.

Heidarieh, M., Mirvaghefi, A.R., Sepahi, A., Sheikhzadeh, N., Shahbazfar, A.A. and Akbari, M. 2013. Effects of dietary aloe vera on growth performance, skin and gastrointestine morphology in rainbow trout (*Oncorhynchus mykiss*). Turkish Journal of Fisheries and Aquatic Sciences, 13, 367-373. https://doi.org/10.4194/1303-2712-v13_2_20.

Heluy, G.M., Ramos, L.R.V., Pedrosa, V.F., Sarturi, C., Figueiredo, P.G.P., Vidal, L.G.P., França, I.F. and Pereira, M.M. 2020. Oregano (*Origanum vulgare*) essential oil as an additive in diets for Nile tilapia (Oreochromis niloticus) fingerlings reared in salinized water. Aquaculture Research, Accepted https://doi.org/10.1111/are.14658.

Hoseinifar, S.H., Doan, H.V. and Ashouri, G. 2019. Galactooligosaccharide effects as prebiotic on intestinal microbiota of different fish species. RUDN Journal of Agronomy and Animal Industries, 14, 266-278**.** https://doi.org/10.22363/2312-797X-2019-14-3-266-278.

Infante, J.Z. and Cahu, C.L. 1994. Influence of diet on pepsin and some pancreatic enzymes in sea bass (*Dicentrarchus labrax*) larvae. Comparative Biochemistry and Physiology Part A: Physiology, 109, 209-212.

Irawan, A., Hidayat, C., Jayanegara, A. and Ratriyanto, A. 2021. Essential oils as growth-promoting additives on performance, nutrient digestibility, cecal microbes, and serum metabolites of broiler chickens: a meta-analysis. Animal Bioscience. 34 (9),1499-1513. https://doi.org/10.5713/ab.20.0668.

Karabulut, A. 1991. Animal feeding physiology and metabolism course note. Bursa, Turkey: Uludag University, Faculty of Agriculture.

Kasapoglu, N., Çankiriligil, E.C., Çakmak, E. and Özel, O.T. (2020). Meristic and morphometric characteristics of the Black Sea salmon (*Salmo labrax* Pallas 1814) culture line: An endemic species for Eastern Black Sea. Journal of Fisheries, 8, 935–939.

Khan, J., Leenoos, L. M., Ruhi, S., Al-Dhalli, S., Kaleemullah, M., Saad, R., Saad, A. H., Ram, S., May, F., Mohamed, R., Sri, B., Shariq, B., Hui, N. C., Nur, S. N., Fadzilah, A., Gamal, O. M., Ibrahim, A., Fadli, A., and Yusuf, E. 2020. Development and evaluation of polyherbal halal ointment using honey and Papaya. International Journal of Medical Toxicology & Legal Medicine, 23 (1and 2), 232–238.

Khattak, F., Ronchi, A., P.Castelli, P. and Sparks, N. 2014. Effects of natural blend of essential oil on growth performance, blood biochemistry, cecal morphology, and carcass quality of broiler chickens. Poultry Science. 93 (1), 132-137. doi.org/https://doi.org/10.3382/ps.2013-03387.

Khojastehfard, Z., Yazdimoghaddam, H., Abdollahi, M. and Karimi, F. 2021. Effect of herbal medicines on postpartum hemorrhage: A systematic review and meta-analysis. Evidence Based Care, 11(1), 62-74. https://doi.org/10.22038/ebcj.2021.58054.2513.

Kilkenny, C., Browne, W.J., Cuthill, I.C., Emerson, M. and Altman, D.G. 2010. Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLoS Biology, 8, e1000412. https://doi.org/10.1371/journal.pbio.1000412

Marques, A., Teixeira, B. and Nunes, M.L. 2016. Bay laurel oils. Essential Oils in Food Preservatşon, Flavor and Safety. Chapter 26. doi.org/https://doi.org/10.1016/B978-0-12-416641-7.00026-2.

Mumford, S., Heidel, J., Smith, C., Morrison, J., MacConnell, B. and Blazer, V. 2007. Fish histology and histopathology. Retrieved from https://training.fws.gov/resources/course-resources/fish-histology/Fish_Histology_Manual_v4.pdf.

Munglue, P., Rattana, K., Sangchanjiradet, S., Jankam, A. and Dasri, H. 2019a. Growth performance and intestinal morphology of hybrid catfish (*Clarias macrocephalus* × *Clarias gariepinus*) fed diet supplemented with rice paddy herb (*Limnophila aromatica*) extract. Asia-Pacific Journal of Science and Technology, 24, 1-12.

Munglue, P. and Dasri, K. 2015. Effects of Bauhinia strychnifolia Craib leaf extract on growth parameters and intestinal morphology of catfish (*Clarias gariepinus*). Proceeding Book of Sakon Nakhon Rajabhat University International Conference. ST50-ST57.

Napora-Rutkowski, L., Kamaszewski, M., Bielawski, W., Ostaszewska, T. and Wegner, A. 2009. Effects of starter diets on pancreatic enzyme activity in juvenile Sterlet (*Acipenser ruthenus*). The Israeli Journal of Aquaculture – Bamidgeh, 61, 143–150.

Nurin, R. Q. and Halim, S. 2019. Potential pharmacotherapy of Olea europaea (Olive) fruit oil against methamphetamine-induced neurotoxicity. 2019. Journal of Cellular and Molecular Anesthesia, 4(2), 67–68.

Odeh, D., Orsolic, N., Berendika, M., Dikic, D., Drozdek, S. D., Balbino, S., Repajic, M., Dragovic-Uzelac, V. and Jurcevic, I. L. 2020. Antioxidant and Anti-Atherogenic Activities of Essential Oils from *Myrtus communis L.* and *Laurus nobilis L.* in Rat. Nutrients. 14, 1465. doi.org/https://doi.org/10.3390/nu14071465.

Othman, Z., Khalep, H. R. H., Abidin, A. Z., Hassan, H. and Fattepur, S. 2019. The Anti-Angiogenic Properties of *Morinda citrifolia. L* (Mengkudu) Leaves using chicken chorioallantoic membrane (CAM) assay. Pharmacognosy Journal, 11(1).

Özcan, M. and Chalchat, J.C. 2005. Effect of Different Locations on the Chemical Composition of Essential Oils of Laurel (*Laurus nobilis L.)* Leaves Growing Wild in Turkey. Journal of medicinal food, 8 (3) 2005, 408–411.

Özel, O.T., Coskun, I., Cakmak, E. and Cimagil, R. 2019. Histomorphological study on the pyloric caeca and intestine of Black Sea trout (*Salmo labrax Pallas, 1814*). Turkish Journal of Agriculture-Food Science and Technology, 7, 2159-2164. https://doi.org/10.24925/turjaf.v7i12.2159-2164.2918

Özel, O.T. and Ertürk-Gürkan, S. 2019. Temporal variation of some digestive enzyme activities of Black Sea trout (*Salmo labrax*). Proceedings of International Erciyes Agriculture, Animal and Food Sciences Conference. Kayseri, Turkey.

Pérez, T., Alba, C., Aparicio, M., Andrés, J., José, A., Quiteria, R.S., Rodríguez, J.M. and Gibello, A. 2019. Abundant bacteria in the proximal and distal intestine of healthy Siberian sturgeons (*Acipenser baerii*). Aquaculture, 506, 325-336. https://doi.org/10.1016/j.aquaculture.2019.03.055

Rampelotto, C., de Lima, J.S., Pinheiro, C.G., Salbego, J., da Silva, L.P. and Emanuelli, T. 2018. Supplementation with microencapsulated lemongrass essential oil improves protein deposition and carcass yield in silver catfish (*Rhamdia quelen*). Acta Scientiarum. Animal Sciences, v. 40, e36517. https://doi.org/10.4025/actascianimsci.v40i1.36517.

Rawling, M.D., Merrifield, D.L. and Davies, S.J. 2009. Preliminary assessment of dietary supplementation of Sangrovit® on red tilapia (*Oreochromis niloticus*) growth performance and health. Aquaculture 294, 118–122. https://doi.org/10.1016/j.aquaculture.2009.05.005.

Ringø, E. and Gatesoupe, F. 1998. Lactic acid bacteria in fish: A review. Aquaculture, 160, 177–203. https://doi.org/10.1016/S0044-8486(97)00299-8

Ringø, E., Hoseinifar, S.H., Ghosh, K., Doan, H.V., Beck, B.R. and Song, S.K. 2018. Lactic Acid Bacteria in Finfish-An Update. Front. Microbiol. 9:1818. https://doi.org/10.3389/fmicb.2018.01818.

Saad, R., Asmani, F., Saad, M., Hussain, M., Khan, J., Kaleemullah, M., Othman, N. B., Tofigh, M. A. and Yusuf, E. 2015. A new approach for predicting antioxidant property of herbal extracts. Int. J. Pharmacogn. Phytochem. Res, 7(1), 166-174.

Salem, M. and Abdel-Ghany, H.M. 2018. Effects of dietary orange peel on growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings. Aquaculture Studies, 18, 127-134. https://doi.org/10.4194/2618-6381-v18_2_06.

Shabana, M.S., Karthika, M. and Ramasubramanian, V. 2019. Effect of dietary *Citrus sinensis* peel extract on growth performance, digestive enzyme activity, muscle biochemical composition, and metabolic enzyme status of the freshwater fish, *Catla catla*. The Journal of Basic and Applied Zoology, 80, 1-9. https://doi.org/10.1186/s41936-019-0119-x.

Shukri, N. S. B., Kaleemullah, M., Khan, J., Al-Dhalli, S., Ruhi, S., Rasny, M., Baber, M. S., Niluga, K. P., Ng, C. H., Elhassan, G. O. and Abdullah, I. 2020. Development and evaluation of halal herbal capsule containing Momordica charantia extract. International Journal of Medical Toxicology & Legal Medicine, 23 (3 and 4), 29–34.

Silva, S.D. and Anderson, T.A. 1995. Digestion and absorption. In: S. S. Silva & T. A. Anderson (Eds.), Fish Nutrition in Aquaculture. (pp. 103-141). London, UK: Publishing of Chapman & Hall.

Snuossi, M., Trabelsi, N., Taleb, S.B., Dehmeni, A., Flamini, G. and De Feo, V. 2016. *Laurus nobilis*, *Zingiber officinale* and *Anethum graveolens* Essential oils: Composition, Antioxidant and antibacterial activities against bacteria isolated from fish and shellfish. Molecules, 21, 1414. https://doi.org/10.3390/molecules21101414.

Tibaldi, E., Hakim, Y., Uni, Z., Tulli, F., Francesco, M., Luzzana, U. and Harpaz, S. 2006. Effects of the partial substitution of dietary fish meal by differently processed soybean meals on growth performance, nutrient digestibility and activity of intestinal brush border enzymes in the European sea bass (*Dicentrarchus labrax*). Aquaculture 261, 182–193.

Tseng, H.C., Grendell, J.H. and Rothman, S.S. 1982. Food, duodenal extracts, and enzyme secretion by the pancreas. American Journal of Physiology-Gastrointestinal and Liver Physiology, 243, G304-G312.

Ture, M., Altinok, I. and Alp, H. 2018. Effects of cage farming on antimicrobial and heavy metal resistance of *Escherichia coli*, *Enterococcus faecium*, and *Lactococcus garvieae*. Microbial Drug Resistance, 24, 1422-1430. https://doi.org/10.1089/mdr.2018.0040.

Vaughn, E.E., Heilig, H.G.H.J., Ben-Amor, K. and de Vos, W.M. 2005. Diversity, vitality and activities of intestinal lactic acid bacteria and bifidobacteria assessed by molecular approaches. FEMS Microbiology Reviews, 29, 477-490.

Versaw, K.W., Cuppet, L.S., Winters, D.D. and Williams, L.E. 1989. An improved colorimetric assay for bacterial lipase in nonfat dry milk. Journal of Food Science, 54, 1557-1558.

Worthington, T.M. 1982. Pepsin enzymes and related biochemicals. Freehold, NJ: Worthington Diagnostic System Inc. Retrieved from http://www.worthington-biochem.com/index/manual.html.

Xu, A., Shang-Guan, J., Li, Z., Gao, Z., Huang, Y. and Chen, Q. 2019. Effects of garlic powder on feeding attraction activity, growth and digestive enzyme activities of Japanese seabass, *Lateolabrax japonicus*. Aquaculture Nutrition. 00: 1-10. https://doi.org/10.1111/anu.13001.

Yousefi, M., Ghafarifarsani, H., Hoseini, S. M., Hoseinifar, S. H., Abtahi, B., Vatnikov, Y. A., Kulikov, E. V. and Van Doan, H. 2022. Effects of dietary thyme essential oil and prebiotic administration on rainbow trout (*Oncorhynchus mykiss*) welfare and performance. Fish & Shellfish Immunology, 120, 737-744.

Yusuf, M.S., Hassan, M.A., Tag, H.M., Sarivistava, K., Reddy, P.G. and Hassan, A.M. 2017. Influence of turmeric (*Curcuma longa*) on performance, histomorphology and microbiota of intestine in juvenile Tilapia (*Oreochromis niloticus*). International Journal of Agricultural Sciences and Veterinary Medicine, 5, 7-16.

Zahran, E., Risha, E., AbdelHamid, F., Mahgoub, H.A. and Ibrahim, T. 2014. Effects of dietary Astragalus polysaccharides (APS) on growth performance, immunological parameters, digestive enzymes, and intestinal morphology of Nile tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology, 1-9.https://doi.org/10.1016/j.fsi.2014.03.002

Zaki, M.A., Ibrahim, M.A.R., Khalafalla, M.M., Mocuta, D., Rahoveanu, A.T., Rahoveanu, M.M.T. and Hassan, A.A. 2016. Physiological and nutritional studies on improving growth of Nile tilapia (*Oreochromis niloticus*) fingerlings using alpinia meal (*Alpinia galangal*) as a feed additive. Agriculture and Agricultural Science Procedia. 10, 604-614. https://doi.org/10.1016/j.aaspro.2016.09.043.

Zharif, N., Santosh, F., Kiran, C. N., Fadli, A., Ibrahim, A., & Nizam, G. 2018. Synergistic effect of ethanolic extract of *melastoma malabataricum* leaves and antibiotics. International Journal of Medical Toxicology & Legal Medicine, 21 (3 and 4), 167–170.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.