



EFFECTS OF MONTEREY CYPRESS (*CUPRESSUS MACROCARPA* HARTW) LEAF ESSENTIAL OIL AS A DIETARY SUPPLEMENT ON GROWTH PERFORMANCE AND HAEMATOLOGICAL AND BIOCHEMICAL PARAMETERS OF COMMON CARP (*CYPRINUS CARPIO* L.)

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Abstract

Common carp (*Cyprinus carpio*) is the most farmed freshwater fish worldwide. In recent years, use of natural products in fish diets has become popular in aquaculture, to improve fish health and growth performance. The present study investigated the effects of essential oil from the leaves of Monterey cypress (*Cupressus macrocarpa*; CMEO) on growth performance and blood parameters in common carp fingerlings. Identification of 96.1% of the CMEO total volatile components was achieved, with the highest contents for terpinen-4-ol and α -pinene, at 22.9% and 47.7%, respectively. After 60 days of feeding of the fingerlings with supplemented diets without CMEO (CMEO 0%) and with CMEO at 0.5%, 0.75% and 1%, the best growth performance was seen for those fish fed with the CMEO 0.5% diet. No significant differences were seen for the haematological parameters and blood cell indices *versus* CMEO 0%. Serum glucose, triglycerides, cholesterol and glutamic pyruvic transaminase were significantly reduced in the fingerlings fed with the CMEO 0.5% diet *versus* CMEO 0%. Thus, CMEO oil as a 0.5% dietary supplement can be used to improve the growth performance and health status of the common carp without any adverse effects seen.

Key words: *Cupressus macrocarpa*, *Cyprinus carpio*, haematology, serum biochemistry, growth performance

Highlights

Plant essential oils can improve fish health and growth performance in aquaculture.

Essential oil from Monterey cypress leaves mainly contains terpinen-4-ol and α -pinene.

Dietary supplementation with this essential oil can provide benefits for common carp.

A 0.5% dietary supplement improves growth performance and health status for this carp.

This essential oil from Monterey cypress leaves has no adverse haematological effects.

Synthetic materials that include hormones, antibiotics, and/or further chemical substances have been widely used in aquaculture to control diseases and promote fish growth (Hernández-Serrano, 2005). However, the use of such synthetic materials in aquaculture products appears to be inappropriate because their heavy use has induced drug resistance and toxic residue in the fish and the environment (Cabello, 2006; Baquero et al., 2008; Koh et al., 2016; He et al., 2017). Due to the known negative effects of synthetic feed additives, several studies in recent years have been focused on the potential use of aromatic oils and extracts of herb origins in fish diets, as growth promoting agents and to improve health status of fish (Baba et al., 2016 a; Gültepe et al., 2014; Dikel, 2015).

The use of a number of dietary additives derived from plants has been reported to provide improvements in growth performance and physiological conditions for different fish species in a range of studies (Acar et al., 2018; Güllü et al., 2016; Gültepe et al., 2014). As these materials are natural and many of them are also edible for humans, they do not appear to pose any hazard to fish or human health, while also not endangering the environment (Gabor et al., 2010).

The Cupressaceae family includes more than 140 tree species that grow under various climatic and regional conditions (Cabrera et al., 2007). *Cupressus macrocarpa* belongs to the gymnosperms, and is known as the Monterey cypress (Bean, 1981). *C. macrocarpa* 'Goldcrest' is an ornamental plant and it is a preferred variant that is used in garden landscaping due to its striking colours (Cabrera et al., 2007). 'Goldcrest' also has high tolerance to temperature and salinity (Luis et al., 2007).

Different parts of trees of the Cupressaceae family, such as leaves, seeds and fruit, have been shown to contain different 'essential compounds', such as α -pinene, β -pinene, camphane and limonene (Badawy and Abdelgaleil, 2014; Emami et al., 2007 a, b). Furthermore, trees belonging to the *Cupressus* genus have been used as part of traditional treatments since ancient times. Twelve different *Cupressus* spp. have been used for application as 'drug treatments' for infections, coughs and inflammation (Ibrahim et al., 2007). An analysis has shown that the volatile components from *Cupressus* spp. are involved in the main mechanisms against pathogenic infestations (De Alwis et al., 2009). The proportions and contents of the essential oils in these trees might also vary depending on season, habitat and pathogenic activities of their region (Zrira and Benjilali, 1996).

Several contributions to the chemical compositions of volatile oils extracted from the leaves of *C. macrocarpa* have been reported in the literature, along with their biological activities (Maliza et al., 2000; El-Ghorab et al., 2007; Al-Sayed et al., 2018). While there have been no reports on the possible effects of *C. macrocarpa* leaf essential oil (CMEO) in fish, it is known that the main components of this essential oil (i.e., terpinen-4-ol, α -pinene) can have anti-cancer (Calcabrini et al., 2004) and anti-microbial (Leite et al., 2007) effects.

Common carp (*Cyprinus carpio*) is the most important species for aquaculture production worldwide (Shirali et al., 2012), and it provides significant economic benefits to the aquaculture sector, especially in Europe and Asia (Mahdavi et al., 2013). This fish is not expensive, and it has an important role in compensating for the protein needs of the world (Liao and Chao, 2009).

To the best of our knowledge, there have been no reports on the effects of the bio-active compounds of CMEO on fish growth performance and blood parameters. The present study was thus conducted to determine the effects of CMEO on these parameters of the common carp.

Material and methods

Fish and feeding unit design

Common carp fingerlings were obtained from the Mediterranean Fisheries Research, Production and Training Institute in Antalya (Turkey). The fish were left to adapt to the experimental conditions for 2 weeks before the beginning of the experiments, during which time they were fed with commercial carp feed. After the adaptation period, the fish were fed with one of four experimental diets (Table 1). The fish (mean \pm standard deviation: weight, 7.86 ± 0.15 g) used in this study were randomly selected, individually weighed, and transferred into the experimental tanks (100 L). Each tank contained 25 fish, with three independent replicates of each of the four experimental diets. The water parameters were measured daily and recorded over the 60 days of the study. During the study, the fish were kept at suitable limits for intensive carp culture (Das et al., 2014), with a mean temperature of $25.45 \pm 1.12^\circ\text{C}$, dissolved oxygen content of 7.56 ± 0.51 mg L⁻¹, and pH of 7.85 ± 0.23 .

Essential oil extraction from *C. macrocarpa* leaves

The leaves used for the essential oil extraction were collected of pruned branches of *C. macrocarpa* trees on the Çanakkale 18 Mart University Terzioğlu Campus. The extraction of the essential oils was carried out by steam distillation. For this method, the fresh leaves (300 g) were cleaned and put into deionised water (1 L) in a round-bottomed flask. The flask was positioned in the Clevenger distillation apparatus, which was operated at 100°C for 4 h. The essential oil produced was filtered and collected (El-Ghorab et al., 2007). This process was continued until a sufficient amount of this CMEO was obtained for the preparation of the diets (~20 mL).

Table 1. Formulation and nutritional content of fish feed ingredients of the experimental diets

Analysis	Detail	CMEO experimental diet content (%)			
		0%	0.5%	0.75%	1%
Component	Fish meal	27	27	27	27
	Soybean meal	30	30	30	30
	Wheat flour	22	22	22	22
	Corn starch	11	11	11	11
	Vitamin-mineral mixture*	4	4	4	4
	Fish oil	6.0	5.5	5.25	5.0
	CMEO	0.0	0.5	0.75	1.0
Nutritional content	Protein	35.44	35.51	35.55	35.47
	Fat	9.16	9.22	9.41	9.54
	Ash	5.68	5.71	5.88	5.90

CMEO, *Cupressus macrocarpa* leaf essential oil.

*Vitamin–mineral mixture: vitamin A, 18000 IU kg⁻¹; vitamin D₃, 2500 IU kg⁻¹; vitamin E, 250 mg kg⁻¹; vitamin K₃, 12 mg kg⁻¹; vitamin B₁, 25 mg kg⁻¹; vitamin B₂, 50 mg kg⁻¹; vitamin B₃, 270 mg kg⁻¹; vitamin B₆, 20 mg kg⁻¹; vitamin B₁₂, 0.06 mg kg⁻¹; vitamin C, 200 mg kg⁻¹; folic acid, 10 mg kg⁻¹; calcium d-pantothenate, 50 mg kg⁻¹; biotin, 1 mg kg⁻¹; inositol, 120 mg kg⁻¹; choline chloride, 2000 mg kg⁻¹; Fe, 75.3 mg kg⁻¹; Cu, 12.2 mg kg⁻¹; Mn, 206 mg kg⁻¹; Zn, 85 mg kg⁻¹; I, 3 mg kg⁻¹; Se, 0.35 mg kg⁻¹; Co, 1 mg kg⁻¹.

Gas chromatography–mass spectrometry analysis

The volatile contents of the CMEO were analysed using gas chromatography–mass spectrometry (QP 2010; Shimadzu) running in electron ionisation mode at 0.70 kW, and with a split/split-less injector system (at 250°C). The device configurations, mobile gas, interface and ion source injection method were applied according to Acar et al. (2018). The column oven temperature ramp programme was as suggested by Kesbiç (2019). The injection volume was 1 µL, with an *m/z* range of 45 to 450. The chromatograms detected were analysed by comparisons with the Wiley library (W9N11) and the literature (Adams, 1997).

Experimental diets and feeding strategy

The CMEO was added to the basal fish diet at the rates of 0% (control), 0.5%, 0.75% and 1% (Table 1). The raw materials for each of the experimental diets were fully mixed using a laboratory-scale feed mixer, and shaped into 1-mm grains in a pelleting machine. The experimental diets were then kept at –18°C until use. During the feeding trial over 60 days, the fish were hand-fed three times each day (09:00, 13:00, 17:00 hours) to satiation, and the weight of the feed consumed was recorded.

Growth performance

At the end of the 60-day feeding experiment, the final weights of the fish in each replicate were determined. The growth parameters were evaluated as feed conversion ratio (FCR), relative growth rate (RGR), specific growth rate (SGR) and daily feed intake (DFI), according to Yiğit et al. (2012), and as defined by Equations (1) to (4). Mortality was checked every day before the fish feeding.

$$FCR = \text{feed intake} / \text{weight gain} \quad (1)$$

$$RGR = 100 \times [(\text{final wet weight} - \text{initial wet weight}) / \text{initial wet weight}] \quad (2)$$

$$SGR = 100 \times [(\ln \text{ final wet weight} - \ln \text{ initial wet weight}) / \text{days}] \quad (3)$$

$$DFI = (\text{air-dried feed intake} / \text{number of fish}) / \text{day} \quad (4)$$

Blood collection and assays

At the end of the experimental feeding, the fish were fasted for 24 h prior to blood collection. Three fish were randomly selected from each tank (i.e., 9 fish/ experimental diet) and were anaesthetised using clove oil (28 ppm). Blood samples were collected using sterile syringes (1 mL) from the caudal blood vessels. Part of each blood sample was transferred in anticoagulant capillary tubes and used for the haematological analyses, while the remainder was placed in gel tubes to produce the serum for the biochemical assays. Red blood cell (RBC) counts ($\times 10^6 \text{ mm}^3$), haematocrit (Hct; %) and haemoglobin (Hb; g dL^{-1}) were determined using the methods described by Blaxhall and Daisley (1973). Mean corpuscular volume (MCV; μm^3), mean corpuscular haemoglobin (MCH; pg) and mean corpuscular haemoglobin concentration (MCHC; %) were determined according to Equations (5) to (7) (Bain et al., 2006):

$$MCV = (\text{Hct} \times 10) / \text{RBC count} \quad (5)$$

$$MCH = (\text{Hb} \times 10) / \text{RBC count} \quad (6)$$

$$MCHC = (\text{Hb} \times 100) / \text{Hct} \quad (7)$$

For the biochemical analyses, the blood samples were first centrifuged at 5000 rpm for 10 min, and the sera were collected in capillary tubes. The biochemical analyses of the serum samples determined the concentrations of glucose, total protein, albumin, globulin, triglycerides and cholesterol, and the activities of glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvic transaminase (GPT), alkaline phosphatase and lactate dehydrogenase. This was achieved with a spectrophotometer (Thermo Multiskan GO) using the appropriate test kits (Bioanalytic Diagnostic Industry, Germany).

Statistical analysis

One-way ANOVA was used to analyse the data after evaluation of the data normality and the variance homogeneity. If ANOVA was significant, further comparisons of the treatment means were carried out using *post-hoc* Tukey's test. The data are expressed as means \pm standard deviation. All of the statistical analyses and summary statistics were determined using the SPSS 16.0 statistical software (IBM, Armonk, NY, USA).

Results

Volatile compounds in the *C. macrocarpa* essential oil

In all, 96.1% of the total volatile components in the CMEO were identified, with the highest contents seen for terpinen-4-ol and α -pinene, at 27.9% and 47.7%, re-

spectively. The full analysis dataset of the 24 compounds identified for the CMEO is given in Table 2.

Table 2. Analytical details of the 24 volatile compounds from the gas chromatography–mass spectrometry of the *Cupressus macrocarpa* leaf essential oil

Compound	Retention time (min)	Concentration (%)
2-Hydroxymethyl-3-methyl-oxirane	3.585	0.97
Vinyl carbinol	3.962	1.48
Isoprenol	4.891	3.74
1-Butenol, 3-methyl	4.991	0.58
n-Pentanol	5.828	0.17
Pent-2-enol	5.914	0.18
Prenol	6.039	1.20
Prenal	6.296	0.61
Caproaldehyde	8.591	0.74
Sabinene	14.460	0.28
Sabinene hydrate	18.265	0.20
Linalool oxide	19.298	1.50
Limonene	19.845	2.67
2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)	21.776	1.95
α -Pinene	22.020	22.90
Dihydrocamphene carbinol	22.216	1.18
Citronella	22.436	0.29
Terpinen-4-ol	23.608	47.70
α -Terpineol	24.249	4.53
2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)	25.06	0.16
β -Citronellol	27.917	0.46
1,4-Dihydroxy-p-menth-2-ene	27.917	0.46
Pulegone	30.196	0.47
p-Menthane-3,8-diol	31.740	1.68
Total		96.10

Growth performance and feed consumption

No fish mortality was recorded during the experiment. The growth performances of these common carp fed for 60 days with the experimental diets are shown in Table 3. The RGR showed significant increase for the fish fed with the CMEO 0.5% diet, compared to the CMEO 0% control group ($P < 0.05$). The FCR showed significant reductions for all of the CMEO supplemented diets, compared to the control group ($P < 0.05$), and the lowest FCR was seen for the fish fed with the CMEO 0.5% and CMEO 0.75% diets. In addition, the CMEO 0.5% group had the highest SGR, which was also significantly greater than the control group ($P < 0.05$). However, the DFI did not show any significant differences between these four experimental groups ($P > 0.05$).

Table 3. Growth performance and feed consumption parameters for the common carp fed with the experimental diets with *Cupressus macrocarpa* leaf essential oil (CMEO) for 60 days

CMEO	Growth performance			Feed consumption		
	Weight (g)		RGR (%)	SGR (% day ⁻¹)	DFI (g day ⁻¹)	FCR
	Initial	Final				
0%	8.015±0.19	14.38±0.20 a	79.44±0.30 a	0.97±0.00 a	0.11±0.00	1.04±0.03 c
0.5%	7.77±0.19	16.25±0.49 b	109.16±4.27 c	1.23±0.03 c	0.10±0.00	0.73±0.05 a
0.75%	7.8 ±0.12	15.44±0.63 ab	96.26±8.09 bc	1.12±0.07 bc	0.10±0.00	0.79±0.06 a
1.0%	7.79±0.12	14.72±0.21 a	89.02±4.38 ab	1.06±0.04 ab	0.10±0.00	0.91±0.03 b

Data are means ±standard deviation.

Different letters within columns indicate significant differences (P<0.05).

RGR, relative growth rate; SGR, specific growth rate; DFI, daily feed intake; FCR, feed conversion ratio.

Haematological parameters

The haematological parameters of these common carp fed with the four experimental diets are given in Table 4. At the end of the experiment, the haematological indices of RBC count, Hct, Hb, MCV, MCH and MCHC did not show any significant differences (P>0.05).

Table 4. Haematological parameters for the common carp fed with the experimental diets with *Cupressus macrocarpa* leaf essential oil (CMEO) for 60 days

CMEO	Haematological parameters					
	RBC count (×10 ⁶ cells mm ⁻³)	Haemoglobin (g dL ⁻¹)	Haematocrit (%)	MCV (µm ³)	MCH (pg)	MCHC (%)
0%	2.25±0.47	8.40±1.42	16.48±3.29	73.27±2.16	37.57±2.58	5.13±0.38
0.5%	2.36±0.43	8.57±0.97	16.88±2.09	72.13±5.60	36.65±2.86	5.08±0.13
0.75%	2.58±0.27	8.65±1.60	18.20±3.40	70.35±10.38	33.59±5.98	4.76±0.32
1.0%	2.22±0.23	8.83±0.62	17.72±1.66	80.72±12.59	40.25±5.79	5.00±0.22

RBC – red blood cell; MVC – mean corpuscular volume; MCH – mean corpuscular haemoglobin; MCHC – corpuscular haemoglobin concentration.

Serum biochemical parameters

At the end of the 60-day feeding period, the albumin concentrations and alkaline phosphatase activities in the serum showed no significant differences between the treatment groups (P>0.05). However, the fish fed with the CMEO 0.5% diet showed significantly lower glucose concentration (P<0.05), compared to the CMEO 0% control diet. There were no significant differences in the serum total protein and globulin concentrations between the fish fed with the CMEO 0%, CMEO 0.5% and CMEO 0.75% diets (P>0.05), although the serum total protein concentration was significantly lower in CMEO 1% group, compared to other treatments (P<0.05). The highest triglycerides concentrations in the serum were seen for the CMEO 0% control group, which was significantly higher than the other three treatments (P<0.05). The fish fed with the CMEO 0.5% diet showed significantly low serum cholesterol concentrations, compared to the other experimental groups (P<0.05) (Table 5).

Table 5. Serum biochemical parameters and enzyme activities for the common carp fed with the experimental diets with *Cupressus macrocarpa* leaf essential oil (CMEO) for 60 days

CMEO	Serum biochemical parameters							Enzyme activities (U L ⁻¹)			
	GLU (mg dL ⁻¹)	TPROT (g dL ⁻¹)	ALB (g dL ⁻¹)	GLO (g dL ⁻¹)	TRIG (mg dL ⁻¹)	CHOL (mg dL ⁻¹)	GOT	GPT	ALP	LDH	
0%	103.83±9.89 b	8.17±0.60 b	0.13±0.04	8.04±0.58 b	32.62±7.68 b	189.20±37.50 b	52.92±10.15 b	5.94±1.59 c	36.04±15.47	532.5±121.6 c	
0.5%	74.42±17.28 a	8.45±0.38 b	0.11±0.03	8.34±0.37 b	22.63±3.32 a	141.45±13.35 a	42.21±5.51 ab	3.45±0.39 a	21.28±3.62	411.2±72.7 bc	
0.75%	87.04 ±14.28 ab	8.16±0.50 b	0.11±0.02	8.04±0.52 b	22.31±4.27 a	168.90±27.10 ab	30.63±5.43 a	4.23±0.47 ab	24.60±12.38	330.3±51.1 b	
1.0%	100.17 ±21.63 ab	6.97±0.51 a	0.11±0.01	6.85±0.50 a	21.66±3.20 a	235.68±18.63 c	50.20±13.32 b	5.53±1.09 bc	34.79±12.45	554.1±184.7 a	

Data are means ±standard deviation (n = 27 fish per treatment).

Different letters within columns indicate significant differences (P<0.05).

GLU – glucose; TPROT – total protein; ALB – albumin; GLO – globulin; TRIG – triglycerides; CHOL – cholesterol; GOT – glutamic-oxaloacetic transaminase; GPT – glutamic-pyruvic transaminase; ALP – alkaline phosphatase; LDH – lactate dehydrogenase.

Enzyme activities

The activities of the liver enzymes are shown in Table 5. The GOT activity was significantly lower in the fish fed with the CMEO 0.75% diet, compared to the control ($P < 0.05$), whereas the lowest GPT activity was seen for the fish fed with the CMEO 0.5% diet, compared to the control ($P < 0.05$). The serum lactate dehydrogenase activity was lowest in the fish fed the CMEO 0.75% diets, and highest in those fed the CMEO 1% diet, which was significantly lower than the CMEO 0% control group ($P < 0.05$). There were no significant differences in alkaline phosphatase activities between these experimental groups.

Discussion

The use of supplementation of the diet with natural products to enhance fish growth performance and health status under rearing conditions is very important, and has been the subject of a number of studies recently (Acar et al., 2018; Yılmaz and Ergün, 2018; Parrino et al., 2019). The data from the present study show that the CMEO 0.5% supplementation significantly increased the growth performance parameters of these common carp. In addition, although there were no significant differences between the diet groups for the DFI, the lowest FCR was seen for the groups fed with the CMEO 0.5% and CMEO 0.75% diets.

A previous study showed that supplementation of the diet with an oregano (*Origanum heracleoticum* L.) essential oil provided improved weight gain and related dose-dependent reduction in FCR for the channel catfish (*Ictalurus punctatus* Rafinesque) (Zheng et al., 2009). Another study reported that for maximum SGR and minimum FCR of angelfish (*Pterophyllum scalare* Schultze), a summer savoury (*Satureja hortensis* L.) essential oil could be added to the diet at 400 mg kg⁻¹ (Ghafari Farsani et al., 2018). In another study that investigated the effects of juniper (*Juniper oxycedrus*) berry oil on the growth performance of common carp, it was reported that the lowest FCR and the highest RGR were obtained when the fish were fed with diets supplemented with 0.62% juniper berry oil (Kesbiç, 2019).

The effects of herbal supplements as growth promoters would presumably act on the intestinal microbiota, which might have direct or indirect effects on growth (Reverter et al., 2014). Indeed, in fish, the intestinal microbiota are affected by microbiological, nutritional and environmental factors (Gómez and Balcázar, 2008; Wong and Rawls, 2012). The essential oils might also stimulate gut secretion, to further assist the microbiota in the control and implementation of digestion and nutrient absorption. This stimulation can also increase the amino-acids content, which is useful for synthesis of proteins, to consequently enhance the body protein content (Freccia et al., 2014). The data obtained here are also useful to underline the crucial role of suitable dosing to obtain the desired effects (Reverter et al., 2014). Further studies need to be carried out to define the active molecules in these plant extracts, along with the correct doses to use for addition to aquafeed.

Haematological parameters can be useful indicators to determine the health status of fish (Harikrishnan et al., 2011; Fazio, 2019). In the present study, the CMEO addi-

tions to the diet did not significantly influence the haematological status of these fish. Similar results were reported for the Mozambique tilapia (*Oreochromis mossambicus*) fed with 0.5% to 1.0% lemon (*Citrus limon*) peel essential oil supplemented diets (Baba et al., 2016 b). However, contrary to the present study, Ngugi et al. (2017) reported that the addition of 1% to 8% essential oil extract from bitter lemon peel to the feed for juvenile ningu (*Labeo victorianus* Boulanger) significantly increases the haematological parameters of these fish. These differences can be explained by the duration of use and the higher doses of the essential oils used.

Serum glucose concentrations act as a stress indicator in fish (Yin et al., 1995). Therefore, it can be concluded here that low concentrations of CMEO in the diet of common carp have stress-relieving effects. de Oliveira Hashimoto et al. (2016) also reported increased serum glucose in Nile tilapia (*Oreochromis niloticus* L.) after water treatment with pepper rosemary (*Lippia sidoides* Cham.) essential oil. This suggests that essential oils can increase serum glucose levels when applied to fish through different methods. Previous studies have reported similar reductions in serum glucose after the use of essential oils as alternative feed additives in fish diets (Gressler et al., 2014; Acar et al., 2018).

The active constituents of CMEO, such as α -pinene, have been shown to have sedative effects in fish (Mercier et al., 2009). In the present study, the α -pinene content of the CMEO was 22%, and therefore the decrease in serum glucose levels might be caused by a sedative effect of this content. These data are similar to those for Mozambique tilapia fed with diets supplemented with 0.5%, 1% and 3% citrus essential oil (Acar et al., 2015), and in juvenile ningu using a diet that contained essential oil from bitter lemon peel (Ngugi et al., 2017).

The total protein concentration in serum shows an enhancement of non-specific immune responses, and these changes in total protein can be influenced by any malnutrition condition during rearing (Melo and Moura, 2009). Alternatively, a decrease in the total protein levels in serum can indicate hepatic dysfunction, difficulties in absorption of protein, and protein loss during metabolism (Bernet et al., 2001). Herbal feed additives can increase non-specific immunity by enhancing the synthesis of these molecules that modulate innate immunity (Immanuel et al., 2009). Albumin and globulin are the major plasma proteins in fish (Gunter et al., 1961). In the present study, the total protein, albumin and globulin findings support the data of Awad et al. (2013), who reported significant increases in their levels in rainbow trout (*Oncorhynchus mykiss*) fed on a diet supplemented with 1%, 2% and 3% black cumin (*Nigella sativa* L.) essential oil, compared with their control group. Güleç et al. (2013) described increases in total protein and albumin concentrations in rainbow trout fed on diets that included various doses of thyme and fennel oils.

Serum triglycerides and cholesterol concentrations are generally used to evaluate liver function and nutritional status of fish (Wagner and Congleton, 2004). In the present study, serum triglycerides and cholesterol were significantly reduced in the fish fed with the CMEO 0.5% diet, compared to the CMEO 0% control. Effects of herbal oils and essential oils for the lowering of cholesterol have been reported in a number of studies. In common carp, juniper berry oil in the diet reduced serum cholesterol concentrations (Kesbiç, 2019), while ginger (*Zingiber officinale*) oil was

shown to decrease the triglycerides and cholesterol concentrations in the same fish species (Immanuel et al., 2009). Rinchar et al. (2003) reported that decreases in serum cholesterol can be due to the presence of phytosterols, whereas inhibition of cholesterol biosynthesis by plant essential oils might also be a cause of the reduction in cholesterol concentrations (Ngugi et al., 2017). Studies on carp reported that toxic product applications can increase serum cholesterol levels (Peyghan and Takamy, 2002; Yang and Chen, 2003). In the present study, the fish fed with CMEO 1% showed increased cholesterol levels. For this reason, it was hypothesised that the use of high doses of CMEO, such as 1%, can have unhealthy effects on these common carp.

The activities of hepatic enzymes have been related to liver damage in fish (Babalola et al., 2009). Increased hepatic enzyme activities can be associated with a deficiency of polyunsaturated fatty acids in the diet, and to liver damage or adiposity (Lanari et al., 1999), although this can also be an indicator of oxidative stress associated with hypothyroidism (Chattopadhyay et al., 2007). The present study showed that the activities of the serum liver enzymes, which included GOT, GPT and lactate dehydrogenase, decreased when CMEO 0.5% and 0.75% were used in the diets. From our data, it is clear that the incorporation of CMEO into the common carp diet resulted in inhibition of liver enzyme activities in a dose-dependent manner. The decline in hepatic enzyme activities here suggests that dietary supplementation with CMEO did not have any harmful effects on the physiological health status of these common carp at the doses associated with high growth rates. Babalola et al. (2009) reported that vegetable oil sources used in the feed of catfish (*Heterobranchus longifilis*) increased the liver enzyme activities; this might have been due to damaged liver cell membranes, which would lead to the release of transaminases from the cytoplasm. Similar data on the effects of high doses of additives on the liver enzymes were reported by Parrino et al. (2019) when rainbow trout were fed with a hot pepper (*Capsicum* spp.) oil supplemented diet at 6‰.

Conclusions

This investigation on the use of CMEO in the common carp diet has shown it to be effective and safe, and that it can be used as a growth promoter and immunostimulant as a 0.5% additive to common carp diets, to minimise the stress associated with aquaculture and increase the welfare of the carp. In future studies, it will be important to determine the effects of CMEO on different fish species and the suitable doses to use. Furthermore, other specific studies are needed to understand the details of the mechanism(s) through which these essential oils have their effects. Also, it is important to determine whether the resulting bioactivity is due to the presence of a single major active constituent, or whether it is the result of synergistic effects between different physiologically active molecules that are present in the essential oil.

Conflict of Interest

The authors declare that this study was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

All of the authors made substantial contributions to each step of the experimental procedure or to the manuscript preparation. The study idea was conceived by Osman Sabri Kesbiç, Vincenzo Parrino and Francesco Fazio. The experiments were designed by Osman Sabri Kesbiç, Ümit Acar and Sevdan Yılmaz. The data were analysed by Francesco Fazio and Giuseppe Lo Paro. The manuscript was written by Osman Sabri Kesbiç, Francesco Fazio, Ümit Acar and Vincenzo Parrino.

Ethics Statement

All of the experimental trials were performed in accordance with the ethical considerations presented by European legislation concerning the protection of animals used for scientific purposes (European Directive 2010/63). The project was carried out according to Kastamonu University Animal Husbandry Ethical Committee Regulations (Decision No: E.14162).

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