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RESEARCH PAPER

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Effects of a Myrrh (*Commiphora myrrha)* Essential Oil Supplemented Diet on Haemato-Biochemical Parameters, Expression of Tissue-Specific Immune- and Stress-Related Genes, and Resistance of *Cyprinus carpio* to *Aeromonas hydrophila* Infection

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Abstract

Plant-based additives or botanicals, have garnered considerable interest in the aquaculture industry for their multifaceted benefits in fish diets. In this study, a 30 days short term feeding experiment was designed to determine the effects of Myrrh (*Commiphora myrrha*) essential oil on hematological, biochemical parameters and tissue specific mRNA expression responses of common carp (*Cyprinus carpio*). Fish were experimentally infected with *Aeromonas hydrophila* and monitored for 20 days after been divided in four groups: a control group without *C. myrrha* and three experimental groups supplemented with 0.25%, 0.50%, and 1.0% *C. myrrha* essential oil (CMO 0.25, CMO 0.50, and CMO 1.0, respectively). Results revealed a significant increase in RBC count, hemoglobin, and hematocrit values compared to the control diet. Positive effects of *C. myrra* supplementation have been observed also in serum biochemistry parameters. mRNA transcripts of il-1ß, tnf-α, il-8, sod, cat, gpx and gst genes significantly increased in spleen and liver tissue of fish fed with *C. myrra* supplemented diets. After the challenge with *Aeromonas hydrophila*, the survival rates were 60%, and 83.33%, respectively, suggesting enhanced fish resistance in the CMO 0.50 and CMO 1.0 groups compared to the control. The results suggest the immunomodulatory roles of *C. myrrha* essential oil.

Introduction

Driven by increasing global demand for aquafood and sustainable food production, aquaculture has rapidly expanded as a key agricultural sector. Among the widely cultivated freshwater species, the common carp (Cyprinus carpio) is particularly valued for its adaptability to diverse climatic conditions and its significant contribution to the industry (Eddy and Underhill, 1974). In addition, common carp is the third most frequently introduced species globally and the most commercially significant freshwater fish species in the world (Saika and Das, 2009). Nonetheless, the rapid growth of common carp aquaculture has resulted in the emergence of numerous diseases and health complications within this culture. Bacterial infections, in particular "motile aeromonas septicemia" caused by *Aeromonas hydrophila*, have significantly affected the culture of common carp in river aquaculture and resulted in significant mortality as well as financial losses for fish farmers (Austin and Adams, 1996). *A. hydrophila* is a facultatively anaerobic, motile, gram-negative, oxidase-positive bacilli or coccobacilli rod (Dias et al*.,* 2012; Semwall et al*.,* 2023). In fish, it is an opportunistic pathogen and causes hemorrhagic septicemia, exophthalmia, infectious abnormal dropsy, fin, hemorrhagic septicemia, exophthalmia, infectious abnormal dropsy, and fin and tail rot (Austin and Austin, 2007; Zhang et al*.,* 2020).

Antibiotics are extensively used to control bacterial diseases in aquaculture. Nevertheless, their long-term use leads to the rise of antibiotic-resistant bacteria and other negative consequences such as the permanence of antibiotic residues in both fish and the environment (Cabello, 2006). Thus, the development of innovative methods for disease control in common carp aquaculture is critically needed.

Essential oils (EOs) may have the potential to be used as an alternative to antibiotics in aquaculture (Romero et al*.,* 2012; Cunha et al*.,* 2018). EOs obtained from plant raw materials are lipophilic, volatile, fragrant, liquid natural multicomponent systems (Edris, 2007) obtained from the flowers, leaves, seeds, roots, peel, wood, herbs, and fruits parts of the plant. EOs are mixtures of low molecular weight substances with a wide variation in their chemical properties (Hussain et al*.,* 2008). More than 3,000 different chemicals with a wide variety of chemical structures have been identified in EOs (Cunha et al*.,* 2018). EOs are generally classified as terpene hydrocarbons, simple and terpene alcohols, aldehydes, phenols, ketones, esters, ethers, peroxides, oxides, organic acids, furans, lactones, coumarins (Swamy et al*.,* 2016). EO compounds have a wide spectrum of inhibitors to the growth of Gram-negative and Gram-positive bacteria (Remmal et al*.,* 1993). Some studies have determined that the mechanism of action of EOs depends on their major functional groups. Due to their antimicrobial properties, these oils have the ability to constitute alternative prophylactic and therapeutic agents in aquaculture (Romero et al*.,* 2012). Previous research has highlighted the benefits of herbal medicine, citing its potential to replace antibiotics in the treatment of disease, availability locally, biodegradability, and lack of adverse effects. Nowadays, the effect of essential oils or extracts on aquaculture pathogens has been reported in some studies (Wu et al*.,* 2010; Wu et al., 2013; Dotta et al*.,* 2014; Tang et al*.,* 2014; Zahran et al*.,* 2014; Gabriel et al*.,* 2015; Acar et al*.,* 2015; Baba et al*.,* 2016; Hashimoto et al*.,* 2016; Fazio et al., 2022; Habib et al., 2024; Naz et al., 2024; Ujan et al., 2025). There are various studies showing that different essential oils in carp, such as *Zataria multiflora (*Soltani et al*.,* 2010), blue gum (Sheikhzadeh et al*.,* 2009), oregano (Abdel-Latif et al*.,* 2020), *Thymus vulgaris* (Ghafarifarsani et al*.,* 2022), increase the immune system and disease resistance. In many studies where plant essential oils were used as feed additives, it was determined that the application produced successful results in cultivation. A number of beneficial effects of essential oils were documented by these earlier researchers, including improved growth performance, feed utilization, and disease resistance; they also strengthened the immune system, prevented stress, and decreased mortality. No studies have examined the use and effects of myrrh (*Commiphora myrrha*) essential oil in fish feed.

Myrrh is an important medicinal plant of the Burseraceae family. It has more than 150 species of plant spread across subtropical and tropical regions, particularly north-east Africa, southern Arabia, and India (Demissew, 1993). Myrrh and myrrh derivatives have long been used as a raw material in the traditional medicines of India, China, Rome, Greece and Babylon (Shen and Lou, 2008). Myrrh is derived from an Arabic term that translates to 'bitter.' One especially valued type of myrrh is referred to as *karam* or Turkish myrrh (Encyclopedia, 2024). Myrrh is composed of several components, which consist of volatile oil ranging from 2 to 8%, resin making up 23 to 40%, gum accounting for 40 to 60%, and bitter compounds that comprise 10 to 25% (Chen et al., 2013).

Myrrh has been used traditionally for the treatment of many diseases, such as mouth ulcers, aches, stomach disorders, fractures, inflammatory diseases, and microbial infections since ancient times (Su et al*.,* 2011; Abdelsalam et al*.,* 2022). *C. myrrha* is utilized for its antiseptic, astringent, anthelmintic, carminative, emmenagogue, and expectorant properties. The primary components of myrrh are terpenoids (monoterpenoids, sesquiterpenoids, and volatile/essential oils), diterpenoids, triterpenoids, and steroids (Batiha et al*.,* 2023). Studies have illustrated its diverse biological activities, including anti-inflammatory, antioxidant, antimicrobial, anti-viral, neuroprotective, antidiabetic, anticancer, analgesic, and antiparasitic properties (Gadir and Ahmet., 2014; Madia et al., 2021; Abdelsalam et al*.,* 2022; Batiha et al*.,* 2023) immune response (Noyal et.al.,2018), hematology-serum biochemistry (Mitsumoto et.al., 2021) and leukocytes proliferation (Haffor, 2009) .

Considering the introduction presented above, this study is specifically designed to investigate the effects of myrrh essential oil on the health components of carp, including the expression levels of immune and antioxidant genes, hematological and serum biochemical responses, and resistance to *A. hydrophila*.

Materials and Methods

This study was approved by the Animal Ethics Committee of Muğla Sıtkı Koçman University (Muğla,

Türkiye, Approval Number: 2024/02-2). Myrrh essential oil was chosen due to its valued for its antimicrobial, anti-inflammatory, and antioxidant properties, supporting immune function and overall health. Myrrh essential oil was purchased from a local herbal medicine store in İzmir, Turkey. Four diets with different concentrations of CMO (0.25%, 0.50%, and 1%) along with a control diet without CMO were prepared. The details of the dietary formulations and ingredient compositions are provided in Table 1.

Crude ash (%), crude protein (CP; %), and crude lipid (CL; %) of the test diets were determined using the AOAC procedures. The experimental fish were supplied by the Central Fisheries Research Institute in Antalya, Türkiye. Upon arrival at the laboratory, 180 clinically healthy juvenile common carp weighing 14.77±0.21 g

were given a 14-day adaptation period before the start of the feeding experiment. Subsequently, they were randomly divided into 12 experimental glass tanks with triplicate (15 fish/tank), with an equal number of fish in each tank. The feeding experiment was carried out at a temperature of 22±0.5°C, following a 12-hour light to 12-hour dark photoperiod. Throughout the feeding experiment, water conditions were maintained at a pH of 7.6±0.1 and a dissolved oxygen level above 5.5 mg/L. The fish were fed twice daily, in the morning and afternoon, with the amount of food given being 2% of their body weight per day. The experimental diets were administered to the fish for a period of 30 days (Ghelichpour et al., 2021). No mortality was recorded during the course of the feeding experiment. The phytonutrients found in the CMO supplement were investigated using the GC–MS analysis method. Chromatograms of the CAO were characterized and then identified after matching with the spectra of the Wiley W9N11 library.

At the ending of the experimental period, the fish were not fed for 24 hours. 15 fish from each experimental group (5 fish from each aquarium) were randomly selected and anesthetized with 50 µL/L of clove oil to collect blood samples (Woody et al., 2002). In order to prevent contamination, the caudal region of the fish was meticulously cleaned with 70% ethanol. Syringes were employed to capture blood samples in the subsequent phase. Following this, the samples were divided into two categories for analysis: lavender-top tubes with anticoagulant (K3EDTA) for hematological analysis and red-top (SST™ II) advance serum separator tubes for biochemical analysis. Finally, the serum separator containers were centrifuged at 4000 g for 10 minutes to separate the serum for biochemical analysis. Serum samples were subsequently stored at -80°C for subsequent analysis. Liver and splenic tissues were extracted from the fish subsequent to blood collection. Until the analysis, the samples were maintained at -80˚C.

Hemoglobin (HGB) content was measured using the cyanmethemoglobin technique after observing red blood cells (RBCs) under a light microscope. The hematocrit (HCT) measurement was conducted by employing a capillary HCT tube subsequent to centrifugation per the procedures outlined by (Hesser, 1960). Commercial test kits from the Bioanalytic Diagnostic Industry in Germany were used for biochemical analysis. The spectrophotometer (OptizenPOP UV/VIS) was utilized to measure various parameters including glucose (GLU), total protein (TPROT), cholesterol (CHOL), albumin (ALB), globulin (GLO), triglyceride (TRIG), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), glutamic pyruvic transaminase (GPT), and glutamic-oxaloacetic transaminase (GOT) (Yilmaz and Ergün, 2012).

Thirty fish from both the control and experimental groups were used to investigate the resistance of the common carp to *A. hydrophila*. Following a period of 30 days during which the fish were given food and had their blood samples taken, they were then injected with 0.1 mL of a suspension containing 1.5 × 106 colony-forming units per milliliter of *A. hydrophila*, using the intraperitoneal route. The suspension was prepared in phosphate buffered saline. The fish were constantly examined for any obvious symptoms of sickness, such as odd behavior, and deceased fish were carefully removed from the tanks to minimize stress. Death occurred in all of the groups within 20 days after infection. The infection was confirmed by re-isolating the germs from the deceased fish. Bacterial identification was achieved by using traditional biochemical methods (Austin and Austin, 2007) and the API 20 Strep kit (Biomerieux, France).

The liver and spleen tissues were collected from 6 fish per group, with 2 fish from each tank, for the purpose of RNA isolation. The GeneJET RNA Purification Kit, made by Thermo Fisher Scientific in the United States, was used in our study. Quantification of RNA and study of RNA integrity was performed using a Multiskan

Table 1. Formulations and chemical composition of experimental diets.

Ingredient	Control	$CMO_{0.25}$	$CMO_{0.50}$	$CMO_{1.0}$
Fish meal	25.00	25.00	25.00	25.00
Soybean meal	35.00	35.00	35.00	35.00
Wheat meal	12.00	12.00	12.00	12.00
Fish oil	5.00	5.00	5.00	5.00
Mineral/vitamin premix[a.b]	4.00	4.00	4.00	4.00
Starch	19.00	18.75	18.50	18.00
Myrrh essential oil	0	0.25	0.50	1.00
Chemical composition				
Protein	36.62	36.84	36.76	36.19
Lipid	7.51	7.34	7.56	7.23
Ash	5.49	5.60	5.47	5.72

aVitamin Mix: Vit. A, 18000 IU; Vit. D3, 2500 IU; Vit. E, 250 mg/kg; Vit. K3, 12 mg/kg; Vit. B1, 25 mg; Vit. B2, 50 mg; Vit. B3, 270 mg; Vit. B6, 20 mg; Vit. B12, 0.06 mg; Vit. C, 200 mg; Folic acid, 10 mg; Calcium d-pantothenate, 50 mg; Biotin, 1 mg; Inositol, 120 mg; Choline chloride, 2000 mg. bMineral Mix: Fe, 75.3 mg; Cu, 12.2 mg; Mn, 206 mg; Zn, 85 mg; I, 3 mg; Se, 0.350 mg; Co, 1 mg

Go instrument manufactured by Thermo Fisher Scientific in the United States. The purified RNA was mixed with RNase-free DNase I (Thermo Fisher Scientific, USA) and then analyzed using Real-time PCR. Specific primer pairs were used to amplify SOD, CAT, GpX, GST, IL-1β, IL-8, TNF-α, and β actin. The cDNA synthesis process included reverse-transcription of 1 µg of total RNA from each sample using the RevertAid™ H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA). The cDNA was stored at a temperature of -20°C to guarantee its long-term preservation. The FastPCR 6.0 program (Kalendar et al., 2009) was used to generate primers (Table 2) that were meant to cover at least one exon boundary.

The data were reported as mean values with standard deviation (SD) and analyzed using SPSS 17.0 software (SPSS Inc., Chicago, USA). The study used one-way analysis of variance (ANOVA) followed by the Tukey's test to see whether there were significant differences in the measured parameters across the treatments. The analyses were conducted with a significance threshold of 0.05.

Results

Bioactive Components of CMO

The primary functional bioactive components in CMO used in the current investigation were also assessed. The GC–MS chromatograms showed 46 peaks representing different metabolites, as shown in Table 3.

The phyto-ingredients that were found in the highest quantities were cis-Ocimene (24.49%), Eucalyptol (34.77%), linalool (13.83%), alpha-Terpineol (4.16%), and Linalyl acetate (5.87%) (Table 3).

Hematological and Serum Biochemical Parameters

Based on the results reported in Table 4, the inclusion of dietary CMO at a dose range of 0.25-1% led to a substantial and measurable increase ($P<0.05$) in the red blood cell count, Hgb concentration, and Hct value of common carp.

Significant changes were seen in the assessed biochemical variables across all experimental groups. When comparing the control group to the CMO1.0 group, it was noticed that the CMO1.0 group had the highest TPROT levels ($P<0.05$). However, the TPROT values in other groups did not exhibit a statistically significant difference compared to the controls ($P>0.05$). The control group exhibited a substantial ($P<0.05$) rise in CHOL and LDH levels compared to the groups supplemented with CMO. No significant variations were seen in the other serum biochemical measures, including GLU, TRIG, ALP, and AST levels, between the experimental groups ($P>0.05$).

Gene Expression Profile

Dietary supplementation with CMO over a period of 30 days elicited notable changes in gene expression levels within the spleen and liver tissues of common carp, particularly affecting genes associated with anti-oxidative responses and immune function (Figure 1).

Specifically, both spleen and liver tissues exhibited a significant upregulation in the mRNA expression levels of SOD, CAT and GpX in groups supplemented with CMO at concentrations of 0.5% and 1%. Concurrently, there was a similar upregulation observed in pro-inflammatory cytokines such as IL-1β, IL-8, and TNF-α within both spleen and liver tissues, compared to the control group.

Table 2. Primer nucleotide sequences used to conduct this research.

Gene		Primer sequence	Product size (bp)	GenBank No
β-Actin	F	CTGGTATCGTGATGGACTCT	204	M24113
	R	CAGAGCTTCTCCTTGATGTC		
SOD	F	GATAGTGACAGACACGTCGGA	180	XM_019111527.1
	R	AAGACTTTCGTCATTGCCTCC		
CAT	F	GAGCACGTAGGGTTCAAGTGC	162	NC_031745.1
	R	TCGCCTTATCTCTGTCTGCCA		
GpX	F	TGTCCTTGATGGGTGATCCCA	144	GQ376155.1
	R	CAATGTCGCTGGTGAGGAACC		
GST	F	CCTCGCTGGAAAGAGCTTCAC	138	XM_019117245.1
	R	ATACTGGGACGGTCCTTCAGC		
TNF-α	F	GTGTCTACAGAAACCCTGGA	109	AJ311800
	R	AGTAAATGCCGTCAGTAGGA		
IL-1ß	F	TTACAGTAAGACCAGCCTGA	89	AJ245635
	R	AGGCTCGTCACTTAGTTTGT		
IL-8	F	GTCTTAGAGGACTGGGTGTA	120	AB470924.1
	R	ACAGTGTGAGCTTGGAGGGA		

*The real-time PCR was performed using the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. The cDNA used was 1 µg. The thermal cycling conditions consisted of 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds, 55 cycles of 95 °C for 30 seconds, and an extension step at 72°C for 10 minutes. The target genes included β-actin, SOD, CAT, GpX, GST, IL1-β, IL-8, and TNF-α. Electrophoresis was performed on the PCR and RNA product using a 2% w/v agarose gel electrophoresis technique, using a 2 µg/ml SYBR Safe DNA Gel Stain (Thermo Fisher Scientific, USA). The length of PCR and RNA products was assessed by administering and checking a DNA ladder Gene Ruler 50 bp Marker Plus (Thermo Fisher Scientific, USA) alongside the samples. The findings were assessed using real-time PCR using the CFX Connect™ RealTime PCR Detection System, manufactured in the United States.

Table 3. Chemical composition of *Commiphora myrrha* essential oil.

Peak#	R.Time	Area%	Name
1	6.020	0.01	4-Octen-3-one (CAS)
2	8.985	0.16	Tricyclene
3	9.242	0.59	Thujene <alpha->
4	9.510	24.49	cis-Ocimene
5	10.012	0.55	Camphene
6	11.046	0.08	Sabinene
7	11.126	1.01	Pinene <beta->
8	11.820	0.44	Myrcene
9	12.051	0.47	Butanoate <butyl->
10	12.272	0.14	Phellandrene <alpha->
11	12.499	0.63	DELTA.3-Carene
12	12.774	0.14	Terpinene <alpha->
13	13.107	1.42	Cymene <para->
14	13.392	34.77	Eucalyptol
15	13.708	0.15	trans-.beta.-Ocimene
16	14.479	0.28	.gamma.-Terpinene
17	15.048	0.23	Linalool oxide <trans->
18	15.670	0.31	Linalool oxide <cis->
19	16.010	0.04	alpha.-Pinene oxide
20	16.237	13.83	Linalool
21	16.385	0.06	Limonene oxide <cis->
22	16.679	0.04	Oct-1-en-3-yl acetate
23	17.496	0.16	Dihydrolinalool
24	17.811	0.92	Camphor
25	17.901	0.16	BETA. TERPINEOL
26	18.211	0.04	Cyclohexanone, 5-methyl-2-(1-methylethyl)-, (2R-cis)- (CAS)
27	18.347	0.29	Isoborneol
28	18.635	0.07	p-Menth-8-en-1-ol, stereoisomer
29	18.707	0.32	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-
30	18.790	0.06	Lavandulol
31	19.146	0.68	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-
32	19.494	0.04	Benzenemethanol, 4-(1-methylethyl)- (CAS)
33	19.693	4.16	.alpha.-Terpineol
34	19.942	0.86	Terpineol <gamma->
35	21.092	0.24	Nerol (CAS)
36	22.102	5.87	Linalyl acetate
37	23.173	0.12	Anisole <para-allyl->
38	23.363	0.24	Lavandulyl acetate
39	25.423	1.30	Terpinyl acetate <alpha->
40	25.490	0.16	Terpineol <gamma->
41	25.926	0.59	Neryl acetate
42	26.586	1.89	Geranyl acetate
43	27.318	0.42	Benzene, 1,2-dimethoxy-4-(2-propenyl)- (CAS)
44	27.838	0.72	Caryophyllene
45	28.969	0.67	alpha.-Humulene (CAS)
46	33.097	0.20	Caryophyllene oxide
		100.00	

Table 4. Hematological and Serum Biochemical Parameters of fish fed with experimental diets

Parameter	**Control**	**$CMO_{0.25}$**	**$CMO_{0.50}$**	**$CMO_{1.0}$**
RBC ($\times 10^6$ per mm^3)	1.93 ± 0.05^{b}	2.09 ± 0.07^{b}	2.73 ± 0.16^{a}	2.68 ± 0.19^{a}
HGB (g/dL)	9.38 ± 0.16^{d}	10.55 ± 0.42^{c}	11.65 ± 0.49^{b}	13.31 ± 0.41^{a}
HCT (%)	26.14 ± 0.62^{c}	28.00 ± 0.55^{c}	30.48 ± 0.70^{b}	33.17 ± 1.87^{a}
TPROT(g/dL	9.80 ± 1.95^{b}	9.83 ± 0.96^{b}	10.98 ± 3.09^{ab}	13.27 ± 1.39^{a}
GLU(mg/dL)	49.83 ± 9.15^{a}	49.71 ± 11.64^{a}	49.40 ± 9.55^{a}	50.54 ± 4.43^{a}
CHOL(mg/dL)	50.27 ± 13.02^{a}	35.67 ± 6.93^{b}	25.33 ± 6.19^{b}	31.60 ± 5.67^{b}
TRIG(mg/dL)	21.19 ± 5.49^{a}	23.14 ± 6.50^{a}	23.14 ± 7.46^{a}	21.86 ± 5.14^{a}
ALP (U L^{-1})	45.00 ± 8.01^{a}	28.63 ± 4.17^{b}	27.37 ± 9.44^{b}	51.00 ± 4.81^{a}
AST (U L^{-1})	47.82 ± 12.77^{a}	35.11 ± 6.93^{a}	40.83 ± 2.20^{a}	40.26 ± 8.37^{a}
LDH (U L^{-1})	165.5 ± 44.5^{a}	113.8 ± 29.9^{ab}	130.3 ± 25.6^{ab}	109.03 ± 23.67^{b}

*The data is shown as the mean value plus or minus the standard deviation, with a sample size of 15. Values with distinct letters in the same row are deemed statistically significant within groups ($P<0.05$). Acronyms: RBC is for red blood cells, HGB stands for hemoglobin, HCT stands for hematocrit, TPROT stands for total protein, GLU stands for glucose, and CHOL stands for cholesterol. TRIG: Triglycerides; AST stands for aspartate aminotransferase, ALT stands for alanine aminotransferase, and LDH stands for lactate dehydrogenase.

Bacterial Pathogen Challenge

Common carp's resistance to *Aeromonas hydrophila* after a 20-day observation period, survival rates recorded in control, CMO0.25, CMO0.50, and CMO1 groups 50, 43.33, 60, and 83.33%, respectively (Table 5).

According to Kaplan–Meier survivorship curves, it was noticed that the highest survival rates were found in CMO1 (P<0.05; Figure 2).

Discussion

Limitation of the use of antibiotics is crucial to prevent the proliferation of antibiotic-resistant bacteria and maintain the health of aquatic environments in aquaculture. For this purpose, incorporating natural additives can be an effective strategy for improving the well-being and resilience of cultured fish (Mathew et al*.,* 2022). Reducing dependence on antibiotics and embracing natural remedies such as herbal extracts and essential oils is in line with sustainable aquaculture practices. These additives not only stimulate appetite, promote growth, and enhance immune responses but also provide a safer alternative to synthetic chemicals. Furthermore, focusing on the immunostimulatory and antioxidant properties of essential oils derived from medicinal plants can contribute to healthier aquatic ecosystems and more resilient fish populations (Zhang et al*.,* 2020; Farag et al*.,* 2023). This study assesses, for the first time, the impact of adding *Commiphora myrrha* essential oil to the diet on physiological reactions, antioxidant levels, enhancement of immune function, and increased resilience of common carp against *Aeromonas hydrophila* infections.

Monitoring parameters such as the count of red blood cells (RBC), levels of hemoglobin (Hb), and levels of hematocrit (Hct) may offer important information about the health of fish, including their ability to transport oxygen and the possibility of hemolytic

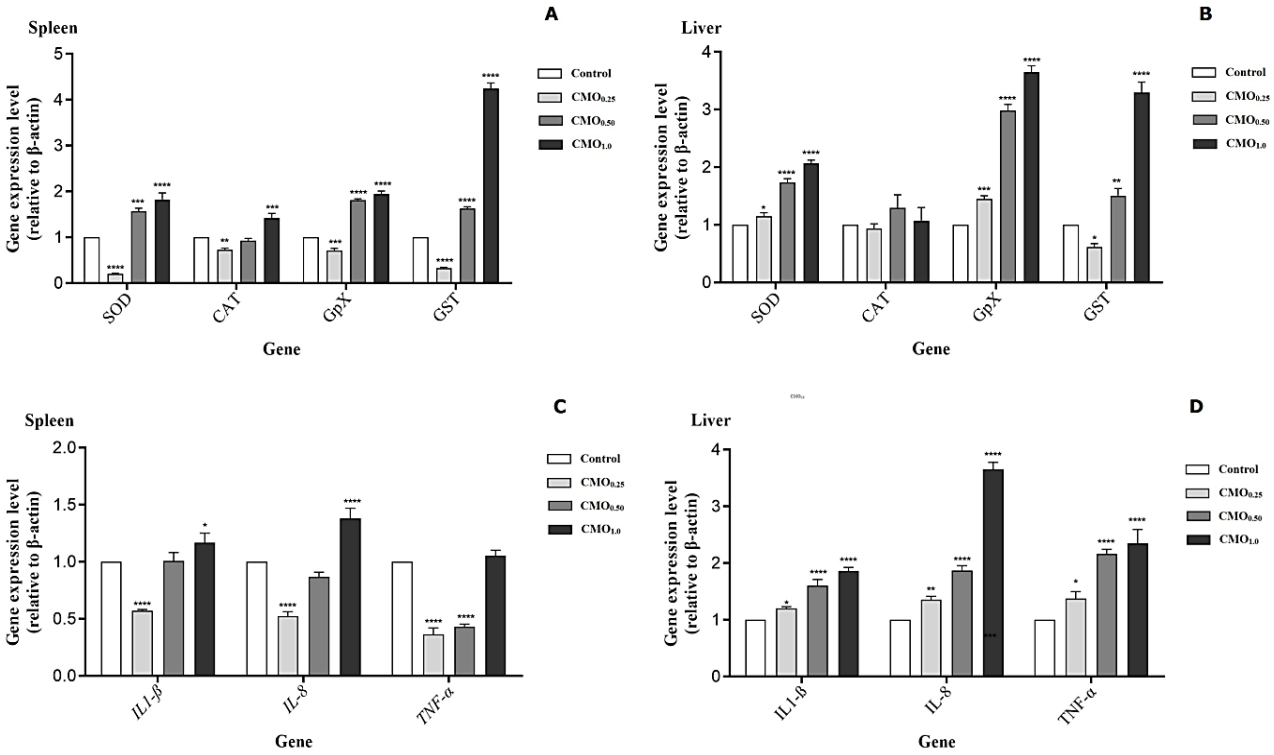

Figure 1. Gene expression responses in spleen (A-C) and liver (B-D) tissue of common carp fed diets supplemented with different levels of CMO. Values are presented as means ± S.D. (n=6) (P<0.05).

Table 5. Mortality rate (MR; %), survival rate (SR; %), and relative percent survival (RPS) of common carp fed with diets containing different levels of CMO for 30 days and then experimentally challenged with *A. hydrophila* infection and observed for 20 days.

Experimental groups	Number of challenged fish	Mortality rate (MR;%)	Survival rate (SR;%)	RPS
Control	30	50.0[a]	50.0[c]	-
$CMO_{0.25}$	30	56.66[a]	43.33[c]	-13.33
$CMO_{0.50}$	30	40.0[b]	60.0[b]	20.0
CMO_{1}	30	16.66[c]	83.33[a]	66.66

*Values with different superscript letters in the same column are considered statistically different within groups (P<0.05). MR (%) = (No. of fish died after challenge / Total No. of challenged fish) × 100. RPS (%) = [1 – (Mortalities of CMO-supplied groups / Mortalities of Control group)] × 100.

processes (Clauss et al*.,* 2008; Fazio et al*.,* 2013). Examination of qualitative and quantitative variations in these parameters may serve as indicators for different illnesses, such as anemia or dehydration, and assist the researchers in accurately diagnosing and treating fish. Moreover, these characteristics aid in assessing the efficacy of the fish's hematological system and its capacity to facilitate oxygen transportation and eliminate carbon dioxide, which are crucial functions for survival and general well-being (Abdel-Tawwab et al*.,* 2006). In the present study, RBC count, Hct, and Hbg values were significantly increased in all groups of fish fed with CMO-supplemented diets compared to the control group. The findings indicate that adding CMO to common carp fish's diet effectively decreases hemolysis. This can be due to the enhancement of antioxidant capacity, which safeguards red blood cells from hemolysis (Gaudet et al*.,* 1975). Nevertheless, eucalyptol, the main constituent of CMO used in the current study, seems to alleviate such harm, resulting in a significant enhancement in hematology when compared to the control group. The potential reason for this protective effect may be the reduction of oxidative stress as for previous studies reporting that eucalyptol has potent inhibitory effects on pro-oxidant-induced oxidative stress [Cho, 2012). As far as we know, this is the first documentation of CMO in diets for common carp. Consistent with these results, gibel carp (*Carassius gibelio*) that were given diets enhanced with thyme essential oil showed improvements in hematological parameters, including higher levels of red blood cells (RBCs), hematocrit (Hct), and hemoglobin (Hb) [(Zadmajid et al*.,* 2017). Moreover, research has shown that adding herbal supplements to the diet might improve hematological parameters in different species of teleost fish (Acar et al*.,* 2015; Rashidian et al*.,* 2020).

Serum biochemical parameters are essential biological markers that aid in evaluating the impact of feed additives on the health of fish. These metrics provide essential data on the metabolic processes, organ function, and general physiological condition of fish [Harikrishnan et al., 2012). This study focused on analyzing many important serum biochemical markers, including TPROT, Glu, Chol, Trig, ALP, AST, and LDH. TPROT has a role in the health of fish (Magnadóttir, 2006; Yilmaz et al*.,* 2024) and indicates the nutritional and health state of fish (El-Houseiny et al*.,* 2022). The findings of our research show that the CMO1.0 group had the highest levels of TPROT compared to the control group. In another study, higher blood protein levels were associated with improved fish survival rates and enhanced immunological effectiveness (Simajuntak et al*.,* 2018). These results were in line with the findings of (Souza et al*.,* 2020), who discovered that adding Cochin grass *(Cymbopogon flexuous)* EO to diets might raise TPROT levels in the blood of Nile tilapia (*Oreochromis niloticus*). Furthermore, addition of 2 ml of Panax ginseng essential oil to the diets of Nile tilapia was reported to significantly increase the TPROT level (Yilmaz et al*.,* 2024). In this study, GLU, note to be a stress indicator in fish (Martínez-Porchas et al., 2009), did not varied between all treatment groups. In contrast, other studies have reported that dietary supplementation with herbal additives can reduce serum GLU levels in fish (Ghafarifarsani et al*.,* 2022; Adeli et al*.,* 2021). However, based on the current

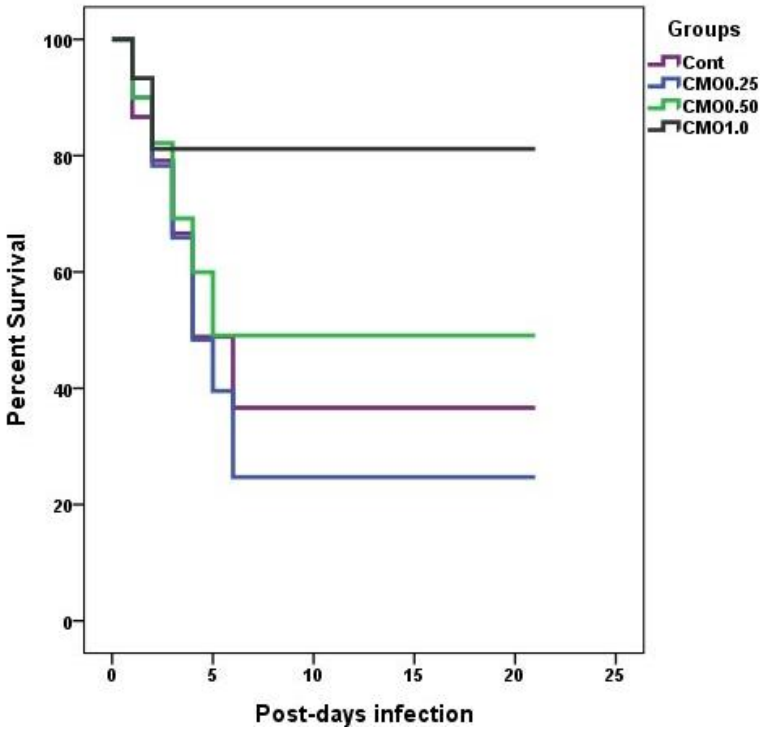

Figure 2. Kaplan–Meier survivorship curves (cumulative survival [%] over time [h]) for common carp after challenge with *Aeromonas hydrophila*; the fish were fed with CMO supplemented diets.

results, it can be concluded that supplementation with CMO did not induce any stress in the fish. This observation suggests that the inclusion of CMO in the diet did not lead to an increase in stress levels as reflected by serum GLU levels. CHOL is essential for the structure of biomembranes, especially in the formation of the outer layer of serum lipoproteins (Dorojan et al*.,* 2015). In addition, CHOL acts as a precursor for the production of steroid hormones (Pourmozaffar et al*.,* 2019), which play a crucial role in numerous physiological processes in organisms. However, TRIG operates largely as an energy storage form in cells and serves as the major source of metabolic and cellular energy (Pourmozaffar et al*.,* 2019). They play a crucial role in energy metabolism and are activated when the body needs more energy. We noticed a substantial reduction in cholesterol levels in those fed with a meal supplemented with CMO and no significant changes in TRIG. Several studies have proven the efficacy of herbal oils and essential oils in reducing cholesterol levels. Similarly, Monterey cypress (*Cupressus macrocarpa* Hartw) leaf essential oil was shown to lower blood cholesterol levels in common carp (Kesbiç et al., 2020). Also, ginger oil was proven to have a similar effect on cholesterol concentrations in the same fish species (Immanuel et al., 2009). Some researchers reported that these lower levels of cholesterol in the blood may be attributed to the presence of phytosterols (Rinchard et al., 2003). Additionally, the suppression of cholesterol production by essential oils derived from plants may also contribute to the decrease in cholesterol levels (Ngugi et al., 2017). Monitoring AST and ALP levels in fish serum is crucial for assessing liver health and detecting potential liver problems, especially when fish are exposed to external stressors or environmental challenges (Yilmaz et al*.,* 2024). Changes in these enzyme levels can provide valuable insights into the impact of stress, diseases, or dietary factors on liver function and overall fish health. Elevated levels of these enzymes in fish serum can indicate liver damage or dysfunction, as these enzymes are primarily found in hepatocytes (liver cells) and are released into circulation when hepatocytes are damaged (Ghelichpour et al., 2021). The current findings suggest hepatoprotective properties of CMO, which may be attributed to its enhanced antioxidant capacity in the liver of the fish. In the study conducted by (Shourbela et al., 2021), Nile tilapia fed with diets containing oregano essential oil did not exhibit any signs of toxicity, stress, inflammatory diseases, or other undesirable effects. Aquaculture researchers are particularly interested in the ability of fish to withstand damaging stress, especially in aquaculture settings. One possible method to mitigate this stress is to use herbal sources that are abundant in physiologically active compounds (Khalil et al., 2022).

The enzymatic antioxidant protectors such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione S-transferase (GST) play a significant role in the detoxification process and in counteracting the adverse effects of reactive oxygen species (ROS) and free radicals (Oberley et al.,1988). These enzymes work synergistically to mitigate oxidative stress by reducing the accumulation of harmful oxidants. In this study, the increase in Myrrh essential oil dosage in the experimental diets led to enhanced expression of antioxidant enzymes such as SOD, CAT, GPX, and GST in the spleen and liver of common carp. This indicates that higher doses of the oil strengthened the fish's defense mechanisms against stress and/or pathogens. The results obtained in the present study may be associated with myrrh essential oils' antioxidant abilities. Similarly, palm fruit extracts (Hoseinifar et al., 2017), *Thymus vulgaris* extract (Hoseini et al., 2019), *Santalum album* essential oil (Mansour et al., 2024), *Lavandula angustifolia* extract (Yousefi et al., 2020) could increase SOD, CAT, GPX and/or GST expression in fish tissue.

In recent years, gene expression analysis has become increasingly important in assessing the status of fish immunity using reliable technological techniques. Interleukin-8 (IL-8) functions as a crucial chemokine in fish, mediating the activation and recruitment of neutrophils to sites of inflammation, and directing immune cell migration, thus playing a pivotal role in regulating immune responses and enhancing the defense against bacterial pathogens (Ahmadifar et al.,2021). Interleukin-1 beta (IL-1β) acts as a central pro-inflammatory cytokine in fish, primarily produced by macrophages and monocytes, it not only mediates inflammatory responses by enhancing the production of other cytokines but also initiates crucial immune processes necessary for combating infections (Dawood, 2021). Tumor Necrosis Factor alpha (TNF-α) is a key cytokine that regulates acute inflammatory responses and immune cell functions in fish, significantly impacting systemic inflammation and playing a central role in both inflammation and apoptosis during pathogenic attacks (Ahmadifar et al.,2021; Dawood, 2021). The present study determined that the IL-8, IL-1β, and TNF-α gene expression levels in the spleen and/or liver were significantly higher in the group supplemented with all myrrh essential oil supplemented groups compared to the control group. Moreover, as the concentration of Myrrh essential oil in the diet increased, there was a proportional rise in Relative Percent Survival (RPS) values, suggesting a dose-dependent reduction in mortality from *Aeromonas hydrophila* infection. Similar to our results, Spirulina platensis (*Arthrospira platensis*) and Sage (*Salvia officinalis*) supplementation significantly increased the expression of IL-1β and TNF-α in head kidney tissue in *Oreochromis niloticus*, and protecting against *Pseudomonas aeruginosa*. Trans-cinnamic acid was reported to increase the head kidney expression levels of immune-related genes, including TNF-α, IL-8, IL-1β, and other cytokines, in rainbow trout (*Oncorhynchus mykiss)*, enhancing their disease resistance against *Yersinia ruckeri* (Yılmaz and Sergün, 2018).

Conclusions

The pharmacological properties of myrrh are due to a variety of phytochemicals, including terpenoids (monoterpenoids, sesquiterpenoids and essential oils), diterpenoids, triterpenoids and steroids (Hanus et al., 2005; Marcotullio et.al., 2009; Batiha et al., 2023). Addition of CMO to the diets of common carp, especially at concentrations of 0.5% or 1.0%, has evidently improved their antioxidant status and immune responses, thereby enhancing their resistance against the pathogen *Aeromonas hydrophila*. This study confirms the potential of Myrrh essential oil as a natural additive to improve disease resistance in aquaculture. As this research represents the first investigation into the culture of various aquatic species, particularly carp, it could provide valuable insights into fish health, blood parameters, and gene expression. However, further studies are required to assess growth performance, feed utilization, and the anti-parasitic effects of myrrh in fish. In particular, long-term trials and investigations involving other fish species are recommended.

Ethical Statement

This study was approved by the Animal Ethics Committee of Muğla Sıtkı Koçman University (Muğla, Türkiye, Approval Number: 2024/02-2)

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Author Contribution

Conceptualization, Ü.A, Ö.Y.; methodology, Ü.A., E.B. and F.Z.N; validation, Ü.A. and S.Y; formal analysis, Ü.A., E.B, M.H. and F.Z.N.; data curation, Ü.A., E.B. and F.Z.N.; writing—original draft preparation, Ü.A., Ö.Y. and S.Y; writing—review and editing, Ü.A. , Ö.Y. All authors have read and agreed to the published version of the manuscript

Conflict of Interest

The author(s) declare that they have no known competing financial or non-financial, professional, or personal conflicts that could have appeared to influence the work reported in this paper.

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