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Impact of Dietary Co-Supplementation with *Schizochytrium* sp. and Lavender Essential Oil on Growth, Digestive Physiology, Biochemical Composition, and Intestinal and Hepatic Histology in Common Carp

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Abstract: This study aimed to evaluate the effects of dietary supplementation with marine microalgae (*Schizochytrium sp.*) and lavender (*Lavandula intermedia*) essential oil on growth performance, biochemical composition, digestive enzyme activity, and histological parameters in common carp (*Cyprinus carpio*). A total of 480 fish were randomly assigned to four dietary treatment groups for 8 weeks: a control group (SL0; 0% *Schizochytrium sp.*, 0% lavender oil), and three experimental groups supplemented with 1% *Schizochytrium sp.* + 0.5% lavender oil (SL1), 2% *Schizochytrium sp.* + 0.5% lavender oil (SL2), and 4% *Schizochytrium sp.* + 0.5% lavender oil (SL4). Growth performance data including final weight, weight gain, and specific growth rate were significantly higher in the SL2 group ($p < 0.05$), which also exhibited the highest survival rate. Compared to the initial values, significant differences were observed in the whole-body biochemical composition among groups ($p < 0.05$), with SL2 showing the lowest protein and ash contents, and SL1 the lowest lipid level. The biochemical composition of the diets showed significant differences in protein, lipid, and ash contents among the experimental groups ($p < 0.05$). Histomorphometric analysis showed significant improvements in villus height, tunica muscularis thickness, and villus width in the SL2 group. Histological evaluation of the liver and intestine demonstrated dose-dependent lipid accumulation, with the 4% group showing increased lipid droplets and with the 4% group showing increased lipid droplets and hepatocellular vacuolization with occasional necrotic changes. While protease activity did not differ significantly among treatment groups ($p > 0.05$), protease inhibition was significantly reduced in the SL2 and SL4 groups ($p < 0.05$), indicating improved digestive efficiency. Overall, the SL2 group enhanced growth, survival, and intestinal structure without compromising enzyme activity in common carp, suggesting its potential as a functional feed additive in sustainable aquaculture.

Keywords: Biochemical composition, *Cyprinus carpio*, histology, *Lavandula intermedia*, *Schizochytrium* sp., protease.

Schizochytrium sp. ve Lavanta Esansiyel Yağının Diyette Birlikte Kullanımının Sazan Balığında Büyüme, Sindirim Fizyolojisi, Bağırsak ve Karaciğer Histolojisi Üzerindeki Etkisi

Öz: Bu çalışma, denizel mikroalg olan *Schizochytrium sp.* ve lavanta (*Lavandula intermedia*) esansiyel yağı ile yapılan diyet takviyesinin, sazan balıklarında (*Cyprinus carpio*) büyüme performansı, biyokimyasal kompozisyon, sindirim enzim aktivitesi ve histolojik parametreler üzerindeki etkilerini değerlendirmeyi amaçlamıştır. Toplam 480 balık, 8 hafta boyunca rastgele olarak dört farklı muamele grubuna dağıtılmıştır: Kontrol grubu (SL0; %0 *Schizochytrium sp.*, %0 lavanta yağı) ve üç deneysel grup SL1 grubu (%1 *Schizochytrium* sp. + %0,5 lavanta yağı), SL2 grubu (%2 *Schizochytrium* sp. + %0,5 lavanta yağı) ve SL4 grubu (%4 *Schizochytrium* sp. + %0,5 lavanta yağı). Büyüme performansı verileri incelendiğinde, nihai ağırlık, ağırlık artışı ve spesifik büyüme oranı bakımından SL2 grup istatistiksel olarak en yüksek değerlere ulaşmış ve aynı zamanda en yüksek yaşama oranını göstermiştir ($p < 0.05$). Başlangıç değerleriyle karşılaştırıldığında, gruplar arasında tüm vücut biyokimyasal bileşiminde anlamlı farklılıklar gözlemlenmiştir ($p < 0.05$); en düşük protein ve kül değerleri SL2 grubunda, en düşük lipit düzeyi ise SL1 grubunda kaydedilmiştir. Yemlerin biyokimyasal bileşimi açısından değerlendirildiğinde, gruplar arasında protein, lipit ve kül içerikleri bakımından anlamlı farklılıklar gözlemlenmiştir ($p < 0.05$). Histomorfometrik analizde, SL2 grubunda villus yüksekliği, tunika muskularis kalınlığı ve villus genişliğinde önemli artışlar gözlenmiştir. Karaciğer ve bağırsak histolojisine yönelik değerlendirmelerde, doza bağlı olarak lipit birikiminin arttığı

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görülmüş; özellikle SL4 grubunda daha fazla lipit damlacıkları ve hafif hepatosit değişimleri saptanmıştır. Proteaz aktivitesi gruplar arasında istatistiksel farklılık göstermemiştir ($p > 0.05$), ancak SL2 ve SL4 gruplarında uygulanan diyetlerde proteaz inhibisyonu anlamlı şekilde azalmıştır ($p < 0.05$). Bu durum, sindirim etkinliğinde iyileşmeye işaret etmektedir. Genel olarak, SL2 grubu sazanlarda, büyüme, yaşama oranı ve bağırsak yapısını geliştirmiş; enzim aktivitesinde herhangi bir bozulmaya yol açmamıştır. Bu sonuçlar, söz konusu kombinasyonun sürdürülebilir su ürünleri yetiştiriciliğinde fonksiyonel yem katkı maddesi olarak potansiyele sahip olduğunu göstermektedir.

Keywords: Biyokimyasal kompozisyon, *Cyprinus carpio*, histoloji, *Lavandula intermedia*, *Schizochytrium* sp, proteaz

INTRODUCTION

Aquaculture has become one of the most promising solutions to meet the growing global demand for high-quality animal protein (Abdel-Rahim et al., 2024). However, the intensification of aquaculture practices has brought new challenges, including disease outbreaks, suboptimal growth, oxidative stress, and compromised gut health, particularly in aquatic species (Fachri et al., 2024). To address these challenges, there is increasing interest in the development of functional feeds that can enhance growth performance, support immune competence, and maintain intestinal integrity while reducing the need for chemotherapeutics and antibiotics (Hoseinifar et al., 2024).

In recent years, natural feed additives such as marine-derived macroalgae, microalgae and plant-based essential oils (EOs) have gained substantial attention due to their bioactive profiles and multifunctional roles (Souza et al., 2020; Yazici et al., 2022). Among these, *Schizochytrium sp.*, a heterotrophic marine microalga, is widely recognized for its high content of omega-3 long-chain polyunsaturated fatty acids (LC-PUFAs), particularly docosahexaenoic acid (DHA), which is essential for fish growth, neural development, reproductive capacity, and immune modulation (Souza et al., 2020; Peng et al., 2024). Studies have reported that dietary inclusion of *Schizochytrium sp.* improves lipid metabolism, enhances antioxidant status, and promotes growth in several aquaculture species (Souza et al., 2020; Ferreira et al., 2022). However, despite its proven potential in marine species, there is limited information on its use in freshwater fish such as common carp, *Cyprinus carpio*, particularly in relation to intestinal morphology and digestive physiology.

Alongside microalgae, EOs from medicinal and aromatic plants have also emerged as potent feed additives due to their natural antimicrobial, anti-inflammatory, antioxidant, and growth-promoting properties (Dawood et al., 2022). Among them, lavender (*Lavandula intermedia*) EO, rich in monoterpenes such as linalool and linalyl acetate, has demonstrated antibacterial, sedative, and immunomodulatory effects in terrestrial animal models and is increasingly being explored for use in aquatic species (Detar et al., 2020; Thabet et al., 2023). Nevertheless, data on its dietary use in fish nutrition remain limited, and potential interactions with marine microalgal supplements such as *Schizochytrium sp.* have not been comprehensively investigated. In functional feed development, it is important not only to assess growth and survival metrics but also to understand the physiological and histological responses, particularly in the digestive system (Ghafarifarsani et al., 2022). Histological indicators such as villus height, crypt depth, and liver morphology provide insight into the absorptive capacity and health status of the fish (Scocco & Felice, 2025). Similarly, digestive enzyme activities, such as protease levels and their inhibition by dietary components, serve as biomarkers of nutritional efficiency and gut functionality (Mazlum et al., 2025).

To the best of our knowledge, no comprehensive study has simultaneously evaluated the combined effects of *Schizochytrium sp.* and lavender EO on growth, biochemical composition, digestive enzyme activity, and intestinal histomorphometry in common carp. Given these considerations, the aim of this study is to investigate the potential synergistic or dose-dependent effects of dietary inclusion of *Schizochytrium sp.* microalgae and lavender EO on growth performance, whole-body composition, histological parameters, and proteolytic enzyme profiles in *Cyprinus carpio*. The findings of this study may offer novel and valuable insights into the formulation of functional aquafeeds by highlighting the synergistic benefits of combining marine microalgae and plant-derived EOs to enhance fish health, nutrient utilization, and overall productivity in sustainable aquaculture systems.

MATERIAL AND METHOD

Ethical Statement: All experimental procedures involving fish were conducted in accordance with institutional guidelines and approved by the Local Ethics Committee for Aquatic Vertebrate Experiments of Iskenderun Technical University (Approval No: E-81218362-050.04-178509/ İSTE-SOCYEK-2025/01/1).

Essential Oil Extraction of Lavandula intermedia: Lavender flowers (*Lavandula intermedia*), collected from the Field Crops Collection Garden at Hatay Mustafa Kemal University, were dried and then cut into smaller pieces. A 25 g sample of the dried flowers was subjected to steam distillation in a Clevenger apparatus to determine the EO content. Afterward, the oil yield was calculated and expressed as a percentage. The EOs were stored in dark-

colored bottles at +4 °C until analyzed (Türkmen & Koçer, 2021).

GC-MS Analysis of Lavandula intermedia: The chemical composition of the lavender EO was determined using a Thermo Scientific ISQ Single Quadrupole Gas Chromatography-Mass Spectrometry (GC-MS) system. A TR-FAME MS model column, 0.25 mm inner diameter × 60 m length, with a 0.25 µm film thickness and 5% Phenyl Polysilphenylene-siloxane, was used as the stationary phase. Helium gas (%99.9) was used as the carrier gas at a flow rate of 1 mL/min. The ionization energy was set to 70 eV, and the mass range was m/z 1.2-1200 amu. Scanning mode was used during data acquisition. The MS transfer line temperature was set at 250°C, the MS ionization temperature at 220°C, and the injection port temperature at 220°C. The column temperature was initially set at 50°C and increased at a rate of 3°C/min until it reached 220°C. The structure of each compound was identified using mass spectra with the Xcalibur program (Wiley 9) (Türkmen, 2021).

Experimental animals and conditions: The experimental trial was conducted at the aquaculture research facility of Iskenderun Technical University. Fish were sourced from the Ministry of Agriculture and transferred to the experimental facility. Healthy fish with no history of bacterial infection were used, and after a 10-day acclimation period, a total of 480 common carp, (*Cyprinus carpio*) with an initial average weight of 1.02±0.07 g, were distributed across twelve square fiberglass tanks (500 L each), with 40 fish per tank. The tanks were supplied with filtered, aerated freshwater through air stones connected to a central pump to maintain near-saturation dissolved oxygen levels and uniform water quality. To maintain optimal culture conditions, approximately 50% of the water in each tank was refreshed every three days with fresh water. The average values of physicochemical parameters, including temperature and pH, were recorded at 21.44 ± 1.42°C and 7.4 ± 0.3, respectively. Fish were fed commercial pellets twice daily at 08:30 and 16:30 to apparent satiation for 8 weeks. Each experimental diet was tested in triplicate. A natural photoperiod was applied throughout the trial.

Experimental Setup and Diet: In this experiment, four dietary treatments were formulated with varying percentages of *Schizochytrium* sp. microalgae (S), sourced from the commercial company Aquamax, and Lavender EO (L): 0% S + 0% L (SL0), 1% S + 0.5% L (SL1), 2% S + 0.5% L (SL2), and 4% S + 0.5% L (SL4). All diets were prepared by thoroughly blending with 8 ml distilled water for 100 g of a basal diet using a 3D-Mixer Alphi 1 (Hexagon Product Development Pvt. Ltd., India), following the method by Yazici et al. (2022). Before adding the lavander oil and distilled water mixture to the feed, the oil and water were continuously stirred to maintain homogeneity. The blended diets were stored in polyethylene bottles until use. Every two weeks, the feed quantity and pellet sizes were adjusted based on the rate of fish growth and feed consumption to ensure they received adequate nutrition.

Growth Performance: Throughout the 8-week feeding trial, fish were fed their designated diets, and individual weights were recorded biweekly to evaluate growth dynamics and feed consumption. Growth performance was assessed using the following parameters:

Weight Gain (WG): Final body weight minus initial body weight

Survival Rate(%)= (Final number of fish/Initial number of fish) * 100

Specific Growth Rate (SGR): [Ln (Final weight) – Ln (Initial weight)] / Number of experimental days

Feed Conversion Ratio (FCR): Total feed consumption / Weight Gain

Biochemical Analysis: Biochemical analyses of the experimental diets and whole fish bodies were conducted using standard protocols: Dry matter analyses are based on the principle of evaporating the moisture in the samples at a temperature of 105 ºC (Korkut et al., 2004). For biochemical assays, fish were relatively small in size; therefore, samples from each treatment group were pooled to obtain sufficient material for analysis. Ash analyses were performed according to Vollenweider et al. (2011) and after the samples are weighed, they were burned at 550 ºC for 4 hours. Then, they were cooled in a desiccator and weighed on a scale with a sensitivity of 0.0001 g. Lipid analyses were performed according to the chloroform–methanol extraction method described by Bligh and Dyer (1959). Protein analyses were carried out according to standard AOAC (1997) procedures, and this process were carried out in three stages: burning, distillation and titration. The burning stage of the samples were carried out at 420 ºC, and then the distillation stage was started. After collecting a sufficient amount of distillate, titration stage was carried out with 0.1 N HCl. The amount of 0.1 N HCl spent in the titration were used in the calculation. All measurements were conducted in triplicate for accuracy and reproducibility.

Histological Examination: Carp (*Cyprinus carpio*) (n = 6) were sampled each experimental group and after the autopsy liver and intestine tissue samples were fixed in 10% neutral buffered formalin. All tissue samples were dehydrated with graded series of ethanol, cleared in xylene, and embedded in paraffin wax, according to the standard histological procedures. Paraffin blocks were cut into serial sections (4–5 µm) with a microtome (Leica RM2125, Germany) and stained with Mayer's haematoxylin and eosin (H&E). Slides were examined under an Olympus BX-51 light microscope equipped with an Olympus DP72 digital camera (Roberts et al. 2001). Intestinal histomorphometry analysis (villus width, villus height, tunica muscularis,

thickness crypt depth) was performed using the ImageJ software version 1.36 according to Abdel-Latif et al. (2020).

Digestive Enzyme Activities

Protease Activities: The samples were homogenized with distilled water and centrifuged at 16,000 G for 30 minutes at +4°C. Then, the supernatants were stored in the deep freezer at –80 °C to be used in analyses. Protease activities were performed according to Walter (1984). Casein 10 g/L solution (buffer: 50 mM Tris HCl, pH: 8.5) were used as substrate. Buffer and sample extracts were incubated at 37 ºC for 30 minutes. Then, 500 µL casein were added and incubated for 60 minutes. The reaction were stopped by the addition of 0.5 mL trichloroacetic acid (TCA, at a concentration of 120 g/L). After applying the Biorad Kit procedures for soluble protein concentrations, measurements were made in the spectrophotometer at 595 nm (Bradford, 1976). Protease activities were given as the amount of tyrosine released per minute expressed in µg (U/mg protein).

Protease Inhibition Analyses: In the protease inhibition analyses, extracts of the feeds used during the feeding stages were used. Feeds used in feeding stages was homogenized in distilled water (100 mg/mL) . Then, extracts were obtained by centrifuging at 15,000 g for 10 minutes.

The inhibitory effects of the feeds used in the feeding stages on protease were determined according to the method described by García-Carreno (1996). The described method is based on measuring the remaining residual protease activity of the feeds used in feeding stage after pre-incubation (30 minutes at 37 °C). The mixtures containing the feeds used in the feeding stages and larval extracts were incubated at 37 °C for 60 minutes after pre-incubation. The reaction was stopped by the addition of 500 µL TCA (120 g/L). One unit of enzyme activity was defined as 1 µg of tyrosine release per minute.

Statistical Analysis: All data obtained from the experiment were subjected to statistical evaluation using IBM SPSS Statistics software (IBM Corp., Armonk, NY, USA). Prior to analysis, normality and equal variance assumptions were confirmed through Shapiro-Wilk and Levene's tests, respectively. For normally distributed data, one-way analysis of variance (ANOVA) was used to evaluate differences among treatment groups. When significant differences were detected ($p < 0.05$), Duncan's multiple range test was applied for post hoc pairwise comparisons to identify which groups differed significantly. All results were expressed as mean ± standard deviation (SD). For histomorphometric and biochemical analyses, each treatment was represented by triplicate measurements, and for digestive enzyme activity, biological replicates were used to ensure consistency. Figures were generated using GraphPad Prism, and significance levels were indicated where applicable.

RESULTS

The GC-MS analysis results of lavender EOs are presented in Table 1.

Table 1. Lavender EO components

RT	Compound Name	SI	RSI	Cas #	Area %
6.59	α-Pinene	958	989	80-56-8	0.4
7.61	Camphene	990	992	79-92-5	0.24
8.43	β-Pinene	984	992	127-91-3	0.16
8.74	Sabinene	964	977	3387-41-5	0.1
8.98	Myrcene	984	988	123-35-3	0.38
10.04	Limonene	982	989	5989-54-8	0.77
10.61	cis-Ocimene	993	996	6874-10-8	0.85
10.97	β-Phellandrene	981	982	555-10-2	0.47
11.35	trans-β-Ocimene	984	995	3779-61-1	0.72
12.07	Eucalyptol	987	990	470-82-6	5.73
12.66	o-Cymene	966	980	527-84-4	0.1
13.71	n-Hexyl acetate	989	990	142-92-7	0.75
14.22	3-Ethyl-1-butanol	962	965	589-35-5	0.11
15.02	3-Octanone	982	995	106-68-3	1.03
15.98	3-Octanol	960	985	589-98-0	0.13
16.29	n-Hexyl isobutyrate;	981	996	2349-07-7	0.17
16.69	1-octen-3-yl acetate	973	986	2442-10-6	0.21
18.84	n-Hexyl butanoate	919	955	2639-63-6	0.65
20.01	Linalool oxide	983	985	5989-33-3	0.65
20.65	**Linalool**	**985**	**988**	**78-70-6**	**33.3**
21.14	1-Octanol	970	986	111-87-5	0.15
22.35	**Linalyl acetate**	**987**	**987**	**115-95-7**	**36.12**
23.49	Hotrienol	962	970	20053-88-7	0.19
24.23	Neryl acetate	871	890	141-12-8	2.25
24.99	Lavandulol	949	954	498-16-8	0.42
25.36	Allyl tiglate	889	965	7493-71-2	1.18
25.99	Camphor	990	993	76-22-2	5.89
26.98	α-Terpineol	993	994	98-55-5	1.24
27.21	Valencene	865	877	4630-07-3	0.11
27.53	Borneol	985	986	10385-78-1	3.05
28.59	Geranyl acetate	989	991	105-87-3	0.41
28.91	Nerol	962	971	106-25-2	0.14
29.2	Geranyl propionate	923	937	105-90-8	0.22
30.32	Geraniol	980	988	106-24-1	0.2
31.89	Cryptone	965	971	500-02-7	0.18
32.46	p-Cumic aldehyde	933	967	122-03-2	0.1
34.04	8-Hydroxylinalool	808	831	64142-78-5	0.45
40.21	Caryophyllene oxide	981	993	1139-30-6	0.32

Growth Performance: The impact of varying inclusion levels of *Schizochytrium* sp. and lavender oil on growth and feed performance is presented in Table 2. The experimental groups did not differ significantly in terms of initial weight (IW) ($p > 0.05$), suggesting uniform starting conditions. However, a significant difference was observed in final body weight ($p < 0.05$), with the SL2 group exhibiting the highest value, which was significantly greater than that of all other groups. Similarly, weight gain (WG) and specific growth rate (SGR) were significantly enhanced in the SL2 group compared to the control and other treatments ($p < 0.05$). Feed conversion ratios (FCR) were statistically similar across all groups ($p > 0.05$). The Survival rate (SR) was also significantly higher in the SL2 group compared to other treatments, while the lowest survival was observed in the 4% group. Overall, dietary supplementation in the SL2 group resulted in the most favourable growth performance and survival outcomes, indicating a dose-dependent response and suggesting this combination as the potential optimal level.

Table 2. Growth performance, survival, and feed efficiency of common carp fed diets supplemented with various levels of *Schizochytrium* sp. and lavender (*Lavandula intermedia*) EO for 8 weeks (n = 50).

Treatments	SL0	SL1	SL2	SL4
Initial WT	1.02±0.07	1.02±0.08	1.05±0.07	1.04±0.02
Final WT	2.18±0.24^{a}	1.94±0.22^{a}	3.28±0.27^{b}	2.54±0.29^{a}
WG	1.16±0.16^{a}	0.92±0.21^{a}	2.23±0.20^{b}	1.50±0.31^{a}
SGR	1.26±0.06^{a}	1.07±0.21^{a}	1.90±0.03^{b}	1.48±0.22^{a}
SR	54.67±1.15^{a}	50.33±10.58^{a}	63.33±10.06^{b}	43.33±4.16^{a}
FCR	2.33±0.19^{a}	2.56±0.18^{a}	2.30±0.24^{a}	2.58±0.10^{a}

Data represent the mean of 40 fish per group and are presented as mean ± SD. Different superscript letters within the same row indicate significant differences between groups (P < 0.05). Dietary treatments: SL0: Control (0% *Schizochytrium* sp. + 0% lavender EO); SL1: 1% *Schizochytrium* sp. + 0.5% lavender EO; SL2: 2% *Schizochytrium* sp. + 0.5% lavender EO; SL4: 4% *Schizochytrium* sp. + 0.5% lavender EO.

Biochemical Compositions: Table 3 summarizes the whole-body biochemical composition of common carp at the initial stage and after 8 weeks of feeding with diets supplemented with *Schizochytrium* sp. and lavender EO. Protein, lipid, and ash levels exhibited statistically significant variation among the groups ($p < 0.05$). Protein content was highest in the SL0 group and significantly higher than in the SL1 group, which showed the lowest protein level ($p < 0.05$). The SL4 and SL2 groups had intermediate values and also, statistically similar to each other. Compared to the initial values, lipid content significantly increased in the SL0, SL2, and SL4 groups ($p < 0.05$), with the highest level observed in the SL0 group. Lipid concentrations in the SL2 and SL4 groups were statistically similar to SL0 ($p > 0.05$), while the SL1 group exhibited the lowest lipid content ($p < 0.05$). Ash content also differed significantly among the experimental groups ($p < 0.05$). The highest ash level was recorded in the SL2 group, whereas the SL1 group had the lowest value ($p < 0.05$).

Biochemical Composition of Diets: At the end of the feeding trial, the biochemical composition of the experimental diets showed significant differences among treatments ($p < 0.05$), as presented in Table 4. The highest crude protein content was recorded in the SL0 group, which was significantly greater than that of SL1, SL2, and SL4 ($p < 0.05$). The lowest protein levels were found in the SL2 and SL4 groups, with no statistical difference between them ($p > 0.05$). Among all treatments, the SL1 group exhibited a significantly higher lipid content ($p < 0.05$), while the remaining groups showed statistically similar values ($p > 0.05$). Regarding ash content, the SL2 group had the lowest value, significantly different from the other treatments ($p < 0.05$), while the SL0, SL1, and SL4 groups indicated no significant differences among them ($p > 0.05$).

Table 3. Biochemical composition of whole-body common carp (% dry weight basis), fed diets supplemented with *Schizochytrium* sp. and lavender EO.

Biochemical Composition	Initial Stage	SL0	SL1	SL2	SL4
Protein	64.45±0.27^{bc}	65.85±0.30^{c}	56.92±0.55^{a}	63.90±0.66^{b}	64.43±0.30^{bc}
Lipid	21.75±1.31^{a}	25.70±0.14^{b}	20.58±0.43^{a}	24.80±0.57^{b}	24.82±0.18^{b}
Ash	6.18±0.08^{d}	3.66±0.1^{b}	2.95±0.01^{a}	6.57±0.07^{e}	4.87±0.07^{c}

Data are presented as mean ± SD (n = 6). Different superscript letters within the same column indicate statistically significant differences among groups (P < 0.05). SL0: Control (0% *Schizochytrium* sp. + 0% lavender EO); SL1: 1% *Schizochytrium* sp. + 0.5% lavender EO; SL2: 2% *Schizochytrium* sp. + 0.5% lavender EO; SL4: 4% *Schizochytrium* sp. + 0.5% lavender EO.

Table 4. Biochemical composition of the experimental diets used in common carp feeding trial (% dry weight basis), including dietary supplements of *Schizochytrium* sp. and lavender EO.

Biochemical Composition	SL0	SL1	SL2	SL4
Protein	53.63±0.34^{c}	51.08±0.49^{b}	48.11±0.28^{a}	48.37±0.21^{a}
Lipid	12.95±0.04^{a}	13.58±0.09^{b}	12.99±0.17^{a}	13.10±0.01^{a}
Ash	10.66±0.12^{b}	10.39±0.05^{b}	9.91±0.11^{a}	10.45±0.07^{b}

Data are presented as mean ± SD (n = 6). Different superscript letters within the same column indicate statistically significant differences among groups ($p < 0.05$). SL0: Control (0% *Schizochytrium* sp. + 0% lavender EO); SL1: 1% *Schizochytrium* sp. + 0.5% lavender EO; SL2: 2% *Schizochytrium* sp. + 0.5% lavender EO; SL4: 4% *Schizochytrium* sp. + 0.5% lavender EO.

Histology:

Intestinal Histomorphometry: The intestinal histomorphometric measurements of common carp are presented in Table 5. Tunica muscularis thickness was significantly increased in the SL1 and SL2 groups compared to SL0 and SL4 ($p < 0.05$). Villus height showed a progressive increase across dietary treatments, with the highest value observed in the SL2 group, followed by SL1, SL4, and SL0 ($p < 0.05$). Similarly, villus width was significantly greater in SL2, followed by SL1 and SL4, compared to SL0 ($p <0.05$). In terms of crypt depth, SL2 and SL0 exhibited significantly higher values than SL4 ($p <0.05$), while no significant differences were observed between the other groups.

Histological Observations: The main histological alterations observed in the liver and intestine of fish from the SL1, SL2, and SL4 groups are presented in Figures 1 and 2. In the liver of the SL4 group, numerous pyknotic nuclei and areas of hepatocellular necrosis were observed compared to the other experimental groups. In addition, the presence of lipid droplets was identified in the liver parenchyma of fish in SL2 and SL4 groups, and it was determined that the increase in the amount of these lipid droplets increased depending on the amount of *Schizochytrium* sp. (Figure 1). Similarly, in the SL4 group, histological examination of the intestinal tissue revealed lipid accumulation along the intestinal folds (Figure 2).

Table 5. Intestinal histomorphometric changes in common carp fed *Schizochytrium* sp. and lavender EO diets.

Treatments	T M T (µm)	V H (µm)	V W (µm)	C D (µm)
SL0	15.80±0.63^{a}	206.43±0.61^{a}	50.39±0.72^{a}	33.28±1.44^{b}
SL1	20.76±0.13^{c}	311.57±0.77^{c}	61.14±0.85^{b}	32.34±0.46^{ab}
SL2	22.11±0.42^{c}	336.26±5.24^{d}	69.61±0.46^{c}	35.40±1.08^{b}
SL4	18.83±0.45^{b}	260.79±1.56^{b}	60.71±0.53^{b}	29.26±1.15^{a}

Data are presented as mean ± SD (n = 6). Different superscript letters within the same column indicate statistically significant differences among groups ($p < 0.05$). SL0: Control (0% *Schizochytrium* sp. + 0% lavender EO); SL1: 1% *Schizochytrium* sp. + 0.5% lavender EO; SL2: 2% *Schizochytrium* sp. + 0.5% lavender EO; SL4: 4% *Schizochytrium* sp. + 0.5% lavender EO. TMT: Tunica Muscularis Thickness; VH: Villus Height; VW: Villus Width; CD: Crypt Depth.

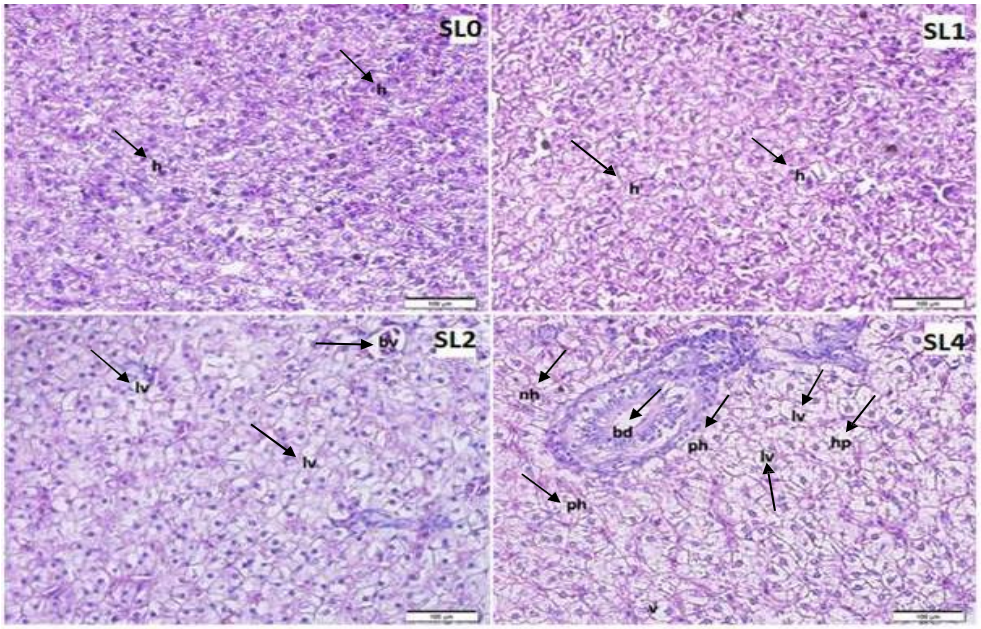

Figure 1. Representative liver histology of common carp (*Cyprinus carpio*) after 8 weeks of feeding with diets containing different levels (1%, 2%, and 4%) of *Schizochytrium sp.* and 0.5% lavender (*Lavandula intermedia*) EO. Hematoxylin and eosin (H&E) staining was performed; scale bar = 100 µm. SL0: Control group (0% *Schizochytrium sp.*, 0% lavender oil), showing dense hepatocyte arrangement (h); SL1: Slightly disorganized hepatocyte structure (h); SL2: Presence of liver vacuoles (lv) and blood vessel (bv); SL4: Well-organized hepatic tissue with normal hepatocytes (nh), prominent portal veins (ph), bile ducts (bd), hepatic plates (hp), and central veins (v). Abbreviations: h – hepatocyte; lv – liver vacuole; bv – blood vessel; nh – normal hepatocyte; bd – bile duct; ph – portal vein; hp – hepatic plate; v – vein.

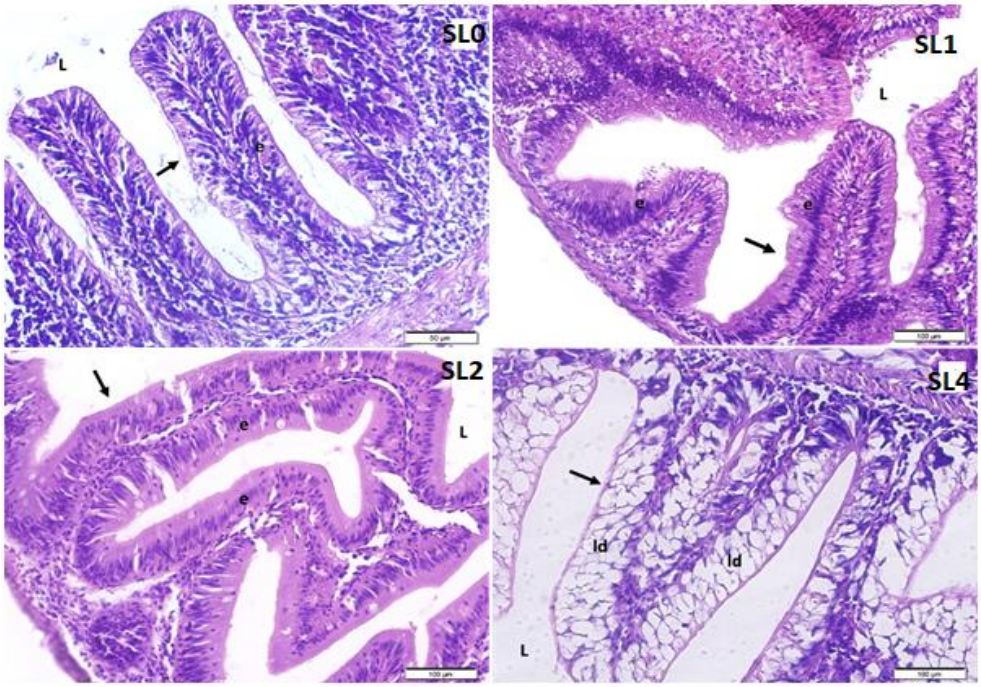

Figure 2. Histological structure of the intestine in common carp fed diets containing different levels (1%, 2%, and 4%) of *Schizochytrium* sp. and 0.5% lavender EO for 8 weeks. Intense lipid accumulation (ld) along the intestinal folds (arrowed) was observed in the group fed 4% *Schizochytrium* sp. and 0.5% lavender oil. Hematoxylin and eosin staining (H &E), scale bar = 100 µm. SL0: Control (0% *Schizochytrium* sp., 0% lavender EO); SL1: 1% *Schizochytrium* sp. + 0.5% lavender EO; SL2: 2% *Schizochytrium* sp. + 0.5% lavender EO; SL4: 4% *Schizochytrium* sp. + 0.5% lavender EO. Abbreviations: e – enterocyte; ld – lipid deposits; L – lumen.

Digestive enzymes: Within the scope of the study, only protease activity was assessed, as protein digestion is a critical determinant of growth performance in common carp. The protease activities of the treatment groups and the protease inhibitions of the feeds used in the feeding stage were tested at the beginning (S) and at the end of the experimental period (Figures 3 and 4).

The highest protease activity value was observed in fish at the beginning of the experiment (727.27±6.8 U/mg protein). No statistical difference was observed between the control group (SL0) and treatment groups (SL1, SL2, SL4) and the lowest and highest protease activity values were 607.53±18.89 U/mg protein (SL1 group) and 649.13±11.51 U/mg protein (SL4 group) ($p > 0.05$), respectively. SL0 (644.33±26.05 U/mg protein) and SL2 (642.02±8.89 U/mg protein) treatment groups showed protease activity at values close to each other. Infante and Cahu (2001) indicated that the decline observed in specific activities of enzymes is not due to a diminution in enzyme synthesis but is the result of an increase in tissue protein concentration.

The differences between the protease inhibition values showing the response of the carp to the feeds tested in the study were found to be statistically significant ($p < 0.05$). The highest protease inhibition value was observed in fish at the beginning of the experiment (14.14±0.62%). According to the protease inhibition values obtained at the end of the experiment, the lowest values were observed in the SL2 (4.39±1.17%) and SL4 (4.49±2.08%) treatment groups, followed by SL1 (7.30±2.32%) and the SL0 group (12.52±2.46%). It can be said that the feed used in the feeding of carp does not have a protease enzyme inhibition effect at high levels.

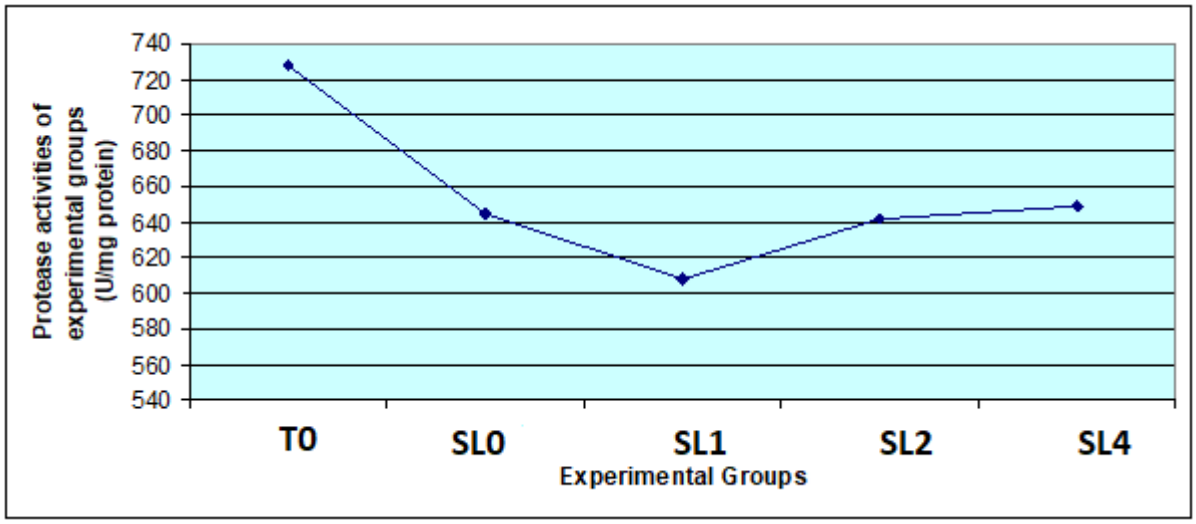

Figure 3. Protease activity (U/mg protein) in the intestinal tissue of common carp before the trial (T0) and after 8 weeks of feeding with experimental diets supplemented with *Schizochytrium* sp. and 0.5% lavender EO. Control (0% *Schizochytrium* sp. + 0% lavender EO); SL1: 1% *Schizochytrium* sp. + 0.5% lavender EO; SL2: 2% *Schizochytrium* sp. + 0.5% lavender EO; SL4: 4% *Schizochytrium* sp. + 0.5% lavender EO.

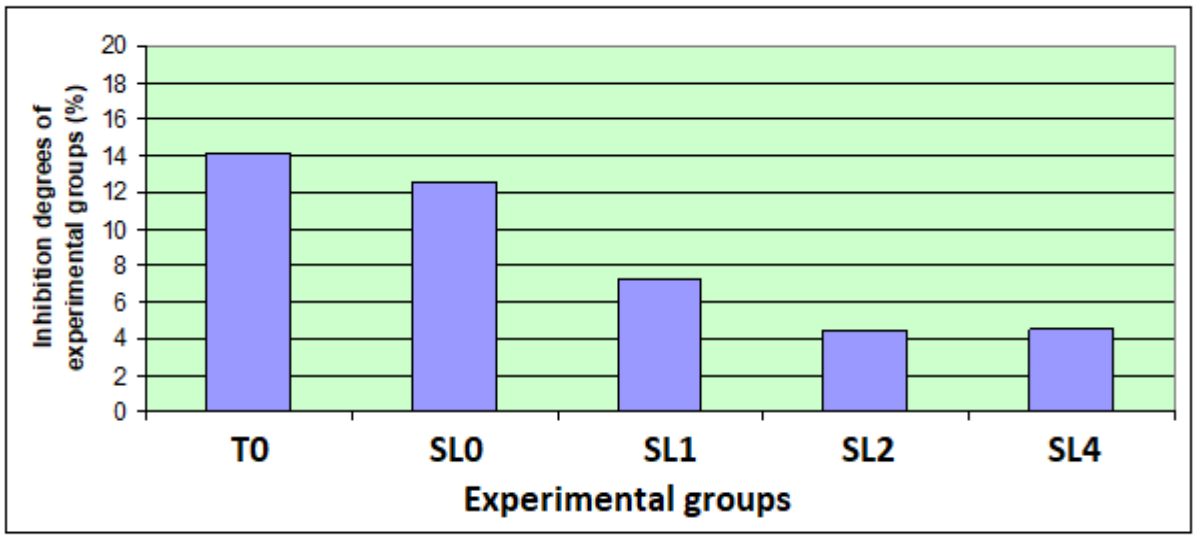

Figure 4. Inhibition degrees (%) in the intestinal tissue of common carp before the trial (T0) and after 8 weeks of feeding with experimental diets supplemented with Schizochytrium sp. and 0.5% lavender EO. SL0: Control (0% Schizochytrium sp. + 0% lavender EO); SL1: 1% Schizochytrium sp. + 0.5% lavender EO; SL2: 2% Schizochytrium sp. + 0.5% lavender EO; SL4: 4% Schizochytrium sp. + 0.5% lavender EO.

DISCUSSION

The integration of herbal EOs and marine algae into aquafeeds has emerged as a promising approach to enhance growth performance, digestive efficiency, and tissue health in farmed fish (Yazici et al., 2022; Mahmoudi et al., 2022).

In the present study, the dietary inclusion of *Schizochytrium sp.* and lavender oil significantly improved the growth performance and survival of common carp, with the SL2 group (2% *Schizochytrium sp.* + 0.5% lavender oil) exhibiting the most favorable results. These findings align with Mahmood et al. (2024), who observed similar growth improvements in *C. carpio* fed lavender oil at 2–4 mL/kg, and with Peng et al. (2024), who reported increased growth and FCR efficiency in fish fed *Schizochytrium*-derived DHA. Likewise, Xiao et al. (2021) found a non-linear growth response in *C. carpio*, where moderate defatted *Schizochytrium* supplementation (0.6%) improved WG and SGR, but higher levels (1.2%) led to decline, mirroring our results in the 4SL group. However, most of the cited studies have employed either *Schizochytrium sp.* or lavender oil as a single additive, whereas our study uniquely investigates the combined effects of these two functional ingredients. Growth performance was significantly improved in the SL2 group, which may reflect a possible synergistic interaction between the two additives. Although the fatty acid composition of the diets was not directly analyzed in this study, previous research has demonstrated that *Schizochytrium* sp. is particularly rich in docosahexaenoic acid ((DHA; ~25–30% of total fatty acids) but contains only negligible amounts of eicosapentaenoic acid (EPA; <1%). DHA, in particular, known to support membrane integrity, energy metabolism, and nutrient utilization (Lee et al., 2022; Peng et al., 2024). Meanwhile, lavender EO, particularly its abundant constituents linalyl acetate (36.12%) and linalool (33.3%), may have contributed to improved digestive efficiency by modulating gut microbiota and reducing intestinal inflammation (Mahmood et al., 2024). The observed enhancement in growth may thus be attributed to more efficient nutrient digestion, reduced metabolic stress, and the supportive physiological effects of bioactive compounds in both additives, which could potentially lead to better feed conversion and growth outcomes. Nevertheless, the absence of direct fatty acid analysis in both diets and fish tissues should be acknowledged as a limitation, as it restricted our ability to confirm the deposition of DHA/EPA and its precise contribution to the observed effects. Future studies are recommended to include fatty acid profiling for a more comprehensive interpretation.

The biochemical composition of fish, particularly protein and lipid levels, serves as a key indicator of nutritional and physiological status, thereby playing a crucial role in determining growth potential and survival in aquaculture species (Yazici et al., 2020). In the current study, significant differences were observed in the whole-body protein and lipid contents among the different experimental groups. The highest protein content was recorded in the SL0 group, whereas the SL1 group exhibited the lowest value, showing a statistically significant difference compared to the SL2 and SL4 groups. Furthermore, an increasing trend in protein levels was noted with higher supplementation levels of *Schizochytrium* sp. These findings support previous reports by Xiao et al. (2021) and Li et al. (2023), which demonstrated that diets enriched with *Schizochytrium sp.* enhance protein synthesis and promote protein deposition in muscle tissue. Based on comparisons with the initial experimental stage, a significant increase in lipid content was observed in the SL0, SL2, and SL4 groups ($p < 0.05$), with the highest lipid level recorded in the SL0 group. In contrast, SL1 showed the lowest lipid content. These results contradict those of Xiao et al. (2021) and Li et al. (2023), who demonstrated that *Schizochytrium* sp. supplementation reduces fat accumulation in fish, as well as with Mahmood et al. (2024), who associated lavender EO with decreased fat content in fish tissues due to its lipolytic and metabolism-regulating properties. Conversely, the findings align with Hassanalizadeh et al. (2020), who demonstrated that dietary incorporation of lavender EO in nanoemulsion form increased both crude protein and lipid contents. Taken together, these results suggest that the combined use of *Schizochytrium sp.* and lavender EO may have complex and possibly interactive effects on lipid metabolism, leading to variable outcomes depending on dosage and formulation. Ash content also differed significantly among the experimental groups, with the highest value observed in the SL2 group and the lowest in the SL1 group. In contrast to our results, Hassanalizadeh et al. (2020) reported that dietary supplementation with lavender essential oil in nanoemulsion form did not affect ash content. The observed differences in our study may therefore be attributed to the synergistic effects of *Schizochytrium* sp. and lavender EO.

The evaluation of digestive enzyme activity serves as an effective indicator for understanding how dietary components influence digestive efficiency and nutrient assimilation (Ghafarifarsani et al., 2022; Peng et al., 2024). In the present study, a significant increase in total protease activity and a concurrent reduction in protease inhibition were observed in the SL2 group, suggesting enhanced protein digestion efficiency. This improvement may reflect the synergistic interaction between *Schizochytrium* sp., a known source of bioactive lipids, and lavender EO, rich in phytochemicals such as

linalool and linalyl acetate, which are believed to modulate gut microbiota and reduce intestinal inflammation.

While Yao et al. (2022) found minimal alterations in digestive enzyme activities with various marine lipid sources, our findings demonstrated a clear enhancement in protease activity and reduction in inhibitory factors. This difference may be attributed to the synergistic action of *Schizochytrium* sp. and lavender oil, which not only provide bioactive lipids but also stimulate digestive processes through phytochemicals such as linalool and linalyl acetate. Similarly, Huang et al. (2023), in a study on *Oreochromis niloticus*, reported time-dependent increases in pepsin, trypsin, amylase, and lipase activities following supplementation with different functional feed ingredients. In our study on *Cyprinus carpio*, only protease activity was analyzed; nevertheless, the results indicate improved digestive function, likely contributing to the observed growth benefits. However, direct comparisons across studies should be interpreted with caution due to species-specific differences and variation in methodological approaches.

Wang et al. (2025) further demonstrated that the combination of *Schizochytrium sp.* and the probiotic *Lactococcus lactis* enhanced multiple digestive enzymes in *Oreochromis niloticus*. In contrast, our study uniquely employed a blend of *Schizochytrium sp.* and lavender EO, offering an alternative strategy to improve digestion via anti-inflammatory and enzymatically supportive mechanisms. Though only protease was evaluated, the elevated activity in the SL2 group supports the efficacy of this novel combination. However, due to limited enzyme analysis, broader comparisons remain speculative.

The reduction in protease inhibition may also be linked to lavender oil's capacity to modulate gut pH and suppress antinutritional effects, as well as to the contribution of microalgal bioactives that enhance enzymatic expression. Collectively, these results suggest that the co-supplementation strategy fosters a favorable digestive environment and optimizes nutrient utilization in common carp. In this context, structural adaptations in the intestinal tissue further support the observed improvements in digestive efficiency.

The intestine plays a central role in nutrient digestion and absorption in fish, and its anatomical and histological features are closely linked to the efficiency of nutrient uptake. Consequently, histopathological assessment, particularly of key indicators such as muscular thickness (MT), villus length (VL), and crypt depth (CD), can provide valuable insights into digestive capacity, overall fish health, and the efficacy of feed supplementation strategies (Yazici et al., 2022; Huang et al., 2023). In our study, common carp fed the SL2 diet exhibited significantly increased VL and MT, reflecting enhanced absorptive capacity. Histological evaluation also revealed better mucosal integrity and greater goblet cell density, aligning with findings by Abdel Rahim et al. (2024) in European seabass (*Dicentrarchus labrax*) fed lavender oil, where villus length and goblet cell numbers increased by over 120%.

In contrast, Souza et al. (2020) found no significant histological improvements in Nile tilapia fed *Schizochytrium* alone, suggesting a possible species-specific effect or the added benefit of co-supplementation with lavender oil in our trial. Similarly, Xiao et al. (2021) observed elevated villus height in mirror carp fed optimal supplementation levels, comparable to our results. Unlike their report of unchanged hepatic histology, our SL2 group showed reduced liver vacuolization, indicating additional hepatoprotective effects. Huang et al. (2023) used a combination of *Schizochytrium limacinum* and *Lactococcus lactis* in *O. niloticus*, leading to increased VL and MT and reduced CD. Our use of *Schizochytrium* sp. with lavender oil produced similar intestinal enhancements in *C. carpio*, without the villus damage reported in the group that received lavender supplementation alone in their study, an outcome likely attributable to the protective synergy between omega-3 PUFAs and monoterpenes such as linalool. However, the presence of lipid vacuolation and signs of hepatocellular necrosis in fish fed the 4SL diet suggest that excessive *Schizochytrium* sp. inclusion may disrupt hepatic lipid metabolism. This indicates that while moderate levels promote digestive efficiency and structural integrity, higher doses may lead to histopathological stress.

The observed protection of intestinal and hepatic tissues in the SL2 group likely reflects the combined anti-inflammatory and antioxidant properties of *Schizochytrium* sp.-derived n-3 PUFAs and lavender oil constituents such as linalool. These compounds are reported to improve tissue integrity and gut morphology, potentially by stabilizing cellular membranes and modulating oxidative and immune responses (Abdel-Rahim et al., 2024; Huang et al., 2023).

A notable strength of our work lies in its dual-supplement strategy, pairing lipid-rich microalgae with phytochemical-rich lavender oil. This potential synergistic combination appeared to enhance intestinal architecture, liver health, enzyme activity, and growth, highlighting a promising multi-targeted dietary approach for improving aquaculture efficiency in *C. carpio* and potentially other species. In this context, both Schizochytrium sp. and lavender oil should be considered as functional feed additives rather than replacement ingredients, supporting fish performance at low inclusion levels. Their combined use diversifies the portfolio of natural additives available to aquaculture, which is an important step toward sustainability by reducing dependence on synthetic

additives and promoting environmentally friendly production practices.

However, a limitation of the present study is that only one inclusion level of lavender essential oil (0.5%) was tested, and therefore potential dose–response effects could not be evaluated. Future studies should examine multiple inclusion levels to better characterize the functional range of lavender essential oil in aquafeeds. Overall, the results of this study suggest a synergistic potential between *Schizochytrium* sp. and lavender EO in promoting growth and gut health in common carp when included at moderate levels. However, the decline in survival and the appearance of hepatic stress markers in the highest inclusion group (4SL) emphasize the importance of dose optimization. Further research is warranted to elucidate the mechanistic basis of these observations, especially regarding hepatic lipid regulation and the immunomodulatory roles of lavender oil components.

CONCLUSION

The present study demonstrates that dietary co-supplementation with *Schizochytrium* sp. and lavender essential oil at moderate levels (SL2 group) significantly enhances growth performance, digestive capacity, and tissue health in *Cyprinus carpio*. The improved feed efficiency and protein deposition observed in the SL2 group can be attributed to enhanced protease activity, reduced enzyme inhibition, and favorable histomorphological adaptations in the intestine and liver. These effects likely stem from the synergistic actions of bioactive omega-3 polyunsaturated fatty acids (PUFAs) and phytochemicals such as linalool and linalyl acetate, which exhibit anti-inflammatory, antioxidant, and gut-protective properties. Histological and biochemical analyses revealed preserved mucosal integrity, elevated villus length and muscular thickness, increased goblet cell density, and reduced hepatic vacuolization in the SL2 group, supporting the physiological benefits of this dual-supplement strategy. However, excessive inclusion in the SL4 group was associated with signs of hepatic stress and reduced survival, underscoring the importance of dose optimization. This study highlights the potential of integrating microalgal and herbal feed additives to improve growth, nutrient utilization, and health in aquaculture species. In practical terms, these findings suggest that co-supplementation of Schizochytrium sp. and lavender essential oil at moderate inclusion levels could be considered in future commercial aquafeed formulation to enhance growth and digestive health in common carp. The findings support the development of multi-targeted functional feeds and pave the way for future research on dose-response relationships and underlying molecular mechanisms in diverse fish models.

Contribution of authors: Metin YAZICI: conceptualization, validation, writing, reviewing, and editing the original draft and supervision. Mehmet NAZ: conceptualization, validation, methodology; enzyme and proximate analyses, writing the original draft, visualization. Yavuz MAZLUM: conceptualization, validation, methodology, data curation, writing the original draft, , software, visualization. Çiğdem ÜRKÜ ATANASOV: methodology, formal analysis. Musa TÜRKMEN: formal analysis, resources, and supply of oils. Kemal DEDE: gathering data, analysis, feeding animals.

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