Journal of Applied Phycology (2026) 38:807–825
https://doi.org/10.1007/s10811-025-03733-4

RESEARCH

Effects of seaweed extract (*Ulva sp.* and *Solieria chordalis*) supplementation in high-lipid diets on the growth performance, digestive enzyme activity, and lipid metabolism in rainbow trout (*Oncorhynchus mykiss*)

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Received: 6 May 2025 / Revised: 8 November 2025 / Accepted: 15 November 2025 / Published online: 5 January 2026

Abstract

High-lipid diets are widely implemented in aquaculture as energy-dense formulations to promote growth and feed efficiency; however, excessive dietary lipid levels may induce metabolic dysregulation, undesirable fat deposition, and impaired flesh quality in fish. Seaweed-derived bioactive compounds have recently gained attention as functional feed additives that can improve lipid metabolism, nutrient utilization, and overall fish health. This study investigated the effects of dietary supplementation with seaweed extracts (*Ulva* sp. and *Solieria chordalis*) in high-lipid diets on the growth performance, digestive enzyme activity, and lipid metabolism of rainbow trout (*Oncorhynchus mykiss*). Over an eight-week feeding trial, fish were randomly assigned to three dietary groups: a control diet with 20% lipid (20L), a high-lipid diet with 23% lipid (23L), and a high-lipid diet supplemented with seaweed extracts (23L + SE). While growth performance parameters, such as final weight and specific growth rate (SGR), were numerically improved in the 23L + SE group, these differences were not significant ($P > 0.05$), highlighting a potential disconnect between molecular and phenotypic responses. However, the feed conversion ratio (FCR) was optimized with seaweed supplementation, indicating better nutrient assimilation. Feed intake remained comparable across groups, suggesting that the seaweed-supplemented diets were well accepted, although palatability was not directly assessed. The 23L + SE group exhibited significantly higher whole-body lipid content ($P < 0.05$) while simultaneously showing a reduced mesenteric fat index (MFI), suggesting more efficient lipid mobilization and utilization. Digestive enzyme analysis revealed increased stomach lipase activity in the 23L + SE group, whereas amylase activity was lower, reflecting a metabolic shift favouring lipid utilization. Additionally, the expression of growth-related genes (*GH* and *IGF-I*) in muscle tissue was significantly upregulated in the 23L + SE group ($P < 0.05$), further supporting the role of seaweed extracts in stimulating growth at the molecular level.. Furthermore, dietary seaweed supplementation significantly increased the expression of *cpt1b1* and *elovl2* ($P < 0.05$), suggesting enhanced fatty acid oxidation and elongation. Histological examination of liver tissues demonstrated reduced hepatic lipid accumulation in the 23L + SE group, indicating potential benefits for liver health. These findings suggest incorporating seaweed extracts into high-lipid diets improves lipid metabolism, promotes nutrient utilization, and contributes to sustainable aquafeed development. Further research is warranted to explore seaweed-derived functional additives' long-term impacts and economic feasibility in aquaculture.

Keywords Aquafeed additives · Functional feed ingredients · Nutritional physiology · Metabolic regulation · Sustainability · Chlorophyceae · Rhodophyceae

Introduction

Aquaculture has become an indispensable solution to address global food security challenges, providing a sustainable and reliable source of protein (FAO 2020). As the

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demand for aquatic products continues to rise, the industry faces significant sustainability challenges, primarily due to the reliance on traditional feed ingredients such as fish meal and fish oil (Tacon and Metian 2008; Naylor et al. 2009). While high-lipid diets offer an efficient energy supply, they can also present challenges, including reduced digestibility, excessive lipid accumulation, and metabolic imbalances. If not properly managed, these issues may compromise fish health and product quality (Gaylord and Gatlin 2000; Wang et al. 2005). These issues highlight the need for strategies that optimize nutrient utilization and lipid metabolism, particularly in high-energy feeds used in modern trout aquaculture.

To address challenges in aquaculture nutrition, functional feed additives play a crucial role in enhancing lipid metabolism, nutrient absorption, and overall fish health while optimizing growth performance and stress resistance (Wangkahart et al. 2022a, b; Mueller et al. 2023). Due to their rich nutritional profile and bioactive compounds, seaweed-derived additives have emerged as promising candidates for sustainable aquafeeds (Güroy et al. 2007).

Seaweeds such as *Ulva* and *Solieria* species rich in polyunsaturated fatty acids, chlorophyll, sulfated polysaccharides, vitamins, minerals, and peptides have been shown to enhance growth performance, lipid metabolism, and immune function in various fish species (Nakagawa et al. 1987, 1993; Wassef et al. 2001, 2005; Ortiz et al. 2006; Valente et al. 2006; Güroy et al. 2007; Mohamed et al. 2012; Lange et al. 2014; Zhu et al. 2016; Shpigel et al. 2017). Among their bioactive components, ulvans (sulfated polysaccharides) and polyphenols are particularly noted for their ability to modulate lipid metabolism, enhance antioxidant defenses, and improve gut health (Kendel et al. 2015; Tharaka et al. 2020). Recent advancements in functional feed formulations have further improved the digestibility and bioavailability of alternative feed ingredients. Functional feeds containing seaweed-derived bioactives enhance lipid metabolism and nutrient assimilation and support sustainability in carnivorous species such as rainbow trout and salmonids, which often require high-energy, high-lipid diets (Ferreira et al. 2020; Sartipiyarahmadi et al. 2023; Hughes et al. 2025). However, excessive dietary lipids can lead to quality issues related to lipid accumulation, impacting flesh composition and processing properties such as freezing and smoking (Güroy et al. 2011). Including seaweed-based functional additives can mitigate these issues by modulating lipid metabolism, improving feed efficiency, and supporting overall fish health. Additionally, compounds such as sulfated polysaccharides and polyphenols have been shown to regulate lipid deposition and metabolic pathways, making seaweed supplementation a promising approach for optimizing aquaculture feeds (Kendel et al. 2015; Tharaka et al. 2020).

MFeed + ®, the additive tested in this study, combines extracts of *Ulva* sp. and *Solieria chordalis* with micronized montmorillonite clay. Montmorillonite has been reported to bind dietary toxins and improve gut integrity, which may indirectly support lipid digestion and nutrient absorption (Kulshreshtha et al. 2020).

In this study, the dietary supplementation of seaweed extracts was limited to high-lipid diets, excluding its addition to low-lipid diets. Given that high-lipid diets are particularly prone to excessive fat accumulation, impaired digestibility, and metabolic stress compared to low-lipid diets, seaweed supplementation was evaluated only in high-lipid formulations. This approach was chosen to maximize the detection of functional benefits under nutritionally challenging conditions, where the additive's lipid-modulating and gut-supportive effects are hypothesized to be most impactful. In contrast, low-lipid diets typically align more closely with the baseline nutritional requirements of fish and do not present the same degree of metabolic stress or imbalance. Therefore, the potential benefits of functional additives like seaweed extracts are hypothesized to be more pronounced in high-lipid diets, where the metabolic demands are higher. Previous studies suggest that seaweed-based feed additives can improve feed conversion ratios (FCR), nutrient absorption, and gut health in various fish species (Kulshreshtha et al. 2020; Tharaka et al. 2020; Liang et al. 2023).

To contribute to the development of sustainable and functional feed strategies for the trout industry by addressing key nutritional limitations in aquafeed formulation. To this end, it investigates the effects of dietary supplementation with seaweed extracts on the growth performance and digestive enzyme activities of rainbow trout (*Oncorhynchus mykiss*), while also examining their correlations with lipid metabolism and the expression of growth-related genes.

Materials and methods

Experimental diets

Three experimental diets were formulated to investigate the effects of varying lipid levels and seaweed extract supplementation on rainbow trout (*Oncorhynchus mykiss*). The diets were designated as the 20L, 23L, and 23L + SE. The numerical codes represent the dietary lipid content: 20% and 23%, respectively, while the suffix " + SE" refers to the inclusion of a commercial seaweed extract product (MFeed + ®, Olmix Group, France). This study primarily aimed to evaluate whether supplementing seaweed extract could enhance lipid utilization in high-energy diets (23% lipid), which are commonly used in trout aquaculture; therefore, a 20L + SE group was not included. This design choice

was made to maximize the detection of metabolic benefits under nutritionally stressful conditions, although the absence of a low-lipid seaweed group may limit the interpretation of seaweed-specific effects across lipid levels. All diets were isonitrogenous (approximately 43% crude protein) and designed to meet the known nutritional requirements of rainbow trout. The 23L + SE diet included MFeed + at 2 g kg^{-1}, and according to the manufacturer (Olmix Group, Bréhan, France) and previous literature, MFeed + ® is a patented algae–clay complex composed of extracts from a green alga (*Ulva* sp.) and a red alga (*Solieria chordalis*), combined with exfoliated micronized montmorillonite clay (Tharaka et al. 2020; Liang et al. 2023). This formulation provides bioactive sulfated polysaccharides, polyphenols, and trace minerals, which have been reported to stabilize and activate digestive enzymes, thereby enhancing nutrient digestibility and supporting gut health. Although the manufacturer has not disclosed the precise concentrations of active compounds, the additive is standardized to contain functional levels of these bioactive molecules, ensuring consistent efficacy across production batches.

The diets were manufactured by Skretting Feed Company (Türkiye) using a Wenger X185 single screw extruder (10 t h^{-1} capacity) under standard commercial conditions. Feed pellets were dried and stored under cool, dry conditions until use. Detailed formulations and proximate compositions of the diets are presented in Table 1.

Table 1 Formulation and proximate composition of experimental diets (%)

	20 L	23 L	23 L + SE
Fish meal	25	25	25
Poultry meal	9.8	9.8	9.8
Soybean meal	15.8	15.8	15.8
Sunflower meal	15	14.7	14.4
Wheat gluten	6.1	6.9	7
Fish oil	7.1	8.7	8.7
Soybean oil	7.1	8.7	8.7
Wheat	8.8	8.1	8.1
Wheat middlings	4	1	1
MFeed +	0	0	0.2
Choline chloride, 70% choline	0.30	0.3	0.3
Vitamin–Mineral Premix	0.30	0.3	0.3
Dicalcium phosphate, DCP, $CaHPO_4$	0.45	0.45	0.45
Mold inhibitor	0.10	0.1	0.1
Mycotoxin binder	0.10	0.1	0.1
Antioxidant	0.05	0.05	0.05
Proximate composition (%)			
Moisture	6.8	6.9	6.8
Crude protein	43.6	43.5	43.5
Crude fat	20	23	23
Ash	8.5	8.3	8.4
Crude fiber	3.1	2.7	2.6
Nitrogen free extract	18.0	15,6	15.7

Nitrogen free extract (NFE) = 100 − (Moisture + Crude Protein + Crude Fat + Crude Fiber + Ash)

Rearing systems and fish

All-female rainbow trout of the same origin and feeding history were provided from Altıparmak Trout Farm (Düzce, Türkiye). Fish brought to the experimental system were acclimated to the experimental conditions in adaptation tanks for two weeks before the feeding trial began. During the adaptation period, fish were fed a 4 mm pellet-sized, sinking extruded commercial rainbow trout diet (Skretting Optiline) containing 44% protein and 21% lipid. Fish, with an average weight of 100 g, were randomly distributed into 600-L cylindrical tanks, 15 per tank, by measuring their length and weight, and were fasted for 24 h before the start of the experiment. A total of 9 tanks were used in the experiment, with three replications for each of the three feed groups. Each tank was covered with a fishnet to prevent fish from jumping out of the tank. Since the system of tanks allowed natural light, fish were exposed to natural photoperiod throughout the experiment. An 8 L min^{-1} freshwater flow was provided throughout the experiment to provide suitable water conditions for each tank. Basic water quality parameters such as temperature, oxygen, and pH were measured and recorded daily during both the adaptation and experimental period; water temperature was kept at 15.30 ± 0.08 °C, dissolved oxygen at 8.30 ± 0.03 ppm, and mean pH at 8.16 ± 0.08. Fish were fed by hand twice daily (09:00 and 16:00) for eight weeks until almost ad libitum (until fish ate the food at the bottom of the tank). Feed intake (FI) was assessed by the difference between the feed given to the fish and the uneaten feed siphoned 20 min after the feeding period. This method allowed estimation of feed consumption but did not directly measure palatability or feed preference. FI was recorded daily to calculate the feed conversion ratio (FCR).

Sampling protocols

Rainbow trout were individually removed from tanks at the beginning and end of the experiment to measure their length and weight. All fish were fasted for 48 h after the feeding experiment to ensure that the digestive tract was clear of feed. Six fish were randomly selected from the beginning of the experiment, and two fish from each tank (six fish per treatment) were sampled at the end of the experiment to determine whole-body proximal analysis.

Samples were stored frozen at − 80 °C until analyzed. Three fish were randomly selected, sacrificed with an extra

dose of clove oil, weighed individually, and dissected to obtain livers to determine biological parameters; the same livers were then used for histomorphological analysis. For histological, qPCR, and digestive enzyme analyses, three fish were sampled from each replicate tank, resulting in a total of nine fish per dietary group.

Calculation of growth performance and somatic indices

The growth performance measurements of fish were:y

$$\textit{Weight Gain (WG)} = [(\textit{final fish weight (g)} - \textit{initial fish weight (g)})/\textit{initial fish weight}] \times 100$$

$$\textit{Specific Growth Rate (SGR)}(\%\ day^{-1}) = [(\textit{ln Final mean weight} - \textit{ln Initial mean weight})/\textit{Trial period})] \times 100$$

$$\textit{Feed Intake (FI)} = \textit{Average of the total feed given to each experimental group during the study (g)}$$

$$\textit{Feed Conversion Ratio (FCR)} = \textit{Feed intake (g)}/\textit{Weight gain (g)}$$

$$\textit{Condition Factor (CF)} = \left(\textit{Fish weight (g)}/\textit{Fish length}^3\ \textit{(cm)}\right) \times 100$$

$$\textit{Hepatosomatic Index (HSI)} = (\textit{Liver weight (g)}/\textit{Fish weight (g)}) \times 100$$

$$\textit{Viscerosomatic Index (VSI)} = (\textit{Viscera weight}/\textit{Final body weight}) \times 100$$

$$\textit{Mesenteric Fat Index (MFI)} = (\textit{Body fat weight}/\textit{Final body weight}) \times 100.$$

Six fish were randomly selected from the initial fish pool at the start of the experiment, and three fish were randomly selected from each tank (nine fish per treatment) at the end of the trial to determine somatic indices.

Proximate analyses

The whole fish and diets were analyzed for crude protein, moisture, fiber, and ash using standard methods (AOAC 2000). The dry matter was determined by drying at 105 °C until constant weight. Ash was measured by burning in a muffle furnace at 525 °C for 12 h. The Gerhardt system acid digested crude protein (N*6.25) for Kjeldahl analysis. Acid/alkali hydrolysis of filtered residue and three hours of drying sample ignition determined crude fiber. Folch et al. (1957) extracted dietary and whole-body lipids with chloroform/methanol (2:1 v/v). Nitrogen-free extract (NFE) was calculated by subtracting 100 from the sum of moisture, crude protein, lipid, ash, and crude fiber (Olvera-Novoa et al. 1994). Protein, lipid, and carbohydrate conversion factors of 23.7, 39.5, and 17.2 kJ g^{-1} were used to calculate dietary gross energy (Brett and Groves 1979).

Digestive enzyme activity

Prior to sampling, fish were fasted for 24 h. Euthanasia was performed using 0.01 mg L^{-1} 2-phenoxyethanol (Sigma-Aldrich, #7699). Intestine and stomach tissues were collected from three randomly selected fish per tank (n = 9 fish per treatment). Samples were diluted 1:10 (w/v) with distilled water, homogenized, and centrifuged at 20,000 × *g* for 45 min at 4 °C, as described by Yılmaz et al. (2018). Supernatants were stored at − 80 °C until analysis. Protein concentrations were determined according to Bradford (1976) and enzyme activities in the stomach and intestinal tissues were expressed as U mg^{-1} protein.

Amylase activity was determined using soluble starch as substrate (Benfeld 1955; DNS method). A reaction mixture containing 5 µL sample, 45 µL distilled water, and 50 µL 1% starch solution in phosphate buffer (pH 6.8, 6.7 mM NaCl) was incubated at 37 °C for 1 h. The reaction was terminated with 50 µL DNS reagent, boiled for 5 min, cooled, diluted, and absorbance measured at 540 nm.

Trypsin activity was measured using N_α-Benzoyl-DL-arginine p-nitroanilide (BAPNA) as substrate (Erlanger et al. 1961). A mixture of 100 µL sample and 100 µL substrate solution (3.5 mg BAPNA in 1 mL DMSO, diluted in 0.1 M Tris buffer, pH 8.2, containing 1 M NaCl, 0.01 M $CaCl_2$) was incubated at 30 °C for 10 min. Absorbance was recorded kinetically at 410 nm every 30 s.

Pepsin activity was determined using hemoglobin as substrate (Worthington 1991). A mixture of 50 µL sample and 250 µL 2% hemoglobin in 0.3 M HCl (pH 2.0) was incubated at 35 °C for 10 min, terminated with 500 µL 5% trichloroacetic acid (TCA), incubated for 5 min, centrifuged (12,000 × *g*, 5 min), and absorbance measured at 280 nm.

Lipase activity was measured using p-nitrophenyl myristate (p-NPM) as substrate (German et al. 2004). A mixture of 20 µL sample, assay buffer (0.1 M Tris–HCl, pH 9.0, with sodium cholate and 2-methoxyethanol), and 18 µL p-NPM solution was incubated at 15 °C for 2 h. The reaction was stopped with acetone/heptane (7:2, v/v), centrifuged (6100 × *g*, 2 min), and absorbance measured at 405

nm. Activity was calculated from a p-nitrophenol standard curve and expressed as U per mg wet tissue weight.

Fatty acid analysis

Dietary and whole-body lipids were extracted according to the procedure of Folch et al. (1957) with chloroform/methanol (2:1 v/v). The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% boron trifluoride-methanol (AOAC 2000). Fatty acid methyl esters (FAMEs) were analyzed using a flame ionization gas chromatograph equipped with an Omegawax 250 capillary column (30 mL × 0.25 mm internal diameter), an FID detector, and a split injection system with nitrogen carrier gas. Injector port and detector temperatures were maintained at 250 and 260 °C, respectively. The column temperature programme was held at 140 °C for 5 min and then elevated at 3 °C min^{-1} to 200°C. The total run time was 60 min per sample. Fatty acids were identified by comparing their retention times with the authentic standard fatty acid standards (Sigma-Aldrich, USA).

Texture

The measurement of the texture properties was determined by modifying the method by Yagiz et al. (2007). Fillet texture was measured by Brookfield CT3 Texture Profile Analyzer (Brookfield Engineering Laboratories, Inc., USA) equipped with TA-RT-KIT base table and TA4/1000 38.1 mm diameter cylindrical probe. The right fillets taken during the pre-rigor period were stored at 4 °C for 24 h, then brought to room temperature and deskinned. For texture measurements, samples with a surface area of 15 × 15 mm taken from the dorsa ventral part were used. Two compression test cycles were conducted at 2 mm s^{-1} pre-test speed, 1 mm s^{-1} test speed, and 2 mm s^{-1} post-test speed to 30% of total deformation. A force of 0.067 N was used as a trigger load. All parameters (hardness, adhesiveness, chewiness, springiness, cohesiveness, and gumminess) were determined from the plot of force compared with deformation.

Histomorphology

Sampled fish livers were fixed in neutral buffered formalin (10%). Dehydration, clearing, and paraffin wax processes were performed manually. Paraffin blocks were sectioned (5 µm) and stained with haematoxylin and eosin (H&E). Light microscopy (Nikon Eclipse E200) and a digital camera (Nikon DS-Fi1) with NIS-Elements Microscope Imaging Software (Nikon Corporation, Japan) were used for the examination of hepatopancreas tissues (Roberts et al. 2012; Güroy et al. 2017). Imsland et al. (2019) evaluation criteria were used for histological analysis of the liver. Accordingly, liver vacuolization means 0: none or minimal, 1: mild, 2: moderate, and 3: severe vacuolization.

Cytokine gene expression analysis

Total RNA was extracted from the gastric, intestinal, liver, and muscle tissues using Hybrid-R (GeneAll Cat. No: 305–101) according to the manufacturer's instructions. The RNA purity and quantity were analyzed via a microplate reader (Multiscan GO; Thermo Fisher Scientific) with absorption at 260 and 280 nm, and samples were used for cDNA synthesis after dilution to 20 ng. cDNA was synthesized from the RNA by reverse transcription using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The synthesized cDNA was immediately stored at − 20 °C for further analysis. The expression level of cytokine genes was determined using a real-time PCR detection system to analyze qRT-PCR. The specific primer sequences and references for the expressed genes used in the study are listed in Table 2. The relative quantification of the target genes of digestive (*Pga, lipf, α-Amy, trp-3, ghr-1, igf-I*) and lipid metabolism (*Cpt1b1, elovl2, Idılr*) was normalized with the beta-actin (*β-act*) gene, using the $2^{-\Delta\Delta CT}$ method. Briefly, 20 µL qPCR reactions were prepared with 10 µL SsoAdvanced Universal SYBR Green Supermix (2x), 1 µl primers (each forward and reverse: 250 nM), 6 µl nuclease-free water, and 2 µL of cDNA(30 ng). The reactions were processed on a Bio-Rad,, with 40 cycles of 95 °C for 30 s, 95 °C for 15 s, and 60 °C for 30 s. Four technical replicates were used for each qPCR gene expression quantification.

Statistical analysis

Before analysis, the Shapiro–Wilk W and Levene tests were used to ensure the variance was normal and homogeneous. All data were subjected to a one-way analysis of variance (ANOVA), and if a significant difference between treatments was discovered, Tukey's multiple range test was used to rank the groups using Minitab 21 (Minitab LLC, 2025) statistical software (Zar 1999). All data are presented as the mean, standard error of the mean calculated from all replicates. Before being analyzed, all percentage data were arcsine transformed. Differences were considered significant at the 95% confidence interval.

Results

Feed intake in the dietary seaweed extracts diet at the same rate as the control diet ($P > 0.05$), suggesting that the diet was well accepted by the fish. However, feed palatability per se was not directly assessed. After the 8-week trial, the fish fed experimental diets did not differ among the groups regarding growth performance (Table 3). The final weight

Table 2 The specific primer sequences

Gene	Abbreviation	Primer	Sequence 5′−3′	Accession Number
Pepsinogene-A	pga	F:	GTTTCGGTGGGAGGCATCT	CX136077
Pepsinogene-A		R:	TGTTTCCGAAAGACACCACA	
Gastric Lipase	lipf	F:	TCAACATGACTCGCACACCT	XM_036946231
Gastric Lipase		R:	TTCATGTCCCGGATGTTGTA	
α-Amylase	α-Amy	F:	ACCGTGGCTTCATTGTCTTC	TC87786
α-Amylase		R:	GTCGGTGTTGCTGATCTTGA	
Trypsinogen	trp-3	F:	CCATGTGTACCGTCTCCTGGA	EE605190.1
Trypsinogen		R:	CACCCTGGCAAGAGTCCTT	
Growth hormone-I	ghr-1	F:	TGGAAGACATCGTGGAACCA	AF403539
Growth hormone-I		R:	ATCAAAGTGGCTCCCGGTTA	
Insulin-like growth factor-I	igf-1	F:	TGGAAGACATCGTGGAACCA	AF403539
Insulin-like growth factor-I		R:	ATCAAAGTGGCTCCCGGTTA	
Carnitine palmitoyltransferase-1b1	Cpt1b1	F:	GATGTTCCGTGAGGGTAGGA	AJ606076
Carnitine palmitoyltransferase-1b1		R:	TTGTCTTGCATGGCTCTGAC	
Elongation of very long chain fatty acids protein	elovl2	F:	GATGCCTGCTCTTCCAGTTC	KM244737
Elongation of very long chain fatty acids protein		R:	CATTGGTGGAGACAGTGTGG	
Low density lipoprotein receptor F:	Idlr	*F:*	CAGCGAAGGACTGGAGAAAC	AF542091
Low density lipoprotein receptor R:		R:	TTCAGCCCACTCTTCTCGAT	

Table 3 Growth performance and nutrient utilization of rainbow trout after 8 weeks of feeding on experimental diets. $n=9$

	20L	23L	23L + SE
Initial mean weight (g)	100.47 ± 5.67	100.70 ± 4.95	100.54 ± 6.54
Final mean weight (g)	345.08 ± 3.69	346.45 ± 4.27	355.27 ± 4.19
Specific growth rate (%/day) (SGR)	2.33 ± 0.02	2.33 ± 0.03	2.38 ± 0.01
Feed conversion ratio (FCR)	0.95 ± 0.01	0.96 ± 0.01	0.93 ± 0.01

* In the same line, values with different superscript letters are significantly different ($P<0.05$). Data are expressed as mean ± S.E

(355.27 g) and specific growth rate (2.38% day^{-1}) in the 23L + SE group were higher compared to the control groups ($P>0.05$), although there were no statistical differences. The feed conversion ratio (FCR) was also improved, indicating enhanced feed efficiency in diets supplemented with seaweed extract.

As depicted in Table 4, the effect of supplementation dietary seaweed extract in rainbow trout was assessed in terms of the whole-body proximate. The whole-body lipid content in the 23L + SE group (9.75%) was significantly higher than in the control groups ($P<0.05$). Protein levels also showed a slight increase in the 23L + SE group, further supporting the nutritional benefits of the experimental diet.

Table 5 describes the effects of dietary seaweed extract on fish biological indices. No significant response was observed for the CF, VSI, HSI, or MFI in fish fed the experimental diets. However, the VSI was slightly lower in the 23L + SE group (10.73%) than in other groups, suggesting minimal

Table 4 Whole body composition of rainbow trout after 8 weeks of feeding on experimental diets. $n=6$

	20L	23L	23L + SE
Moisture	69.51 ± 0.60	68.69 ± 0.30	68.55 ± 0.28
Protein	17.97 ± 0.195	18.18 ± 0.242	18.35 ± 0.185
Lipid	8.52 ± 0.06^{b}	8.96 ± 0.23^{b}	9.75 ± 0.09^{a}
Ash	1.49 ± 0.03	1.53 ± 0.02	1.48 ± 0.04

* In the same line, values with different superscript letters are significantly different ($P<0.05$). Data are expressed as mean ± S.E

Table 5 Biological index of rainbow trout after 8 weeks of feeding on experimental diets. $n=6$

	20L	23L	23L + SE
Condition factor (%)	1.51 ± 0.01	1.53 ± 0.02	1.54 ± 0.01
Viscerosomatic index (%)	11.00 ± 0.54	11.14 ± 0.63	10.73 ± 0.66
Hepatosomatic index (%)	1.22 ± 0.10	1.07 ± 0.04	1.14 ± 0.05
Mesenteric fat index (%)	1.97 ± 0.40	2.52 ± 0.44	1.93 ± 0.49

* In the same line, values with different superscript letters are significantly different ($P<0.05$). Data are expressed as mean ± S.E

effects of seaweed extract supplementation on visceral development. The MFI was notably lower in the 23L + SE group (1.93%) compared to 23L (2.52%).

Fatty acid parameters of rainbow trout are shown in Table 6. Significant differences were determined in C16:0, C17:0, C18:0, C18: 2n6, C18:3n3, C20:3n3, C22:1n9, C22:5n3, n-6 and n-6/n-3 fatty acids among the groups.

The texture parameters of fish fed these different experimental diets showed no statistically significant difference (Table 7). These findings indicate that the tested diet types did not significantly affect the textural properties of rainbow trout, providing valuable insights into diet formulation and its effects on fish quality.

The effects of experimental diets on fish digestive enzyme activities in the intestine and stomach are described in Fig. 1. The specific activity of lipase in the intestine of fish fed with the 23L was higher than in those diets with the 20L and 23L + SE ($P < 0.05$). However, lipase activity in the stomach was higher in fish in the 23L + SE group than in the other groups ($P < 0.05$). The pepsin activities in the intestine and stomach were significantly higher in groups 23L and 23L + SE than in 20L ($P < 0.05$). In fish fed the experimental diets, trypsin activities in the intestine and stomach were unaffected. The amylase activity in the intestine of 23L treatment markedly increased compared to that of the 20L and 23L + SE ($P < 0.05$). 23L treatment markedly increased amylase activity in the intestine compared to the 20L and 23L + SE, while the 20L and 23L showed the highest amylase activity values in the stomach ($P < 0.05$).

Table 6 Fatty acid profile (% of total identified fatty acids, mean ± SE) of rainbow trout after 8 weeks of feeding on experimental diets. $n = 9$

Fatty acid	20L	23L	23L + SE
C14:0	2.05 ± 0.08	2.06 ± 0.07	2.03 ± 0.09
C15:0	0.34 ± 0.02	0.36 ± 0.02	0.33 ± 0.03
C16:0	17.98 ± 0.41[a]	18.25 ± 0.38[a]	16.75 ± 0.45[b]
C16:1n9c	2.81 ± 0.10	2.64 ± 0.09	2.60 ± 0.11
C17:0	0.34 ± 0.02[b]	0.38 ± 0.03[a]	0.32 ± 0.02[b]
C18:0	4.99 ± 0.27[a]	4.80 ± 0.24[a]	4.46 ± 0.30[a]
C18:1n9	25.72 ± 0.61	25.62 ± 0.56	26.62 ± 0.64
C18:2n6	22.43 ± 0.74[a]	23.68 ± 0.80[ab]	25.19 ± 0.88[a]
C18:3n3	2.22 ± 0.09[b]	2.23 ± 0.08[b]	2.51 ± 0.10[a]
C20:0	0.27 ± 0.02	0.28 ± 0.01	0.27 ± 0.02
C20:1n9	1.05 ± 0.05	1.03 ± 0.04	1.14 ± 0.06
C20:2n6	0.16 ± 0.01	0.18 ± 0.02	0.21 ± 0.02
C20:3n3	1.35 ± 0.08[b]	1.34 ± 0.09[b]	1.63 ± 0.10[a]
C20:4n6	0.73 ± 0.04	0.77 ± 0.05	0.61 ± 0.04
C20:5n3	2.10 ± 0.11	1.99 ± 0.10	1.78 ± 0.09
C22:0	0.21 ± 0.01	0.21 ± 0.01	0.21 ± 0.01
C22:1n9	0.14 ± 0.01[a]	0.11 ± 0.01[b]	0.14 ± 0.01[a]
C23:0	0.85 ± 0.04	0.56 ± 0.03	0.85 ± 0.04
C22:5n3	0.86 ± 0.05[a]	0.75 ± 0.04[ab]	0.72 ± 0.05[b]
C22:6n3	11.94 ± 0.55	11.89 ± 0.50	9.23 ± 0.48
C24:0	0.33 ± 0.02	0.27 ± 0.02	0.31 ± 0.02
C24:1n9	0.98 ± 0.05	0.62 ± 0.04	1.06 ± 0.06
Σ n-6	23.32 ± 0.58[b]	24.63 ± 0.61[ab]	26.23 ± 0.67[a]
Σ n-3	18.35 ± 0.47	18.20 ± 0.53	15.81 ± 0.59
n-6/n-3	1.32 ± 0.05[b]	1.35 ± 0.06[ab]	1.66 ± 0.07[a]

Table 7 Texture parameter of rainbow trout after 8 weeks of feeding on experimental diets. $n = 9$

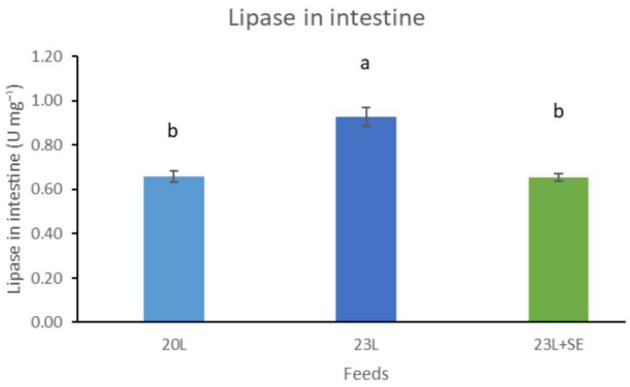
	20L	23L	23L + SE
Hardness, N	4.63 ± 0.59	4.47 ± 0.10	4.34 ± 0.16
Adhesiveness, mJ	0.34 ± 0.04	0.27 ± 0.08	0.30 ± 0.02
Springiness, mm	2.26 ± 0.04	2.54 ± 0.15	2.63 ± 0.01
Gumminess, N	1.84 ± 0.28	1.60 ± 0.10	1.67 ± 0.12
Chewiness, N	4.17 ± 0.67	4.07 ± 0.36	4.23 ± 0.39
Cohesiveness	0.39 ± 0.02	0.34 ± 0.01	0.38 ± 0.02

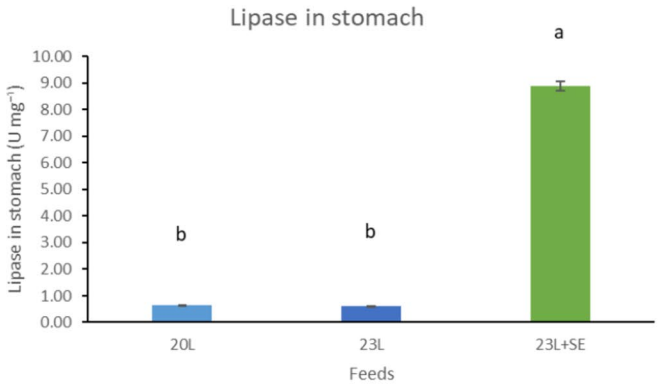
[*] In the same line, values with different superscript letters are significantly different ($P < 0.05$). Data are expressed as mean ± S.E

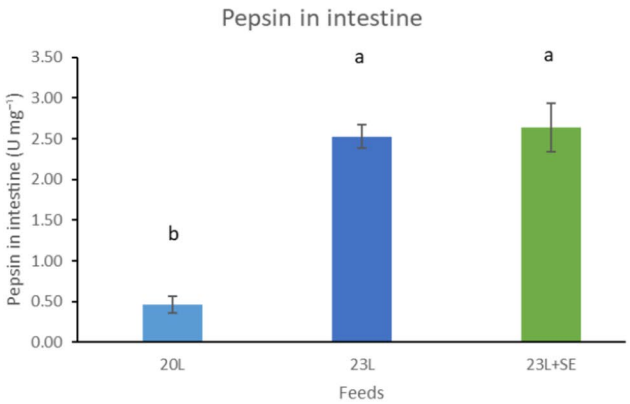
Gene expression levels in the intestine and stomach among different treatments are presented in Fig. 2. The relative mRNA expression level of lipase in the intestine showed the highest values for fish fed with the 23L ($P < 0.05$). The relative mRNA expression level of lipase in the stomach was significantly upregulated in fish fed with the 23L + SE compared with other groups ($P < 0.05$). The relative mRNA expression level of *amylase* in the intestine was significantly upregulated in fish fed the 20L diet ($P < 0.05$). In contrast, dietary treatments did not significantly affect *amylase* expression in the stomach. The expression levels of *pepsinogen* in both the stomach and intestine were significantly higher in fish receiving the 23L and 23L + SE diets ($P < 0.05$). In addition, intestinal *trypsinogen* expression was significantly elevated in the 23L group compared to the 20L and 23L + SE groups ($P < 0.05$). Similarly, *trypsinogen* expression in the stomach was significantly higher in the 23L group than in the other groups ($P < 0.05$).

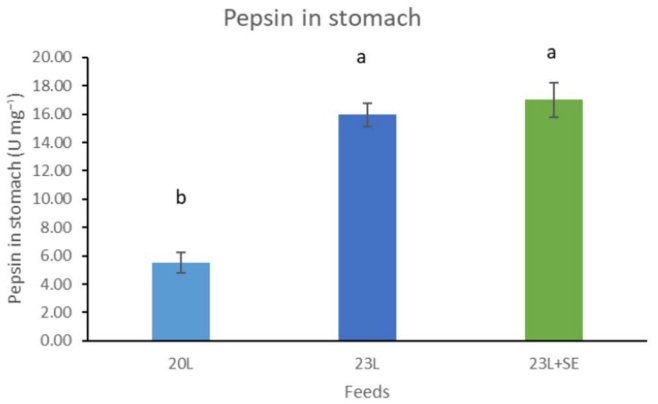
The expression levels of growth-related genes in the muscle tissue of fish fed experimental diets are presented in Fig. 3. A significant modulation in the *GH* and *IGF-I* gene expression was observed after eight weeks of feeding in fish receiving the 23L + SE diet ($P < 0.05$; Fig. 3). The relative mRNA expression level of the *GH* was significantly higher in the 23L + SE group (8.93) compared to the 20L (0.26) and 23L (0.83) groups ($P < 0.05$). Similarly, the expression level of the *IGF-I* was significantly upregulated in the 23L + SE group compared to the other groups ($p < 0.05$).

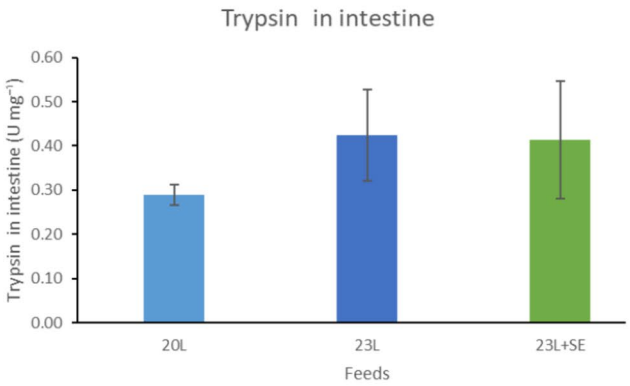
The dietary treatments significantly affected the relative mRNA expression levels of the *cpt1b1*, *elovl2*, and *ldlr* genes in the liver of rainbow trout (Figs. 4, 5, 6). The

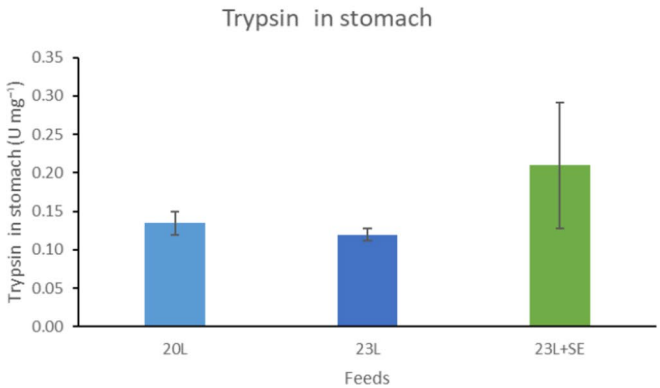

Lipase in intestine
Lipase in intestine (U mg⁻¹)
1.20
1.00
0.80
0.60
0.40
0.20
0.00
b
a
b
20L
23L
23L+SE
Feeds

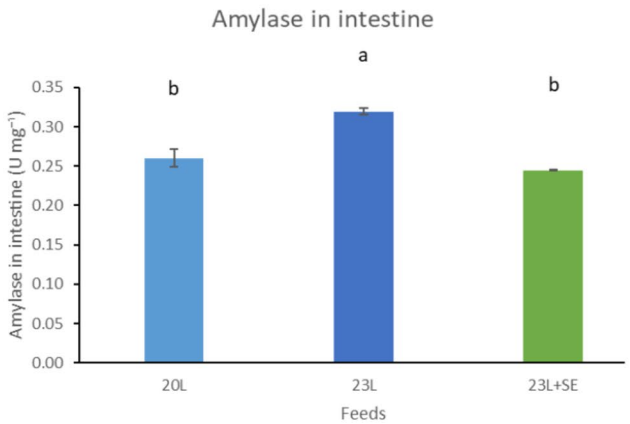

Lipase in stomach
Lipase in stomach (U mg⁻¹)
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
b
b
a
20L
23L
23L+SE
Feeds

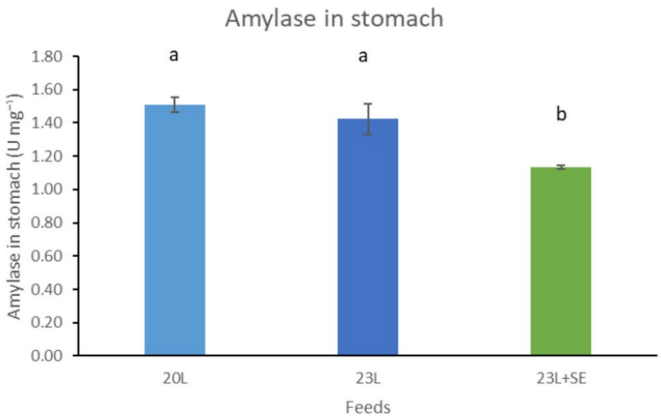

Pepsin in intestine
Pepsin in intestine (U mg⁻¹)
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
b
a
a
20L
23L
23L+SE
Feeds


Pepsin in stomach
Pepsin in stomach (U mg⁻¹)
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
b
a
a
20L
23L
23L+SE
Feeds


Trypsin in intestine
Trypsin in intestine (U mg⁻¹)
0.60
0.50
0.40
0.30
0.20
0.10
0.00
20L
23L
23L+SE
Feeds


Trypsin in stomach
Trypsin in stomach (U mg⁻¹)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
20L
23L
23L+SE
Feeds


Amylase in intestine
Amylase in intestine (U mg⁻¹)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
b
a
b
20L
23L
23L+SE
Feeds


Amylase in stomach
Amylase in stomach (U mg⁻¹)
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
a
a
b
20L
23L
23L+SE
Feeds

◂**Fig. 1** Effects of dietary treatments on digestive enzyme activities (U mg^{-1} protein) in the intestine and stomach of rainbow trout after 8 weeks of feeding. Values are mean ± standard error (n = 9). Bars with different letters (**a**, **b**) indicate statistically significant differences ($P < 0.05$) among dietary groups

23L + SE group exhibited a significant upregulation in the mRNA expression level of *cpt1b1* compared to the 20L and 23L groups ($P < 0.05$). The expression of *elovl2* was also significantly higher in the 23L + SE group than in the 23L group ($P < 0.05$). No significant difference in *ldlr* expression was observed between the 23L + SE and 23L groups ($P > 0.05$), while expression in the 20L group was significantly lower than in both ($P < 0.05$).

The effects of the SE supplementation on the liver were examined in groups fed diets with two different fat levels, including one with high-fat content. In the 20L group, hepatic cords and hepatic sinusoids appeared normal, with low levels of lipid vacuolization. In contrast, in the 23L group, the hepatic cord and sinusoidal structure were considered normal, but a moderate level of lipid accumulation was noted. In the 23L + SE group, the effectiveness of the functional feed additive was demonstrated by the normal hepatic cords and sinusoids, along with low lipid vacuolization. Accordingly, the grading was determined as follows: 20L: 1, 23L: 2, and 23L + SE: 1 (Fig. 7).

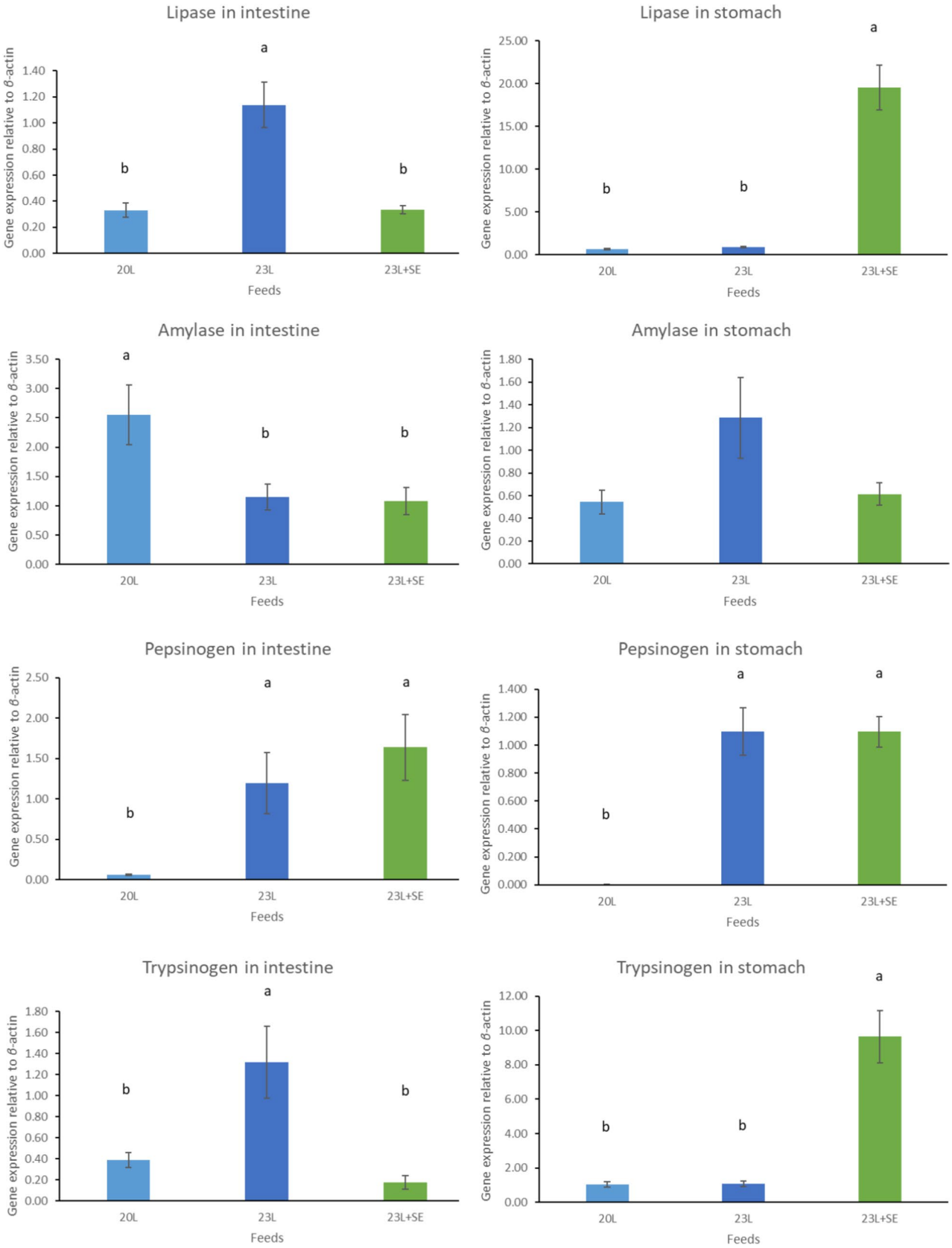
The histological sections of the small intestine, covering the region following the pyloric section of the stomach, were similar and normal across all groups. The intestinal sections' serosa, muscularis externa, muscularis interna, submucosa, and mucosa layers showed no deviations from normal tissue structure. Before the experiment, the villus lengths of the fish were measured as 436.79 ± 97.06 µm, and villi widths were recorded as 96.56 ± 14.12 µm ($P < 0.05$). At the end of the experiment, the villus lengths of the trout fed the 20L diet were statistically longer than those of the 23L group. However, it was determined that the fat content of the diet and the functional feed additive did not statistically affect villus width ($P > 0.05$) (Table 8).

Discussion

The present study demonstrated that dietary supplementation with seaweed extracts enhanced growth performance and feed conversion efficiency in rainbow trout (*Oncorhynchus mykiss*). Feed intake results indicated that experimental diets were equally palatable compared to the control diets ($P > 0.05$), suggesting that including seaweed-derived additives did not adversely affect feed acceptance. Palatability is a critical factor in aquaculture, as reduced feed intake can lead to suboptimal growth and inefficient feed utilization. The absence of significant differences in feed intake among fish fed ad libitum throughout the experimental period indicates that seaweed-based additives did not exert any antinutritional effects. Although this finding does not provide direct evidence of feed-attractant properties, it confirms that the fish tolerated and accepted the additives. Notably, fish in the 23L + SE group achieved the highest final weight (355.27 g) and specific growth rate (SGR: 2.38% day^{-1}), along with an improved feed conversion ratio (FCR), compared to other groups. These findings highlight the potential of seaweed-derived additives to enhance growth performance and feed efficiency while mitigating the adverse effects of high-lipid diets.

High-lipid diets are commonly used in aquaculture for their protein-sparing and cost-effective benefits (Wangkahart et al. 2022a, b), but excessive dietary fat can lead to fat accumulation in the liver and metabolic disorders in fish (Naiel et al. 2023). Functional feed additives have been explored as a strategy to counteract these issues (Naiel et al. 2023). Similarly, studies involving seaweed supplementation in high-lipid diets have also demonstrated improved growth performance, feed utilization, and metabolic health across fish species such as gilthead seabream (*Sparus aurata*), Nile tilapia (*Oreochromis niloticus*), and common carp (*Cyprinus carpio*) (Ergün et al. 2009; Emre et al. 2013; Sabzi et al. 2023). Consistent with prior studies, the seaweed-supplemented high-lipid diet in our study led to improved growth and metabolic health in rainbow trout (*O. mykiss*). Similar enhancements in growth performance and feed utilization under high-lipid conditions have been reported with various supplements, including seaweed in other species (Ergün et al. 2009; Emre et al. 2013; Sabzi et al. 2023), berberine (Wang et al. 2021), curcumin (Ji et al. 2021), and prebiotic xylooligosaccharides (Abasubong et al. 2022). In line with those findings, the 23L + SE group in our study showed superior growth and feed conversion, accompanied by reduced hepatic lipid vacuolization and a lower mesenteric fat index. Thus, seaweed extracts may serve as a natural, functional alternative to synthetic additives for improving fish tolerance to lipid-rich diets.

At the molecular level, seaweed supplementation modulated growth-related gene expression. The 23L + SE group showed significantly higher hepatic mRNA levels of growth hormone (*GH*) and insulin-like growth factor I (*IGF-I*) compared to both the unsupplemented high-lipid diet (23L) and the low-lipid control (20L) ($P < 0.05$). The *GH* and *IGF-I* are pivotal endocrine regulators of somatic growth (Reindl and Sheridan 2012), so their upregulation in the seaweed-fed fish (with GH nearly ninefold higher) indicates a strong stimulatory effect of the additive on growth-promoting pathways. This finding underscores the synergistic relationship between these genes in regulating growth. Importantly, these molecular findings may be closely linked to enhanced digestive capacity observed in the 23L + SE group. The increased


Lipase in intestine
Gene expression relative to β-actin
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
a
b
b
20L
23L
23L+SE
Feeds
Lipase in stomach
Gene expression relative to β-actin
25.00
20.00
15.00
10.00
5.00
0.00
a
b
b
20L
23L
23L+SE
Feeds
Amylase in intestine
Gene expression relative to β-actin
3.50
3.00
2.50
2.00
1.50
1.00
0.50
0.00
a
b
b
20L
23L
23L+SE
Feeds
Amylase in stomach
Gene expression relative to β-actin
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
20L
23L
23L+SE
Feeds
Pepsinogen in intestine
Gene expression relative to β-actin
2.50
2.00
1.50
1.00
0.50
0.00
a
a
b
20L
23L
23L+SE
Feeds
Pepsinogen in stomach
Gene expression relative to β-actin
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
a
a
b
20L
23L
23L+SE
Feeds
Trypsinogen in intestine
Gene expression relative to β-actin
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
a
b
b
20L
23L
23L+SE
Feeds
Trypsinogen in stomach
Gene expression relative to β-actin
12.00
10.00
8.00
6.00
4.00
2.00
0.00
a
b
b
20L
23L
23L+SE
Feeds

◂**Fig. 2** Relative mRNA expression levels of digestive enzyme-related genes (normalized to *β-actin*) in the intestine and stomach of rainbow trout fed different diets after 8 weeks. Values are mean ± standard error (n = 9). Bars with different letters (**a**, **b**) indicate statistically significant differences ($P < 0.05$) among dietary groups

activity and gene expression of pepsin and lipase in the stomach and higher pepsinogen expression in the intestine suggest improved protein and lipid digestion, which could have facilitated better amino acid absorption and hormonal stimulation of the *GH–IGF* axis. The enhanced gene expression in the 23L + SE group is likely attributable to the bioactive compounds in seaweed, such as sulfated polysaccharides and trace elements derived from *Ulva* sp. and *S. chordalis*, which are known to modulate endocrine signaling pathways that promote the *GH* and *IGF-I* synthesis and activity (Mohamed et al. 2012; Zhu et al. 2016). At the molecular

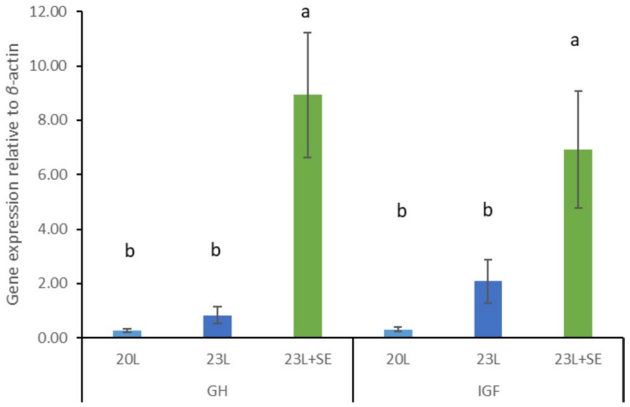


Fig. 3 Relative mRNA expression levels of growth-related genes (GH and IGF-I) in the liver of rainbow trout fed diets with different lipid levels and MFeed + after 8 weeks. Different letters above bars indicate significant differences between groups ($P < 0.05$). Values are mean ± SE

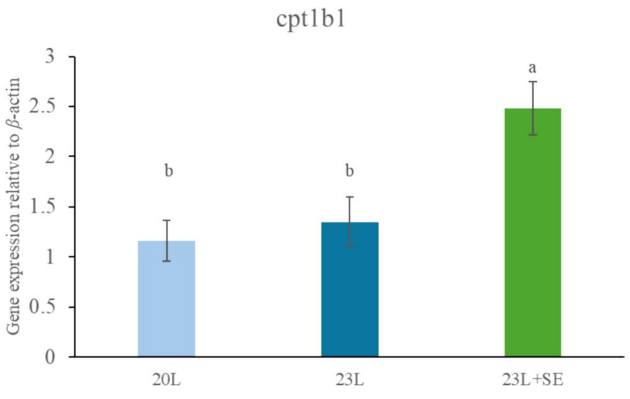


Fig. 4 Relative gene expression of *cpt1b1* in the liver of rainbow trout fed the experimental diets. Different letters above bars indicate significant differences between groups ($P < 0.05$). Values are mean ± SE

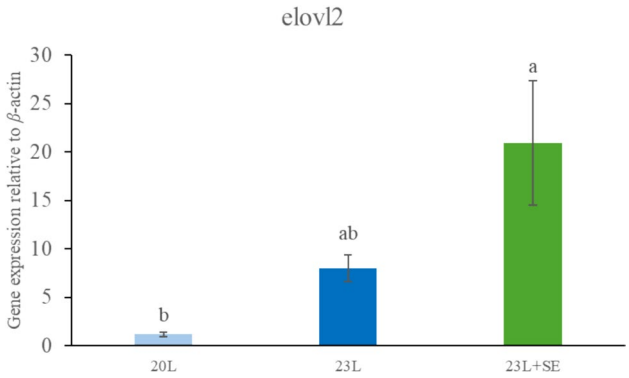


Fig. 5 Relative gene expression of *elovl2* in the liver of rainbow trout fed the experimental diets. Different letters above bars indicate significant differences between groups ($P < 0.05$). Values are mean ± SE

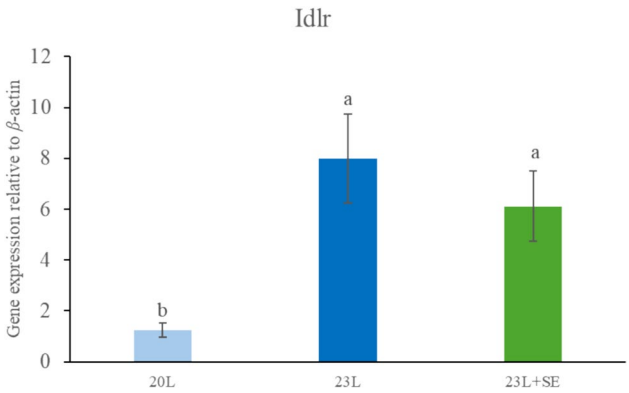


Fig. 6 Relative gene expression of *Idlr* in the liver of rainbow trout fed the experimental diets. Different letters above bars indicate significant differences between groups ($P < 0.05$). Values are mean ± SE

level, seaweed supplementation modulated growth-related gene expression. The 23L + SE group showed significantly higher hepatic mRNA levels of growth hormone (*GH*) and insulin-like growth factor I (*IGF-I*) compared to both the unsupplemented high-lipid diet (23L) and the low-lipid control (20L) ($P < 0.05$). The *GH* and *IGF-I* are pivotal endocrine regulators of somatic growth (Reindl and Sheridan 2012), so their upregulation in the seaweed-fed fish (with the *GH* nearly ninefold higher) indicates a strong stimulatory effect of the additive on growth-promoting pathways. Our findings are in line with a body of literature showing that functional feed additives can activate the *GH–IGF* axis, particularly under high-fat dietary conditions (Valente et al. 2006). For example, Tharaka et al. (2020) reported that supplementing an algae-clay blend (*Ulva lactuca* and *S. chordalis*) improved growth performance, feed utilization, and intestinal morphology in olive flounder. Likewise, other studies have noted the benefits of seaweed-based additives: algae extracts enhanced antioxidant enzyme activities and

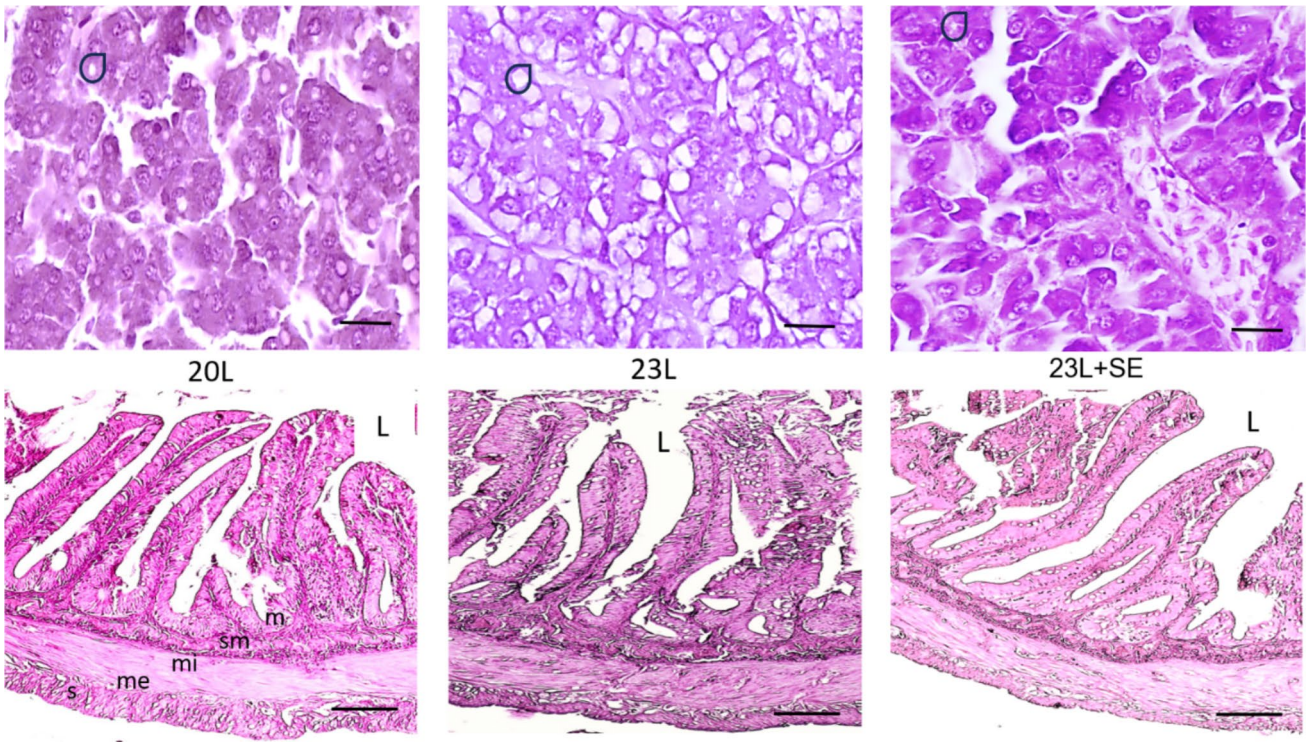


Fig. 7 Liver and small intestine sections of rainbow trout fed with the 20L, 23L, and 23L + SE. The hepatic cord and sinusoidal structure were normal. Low levels of lipid accumulation and mild hepatocyte swelling were observed and considered histologically normal. The liver of the 23L group showed moderate macrovesicular lipid accumulation. Intestinal tissues of all groups were determined to have normal histomorphology. Circle: lipid accumulation; L: lumen; s: serosa; me: muscularis externa; mi: muscularis interna; sm: submucosa; m: mucosa; bar: for liver =20 μm and for intestine =100 μm (7 μm, H&E)

Table 8 Villus measurement of rainbow trout fed with experimental diets

	Initial	20L	23L	23L + SE
Villus length (μm)	436.79 ± 97.06	988.43 ± 162.44[a]	878.15 ± 1071.15[b]	899.04 ± 113.07[ab]
Villus width (μm)	96.56 ± 14.12	147.96 ± 39.40	145.19 ± 28.25	144.52 ± 40.26

*Different letters in the lines indicate significant differences ($p < 0.05$)

growth in fish subjected to fishmeal-reduced diets (Liang et al. 2023), diets supplemented with the green algae *Ulva rigida* boosted growth rate and the FCR in rainbow trout (Güroy et al. 2011), and *Ulva intestinalis* supplementation improved somatic growth and feed efficiency in various aquaculture species (Safavi et al. 2019). Collectively, these reports support the hypothesis that bioactive compounds in seaweeds (e.g., sulfated polysaccharides and antioxidants) positively influence metabolic and endocrine responses, ultimately enhancing protein synthesis and nutrient partitioning. This aligns with our observations of improved growth-related gene expression, nutrient utilization, and reduced visceral fat deposition in seaweed-fed trout (Soler-Vila et al. 2009; Ferreira et al. 2020; Vazirzadeh et al. 2022; Yazici et al. 2024). Notably, the significant upregulation of the *IGF-I* in the 23L + SE group may have contributed to its lower mesenteric fat, given IGF-I's role in stimulating lipolysis and inhibiting lipid accumulation (Reindl and Sheridan 2012). In line with the endocrine changes above, we observed significant effects of seaweed inclusion on whole-body lipid deposition. Fish on the seaweed-supplemented diet had a higher whole-body lipid content (9.75%) than those on the unsupplemented diets ($P < 0.05$). Interestingly, this increase in overall lipid levels was accompanied by a reduced mesenteric fat index (1.93% vs. 2.52% in the 23L group), suggesting that seaweed supplementation may redirect lipid storage away from visceral tissues. This apparent paradox—elevated whole-body lipid content alongside reduced visceral fat—may reflect a redistribution of lipids toward other tissues such as muscle or liver. This interpretation is supported by histological observations and the upregulation of lipid metabolism–related genes (e.g., *elovl2* and *cpt1b1*) in the 23L + SE group, indicating enhanced lipid mobilization and metabolic efficiency. Similar upregulation of the *cpt1b1* has

been observed in rainbow trout supplemented with *Saccharina latissima*, suggesting stimulation of β-oxidation pathways, which could underlie such redistribution (Ferreira et al. 2020). These findings underscore the functional value of seaweed supplementation in aquaculture—promoting leaner carcass quality while maintaining growth performance and supporting healthier lipid distribution.

Seaweed extracts also influenced digestive physiology. The 23L + SE group exhibited significantly higher gastric lipase activity than the high-fat control ($P < 0.05$), indicating an earlier onset of lipid digestion in the stomach. Early lipid hydrolysis is advantageous for lipid-rich feeds, as it accelerates fat breakdown and improves overall fat absorption under energy-dense dietary conditions (Glencross 2009; Tocher 2010), thereby reducing the digestive burden on the intestine (García-Meilán et al. 2025). Correspondingly, intestinal lipase activity was lower in the 23L + SE group ($P < 0.05$), supporting the hypothesis that seaweed supplementation shifts part of the lipid digestive process to the stomach. This shift is consistent with feedback mechanisms reported for other functional feed additives, where enhanced proximal digestion can downregulate pancreatic enzyme secretion in later gut segments (García-Meilán et al. 2025). A similar phenomenon was observed by Tharaka et al. (2020) in olive flounder fed *Ulva* and *Solieria* supplements, which elevated gastric digestion and reduced distal gut enzyme demand. The bioactive compounds in these seaweeds – particularly sulfated polysaccharides – likely play a key role in this modulation of digestive physiology. *U. lactuca* and *S. chordalis* are rich in sulfated polysaccharides that can influence enzyme activity and gut function. Indeed, Liang et al. (2023) noted that seaweed-derived polysaccharides act as natural additives that improve digestive enzyme activities (e.g., amylase, lipase) and nutrient uptake in fish. Thus, the enhancement of stomach lipase (with a concomitant reduction in intestinal lipase) observed in our study can be attributed to the seaweed's bioactive components. By shifting part of lipid digestion to the stomach, the seaweed additive promoted more efficient lipid utilization, which aligns with previously reported benefits of algae-based functional additives in aquafeeds. Interestingly, pepsin activity (the main gastric protease) remained unchanged across all treatments, suggesting that protein digestion in the stomach was inherently stable and not significantly influenced by the dietary additive (Tharaka et al. 2020). Trypsin, a key intestinal protease, was also not affected by seaweed supplementation, reinforcing that the additive selectively modulated lipid and carbohydrate digestion without disrupting essential protein hydrolysis. While elevated protease activity is often linked to improved protein utilization and growth (Tharaka et al. 2020), the lack of change in trypsin in our study implies that any direct effect of the seaweed extract on proteolytic enzymes was minimal.

Carbohydrate digestion showed a distinct response to the diets. Intestinal amylase activity was significantly higher in fish fed the low-lipid diet (20L) but was reduced in the seaweed group (23L + SE) ($P < 0.05$). This indicates a metabolic shift in the 23L + SE fish that favoured lipid and protein utilization over carbohydrates. In the stomach, amylase activity also tended to decrease with seaweed supplementation. Such enzyme activity patterns may result from a prioritization of digestive efforts: the bioactive compounds in the seaweed could be enhancing pathways for lipid and protein metabolism at the expense of carbohydrate breakdown. This adaptation is logical under a fat-rich diet where a greater proportion of energy comes from lipids (Onomu and Okuthe 2024). The reduction in amylase activity in seaweed-fed trout underscores the targeted role of the additive in optimizing digestion for a high-lipid diet.

Seaweed-derived additives likely enhance overall digestive efficiency through multiple mechanisms. Bioactive polysaccharides (ulvans, carrageenans, etc.) and other compounds in seaweed can stabilize gut health, stimulate digestive enzyme secretion, and improve nutrient absorption (Liang et al. 2023). Micronutrients like zinc, copper, and manganese act as enzyme cofactors, while sulfated polysaccharides stimulate lipase and amylase activity, improving lipid and carbohydrate metabolism (Sheikhzadeh et al. 2022). Morais et al. (2020) further emphasized the nutritional potential of seaweed-based supplements, demonstrating their ability to improve lipid utilization and growth performance in aquaculture species. In the present study, this effect is supported by the increased stomach lipase activity and improved feed conversion ratio observed in the 23L + SE group, indicating enhanced digestion and nutrient assimilation. These findings collectively reinforce the potential of seaweed-derived additives to optimize aquaculture feeds by enhancing digestion and nutrient absorption.

The fatty acid composition of fish is a key indicator of dietary lipid metabolism, influencing growth, health, and final product quality. In this study, significant differences were observed in C16:0, C17:0, C18:0, C18:2n6, C18:3n3, C20:3n3, C22:1n9, C22:5n3, n-6, and the n-6/n-3 ratio, indicating that seaweed supplementation affected lipid metabolism in rainbow trout (*O. mykiss*).. Among saturated fatty acids (SFA), palmitic acid (C16:0) was significantly lower in the 23L + SE group compared to the 20L and 23L groups ($P < 0.05$). Similarly, stearic acid (C18:0) and heptadecanoic acid (C17:0) decreased, suggesting that seaweed extracts influenced the SFA metabolism by enhancing lipid oxidation and reducing fat deposition. These results align with findings showing that bioactive compounds in seaweed modulate lipid transport and utilization.

A significant increase in erucic acid (C22:1n9) was observed in the 23L + SE group ($P < 0.05$), which may be linked to enhanced lipid metabolism and energy storage mediated by sulfated polysaccharides (Galindo et al. 2024). These compounds regulate lipid transport and deposition in fish tissues, improving metabolic efficiency (Güroy et al. 2007; Norambuena et al. 2015). Seaweed supplementation significantly increased linoleic acid (C18:2n6) and alpha-linolenic acid (C18:3n3) in the 23L + SE group ($P < 0.05$), confirming that seaweed extracts are rich in essential fatty acids, particularly omega-3 and omega-6 PUFAs (Khonji et al. 2023). The increase in eicosatrienoic acid (C20:3n3) highlights the role of seaweed-derived bioactive in supporting n-3 fatty acid biosynthesis, which is critical for aquaculture nutrition and human health benefits (Siddik et al., 2023). The overall n-6/n-3 fatty acid ratio was slightly higher in the 23L + SE fish than in the other groups ($P < 0.05$). While a lower n-6/n-3 ratio is generally preferred for human dietary balance, a moderate increase in this ratio in fish can reflect shifts in metabolism and is not inherently negative for fish physiology (Dong et al. 2023). Taken together, these alterations in fatty acid composition confirm that seaweed-derived additives can modulate lipid metabolism in rainbow trout (*O. mykiss*). The increases in omega-3 and omega-6 PUFA levels, along with changes in fatty acid saturation and ratios, point to a more efficient utilization and incorporation of dietary lipids when seaweed extracts are included. Consistent with these findings, molecular indicators of lipid metabolism were also affected by the seaweed diet. The 23L + SE group showed a marked upregulation of hepatic *cpt1b1* and *elovl2* gene expression ($P < 0.05$). The c*pt1b1* (carnitine palmitoyltransferase 1b1) is crucial for transporting long-chain fatty acids into mitochondria for β-oxidation; its increased expression implies an enhanced capacity for fatty acid catabolism in seaweed-fed trout. The e*lovl2* (elongation of very long-chain fatty acids protein 2) catalyzes the elongation of polyunsaturated fatty acids, and higher *elovl2* levels suggest improved synthesis of long-chain fatty acids for cellular functions. These gene expression changes indicate a metabolic shift favouring greater lipid utilization and energy production, which likely contributed to the reduced visceral fat and improved feed conversion observed in the seaweed group. Similar regulatory effects of macroalgae on lipid metabolism have been documented in other species; for instance, *Porphyra* polysaccharide supplementation in rabbitfish significantly decreased lipid accumulation by downregulating lipogenic genes and upregulating lipid oxidation pathways (Li et al. 2024).

Interestingly, the *Idlr* expression did not differ significantly between the 23L + SE and 20L groups ($P > 0.05$), indicating that seaweed supplementation did not strongly regulate insulin-dependent lipid uptake mechanisms. However, the *Idlr* was significantly lower in the 23L group than in both other groups ($P < 0.05$), suggesting that a high-fat diet alone may suppress hepatic lipid uptake. This observation is consistent with the lipid-lowering effects of dietary macroalgae, as previously reported in studies on *Saccharina latissima* supplementation in rainbow trout, (*O. mykiss*) which led to the downregulation of fatty acid synthase (FAS) and a general reduction in lipid accumulation in fillets (Ferreira et al. 2020). These findings indicate that seaweed supplementation does not impair lipid uptake but may counteract excessive hepatic lipid accumulation caused by high-fat diets. Moreover, they are consistent with previous studies demonstrating that dietary bioactive compounds, particularly sulfated polysaccharides, modulate lipid metabolism by activating mitochondrial β-oxidation pathways and increasing the availability of bioactive long-chain fatty acids for cellular processes (Liu et al. 2023; Mayulu et al. 2023).

One of the physiological manifestations of these metabolic changes was the observed increase in whole-body lipid content in the 23L + SE group. At first glance, this may appear paradoxical when considered alongside the reduced mesenteric fat index (MFI). However, this outcome can be explained by a redistribution of lipids and more efficient retention of dietary energy within the body. Seaweed supplementation likely improved nutrient utilization and metabolic efficiency, allowing fish to absorb and incorporate more dietary lipids into functional tissues such as muscle and liver for structural functions and energy metabolism, rather than excreting them or storing them excessively as visceral fat (Güroy et al. 2013; Galindo et al. 2022; Liang et al. 2023).

Specifically, although total body lipid content was higher in the 23L + SE fish, MFI was lower, indicating less lipid accumulation in the "overflow" stores surrounding the organs. Instead, lipids were likely redirected toward productive metabolic pathways – supporting cell membrane structure, meeting energy demands via β-oxidation, and contributing to intramuscular fat deposition, which can enhance growth and fillet quality. The enhanced expression of *cpt1b1* (mitochondrial fatty acid oxidation) and the *elovl2* (fatty acid elongation) in seaweed-fed fish confirms that lipid turnover and utilization were accelerated, increasing the flux of dietary fat toward metabolism and growth.

Nevertheless, if dietary lipid energy exceeds the capacity for oxidation, the surplus is stored in the body, explaining the rise in total lipid content. Seaweed extracts helped fish handle a high-fat diet more efficiently—promoting lean growth and reducing visceral fat, while still increasing total lipid deposition through better energy capture. Thus, improved feed conversion and metabolic health enhanced tissue lipid retention, yielding fish that were "fatter" in a healthier way, with more lipid in muscle and less around organs.

However, the expression of lipid uptake genes like ldlr followed a different pattern. Hepatic ldlr was mainly

affected by dietary lipid level, not seaweed extract: both high-lipid diets (23L, 23L + SE) showed higher ldlr mRNA than the low-lipid control (20L), with no difference between the two high-fat groups. This indicates that dietary fat upregulated ldlr as an adaptive response to excess circulating lipids, while seaweed did not modify this effect. Similar trends have been reported in large yellow croaker (Yan et al. 2015). Thus, seaweed's benefits arose from enhanced lipid oxidation and redistribution (e.g., increased *cpt1b1* expression), rather than ldlr-mediated lipid uptake, highlighting distinct effects of dietary fat level versus seaweed bioactives.

At the organ level, histological analyses provided further insights into these physiological changes. In the liver, fish fed the high-lipid diet without seaweed (23L) showed moderate lipid vacuolization in hepatocytes and some lipid presence in hepatic veins, but overall tissue architecture (e.g., sinusoid structure and hepatocyte arrangement) remained intact. These changes were within normal limits for teleost liver plasticity (Hibiya 1982; Genten et al. 2009; Gelibolu et al. 2018; McMillan and Harris, 2018; Mokhtar 2021; Güroy et al., 2022; Hersi et al. 2023; Tefal et al. 2023; Vélez-Calabria et al. 2023; Tampou et al. 2024). Diets rich in certain vegetable oils are known to induce hepatic steatosis in trout, with linoleic acid having particularly strong effects, but such lipid accumulation is often reversible when a balanced diet is restored (Caballero et al. 2004). The liver histology in our study aligns with these observations: the fatty changes seen in the 23L fish were not extreme and are likely a reversible, adaptive response to high dietary lipid, rather than an indication of permanent liver damage. Beyond hepatic effects, dietary treatments also modulated intestinal structure, which plays a critical role in nutrient absorption and maintaining overall physiological balance. Carnivorous fish like rainbow trout naturally have shorter intestines with prominent mucosal folds, and their gut structure can be influenced by diet (Hibiya 1982; Genten et al. 2009; Mokhtar 2021). In our experiment, the low-lipid control (20L) had significantly longer intestinal villi than the high-lipid group (23L), suggesting that a high-fat diet may have an atrophying effect on gut villi length. The seaweed-supplemented group (23L + SE) showed intermediate villus lengths that were not significantly different from either the 20L or 23L groups. While this means the seaweed extract did not produce a statistically significant improvement in villus height, the trend (longer villi than the high-fat control) hints at a potential protective or mitigating effect on the intestine. Longer villi generally provide more surface area for nutrient absorption and are associated with better feed efficiency. Therefore, even a modest retention of villus length in the 23L + SE fish could be beneficial for nutrient uptake, suggesting that the seaweed additive may help preserve gut morphology under high-fat dietary stress.

This study underscores the potential of *Ulva* sp. and *S. chordalis* as functional feed additives in high-lipid diets for rainbow trout (*O. mykiss*). These seaweed extracts appear to enhance growth-related gene expression, digestive enzyme activity, and lipid metabolism based on the observed trends and significant findings, while potentially contributing to reduced visceral fat accumulation and improving feed efficiency. However, some of these effects were not statistically significant and should be interpreted with caution. These findings highlight the role of seaweed-derived bioactive compounds in mitigating metabolic imbalances and quality deterioration associated with high-lipid diets, thereby contributing to more sustainable and nutritionally optimized aquaculture practices. In addition to seaweed extracts, MFeed + ® contains montmorillonite, a natural clay known for its adsorption properties and ability to bind harmful substances in the gastrointestinal tract. Montmorillonite has been reported to improve gut health by modulating the intestinal microbiota and enhancing nutrient absorption efficiency (Shi et al., 2022; Zhang et al., 2022). Although not the main focus of this study, its presence in the additive may have contributed synergistically to the observed improvements in digestive efficiency and lipid metabolism.

Future research should explore long-term feeding trials, economic feasibility, and consumer acceptance to further enhance the integration of seaweed-based additives into commercial aquafeeds. Additionally, elucidating the specific mechanisms of bioactive compounds, particularly their influence on lipid metabolism pathways and potential synergistic effects with other dietary components, will provide deeper insights into their functionality and broader applicability in aquaculture.

Acknowledgements The authors would like to thank Olmix Group France and Olmix Türkiye for providing the MFeed+® product used in this study. We also gratefully acknowledge Tarkan Cemalettin Erkut and Caner Berk for their valuable technical assistance and support throughout the experimental process.

Author contribution DG (Derya Güroy): Conceptualization, Project administration, Supervision, Writing – original draft SM (Serhan Mantoğlu): Whole-body proximate analysis, Data curation İŞ (İzzet Şahin): Growth trial setup, Feed intake monitoring, Sampling logistics UU (Umut Uyan): Coordination with the feed industry, Product sourcing, Technical validation ONK (Osman Nezih Kenanoğlu): RNA extraction, qPCR assays, Gene expression data analysis EG (Ercument Genc): Tissue sampling, Histology sectioning and staining EK (Emre Kabil): Fatty acid analysis, GC-FID method optimization, DK (Doğukan Kaya): Photography, Microscopy documentation, Image editing OE (Onur Erdem): Feed formulation, Technical resources, Quality control of ingredients OK (Onur Karadal): Laboratory processing BG (Betül Güroy): Results interpretation, Literature review, Writing – review & editing, Nutritional insight SB (Soner Bilen): Enzymatic analysis (lipase, amylase, pepsin) GA (Gökhan Arslan): Aquaculture systems monitoring, Fish welfare oversight MP (Mustafa Pekcan): Laboratory processing AG (Ahmet Gürler): Photography, Microscopy documentation, Image editing VN (Vahid Naseh): Industrial applicability insight, Feed manufacturing optimization, Final review.

Funding The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Data availability The data generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethical statement This study was approved by the Atatürk University Local Ethics Committee for Animal Experiments under decision number HADYEK-240.

Conflict of interest The authors declare no competing interests.

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