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Effective immunostimulant for rainbow trout after short term usage: *Lepidium sativum* and its by-product

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ABSTRACT

In the present study, garden cress (*Lepidium sativum*) cold-pressed seed oil and its pulp ethanolic extract, obtained as a by-product, were evaluated as dietary immunostimulants in rainbow trout (*Oncorhynchus mykiss*). Fish were fed an unsupplemented control diet or diets containing garden cress seed oil (LS) or pulp ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1} for 21 days. The seed oil groups were designated LS01, LS05, and LS1, and the pulp extract groups were designated LSE01, LSE05, and LSE1. Sampling was performed at 7-day intervals to assess growth performance, hematological parameters, humoral immune responses, and immune-related gene expression. Digestive enzyme activities and antioxidant status were assessed at the end of the feeding trial, after which fish were subjected to a 10-day experimental challenge with *Yersinia ruckeri*.

Growth performance was significantly enhanced in the LS1 group compared with the control group. Humoral immune responses, including respiratory burst activity and lysozyme levels, increased in both LS and LSE groups compared with the control. Cytokine gene expression was also significantly upregulated, particularly in the LSE-fed groups. Digestive enzyme activities increased notably in the LS1 group and in the higher-dose LSE groups. Antioxidant enzyme activities, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), were significantly higher in the supplemented groups than in the control group. Survival rates after bacterial challenge were higher in all experimental groups than in the control, with the highest survival observed in the LSE1 group. Overall, these results suggest that both garden cress seed oil and its pulp ethanolic extract have immunostimulatory and health-promoting potential in rainbow trout, while the pulp extract showed more pronounced effects in some immune-related responses.

Introduction

Aquaculture has expanded rapidly over recent decades and has become one of the fastest-growing food production sectors worldwide. However, rapid intensification has been associated with an increased incidence of infectious diseases, particularly under high stocking densities and intensive farming conditions, leading to substantial economic losses [1]. Traditionally, disease prevention and control in aquaculture have largely relied on antibiotics and other chemotherapeutic agents. Nevertheless, the extensive use of antibiotics has raised concerns related to antimicrobial resistance, environmental contamination, and potential risks to animal and human health [2–5].

In response to these challenges, research efforts over the past decade have focused on identifying natural and sustainable alternatives to antibiotics in disease prevention. Among these approaches, plant-derived immunostimulants have received increasing attention because of their ability to enhance immune responses in fish without inducing antimicrobial resistance [6,7,3]. Many medicinal plants contain diverse bioactive phytochemicals, such as flavonoids, terpenoids, phenolic compounds, and tannins. These compounds are known to exhibit antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties [8,3,9]. Dietary supplementation with phytogenic compounds has been shown to enhance innate immune responses, improve antioxidant status, and increase resistance to bacterial pathogens in a

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range of fish species [10–12].

Garden cress (*Lepidium sativum*) is a medicinal herb widely recognized for its rich nutritional profile and high content of biologically active compounds. The seeds of *L. sativum* contain substantial amounts of flavonoids, terpenoids, tannins, and other phytochemicals. Extracts derived from the seeds have also been shown to possess strong antioxidant activity [13].

Previous studies have demonstrated that plant-based immunostimulants, including garden cress, can enhance immune responses and improve disease resistance against several important fish pathogens, including *Yersinia ruckeri* [14]. Bacterial infections, particularly those caused by *Y. ruckeri*, remain a major challenge in rainbow trout and other salmonid aquaculture. When not effectively controlled, such infections often result in significant economic losses [15].

Strengthening the innate immune system of fish through dietary strategies is therefore considered a promising approach to reducing disease outbreaks and limiting antibiotic use.

In this context, plant-derived by-products may represent a valuable source of functional feed additives that contribute to sustainable aquaculture practices. Cold-press extraction of plant oils generates substantial amounts of pulp residues. These residues may still contain considerable levels of immunologically active compounds. However, information on the comparative immunostimulatory effects of garden cress seed oil and pulp-derived ethanolic extracts in fish remains limited.

Therefore, the present study aimed to evaluate the immunostimulant potential of garden cress (*Lepidium sativum*) cold-pressed seed oil and its pulp ethanolic extract when included in the diets of rainbow trout (*Oncorhynchus mykiss*). Growth performance and hematological parameters were assessed, together with innate immune responses and immune-related gene expression. In addition, digestive enzyme activities, antioxidant status, and resistance to *Yersinia ruckeri* were investigated.

Materials and methods

Experimental diets

Rainbow trout with an average initial weight of approximately 5 g were fed six experimental diets formulated with garden cress (*Lepidium sativum*) cold-pressed seed oil and a pulp ethanolic (96 %) extract obtained following the oil extraction process. Garden cress (*Lepidium sativum*) seeds were purchased from local herbalists. The purchased seeds were oiled using a cold-pressing extraction machine (OZERGRUP Oz-10). Extraction was also performed on the collected pulp according to [16]. The only change in the method was the purity of the ethanol (96 %) used. The dietary treatments included a non-supplemented control diet (C) (Moyfeed ™, %42 protein, %18 fat), diets supplemented with garden cress seed oil at inclusion levels of 0.1, 0.5, and 1 g kg^{-1} (LS01, LS05, and LS1), and diets supplemented with the ethanolic extract of garden cress seed pulp at the same inclusion levels (LSE01, LSE05, and LSE1) using spraying methot [16]. The selected inclusion levels were chosen to represent low, moderate, and relatively high supplementation levels for evaluating the dietary effects of garden cress seed oil and pulp extract in rainbow trout. All additives were incorporated into the basal diet using a spray-coating method to ensure uniform distribution. After supplementation, the feeds were air-dried at room temperature and stored at −20°C until use.

Composition of garden seed oil and its pulp ethanolic extract by GC-MS

The chemical composition of garden cress (*Lepidium sativum*) cold-pressed seed oil (LS) and its pulp ethanolic extract (LSE) was determined using gas chromatography–mass spectrometry (GC–MS). GC–MS analyses were conducted to characterize both major and minor bioactive constituents present in the oil and the extract prior to their incorporation into the experimental diets.

Following characterization, the cold-pressed oil and the ethanolic extract were incorporated into the experimental diets at different inclusion levels, as described above. The identified compounds and their relative peak area percentages are presented in Table 1 for the garden cress seed oil (LS) and in Table 2 for the garden cress seed pulp ethanolic extract (LSE).

Rearing systems and fish

Approximately 550 rainbow trout (*Oncorhynchus mykiss*), with an average initial weight of about 5 g, were transferred to the experimental facility and acclimated to experimental conditions for two weeks prior to the start of the feeding trial. During this period, fish were maintained in adaptation tanks.

Following acclimation, fish were randomly distributed into experimental groups of 40 individuals per tank based on body weight and stocked into 150-L glass aquaria used for the feeding experiment. All fish were fasted for 24 h before the initiation of the trial. In total, 21 tanks were used, with each dietary treatment conducted in triplicate (three

Table 1
Chemical composition of cress seed cold press oil analyzed by GC-MS.

	R. Time (Min)	Peak Area %	Name of the peak
1	4.611	0.09	2-Hexanone (CAS)
2	4.911	0.48	Hydroperoxide, 1-methylpentyl (CAS)
3	9.919	0.25	Hexane, 2-nitro-
4	10.141	0.08	Hept-2(E)-enal
5	10.296	0.21	2-Pentanone, 3-ethyl-3-methyl-
6	11.715	0.30	Heptandienal <2,4-trans,trans->
7	12.893	0.07	D-Limonene
8	21.836	0.38	Dec-2(E)-enal
9	22.721	0.07	Anethole <(E)->
10	22.882	0.12	1,11-Dodecadiyne
11	22.997	0.07	2,4-Decadienal, (E,E)- (CAS)
12	23.398	0.05	Carvacrol
13	23.806	0.30	endo-Dicyclopentadiene dioxide
14	25.845	0.04	Copaene <alpha->
15	27.332	0.10	Caryophyllene
16	29.363	0.04	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
17	30.370	0.54	Phenol, 2,4-bis(1,1-dimethylethyl)-
18	30.675	0.05	Cedrene <beta->
19	31.916	0.07	Lauric acid
20	32.037	0.19	Acetophenone <3′,4′-dimethoxy->
21	32.900	0.05	Diethyl Phthalate
22	35.835	0.04	Tridecanal
23	37.634	0.29	Tetradecanoic acid
24	41.915	0.18	Hexadecanoic acid, methyl ester
25	42.380	0.27	1,13-Tetradecadien-3-one
26	42.968	6.16	Palmitic acid
27	43.119	4.22	Unidentified peak
28	45.155	6.38	Pentacosane
29	46.081	0.28	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
30	46.224	0.79	8,11,14-Docosatrienoic acid, methyl ester
31	46.523	0.12	2-Hexadecen-1-ol, 3,7,11,15-tetramethyl-, [R-[R*,R*-(E)]]- (CAS)
32	47.346	25.11	7-Tetradecenal, (Z)-
33	47.766	5.23	Octadecanoic acid
34	48.195	1.72	Unidentified peak
35	48.454	2.89	1,2-Benzenedicarboxylic acid, dinonyl ester (CAS)
36	48.823	2.35	9-Octadecenoic acid, (E)-
37	49.143	1.41	Geranyl Lınalool Isomer B
38	49.570	1.97	Unidentified peak
39	49.836	2.20	Hexadecanoic acid, tetradecyl ester
40	50.170	0.76	Unidentified peak
41	50.395	0.77	Unidentified peak
42	50.720	1.47	1-Eicosanol (CAS)
43	50.899	3.14	Hexacosane (CAS)
44	51.642	1.50	Oleic Acid
45	52.437	26.61	Tetracosanal
46	52.770	0.61	Heneicosane
		100.00	Total peak area

Table 2
Chemical composition of cress seed pulp ethanolic extract analyzed by GC-MS.

	R. Time (Min)	Peak Area %	Name of the peak
1	3.697	0.77	2,2-dimethoxybutane
2	6.571	0.33	Unidentified peak
3	8.625	0.25	Unidentified peak
4	9.050	0.39	Unidentified peak
5	9.209	0.21	Unidentified peak
6	10.966	0.25	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one
7	11.740	0.38	Decane
8	12.928	0.23	Unidentified peak
9	15.015	0.74	Cyclopentane, 1-acetyl-1,2-epoxy-
10	15.383	0.28	Unidentified peak
11	16.173	0.28	Unidentified peak
12	17.121	0.31	Unidentified peak
13	17.278	52.05	Benzyl nitrile
14	17.407	0.96	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one
15	17.525	0.62	Unidentified peak
16	17.585	0.25	Unidentified peak
17	18.389	0.88	Benzoic acid
18	20.468	0.45	Benzeneacetonitrile, 4-chloro-
19	20.773	0.42	Hydroxy methyl furfural
20	22.913	0.29	Unidentified peak
21	25.519	13.92	Benzene, (isothiocyanatomethyl)-
22	26.279	0.22	Unidentified peak
23	27.531	0.32	Unidentified peak
24	27.925	0.55	Unidentified peak
25	27.970	0.45	Unidentified peak
26	28.069	1.15	Unidentified peak
27	28.105	0.21	Unidentified peak
28	29.279	0.70	Benzoic acid, 2-(dimethylamino)ethyl ester
29	30.160	0.32	Unidentified peak
30	30.353	0.97	Phenol, 2,4-bis(1,1-dimethylethyl)- (CAS)
31	32.032	10.13	Acetophenone <3′,4′-dimethoxy->
32	32.680	0.29	Unidentified peak
33	33.921	0.22	Unidentified peak
34	38.414	0.27	Unidentified peak
35	41.479	0.75	Lidocaine
36	42.073	0.30	Unidentified peak
37	42.820	1.07	Palmitic acid
38	45.839	0.41	Unidentified peak
39	46.577	0.22	Unidentified peak
40	46.988	0.53	9,12-Octadecadienoic acid (Z,Z)-, methyl ester (CAS)
41	47.099	1.82	cis-Vaccenic acid
42	47.248	0.78	mic acid, 1-(4,7-dihydro-2-methyl-7-oxopyrazolo [1,5-a]pyrimidin-5-yl)-, methyl e
43	47.520	0.63	Unidentified peak
44	47.569	0.32	Unidentified peak
45	47.633	0.89	9-Octadecenoic acid (Z)- (CAS)
46	48.685	0.34	Unidentified peak
47	49.273	0.55	Unidentified peak
48	49.767	0.34	Unidentified peak
49	49.908	0.52	Unidentified peak
50	52.475	0.48	Unidentified peak
		100.00	Total peak area

tanks per treatment).

Throughout the experimental period, key water quality parameters, including temperature, dissolved oxygen, and pH, were monitored and recorded daily. Water temperature was maintained at 16.5 ± 0.05°C, dissolved oxygen at 8.7 ± 0.07 mg L^{-1}, and pH at 8.23 ± 0.14. Fish were hand-fed the experimental diets twice daily (09:00 and 17:00) to apparent satiation for a period of three weeks. The amount of feed offered to each tank was recorded daily, and feed intake was estimated on a tank basis for calculation of feed conversion ratio (FCR).

Growth performance

Growth performance was evaluated based on changes in body weight recorded at the beginning and at the end of the feeding trial. Fish in each tank were bulk-weighed, and mean body weight values were used for growth performance calculations.

Weight gain (WG, %) was calculated using the following equation:

$$WG\ (\%) = [(\text{final body weight (g)} - \text{initial body weight (g)}) \ / \ \text{initial body weight (g)}] \times 100.$$

Specific growth rate (SGR, % day^{-1}) was calculated as:

$$SGR\ (\%\ day^{-1}) = [(\ln \text{final body weight} - \ln \text{initial body weight}) \ / \ \text{experimental period (days)}] \times 100.$$

Feed conversion ratio (FCR) was calculated according to the following formula:

$$FCR = \text{feed intake (g)} \ / \ \text{weight gain (g)}.$$

Sampling protocols

On days 7, 14, and 21 of the feeding trial, three fish were randomly sampled from each replicate tank and anesthetized using spurge (*Euphorbia* sp.), according to the anesthetic procedure described by Alagöz et al. [17]. After anesthesia, blood samples were collected, and fish were subsequently dissected to obtain spleen, head kidney, intestine, stomach, and liver tissues. Thus, each dietary treatment was represented by nine fish (three fish × three replicate tanks) at each sampling point. At each sampling time, newly selected fish were used, and no individual fish was sampled more than once.

For gene expression analyses, tissue samples were immediately immersed in RNAlater™ solution (Invitrogen, Cat. No. AM7020) and stored at −20°C until further processing. For digestive enzyme analyses, the relevant tissues were transferred into cryogenic tubes and stored at −80°C until analysis.

All sampling procedures were performed at each sampling time point to ensure consistency across experimental groups.

Hematological assays

Red blood cell count (RBC), hemoglobin concentration (Hb), and hematocrit value (Hct) were determined using an automated hematology analyzer (Mindray BC-3000 Plus), following standard procedures as described by Hrubec et al. [18].

Hematological indices were calculated according to the following equations:

Mean corpuscular volume (MCV, fL):

$$MCV = (Hct \times 10) \ / \ RBC$$

Mean corpuscular hemoglobin (MCH, pg):

$$MCH = (Hb \times 10) \ / \ RBC$$

Mean corpuscular hemoglobin concentration (MCHC, g dL^{-1}):

$$MCHC = (Hb \times 100) \ / \ Hct$$

Immunological assays

Respiratory burst activity in peripheral blood samples was determined using the nitroblue tetrazolium (NBT) reduction assay (Sigma–Aldrich, St. Louis, MO, USA), following the method described by Siwicki et al. [19]. This assay was used to assess the oxidative activity of phagocytic cells as an indicator of innate immune function.

Lysozyme activity (LA) was measured using the turbidimetric method described by Ellis [20]. Myeloperoxidase (MPO) activity was determined according to the procedure reported by Sahoo et al. [21]. All immunological assays were performed using blood samples collected at each sampling time point.

RNA extraction and gene expression analysis

Total RNA was isolated from spleen, intestine, and head kidney tissues using a Tissue Total RNA Extraction Kit (FavorPrep™, Cat. No. FATRK 001-1), according to the manufacturer's instructions. RNA quality and concentration were assessed spectrophotometrically by measuring absorbance ratios at 260 and 280 nm (A260/A280) using a microplate reader (Epoch™, Agilent). RNA samples were subsequently diluted to a final concentration of 15 ng and used for complementary DNA (cDNA) synthesis. cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems™), following the manufacturer's protocol. The synthesized cDNA was stored at −20°C until further analysis. Gene expression analyses were conducted using a quantitative real-time PCR (qRT-PCR) detection system (Bio-Rad, USA). Specific primer sequences and corresponding references for all target genes are listed in Table 3.

The relative expression levels of immune-related genes, including *IL-1β, TNF-α, IFN-1, COX-2, IL-6, TGF-β, IL-10*, and *IL-4*, were calculated using the $2^{-\Delta\Delta Ct}$ method, with βeta-actin (*β-act*) used as the reference gene. Briefly, qPCR reactions were performed in a total volume of 20 μL, containing 10 μL of SsoAdvanced™ Universal SYBR® Green Supermix (2 ×), 1 μL of each primer (final concentration 250 nM), 6 μL of nuclease-free water, and 2 μL of cDNA (15 ng).

Amplification was carried out with an initial denaturation step at 95°C for 30 s, followed by 40 cycles of denaturation at 95°C for 15 s and annealing/extension at 60°C for 30 s. All reactions were performed in triplicate.

Melt curve analysis was performed after each qRT-PCR run to confirm amplification specificity. Primer efficiency was evaluated before gene expression analysis, and only primers showing acceptable amplification performance were used for relative quantification.

Digestive enzyme analyses

Digestive enzyme analyses were conducted at the end of the feeding trial. Prior to sampling, fish were fasted for 24 h and subsequently euthanized using an anesthetic solution of 2-phenoxyethanol (Sigma-Aldrich™, Cat. No. 7699) at a concentration of 0.2–0.4 mL L^{-1}. Intestinal and stomach tissues were collected from three randomly selected fish per tank.

Following the method described by Yılmaz et al. [22], collected tissues were diluted tenfold with distilled water, homogenized, and centrifuged at 20,000 × g for 45 min at 4°C. The resulting supernatants were carefully collected and stored at −80°C until further enzymatic analyses. Protein content required for the calculation of digestive enzyme activities was determined according to the method of Bradford [23].

Enzyme activities were expressed as units per milligram of protein (U mg^{-1}). Spectrophotometric measurements were performed using a microplate reader (Thermo Scientific Multiskan™ GO) and calculated according to the methods described by Bernfeld [24] and Worthington [25] for amylase, Erlanger et al. [26] for trypsin, Worthington [25] for pepsin, and Furné et al. [27] for lipase.

Table 3
Gene specific primers with their sequences and references used for qRT-PCR in the study.

Gene	Primer	Primer sequence (5'-3')	Accession No.
β-actin	F:	ATGGAAGGTGAAATCGCC	AF157514
	R:	TGCCAGATCTTCTTCATG	
IL-1β	F:	CGTCACTGACTCTGAGAACAAGT	NM001124347
	R:	TGGCGTGCAGCTCCATAG	
TNF-α	F:	GGATGACGGCCAGGCTTTT	AJ278085
	R:	TGTGTGGGATGAGGATTTGGTT	
IFN-1	F:	AGAATGCCCCAGTCCTTTTCC	AJ580911
	R:	GACTTTGTCCTCAAACTCAGCATCA	
COX-2	F:	ACAAAGGGTTGTGGAACGTC	AJ238307
	R:	CCAAATGTGAGCGGGGTGTC	
IL-6	F:	TGAGGCACAAAGCTTCTC	DQ866150
	R:	TCTTCAGCACGTTAAGGC	
TGF-β	F:	GGAAGAAACGACAAACCACTACTGA	NM001124347
	R:	GTTTTCGCACACAGCAACTCT	
IL-10	F:	GCTGGACGAAGGGATTCTACA	AB118099
	R:	AACTTCAGGATGCTGTCCATAGC	
IL-4	F:	ATGTTTCTATTGATTATTATATTCACAAC	KY421676.1
	R:	TCACACACAATGATAAGAGCTGT	

Antioxidant enzyme analyses

Antioxidant defense status was evaluated by determining the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx), together with the level of lipid peroxidation, assessed by malondialdehyde (MDA) content. Liver tissues were aseptically collected using sterile dissection instruments, transferred into individual Eppendorf tubes, and stored at −80°C until analysis.

Prior to analysis, liver samples were washed with sterile physiological saline, weighed (0.1 g), and homogenized in 1 mL of phosphate buffer containing EDTA. The homogenates were centrifuged at 20,000 × g for 45 min, and the resulting supernatants were carefully collected for subsequent analyses.

Antioxidant enzyme activities and lipid peroxidation levels were measured using commercially available assay kits according to the manufacturers' instructions: CAT (Cayman Chemical, Product No. 707002), GPx (Cayman Chemical, Product No. 703102), SOD (Sigma-Aldrich, Kit No. 19160), and MDA (Cayman Chemical, Product No. 10009055).

Challenge test

A 10-day bacterial challenge test was conducted to evaluate the resistance of rainbow trout (*Oncorhynchus mykiss*) to *Yersinia ruckeri* infection. Previously decribed Y. *ruckeri* bacteriaal strain [15] was inoculated into TSB, and incubated at 28°C for a day. The fish were intraperitoneally injected with the *Y. ruckeri* suspension that previously determined as the lethal $dose_{50}$ at a concentration of 1×10^8 CFU mL^{-1}, using an injection volume of 0.1 mL per fish. Fish in the control group received an equal volume (0.1 mL) of sterile phosphate-buffered saline (PBS). Mortality was recorded daily throughout the challenge period, and cumulative survival rates were calculated at the end of the experiment.

Statistical analysis

All data were analyzed using SPSS software (version 22.0) and are presented as mean ± standard error (SEM). The experiment was conducted with three replicate tanks per dietary treatment. For growth performance variables, fish in each tank were bulk-weighed and the tank was considered the experimental unit. At each sampling point, three fish were collected from each replicate tank, resulting in nine fish per treatment for hematological, immunological, enzymatic, and gene expression analyses. Differences among experimental groups were evaluated using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test when significant differences were detected. Statistical significance was accepted at $p < 0.05$. Prior to ANOVA, data were examined for normality and homogeneity of variances.

Results

GC-MS characterization of garden cress seed oil and pulp ethanolic extract

GC-MS analysis provided a clearer chemical characterization of both feed additives. In the cold-pressed garden cress seed oil, the major detected compounds were oleic acid (26.61 %), 2-hexadecen-1-ol, 3,7,11,15-tetramethyl- (25.11 %), pentacosane (6.38 %), 1,13-

tetradecadien-3-one (6.16 %), palmitic acid (4.22 %), and 1-eicosanol (3.14 %). In the pulp ethanolic extract, the predominant detected compounds were benzyl nitrile (52.05 %), benzene (isothiocyanatomethyl)- (13.92 %), acetophenone <3′,4′-dimethoxy-> (10.13 %), cis-vaccenic acid (1.82 %), palmitic acid (1.07 %), and 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (0.96 %). These data were included to better describe the chemical profiles of the additives used in the feeding trial and to improve the reproducibility of the dietary supplementation procedure. These findings indicate that the seed oil and pulp extract differed markedly in their chemical composition.

Growth performance

Growth performance parameters were evaluated based on changes in body weight and feed utilization during the feeding trial. Significant differences were observed among dietary treatments for all growth-related parameters (Table 4).

Final body weight differed significantly among the experimental groups ($p < 0.05$). The highest final body weight was recorded in the LS1 group, whereas the lowest value was observed in the LS05 group. A similar pattern was evident for weight gain percentage. Fish fed the LS1 diet exhibited significantly higher weight gain compared with the control and other dietary treatments ($p < 0.05$), while the LS05 group showed a marked reduction in weight gain.

Feed conversion ratio (FCR) was also significantly affected by dietary treatments ($p < 0.05$). The most efficient feed utilization, indicated by the lowest FCR value, was observed in the LSE05 group, whereas the control group exhibited the highest FCR. Specific growth rate (SGR) showed a comparable response to dietary supplementation ($p < 0.05$). The highest SGR was recorded in the LS1 group, consistent with its superior final body weight and weight gain, while the lowest SGR was observed in the LS05 group.

Overall, dietary supplementation with garden cress seed oil or its pulp extract influenced growth performance in a generally dose-related manner, with the most pronounced improvements in final body weight, weight gain, and SGR observed in the LS1 treatment.

Hematological parameters

Hematological parameters were significantly influenced by dietary supplementation with garden cress seed oil and its pulp extract (Table 5). Red blood cell (RBC) counts differed significantly among dietary treatments ($p < 0.05$). A similar trend was observed for hemoglobin (Hb) concentrations, which were significantly higher in several supplemented groups compared with the control group ($p < 0.05$).

Hematocrit (Hct) values were significantly elevated in the LS01 group; however, this effect was evident only at day 7 of sampling ($p < 0.05$). Mean corpuscular volume (MCV) values increased significantly toward the end of the experimental period ($p < 0.05$). Mean corpuscular hemoglobin (MCH) values were also significantly affected by dietary treatments and showed significant differences at both the initial and final sampling points ($p < 0.05$). In addition, mean corpuscular hemoglobin concentration (MCHC) values were significantly higher in fish fed LSE-supplemented diets than in the control group ($p < 0.05$).

Immunological assays

Respiratory burst activity showed significant variation among dietary treatments (Fig. 1). An early increase was observed in the LSE01 group. More pronounced responses emerged at later sampling times, particularly in fish fed diets containing the pulp ethanolic extract. At the end of the experiment, respiratory burst activity remained significantly elevated in the LSE05 and LSE1 groups compared with the control ($p < 0.05$).

Lysozyme activity also differed significantly among dietary treatments throughout the experimental period (Fig. 1). Increased lysozyme activity was evident from the early sampling times in fish fed diets supplemented with garden cress seed oil and the pulp ethanolic extract. By the end of the experiment, lysozyme activity was significantly higher in all supplemented groups than in the control group ($p < 0.05$).

Myeloperoxidase (MPO) activity varied significantly among dietary treatments (Fig. 1). Dietary inclusion of garden cress seed oil and the pulp ethanolic extract resulted in a consistent reduction in MPO activity. This effect was more pronounced in fish fed diets containing the pulp extract. At the end of the experiment, MPO activity was significantly lower in all supplemented groups compared with the control, with the lowest values recorded in the LSE05 and LSE1 groups ($p < 0.05$).

Gene expression

Overall, dietary supplementation with garden cress seed oil and, more markedly, the pulp ethanolic extract modulated immune-related gene expression in a tissue- and time-dependent manner. The most consistent responses were observed in the LSE-supplemented groups, particularly for pro-inflammatory genes such as *IL-1β, TNF-α, IL-6*, and *COX-2*, as well as for regulatory cytokines including *TGF-β, IL-10*, and *IL-4*. In general, the spleen and kidney responded more clearly than the intestine, suggesting a stronger immunomodulatory effect of the pulp extract than of the seed oil under the present experimental conditions.

IL-1β gene expression patterns in spleen, intestine, and kidney tissues at different sampling times are shown in Fig. 2. In the spleen, *IL-1β* expression was significantly increased only in the LS01 group on day 7 of the feeding trial ($p < 0.05$). On day 14, *IL-1β* expression was significantly higher in the LSE05, LSE1, and LS1 groups than in the control group ($p < 0.05$). By the end of the study (day 21), *IL-1β* expression was significantly elevated in all experimental groups except LS01 compared with the control ($p < 0.05$). In the intestine, *IL-1β* gene expression was significantly higher in the LSE05 and LSE1 groups on day 7 than in all other groups ($p < 0.05$). However, no significant differences among dietary treatments were detected on days 14 and 21 ($p > 0.05$). In the kidney, no significant differences in *IL-1β* expression were observed among groups on day 7 ($p > 0.05$). In contrast, on days 14 and 21, *IL-1β* expression was significantly higher in the LS1 group and all LSE groups than in the control group ($p < 0.05$).

Table 4
Effect of cress seed (*Lepidium sativum*) oil and pulp extracts on the growth performance of rainbow trout fry.

	Growth Performance						
	Control	LS01	LS05	LS1	LSE01	LSE05	LSE1
IW (g)	5.17±0.52	5.14±0.18	5.31±0.24	5.48±0.17	5.49±0.11	5.46±0.29	5.79±0.13
FW (g)	10.44±0.65^{b}	11.14±0.79^{b}	10.22±0.42^{b}	14.24±1.2^{a}	11.47±0.23^{b}	12.81±0.59^{ab}	12.88±1.38^{ab}
WG (%)	101.82±2.22 ^{de}	116.59±4.08 ^{cd}	92.45±6.34 ^{e}	160.01±12.41^{a}	108.99±4.34 ^{d}	134.58±0.22^{b}	122.39±0.11^{c}
FCR	1.30±0.33^{c}	1.14±0.22^{b}	1.22±0.14^{bc}	1.06±0.05^{ab}	1.18±0.13^{b}	0.95±0.87^{a}	1.10±0.20^{b}
SGR (% day^{-1})	3.34±0.28^{b}	3.68±0.95^{ab}	3.12±0.12^{b}	4.55±0.03^{a}	3.51±0.24^{ab}	4.06±0.44^{a}	3.81±0.14^{ab}

Values are presented as mean ± SE. Different lowercase letters within the same column indicate significant differences among groups ($p < 0.05$). Diets were supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1}; the control group (C) received the basal diet without supplementation. IW, initial weight; FW, final weight; WG, weight gain; FCR, feed conversion ratio; SGR, specific growth rate.

Table 5
Hematological profiles of the rainbow trout fry fed with different doses of cress seed (*Lepidium sativum*) oil and extract supplemented diets for 21 days.

Day		RBC (10^6/mm^3)	Hb (g/dL)	Hct (%)	MCV (μm^3)	MCH (pg)	MCHC (%)
7	Control	0.36±0.03^{b}	13.50±0.87^{b}	7.47±0.82^{c}	208.17±3.76	378.07±25.78^{c}	183.40±15.55^{b}
	LS01	0.56±0.11^{a}	15.00±1.57^{ab}	11.63±2.46^{a}	209.40±1.00	283.70±34.70^{d}	136.37±17.64^{b}
	LS05	0.45±0.06^{ab}	16.10±0.25^{a}	9.53±1.16^{b}	210.20±1.47	374.33±50.52^{c}	179.47±23.66^{b}
	LS1	0.18±0.01^{c}	13.90±1.06^{b}	3.50±0.15^{d}	194.74±3.04	779.93±86.52^{a}	399.32±38.42^{a}
	LSE01	0.18±0.03^{c}	11.17±0.77^{c}	3.47±0.62^{d}	192.06±2.44	646.73±91.45^{b}	336.99±47.90^{a}
	LSE05	0.16±0.01^{c}	11.10±1.16^{c}	3.20±0.23^{d}	195.56±4.44	690.74±103.75^{b}	355.29±59.13^{a}
	LSE1	0.21±0.05^{c}	13.10±0.21^{bc}	4.17±1.07^{d}	196.89±3.17	647.30±53.45^{b}	329.70±31.73^{a}
14	Control	0.39±0.04^{ab}	12.10±0.00^{b}	7.90±0.82^{a}	205.43±0.12	320.13±35.56^{b}	156.80±17.82^{b}
	LS01	0.43±0.05^{a}	11.67±0.77^{b}	8.67±1.14^{a}	203.97±0.59	278.60±21.56^{b}	137.53±11.54^{b}
	LS05	0.42±0.04^{a}	13.30±0.81^{ab}	8.67±0.87^{a}	207.80±2.37	323.17±29.18^{b}	155.70±14.60^{b}
	LS1	0.42±0.08^{a}	14.40±1.05^{a}	8.63±1.79^{a}	205.20±3.40	362.47±55.01^{ab}	178.12±29.31^{ab}
	LSE01	0.28±0.03^{c}	11.00±0.40^{bc}	5.67±0.55^{b}	201.23±0.63	396.67±45.27^{ab}	198.33±22.64^{a}
	LSE05	0.25±0.04^{c}	11.43±2.27^{b}	4.83±0.84^{b}	196.07±5.11	457.63±27.46^{a}	234.63±8.42^{a}
	LSE1	0.28±0.07^{c}	13.73±2.33^{ab}	5.57±1.34^{b}	200.70±2.12	386.75±90.10^{ab}	192.60±42.78^{a}
21	Control	0.52±0.05^{ab}	12.10±0.36^{bc}	10.57±0.96^{a}	145.17±6.01^{b}	238.20±21.71^{c}	139.17±27.99^{c}
	LS01	0.58±0.11^{a}	14.50±0.29^{ab}	11.97±2.11^{a}	209.07±2.37^{a}	271.23±53.83^{c}	129.57±24.37^{c}
	LS05	0.32±0.01^{b}	13.57±1.47^{b}	6.40±0.25^{b}	143.67±6.18^{b}	427.07±35.01^{b}	211.43±18.52^{b}
	LS1	0.16±0.05^{c}	14.47±0.35^{ab}	3.20±1.01^{bc}	205.77±1.33^{a}	622.71±1.87^{a}	305.61±0.40^{a}
	LSE01	0.26±0.05^{bc}	15.13±0.45^{a}	5.13±0.92^{b}	201.00±1.22^{a}	499.70±13.39^{b}	251.90±8.41^{ab}
	LSE05	0.27±0.06^{bc}	14.63±1.84^{ab}	5.57±1.43^{b}	206.95±0.53^{a}	497.75±41.19^{b}	241.45±19.23^{ab}
	LSE1	0.10±0.01^{c}	11.70±0.82^{c}	1.63±0.23^{c}	201.75±1.22^{a}	509.44±3.28^{b}	252.25±1.72^{ab}

Data are presented as mean ± SE of three replicates (n = 3). Means within the same sampling time bearing different superscript letters in a row are significantly different ($p < 0.05$). Diets were supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1}; the control group (C) received the basal diet without supplementation. RBC, red blood cells; Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

TNF-α gene expression results are shown in Fig. 3. In the spleen, *TNF-α* expression showed an increasing trend in all experimental groups on day 7 compared with the control, although the differences were not statistically significant ($p > 0.05$). On days 14 and 21, *TNF-α* expression was significantly elevated in all experimental groups, with more pronounced increases in the LSE-supplemented groups ($p < 0.05$). In the intestine, *TNF-α* expression was significantly increased in all experimental groups at all sampling times ($p < 0.05$). In the kidney, *TNF-α* expression was significantly elevated in all LSE groups at all sampling times compared with the control group ($p < 0.05$).

IFN-1 gene expression levels measured on days 7, 14, and 21 are shown in Fig. 4. In the spleen, *IFN-1* expression was significantly increased in all LSE-fed groups at all sampling times compared with the control group ($p < 0.05$). In the intestine, *IFN-1* expression was significantly elevated in all groups except LSE01 on day 7 ($p < 0.05$). This increase continued on days 14 and 21, particularly in the LS1 group and all LSE groups ($p < 0.05$). In the kidney, *IFN-1* expression was significantly increased in all experimental groups at all sampling times compared with the control, except for the LS01 group ($p < 0.05$).

COX-2 gene expression results are shown in Fig. 5. In the spleen, *COX-2* expression was significantly increased on days 7 and 21 in all experimental groups compared with the control group ($p < 0.05$). On day 14, *COX-2* expression in the LS05 group did not differ from the control, whereas all other experimental groups showed significantly elevated expression levels ($p < 0.05$). In the intestine, no significant differences in *COX-2* expression were observed among groups at any sampling time ($p > 0.05$). In the kidney, *COX-2* expression was significantly increased in all experimental groups on days 7 and 21, with particularly pronounced upregulation in the LSE05 and LSE1 groups ($p < 0.05$).

IL-6 gene expression patterns are shown in Fig. 6. In the spleen, *IL-6* expression was significantly increased in the higher-dose LSE groups at all sampling times compared with the control group ($p < 0.05$). No significant differences in *IL-6* expression were observed in the intestine across sampling times ($p > 0.05$). In the kidney, *IL-6* expression was significantly elevated on days 7, 14 and 21, with the highest levels observed in the LSE-supplemented groups compared with all other groups ($p < 0.05$).

TGF-β gene expression results are shown in Fig. 7. In the spleen, *TGF-β* expression was significantly higher in the LSE05 and LSE1 groups on days 7 and 14 ($p < 0.05$). On day 21, *TGF-β* expression was significantly increased in all experimental groups except LSE01 compared with the control group ($p < 0.05$). In the intestine, no significant differences were detected on day 7; however, on days 14 and 21, *TGF-β* expression was significantly increased in all groups except LS01 ($p < 0.05$). In the kidney, *TGF-β* expression was significantly elevated in all experimental groups at all sampling times compared with the control group ($p < 0.05$).

IL-10 gene expression results are shown in Fig. 8. In kidney, *IL-10* expression was significantly increased on day 7 in the LS1, LSE05, and LSE1 groups ($p < 0.05$). Although *IL-10* expression remained elevated at the end of the study, these increases were not statistically significant compared with the control group ($p > 0.05$). In intestinal, *IL-10* expression was significantly increased on day 7 in the LSE-supplemented groups ($p < 0.05$), whereas no significant differences were observed on days 14 and 21 ($p > 0.05$). In spleen, *IL-10* expression was significantly increased in all experimental groups except LS1 on day 7 compared with the control group ($p < 0.05$).

IL-4 gene expression results are shown in Fig. 9. *IL-4* expression was significantly higher in the LSE05 and LSE1 groups, and marked increases observed in all LSE-supplemented groups across all sampled tissues and sampling times compared with the control group ($p < 0.05$).

Digestive enzymes

In the present study, dietary supplementation resulted in significant changes in digestive enzyme activities among the experimental groups (Table 6). These changes were enzyme-specific rather than reflecting a uniform increase across all digestive enzymes.

Pepsin, a key proteolytic enzyme responsible for protein digestion in the stomach under acidic conditions generated by hydrochloric acid secretion [28], showed significantly higher activity in the LS1, LSE05, and LSE1 groups compared with the control group ($p < 0.05$). Notably, increased pepsin activity was predominantly associated with diets containing higher inclusion levels of the pulp ethanolic extract. Although elevated pepsin activity may indicate enhanced gastric protein

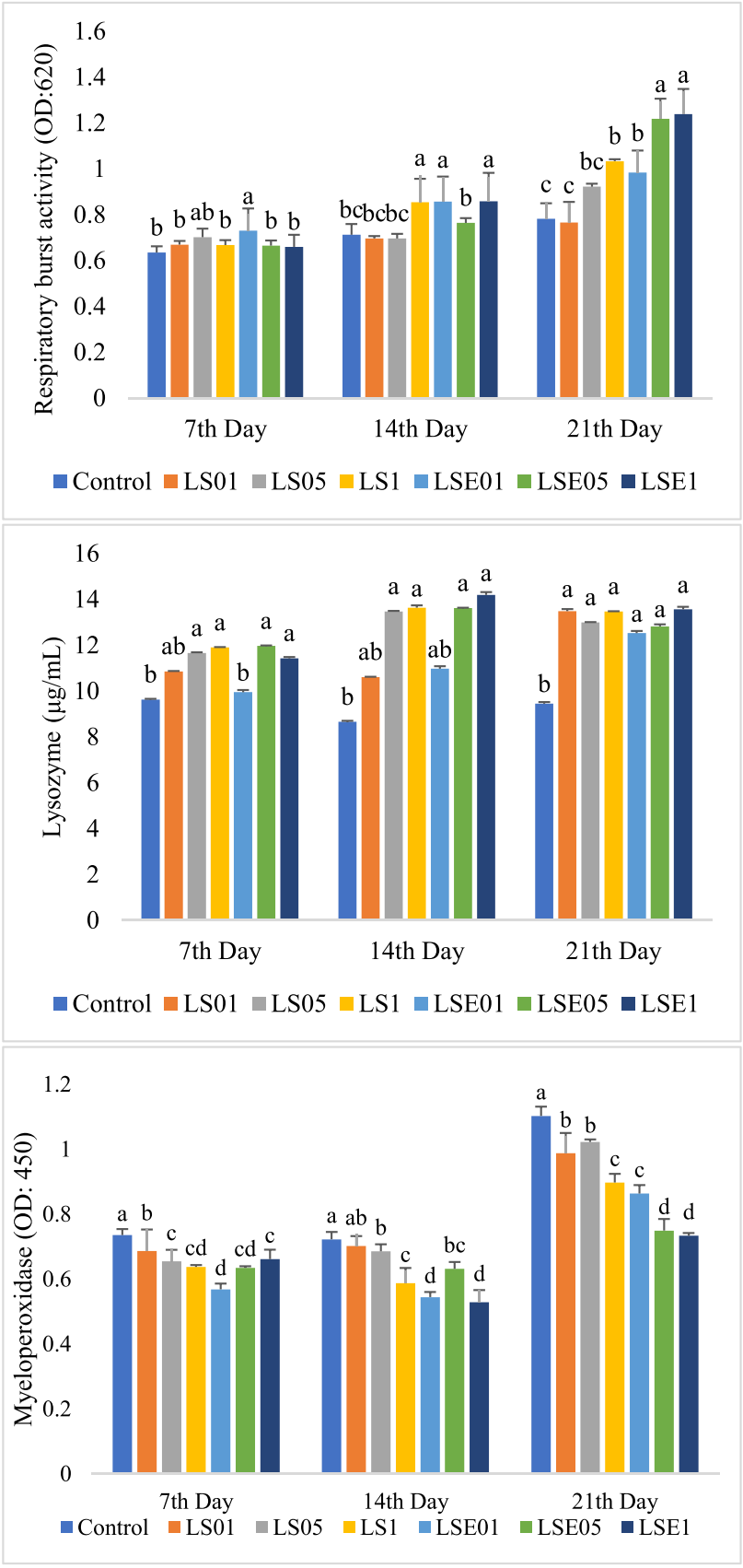

Fig. 1. Changes in respiratory burst activity (mg NBT mL^{-1}), lysozyme activity (µg mL^{-1}), and myeloperoxidase activity (U L^{-1}) in rainbow trout fed diets supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1} for 21 days. Different letters indicate significant differences among groups ($p < 0.05$).

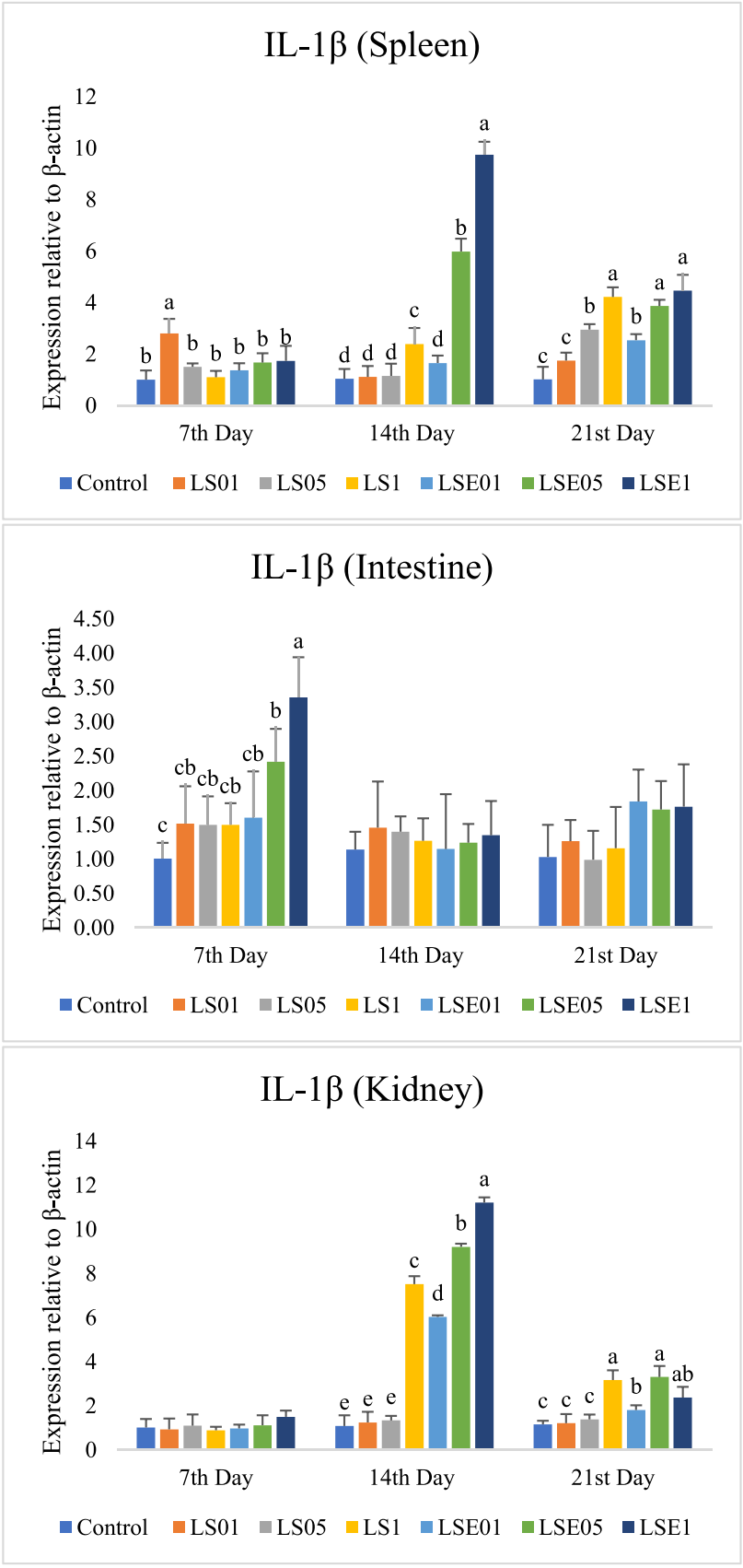

Fig. 2. Relative expression of IL-1β in spleen, intestine, and head kidney tissues of rainbow trout fed diets supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1} for 21 days. Different letters indicate significant differences among groups ($p < 0.05$).

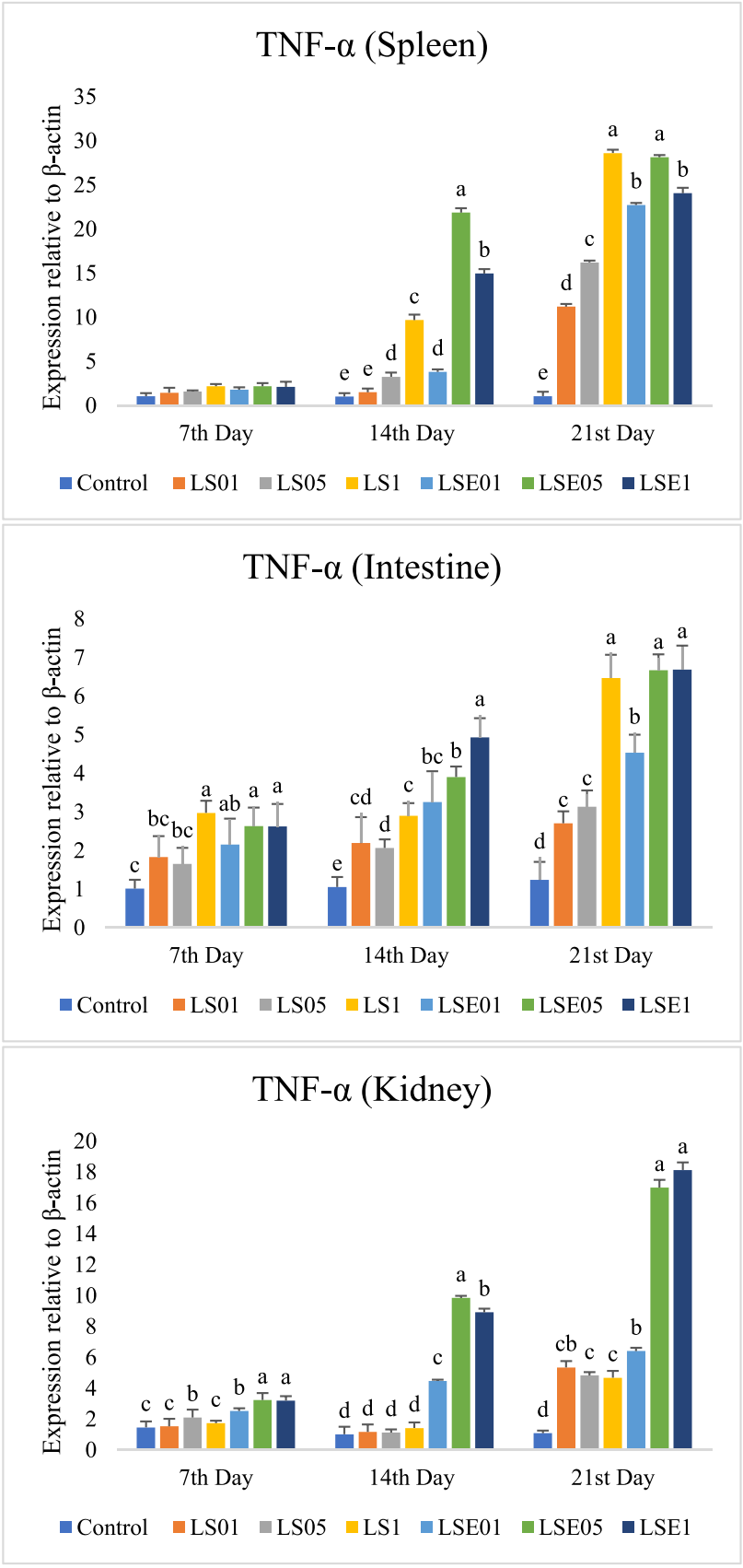

Fig. 3. Relative expression of TNF-α in spleen, intestine, and head kidney tissues of rainbow trout fed diets supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1} for 21 days. Different letters indicate significant differences among groups ($p < 0.05$).

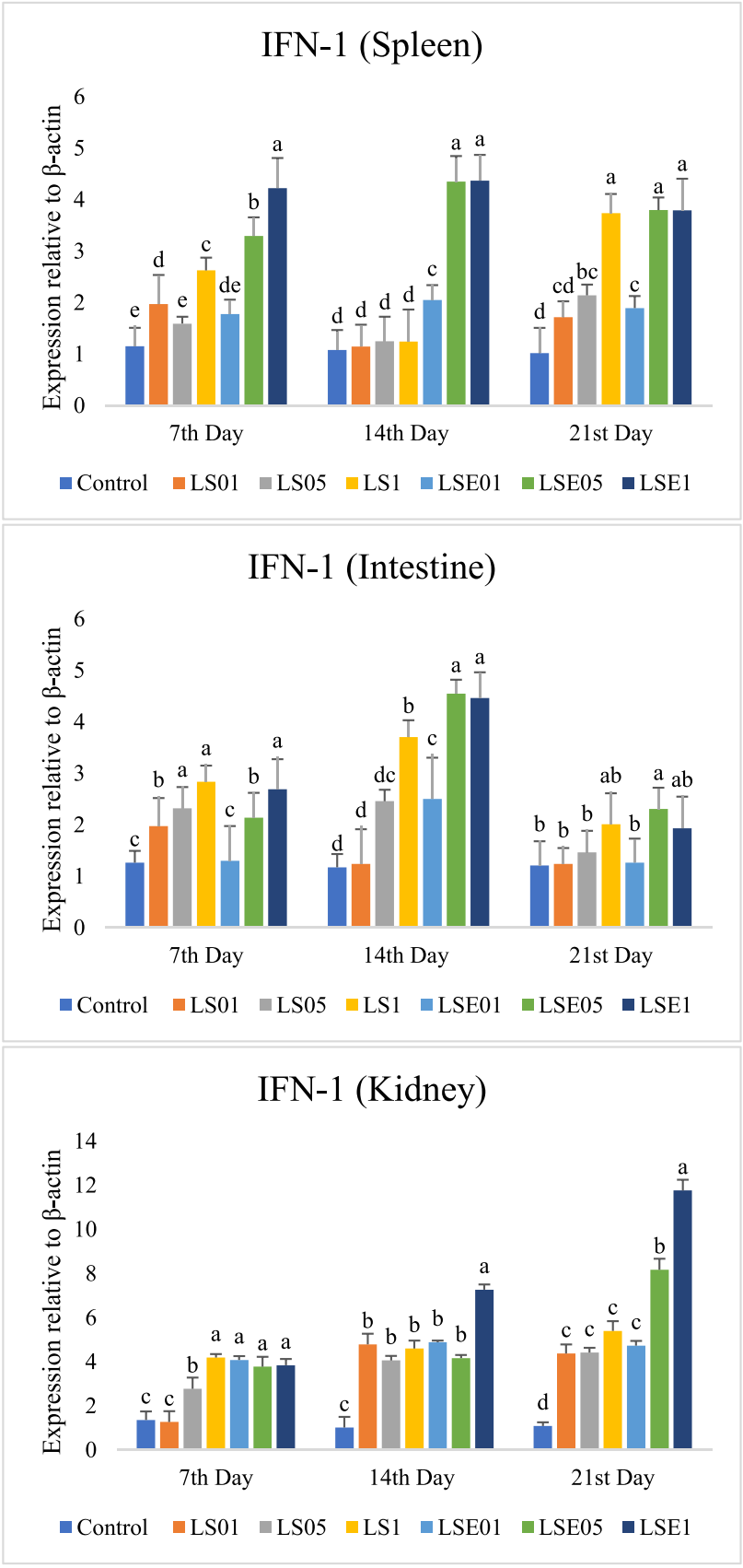

Fig. 4. Relative expression of IFN-1 in spleen, intestine, and head kidney tissues of rainbow trout fed diets supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1} for 21 days. Different letters indicate significant differences among groups ($p < 0.05$).

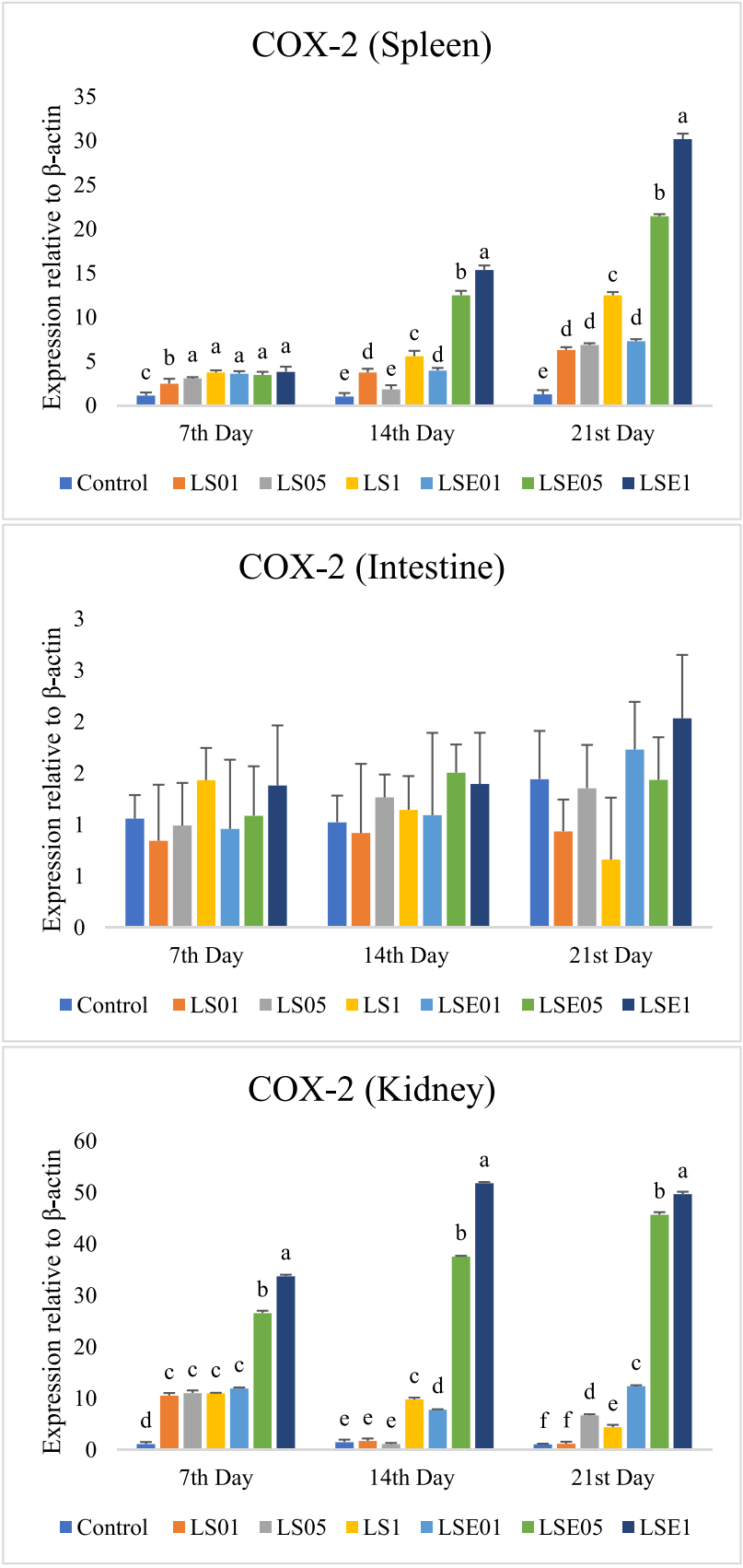

Fig. 5. Relative expression of COX-2 in spleen, intestine, and head kidney tissues of rainbow trout fed diets supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1} for 21 days. Different letters indicate significant differences among groups ($p < 0.05$).

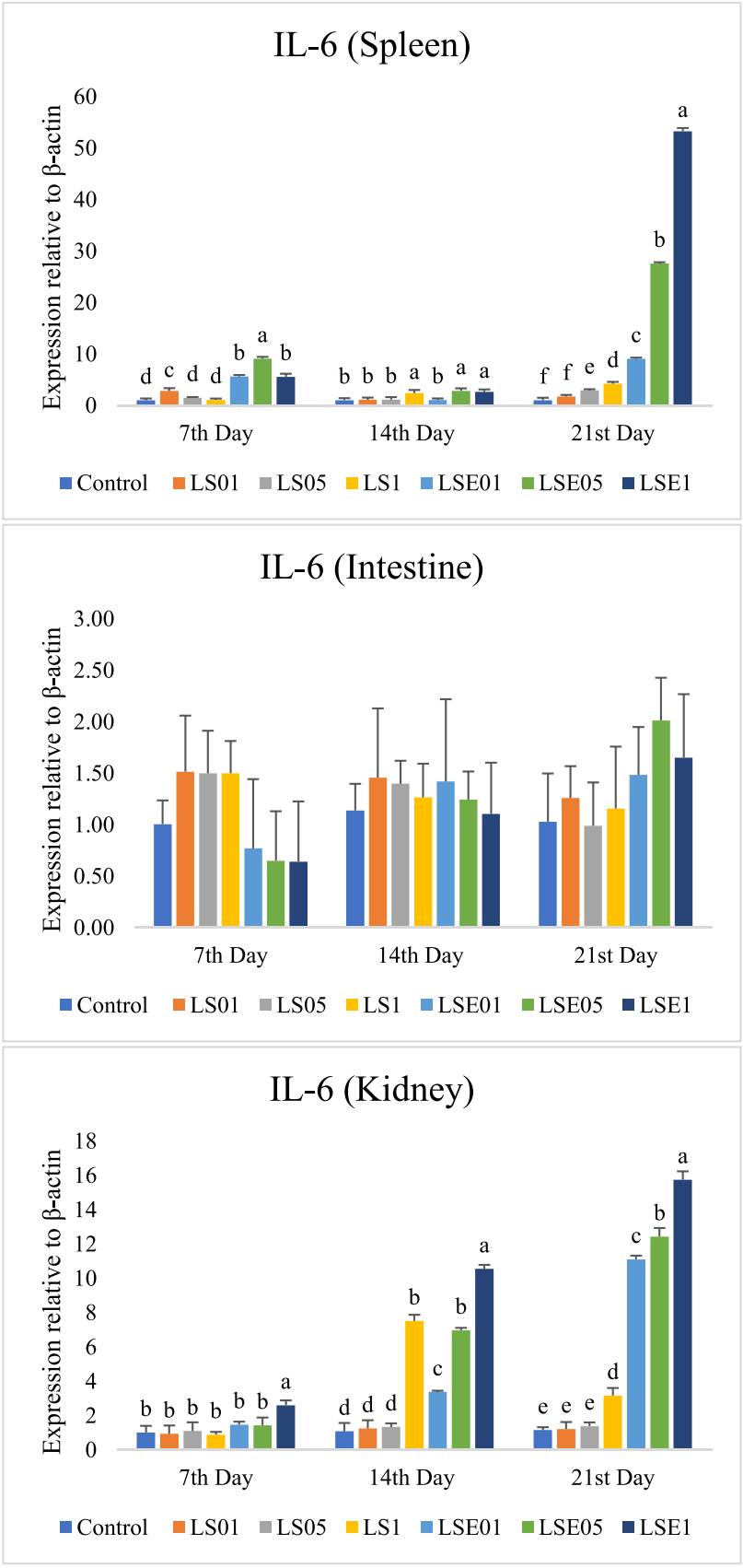

Fig. 6. Relative expression of IL-6 in spleen, intestine, and head kidney tissues of rainbow trout fed diets supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1} for 21 days. Different letters indicate significant differences among groups ($p < 0.05$).

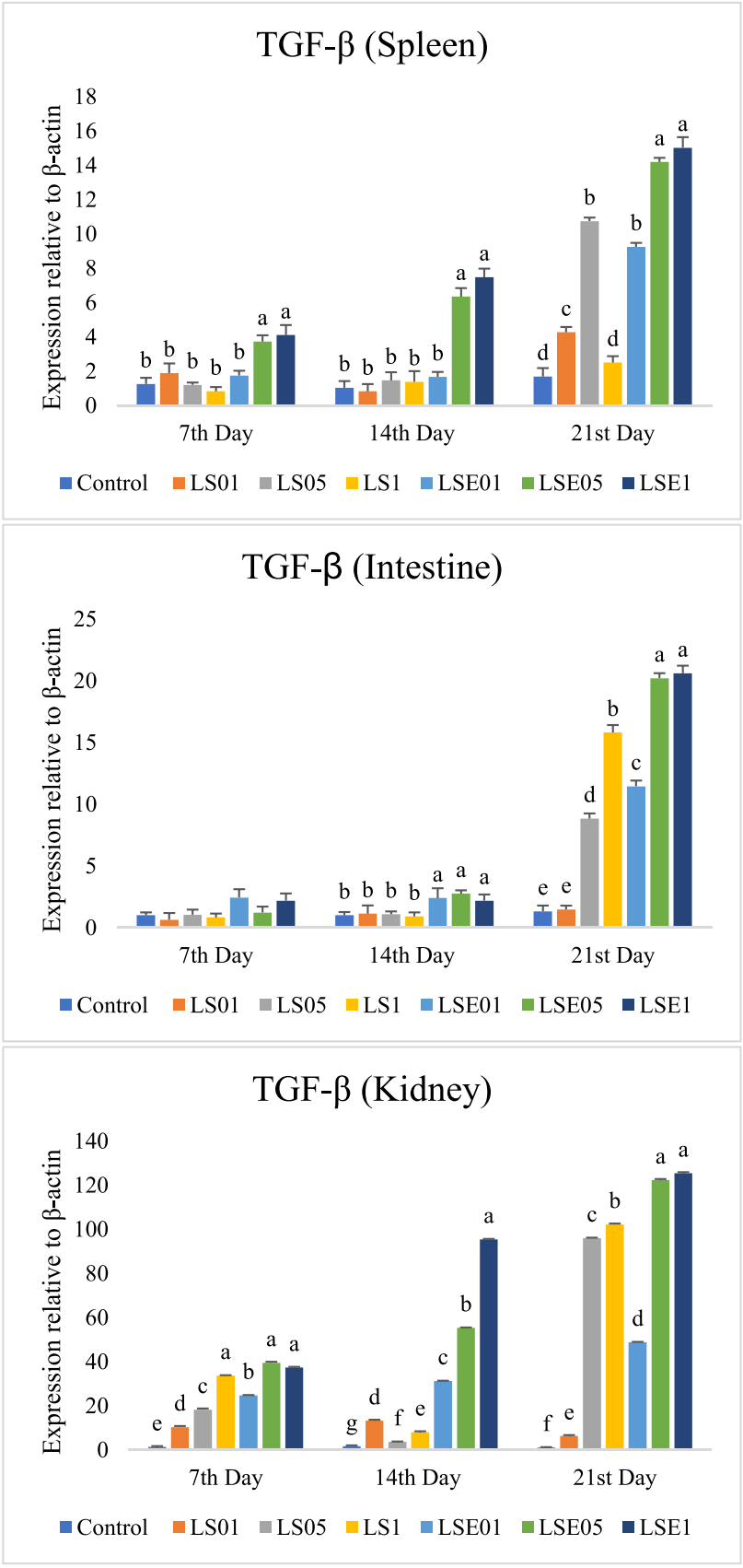

Fig. 7. Relative expression of TGF- β in spleen, intestine, and head kidney tissues of rainbow trout fed diets supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1} for 21 days. Different letters indicate significant differences among groups ($p < 0.05$).

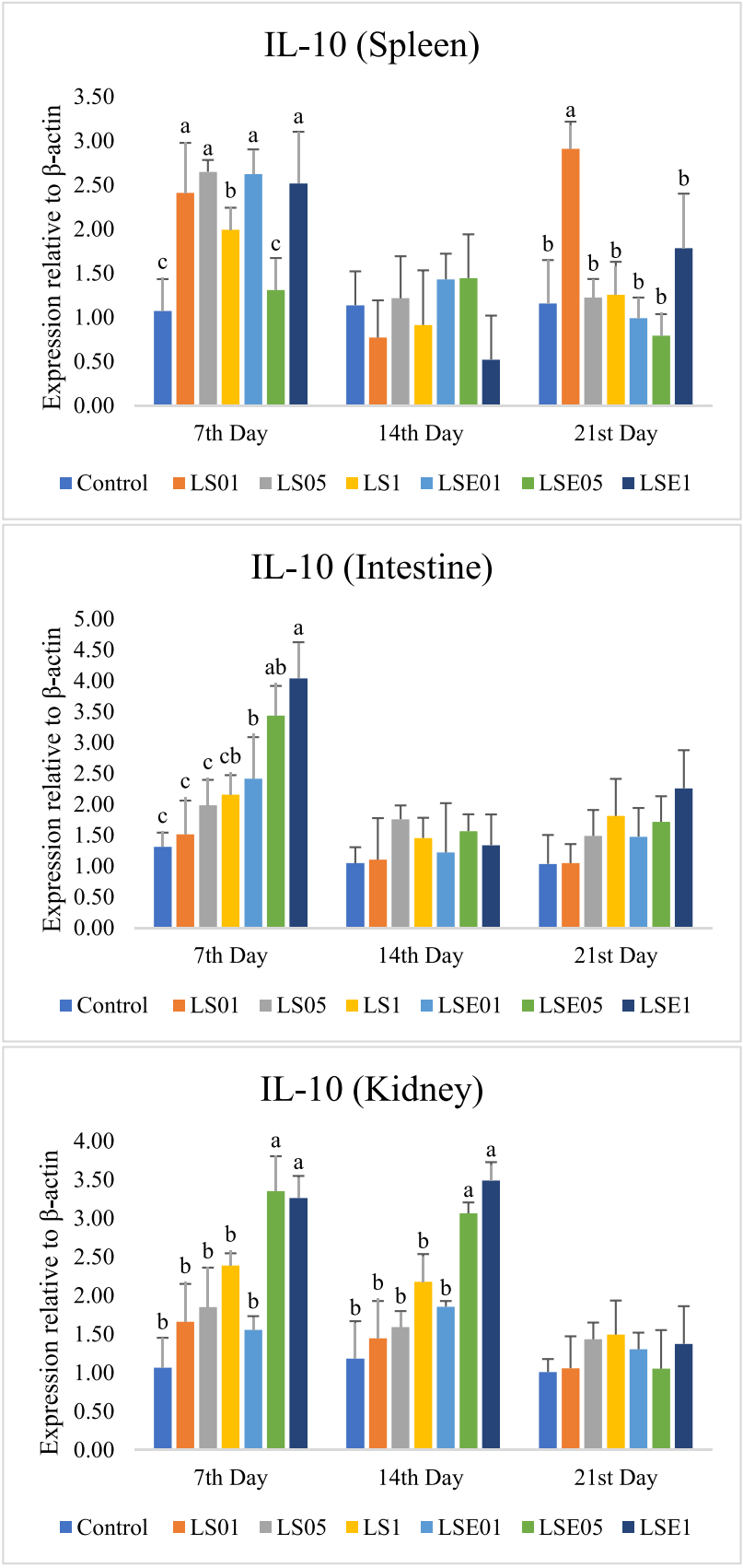

Fig. 8. Relative expression of IL-10 in spleen, intestine, and head kidney tissues of rainbow trout fed diets supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1} for 21 days. Different letters indicate significant differences among groups ($p < 0.05$).

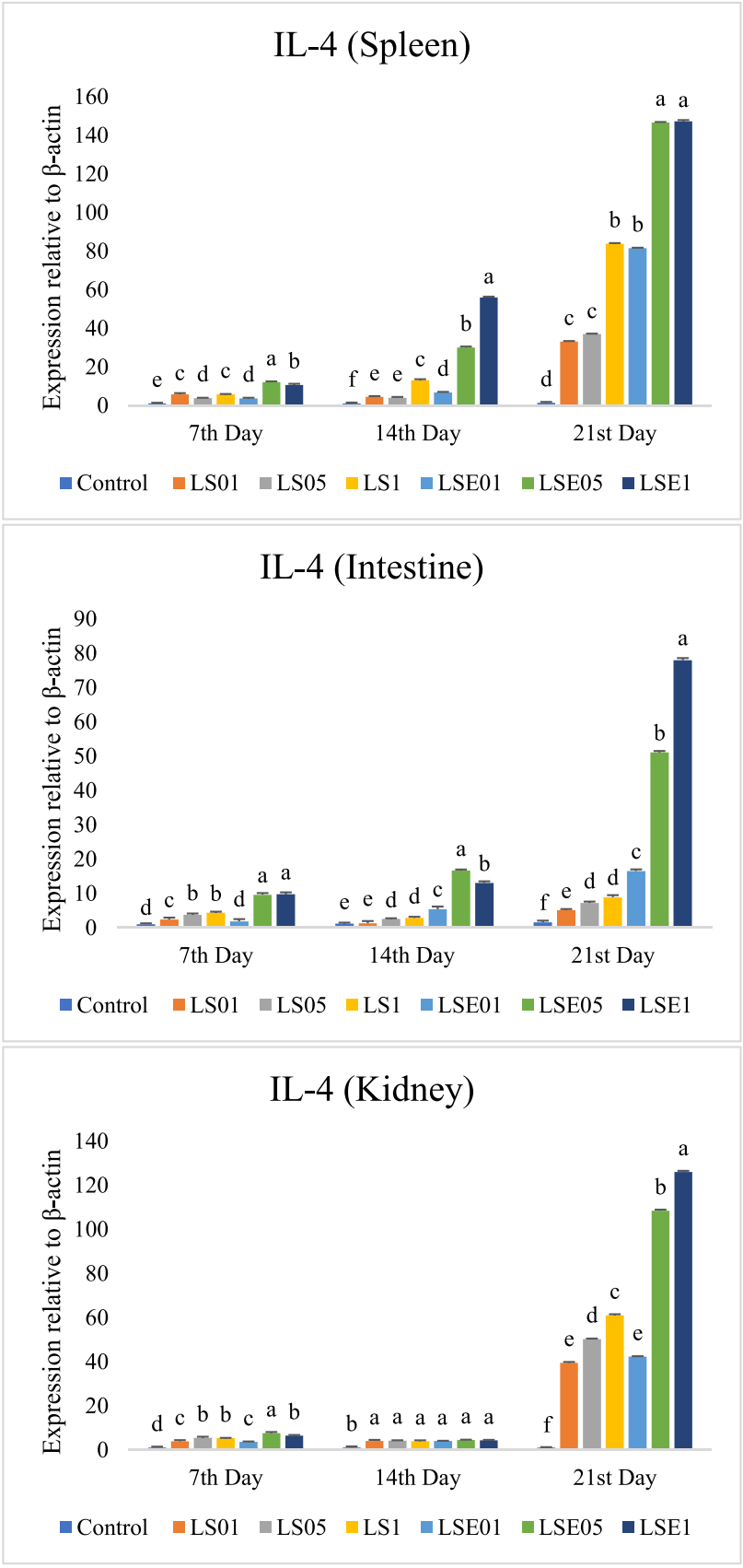

Fig. 9. Relative expression of IL-4 in spleen, intestine, and head kidney tissues of rainbow trout fed diets supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1} for 21 days. Different letters indicate significant differences among groups ($p < 0.05$).

Table 6
The digestive enzyme activities in rainbow trout fry fed with different doses of cress seed (*Lepidium sativum*) oil and extract supplemented diets for 21 days.

	Lipase	Amylase	Trypsin	Pepsin
Control	0.039±0.007^{c}	0.008±0.006^{b}	0.133±0.51^{a}	2.565±0.466^{b}
LS01	0.040±0.003^{c}	0.016±0.000^{a}	1.052±0.783^{a}	4.047±0.163^{b}
LS05	0.056±0.029^{b}	0.011±0.002^{b}	2.990±0.307^{a}	6.655±0.677^{b}
LS1	0.041±0.007^{c}	0.016±0.000^{a}	5.976±0.244^{a}	11.54±0.727^{a}
LSE01	0.050±0.003^{c}	0.012±0.002^{b}	0.191±0.132^{a}	5.907±0.592^{b}
LSE05	0.039±0.002^{c}	0.014±0.001^{a}	2.319±0.125^{a}	11.835±0.76^{a}
LSE1	0.083±0.003^{a}	0.019±0.000^{a}	2.579±0.269^{a}	10.494±0.14^{a}

Values are presented as mean ± SE of 9 fish. Different lowercase letters within the same column indicate significant differences among groups ($p < 0.05$). Diets were supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1}; the control group (C) received the basal diet without supplementation.

digestion, direct effects on growth performance cannot be inferred solely from enzyme activity data.

Lipase activity was significantly increased in the LS05 and LSE1 groups compared with the control ($p < 0.05$), whereas no significant changes were observed in the remaining experimental groups ($p > 0.05$). Amylase activity was significantly higher in the LS01, LS1, LSE05, and LSE1 groups ($p < 0.05$), while no differences were detected among the other dietary treatments ($p > 0.05$). Trypsin activity showed an increasing trend in the LS1 group; however, this increase was not statistically significant ($p > 0.05$).

Antioxidant activity

SOD, CAT, and GPx activities differed significantly among dietary treatments (Table 7). CAT activity was significantly higher in the LS1, LSE01, and LSE1 groups compared with the control group ($p < 0.05$). SOD activity was significantly increased in the LS05, LS1, and LSE01 groups relative to the control ($p < 0.05$). GPx activity showed a similar pattern, with significantly higher values observed in the LSE05 and LSE1 groups compared with the control group ($p < 0.05$).

Lipid peroxidation levels, expressed as malondialdehyde (MDA) content, were significantly lower in the LS01, LS1, and LSE01 groups than in the control group ($p < 0.05$). Overall, antioxidant enzyme responses and lipid peroxidation levels varied in a treatment-dependent manner.

Table 7
Catalase (CAT), Superoxide dismutase (SOD), glutathione peroxidase (GPx), and lipid peroxidation (MDA) in rainbow trout juveniles fed diets containing different doses of cress seed (*Lepidium sativum*) oil and extract.

	CAT (consumed nmol H_2O_2/min/mg protein)	SOD (U/mg protein)	GPX (U/mg protein)	MDA malondialdehyde content nmol/g
Control	53.05±1.48^{c}	3.00 ±0.17^{b}	70.16±1^{c}	91.99±1.73^{a}
LS01	60.37±1.31^{a}	3.09 ±0.27^{b}	94.09 ±1.29^{b}	71.03±1.54^{b}
LS05	49.56±1.06^{b}	4.06 ±0.23^{a}	93.81 ±1.34^{b}	91.05±1.69^{a}
LS1	78.04±1.27^{a}	5.16 ±0.18^{a}	107.49 ±1.04^{b}	70.77±1.16^{b}
LSE01	68.17±1.59^{a}	4.93 ±0.38^{a}	111.96 ±1.44^{ab}	69.07±1.30^{b}
LSE05	56.27±1.85^{a}	3.62 ±0.30^{b}	128.24 ±0.96^{a}	91.66±1.56^{a}
LSE1	66.63±1.01^{a}	3.88 ±0.25^{b}	120.34 ±1.31^{a}	98.75±2.17^{a}

Values are presented as mean ± SE of 9 fish. Different lowercase letters within the same column indicate significant differences among groups ($p < 0.05$). Diets were supplemented with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1}; the control group (C) received the basal diet without supplementation.

Survival rate

At the end of the 10-day survival test following *Yersinia ruckeri* challenge, the highest survival rate was observed in the LSE1 group. Survival rates in the LSE1 and LS1 groups did not differ significantly from each other. All experimental groups exhibited significantly higher survival rates compared with the control group ($p < 0.05$) (Fig. 10).

Discussion

In the present study, garden cress (*Lepidium sativum*) seed oil and its pulp ethanolic extract were evaluated as dietary feed additives for rainbow trout. Dietary supplementation with both LS and LSE resulted in improved growth performance, while a marked reduction in feed conversion ratio (FCR) was particularly evident in the LSE05 group. In addition to growth-related improvements, enhanced digestive enzyme activities and modulation of immune responses were observed, indicating that garden cress-derived products exert multifaceted physiological effects in rainbow trout.

Growth performance was positively influenced by higher inclusion levels of LS and LSE, consistent with previous studies reporting improved growth following dietary administration of medicinal plant-derived additives [29–31]. Enhanced digestive enzyme activity, particularly pepsin activity, may partially explain the observed growth responses. Elevated pepsin activity in fish fed higher doses of the pulp ethanolic extract suggests improved gastric protein digestion; however, enzyme activity alone cannot fully account for growth enhancement, indicating that additional metabolic and immunological mechanisms may be involved.

Dietary supplementation also resulted in improved humoral immune responses, including enhanced respiratory burst and lysozyme activities. Similar immunostimulatory effects have been reported for various medicinal plants in aquaculture species. For instance, Altunoğlu et al. [32] demonstrated that *Nigella sativa* extract significantly increased respiratory burst activity and serum lysozyme levels in rainbow trout, while Bilen et al. [16] reported enhanced growth and immune responsiveness in trout fed *Capparis spinosa* extract. Likewise, Salem et al. [33] showed that dandelion and lichen extracts improved immune responses in fish. These findings support the use of medicinal plants as prophylactic agents that prime the innate immune system and enhance disease resistance.

The present study further demonstrated significant modulation of

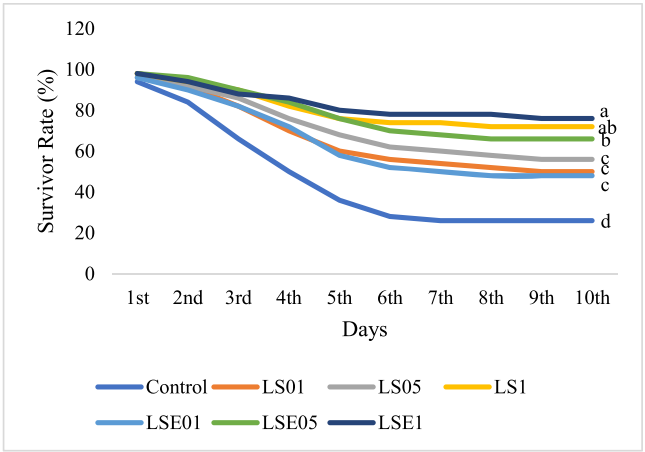

Fig. 10. Survival rate of rainbow trout following *Yersinia ruckeri* challenge after dietary supplementation with cress seed oil (LS) or its ethanolic extract (LSE) at 0.1, 0.5, and 1 g kg^{-1}. Different letters indicate significant differences among groups ($p < 0.05$).

cytokine gene expression, reflecting activation of innate immune pathways. *IL-1β*, a highly conserved proinflammatory cytokine in teleosts, plays a central role in the initiation of innate immune responses through leukocyte activation and pathogen recognition [34,35]. Elevated *IL-1β* and *TNF-α* expression levels, particularly in LSE-supplemented groups, suggest activation of early immune-related inflammatory signaling. Similar increases in *IL-1β* and *TNF-α* expression have been reported in fish fed diets supplemented with capsicum extract [12] and marine polysaccharides [36]. It should be noted, however, that increased expression of pro-inflammatory cytokines such as *IL-1β* and *TNF-α* does not necessarily indicate only a beneficial immune enhancement, since such changes may also reflect inflammatory activation. In the present study, these responses were measured before the bacterial challenge and in fish that showed no apparent clinical signs of disease during the feeding period. Therefore, the observed cytokine upregulation is more likely to reflect dietary immune stimulation or immune modulation rather than disease-associated inflammatory pathology. In addition, the concurrent regulation of anti-inflammatory cytokines such as *IL-10* and *TGF-β* suggests that these responses were controlled rather than excessive.

IFN-1, a key mediator of antiviral defense and immune regulation, showed sustained upregulation in spleen and kidney tissues of fish fed LSE diets. This pattern is consistent with previous reports demonstrating enhanced *IFN-1* expression following dietary administration of *Astragalus caudiculosus* extracts [37]. The observed *IFN-1* responses suggest that LSE supplementation may strengthen antiviral readiness and immune surveillance in rainbow trout.

COX-2 expression was also significantly elevated, particularly in kidney tissues of LSE05 and LSE1 groups. *COX-2* is an inducible enzyme involved in prostaglandin synthesis and inflammatory signaling, contributing to leukocyte migration and vascular permeability [38,39]. The coordinated upregulation of *IL-1β, TNF-α, IL-6*, and *COX-2* suggests activation of inflammatory signaling cascades, which may contribute to pathogen defense when appropriately regulated.

Anti-inflammatory cytokines were also modulated by dietary treatments. *TGF-β*, a pleiotropic cytokine with immunoregulatory and immunosuppressive functions [40], was significantly upregulated in spleen and kidney tissues of fish fed higher LSE doses, with intestinal expression also increased at later sampling times. Similar increases in *TGF-β* expression have been reported following dietary supplementation with *Nigella sativa* [32] and *Capsicum annuum* [12], indicating that phytogenic additives can balance pro- and anti-inflammatory immune responses.

IL-10, a key anti-inflammatory cytokine involved in suppressing excessive inflammatory reactions and oxidative stress [41–43], showed increased expression particularly in kidney tissues of LSE-fed fish during early sampling periods. This response may be linked to the enhanced antioxidant status observed in these groups. Similar *IL-10* induction has been reported in rainbow trout treated with black mustard oil [44] and *Astragalus caudiculosus* supplementation [37].

IL-4 expression was also elevated, particularly in LSE05 and LSE1 groups across multiple tissues. *IL-4* plays a role in macrophage activation and immune regulation [45], suggesting that LSE supplementation may promote macrophage-mediated immune functions. Comparable *IL-4* upregulation has been reported in Nile tilapia fed turmeric (*Curcuma longa*) [46].

Digestive enzyme activity and antioxidant defense were further enhanced by dietary treatments. Increased activities of SOD, CAT, and GPx, accompanied by reduced lipid peroxidation, indicate improved oxidative balance, particularly in LSE-fed groups. Similar antioxidant responses have been reported in rainbow trout fed medicinal plant-based diets [47], and these effects may also be influenced by the lipid composition of the diets [48].

Finally, dietary supplementation significantly improved survival following *Yersinia ruckeri* challenge. Higher survival rates observed in all experimental groups, particularly in LSE-fed fish, suggest enhanced disease resistance. Comparable increases in survival have been reported in rainbow trout fed various medicinal plant extracts [49,50], as well as in seabass challenged with *Vibrio anguillarum* [3]. The garden cress seed by-product is known to contain benzyl isothiocyanate, a bioactive compound with antimicrobial and immunomodulatory properties, which may contribute to the enhanced survival observed in LSE groups. A limitation of the present study is the relatively short feeding period of three weeks. Although clear effects on growth, immune responses, antioxidant status, and post-challenge survival were observed within this period, longer-term studies are needed to determine the persistence and practical applicability of these effects under extended feeding conditions.

Conclusion

In conclusion, both garden cress seed oil and its pulp ethanolic extract showed beneficial effects on physiological and immunological responses in rainbow trout. Among the tested additives, the pulp extract, particularly at 0.5 g kg^{-1} feed, produced the most consistent immunostimulatory response. These findings suggest that garden cress seed by-products may be considered promising functional ingredients in aquafeeds, with potential benefits for fish health and aquaculture sustainability.

Declarations

Use of animals

The study protocol was pre-approved by the Kastamonu University Local Ethics Committee for Animal Experiments KUHADYEK 2016-16.

Data availability

Data available upon reasonable request from the corresponding author.

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CRediT authorship contribution statement

Mabrokah Adrees Rafallah Saed: Writing – review & editing, Writing – original draft, Software, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Soner Bilen:** Writing – original draft, Methodology. **Osman Nezih Kenanoğlu:** Writing – review & editing, Formal analysis. **Kerim Güney:** Resources, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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