Wiley
Aquaculture Research
Volume 2025, Article ID 7950238, 17 pages
https://doi.org/10.1155/are/7950238

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Research Article

Impact of Valerian (*Valeriana officinalis*) Extract Supplementation on Common Carp (*Cyprinus carpio* L.) Growth Performance, Immune Responses, and Gene Expression

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Received 5 October 2024; Accepted 17 February 2025

This study evaluated the effects of dietary supplementation with valerian (*Valeriana officinalis*) extract on growth performance, immune responses, and gene expression in common carp (*Cyprinus carpio*). Four experimental diets (V-0, V-0.25, V-0.5, and V-1) containing 0%, 0.25%, 0.5%, and 1% valerian extract were administered for 30 days. Dietary supplementation did not significantly influence growth performance ($p > 0.05$). Glutathione peroxidase (GPX) activity remained unchanged except in the high-dose group (V-1), while malondialdehyde (MDA) activity was significantly reduced in the V-0.25 group ($p < 0.05$). Aspartate aminotransferase (AST) activity increased in the V-1 group ($p < 0.05$), whereas alanine aminotransferase (ALT) activity decreased in the V-0.25 group ($p < 0.05$). Albumin (ALB) levels were elevated in the V-1 group ($p < 0.05$), while globulin (GLB) levels increased in all valerian-supplemented groups ($p < 0.05$). Creatinine (CRT) levels rose significantly only in the V-1 group ($p < 0.05$), and cholesterol levels decreased in the 0.5% and 1% groups ($p < 0.05$). Gene expression analysis revealed an increase in interleukin-1 beta (*IL-1β*) expression in the V-0.5 group ($p < 0.05$) but reductions in *IL-10* and heat shock protein 70 (*HSP70*) in the V-0.25 group ($p < 0.05$). Superoxide dismutase (SOD) and catalase (CAT) gene expression levels significantly decreased in the groups fed with 0.25% and 0.5% valerian extract ($p < 0.05$), while catalase (*CAT*) gene expression levels significantly decreased in the groups fed with 0.25% and 0.5% valerian extract ($p < 0.05$). *GPX* gene expression levels declined across all experimental groups ($p < 0.05$). Tumor necrosis factor alpha (*TNF-α*) gene expression showed no significant changes ($p > 0.05$). These findings highlight the potential of valerian extract as a functional feed additive. However, further research with extended feeding durations and varying doses is required to understand its physiological effects in aquaculture better.

Keywords: common carp; gene expression; immune responses; *Valeriana officinalis*

1. Introduction

Aquaculture represents a significant component of the global food and nutrition industry, with an annual output of tens of millions of tonnes [1]. The dissemination and proliferation of antibiotic-resistant genetic elements and microbial strains have become increasingly apparent due to the excessive use of antibiotics [2]. Further reduction in the growth and immunity of farmed fish is caused by endocrine disruptions, abnormalities in intestinal microflora, and a weakened ability to break down starch and fibers [3, 4]. Therefore, research and development must be undertaken to create feed additives that will accelerate fish growth, improve intestinal health, strengthen immunity, and enhance disease resistance. This important issue urgently needs to be resolved in the aquaculture industry and is increasingly receiving attention [5]. Many studies have shown that plant-derived compounds can cause different physiological responses in farmed fish. These

natural compounds have many beneficial effects for aquaculture, such as reducing stress, supporting growth, stimulating appetite, protecting liver health, strengthening the immune system, and providing protection against pathogens. These properties can be attributed to the presence of many active compounds, such as alkaloids, saponins, terpenoids, glycosides, tannins, steroids, flavonoids, phenolic compounds, and essential oils. In addition, these extracts are usually locally available, economical, effective against various pathogens, easily biodegradable, and environmentally friendly. Considering all these properties, plant extracts are considered as a strong alternative to traditional antibiotics, chemotherapeutic agents, and vaccines [6, 7].

Common carp (*Cyprinus carpio*) plays a significant role in global aquaculture due to its ability to adapt to various conditions, rapid growth rates, and high tolerance to different farming environments [8]. This species among the most commonly farmed carp species in many Asian and some European countries accounts for 8.6% of global finfish aquaculture production [9]. The increasing demand for *C. carpio* farming may expose the industry to several challenges, including malnutrition, environmental stress, and infectious disease outbreaks [10]. As a result, there is growing interest in plant-based compounds [11].

The *Valeriana* genus includes 425 species belonging to the Valerianaceae family [12, 13]. *Valeriana officinalis*, commonly known as "valerian," naturally occurs in environments with high humidity, such as humid and temperate deciduous forests, ditches, coastal areas, scrublands, and meadow ecosystems. Due to its robust structure, the valerian plant can also grow in mountainous areas at altitudes of up to 1800 m [14]. Valerian is known to have therapeutic properties, such as improving sleep quality and relieving anxiety [15].

This plant contains at least 150 active compounds, including volatile oils (e.g., bornyl acetate, sesquiterpenes, and valerenic acid), valepotriates, hydroxy pinoresinol, alkaloids (actinidine, catnine, valerin, and valerin), and glutamine. Glutamine crosses the blood–brain barrier and plays a role in the production of gamma-aminobutyric acid (GABA) and has calming effects on GABAergic neurons. Additionally, valerian contains another class of compounds known as valepotriates, which are unique to the plant. Valerian root extracts have been found to contain a mixture of valepotriates, which have a bicyclic monoterpene structure. However, these compounds are unstable and can degrade rapidly under acidic, alkaline, or high-temperature conditions. Specifically, extracts derived from the roots of *V. officinalis* L. have been found to contain up to 2% valepotriate mixture [16–18]. The principal constituent of *V. officinalis*, valerenic acid, comprises acetone group derivatives and hydroxyl. Recent studies have indicated that valerenic acid may act as a partial agonist at specific subtypes of GABAA receptors, thereby inducing a hypnotic effect [19–21]. Murphy et al. [22] determined that valerenic acid exhibited the most potent anxiolytic effect among the compounds found in valerian. Additionally, acetoxyvalerenic acid has been observed to bind to GABA receptors, yet it has been demonstrated to diminish the potential anxiolytic effect of valerenic acid [23]. Moreover, this acid impedes the metabolism of GABA by inhibiting the activity of enzymes in the brain, which results in sedation [24]. Additionally, valerian contains a variety of other compounds, including flavonoids, phenolic acids, valerosidatum, caffeic acid, chlorogenic acid, β-sitosterol, choline, and various minerals [25]. Abasali and Mohamad [26] reported that a root extract of *V. officinalis* is effective in suppressing stress responses in swordtails (*Xiphophorus hellerii*). Other studies have indicated that compounds present in valerian exhibit anxiolytic and anticonvulsant effects in zebrafish (*Danio rerio*) [27, 28], sedative effects in juvenile Nile tilapia (*Oreochromis niloticus*) [29], and *C. carpio* [30].

Valerian extract has shown potential not only as a sedative but also as a functional feed additive in aquaculture, where improving fish health and stress tolerance is critical. Its active compounds may contribute to stress mitigation and immune enhancement, addressing some of the challenges faced in intensive fish farming systems. However, despite extensive research on the aquatic applications and anesthetic effects of *V. officinalis*, limited information is available on its physiological effects when incorporated into fish diets at varying doses. This study aimed to evaluate the effects of dietary supplementation with different doses of *V. officinalis* extract on growth performance, immune responses, and gene expression in common carp.

2. Materials and Methods

2.1. Component Analysis of Commercial Valerian Extract. The commercial valerian extract was supplied by Zardband Commercial Company (Product code: 764023, Iran). A precisely measured 2-g extract sample was meticulously transferred into a 15-mL glass bottle securely sealed with a silicone septum. The sample-containing bottle was then placed on a preheated block set to 45°C, allowing it to reach thermal equilibrium over the course of 15 min. Carboxen/polydimethylsiloxane (CAR/PDMS) SPME fiber was introduced into the container and permitted to adsorb the volatile compounds from the sample at 45°C for 30 min. The fiber was then inserted into the gas chromatograph's injection port at 250°C for 5 min to desorb the components. GC/MS analyses were conducted using a Shimadzu GC-2010 Plus system along with a Shimadzu GCMS-QP2010 SE detector. The analytical parameters were as follows: aRxi-5Sil MS column was utilized; the temperature program increased from 40 to 250°C at a rate of 4°C per minute; the injection temperature was maintained at 250°C; the inlet pressure was set to 83.5 kPa; helium was used as the carrier gas; the injection mode was configured to split; the temperature of the MS interface was 250°C; electron ionization was employed in the MS mode; the detector voltage was set to +0.00 kV; the mass range scanned was from 35 to 450 m/z; the scan rate was 1666 u/s; and the interval between scans was 0.30 s. The components were identified by meticulously comparing the retention times

Table 1: Composition and nutrient profiles of the experimental feeds.

Ingredients (dry matter, %)	Control	Valerian extract (0.25%)	Valerian extract (0.5%)	Valerian extract (1%)
Fish meal[a] (60% protein)	23.00	23.00	23.00	23.00
Soybean meal[a] (44% protein)	37.00	37.00	37.00	37.00
Corn starch[a]	19.00	18.75	18.50	18.00
Wheat flour[a]	12.00	12.00	12.00	12.00
Fish oil[a]	5.00	5.00	5.00	5.00
Vitamin, mineral mix[b,c]	4.00	4.00	4.50	4.25
Valerian extract	0	0.25	0.50	1.00
Total	100	100	100	100
Chemical analyses (DM, %)				
Dry matter	94.53	94.22	94.27	94.22
Crude protein	35.27	35.78	35.73	35.78
Crude lipid	7.62	7.71	7.58	7.71
Crude ash	5.88	5.68	5.96	5.68
NFE[d]	1.911	1.914	1.911	1.914
Gross energy (kj/g)[e]	45.76	45.05	45.00	45.05

[a]Saramad Fish Aquafeed Co, Iran.
[b]Kimia Roshd Co., Iran.
[c]Vitamin–mineral mix kindly provided by Ariana Knowledge-Based Company, Mashhad, Iran. The mineral premix includes the following components (mg/kg): Mg, 100; antioxidant (BHT), 100; Zn, 60; Fe, 40; Cu, 5; I, 0.1; Co, 0.1. The vitamin premix includes the following components (mg/kg): choline, 600; niacin, 40; E, 30; pantothenic acid, 18; riboflavin, 7; K, 3; pyridoxine, 3; thiamine, 2; folacin, 1.5; biotin, 0.7; and cyanocobalamin, 0.02.
[d]Nitrogen-free extract (NFE) was calculated as this equation NFE = dry matter − (crude lipids + crude ash + crude protein).
[e]GE was assessed upon 23.6 kJ/g protein, 39.5 kJ/g lipid, and 17.0 kJ/g.

and mass spectra against comprehensive reference libraries, including NIST, Wiley, Tutor, and FFNSC databases [31].

2.2. Experimental Design and Diet Preparation. The Aquatic Resources Production Unit, Department of Fisheries, Mashhad Ferdowsi University, Iran, was the site of this feeding study. A total of 144 common carp with a mean weight of 29.77 ± 0.25 g were used in the trial. The fish were allowed a 15-day acclimation period to the experimental environment prior to the initiation of the study. The study was carried out in a partially enclosed system. Twelve 150-L aerated plastic sponge biofilter tanks made up the system. Every day, about 10% to 15% of the water in the experimental system was replaced. In the experiment, the pH measurements of the water will be taken using a HANNA (HI 2221) desktop pH meter. A water analysis device (YSI Pro 2030) will be used for temperature, dissolved oxygen, and total hardness analyses. pH, temperature, total hardness, and dissolved oxygen were 7.3 ± 0.21, 27 ± 1.5°C, 273.98 ± 33.20 mg/L, and 7.12 ± 0.33 mg/L during the feeding session, in that order.

The experimental diets for carp were formulated according to the composition given in Table 1. Valerian (*V. officinalis*) extract was procured from Zardband, a commercial company, and used in preparing these diets. The different concentrations of the extract (0%, 0.25%, 0.5%, and 1.0%) used in the experimental diets were selected based on the methodology described in Yilmaz et al. [32]. Valerian extract was mixed with commercial fish oil and added to the feed. The control diet did not include any herbal supplements. The dietary ingredients were then thoroughly mixed, and water was added. The mixture was dried in an oven at 40°C and then processed into pellets using a laboratory pellet machine with a 2 mm grain size. The experimental diets were maintained at a temperature of −20°C prior to the commencement of the feeding trial. The pellets obtained were analyzed for moisture, crude protein, ash, and lipid content using standardized methods [33, 34]. The fish were fed the test diets in an unrestricted manner, with three feeding events occurring per day over the 30-day experimental period. At the conclusion of the feeding experiment, the researchers enumerated and measured the fish in each experimental tank to assess the parameters of fish survival and growth.

2.3. Growth Performance. The growth performance parameters were calculated utilizing the applicable mathematical equations [35, 36]:

$$\text{Weight gain}\,(\%) = (\,\text{final body weight} - \text{initial body weight})/\text{initial body weight} \times 100,$$

$$\text{Specific growth rate}\,(\text{SGR}\%\text{day}^{-1}) = [\text{Ln}\,(\,\text{final body weight}) - \text{Ln}\,(\text{initial body weight}) \times 100]/\text{the length of the experimental period},$$

$$\text{Feed conversion ratio (FCR)} = \text{dietary consumption (g, dry weight)}/\text{weight gain (g)},$$

$$\text{SR (\%)} = (\text{number of live fish at the end of the experiment}/\text{number of live fish at the beginning of the experiment}) \times 100.$$

Following the conclusion of the trial, the fish were subjected to a 24-h fasting period, after which three individuals from each tank were randomly selected for blood sample collection and analysis. The fish were anesthetized with clove oil, a natural product commonly used, at a concentration of 150 mg L^{-1} to prevent mucus from mixing with the blood. To prevent mucus contamination, the area just behind the anal fin was thoroughly cleaned with alcohol, and blood samples were taken from the caudal vein of the fish without damaging it. The collected 1.5 mL blood samples were transferred to gel serum tubes without using any anticoagulant. For serum analysis, blood samples collected in gel tubes were centrifuged at a speed of 1350 g for 10 min. The serum obtained was subsequently stored at −80°C until it was analyzed for biochemical purposes [37]. Upon completion of the blood sampling procedure, the fish were subjected to euthanasia through the administration of an excessive amount of clove oil (200 mg/L). Head kidney tissues were collected in PCR tubes, and RNA solution (Qiagen GmbH) was added to preserve the samples; they were then stored at −80°C for further analysis [38].

Mucus samples were obtained from three fish randomly chosen from each experimental group. The fish were treated with clove oil at a dosage of 20 mg L^{-1} for anesthesia and subsequently placed in polyethylene bags [39]. Then, 10 mL of NaCl (50 mM) was added to each bag, and the fish were gently rubbed to collect mucus samples. Later, the mucus samples from the fish skin were placed into sterile 15 mL centrifuge tubes and centrifuged at 1500× *g* for 10 min at 4°C. To prevent external bacterial contamination, the skin mucus samples were immediately frozen and subsequently lyophilized. They were finally stored at −80°C for future analysis [40].

2.4. Immunological Analysis of Mucus Samples. The alkaline phosphatase (ALP) activity in the mucus samples was measured using the Pars-Azmoon kit (Alborz, Iran) and a biochemical autoanalyzer (Prestige 24i), following the manufacturer's instructions [41]. The total immunoglobulin (Ig) concentrations in the mucus samples were assessed using the approach outlined by Siwicki and Anderson [42]. In short, serum samples were diluted 100-fold using a sodium chloride solution (0.005%). Protein levels in these samples were assessed using the Biuret method. Then, 0.1 mL of each serum sample was mixed with 12% polyethylene glycol by volume and incubated for 2 h to allow the accumulation of Ig molecules. The samples were centrifuged at 5000 rpm for 5 min at 4°C. The resulting supernatant was diluted 50-fold with 0.85% NaCl.

Total Ig concentration was obtained by subtracting the protein content detected in the original sample from the protein content measured after adding polyethylene glycol. In this study, lysozyme activity against the bacterial species *Micrococcus lysodeikticus* was evaluated, according to the method described by Sankaran and Gurnani [43]. First, a suspension of *Micrococcus lysodeikticus* (75 mg/100 mL, Sigma) was prepared in phosphate-buffered saline. Then, a 200 µL portion of this suspension was extracted, and its absorbance was measured at 450 nm. Afterward, 15 µL of the mucus sample was added to the same tube, and the absorbance was recorded after a 20-min incubation period. The absorbance values of the samples were compared against a standard curve prepared using egg white lysozyme (Sigma), and the concentration of lysozyme in the samples was measured using spectrophotometric techniques [43]. The total protein (TP) content of the mucus samples was measured using the Bionik kit (Tehran, Iran) and the Biuret colorimetric method, with bovine serum albumin (ALB) employed as the calibration standard. The analysis was conducted at a wavelength of 560 nm using the automated Prestige 24i biochemical analyzer from Biomedical Ltd. [44].

2.5. Oxidative Biomarkers. The total antioxidant capacity (TAC) of the sample was evaluated using the ferric reducing ability of plasma (FRAP) assay developed by Benzie and Strain [45], which serves as an indicator of antioxidant activity. To prepare the FRAP reagent, the following constituents were freshly combined: 5 mL of a 10 mmol/L TPTZ solution in 40 mmol/L hydrochloric acid, 5 mL of a 20 mmol/L ferric chloride solution, and 50 mL of an acetate buffer. Subsequently, a 100 µL aliquot of the supernatant sample was mixed with 3 mL of the FRAP reagent. The reduction of the ferric tri-pyridyl-s-triazine complex to the ferrous tripyridyl-s-triazine complex at pH 3.6 and 25°C is directly proportional to the TAC of the sample. Consequently, measurements were conducted in accordance with this correlation. The formation of the Fe^{2+}–TPTZ complex results in the generation of a pronounced blue hue, which was monitored at 593 nm using a UV/VIS spectrophotometer over a 5-min period. Additionally, calculations were made using a calibration curve established with $FeSO_4{\cdot}7H_2O$, which ranged from 100 to 1000 µM/L. Catalase (CAT) activity was evaluated using the technique described by Goth [46], which utilizes hydrogen peroxide as the substrate and ammonium molybdate as the reaction-terminating agent. The glutathione peroxidase (GPX) activity was assessed in accordance with the protocol described in the biochemical kit instructions procured from the commercial supplier Biorexfars Co., located in Iran [47]. The levels of malondialdehyde (MDA), a biomarker of oxidative stress, were quantified using a revised thiobarbituric acid-based analytical method and presented as micromoles per gram of the assessed tissue sample. The

Table 2: Real-time PCR primers for detecting gene expressions.

Gene name	FWD or REV	Sequence (5′–3′)	Efficiency (%)	Accession No. and references
IL-1β	Forward	ACCAGCTGGATTTGTCAGAAG	98	AB010701.1 (https://www.ncbi.nlm.nih.gov/nuccore/AB010701)
	Reverse	ACATACTGAATTGAACTTTG		
TNF-α	Forward	GGTGATGGTGTCGAGGAGGAA	95	AJ311800.1 (https://www.ncbi.nlm.nih.gov/nuccore/AJ311800.1)
	Reverse	TGGAAAGACACCTGGCTGTA		
IL-10	Forward	CGCCAGCATAAAGAACTCGT	96	JX524550.1 (https://www.ncbi.nlm.nih.gov/nuccore/JX524550.1)
	Reverse	TGCCAAATACTGCTCGATGT		
SOD	Forward	TGGCGAAGAAGGCTGTTTGT	99	JF342355 (https://www.ncbi.nlm.nih.gov/nuccore/JF342355)
	Reverse	TTCACTGGAGACCCGTCACT		
HSP70	Forward	TGTGAGCGAGCCAAGAGAACCC	98	XM_019074376.1 (https://www.ncbi.nlm.nih.gov/nuccore/XM_019074376.1)
	Reverse	AAGCGAGCTCTGGTGATGGACG		
CAT	Forward	CTGGAAGTGGAATCCGTTTG	100	JF411604 (https://www.ncbi.nlm.nih.gov/nuccore/JF411604)
	Reverse	CGACCTCAGCGAAATAGTTG		
GPX	Forward	AGGAGAATGCCAAGAATG	98	GQ376155.1 (https://www.ncbi.nlm.nih.gov/nuccore/300676298)
	Reverse	GGGAGACAAGCACAAGG		
β-Actin	Forward	CCTGTATGCCAACACCGTGCTG	97	JQ619774.1 (https://www.ncbi.nlm.nih.gov/nuccore/JQ619774.1)
	Reverse	CTTCATGGTGGAGGGAGCAAGG		

Abbreviations: *CAT*, catalase; *GPX*, glutathione peroxidase; *HSP70*, heat shock protein 70; *IL-10*, interleukin-10; *IL-1β*, interleukin-1β; *SOD*, superoxide dismutase; *TNF-α*, tumor necrosis factor alpha.

experimental procedure entailed combining 500 μL of the supernatant with 2500 μL of a 20% trichloroacetic acid solution and 1000 μL of a 67% thiobarbituric acid solution in a Pyrex test tube. The mixture was then heated in boiling water at 100°C for 15 min. Thereafter, the organic layer containing the chromogenic substrate was extracted using a solution comprising 1000 μL of deionized water and 5000 μL of n-butanol. The resulting mixture was subjected to centrifugation at 2000 rpm for a period of 15 min at a temperature of 4°C. The concentration of MDA in the samples was determined by measuring the absorbance of the pink-colored complex in the organic phase at 532 nm using a spectrophotometer. A standard solution was prepared using tetraethoxypropane and absolute ethanol in order to determine the MDA concentration.

2.6. Biochemical Parameters. The enzymatic activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and ALP were measured in accordance with the protocols described by Moss and Henderson [48]. The analytical procedures were performed utilizing commercially available biochemical kits supplied by ParsAzemun Co., based in Iran, as well as a UV-Vis spectrophotometer. The enzymatic activities of AST and ALT were measured by monitoring absorbance changes at 340 nm over a 3-min period. LDH enzyme activity was determined by concurrently observing the transformation of pyruvate into L-lactate and the oxidation of NADH, with the changes in absorbance at 340 nm over a 3-min period captured using a spectrophotometer. ALP activity was quantified by spectrophotometric assessment of the conversion of the p-nitrophenol phosphate substrate into the p-nitrophenol product, which was measured at 405 nm. The total plasma protein concentration was determined through the Biuret colorimetric assay, with measurements conducted at a wavelength of 540 nm. The plasma ALB concentration was determined by a colorimetric method that quantified the binding of a bromocresol green dye to the protein, producing a blue–green color measured at 630 nm.

Plasma globulin (GLB) levels were determined by calculating the difference between total plasma protein and ALB concentrations [49]. The plasma glucose concentration was determined using the glucose oxidase method at 500 nm, as described by Burtis and Ashwood [50]. The concentrations of plasma cholesterol and triglycerides were quantified using enzymatic colorimetric assays. Specifically, the CHOD-PAP method was employed to determine cholesterol levels, with absorbance measured at 510 nm. Triglyceride concentrations were assessed via the GPO-PAP technique, with absorbance quantified at 546 nm [51]. The creatinine (CRT) concentration was determined using the Jaffe reaction, in which a visible color complex was formed and detected using a spectrophotometer at 510 nm in an alkaline solution containing picric acid [52].

2.7. Gene Expression Analyses. Total RNA was obtained from the head kidney tissue using the A.B.T. Blood/Tissue RNA Purification Kit (catalog number: I04-01-10 100 preps). The quality and quantity of the RNA were ascertained through the utilization of a NanoDrop spectrophotometer (Thermo Fisher), following the manufacturer's instructions. Subsequently, cDNA synthesis was conducted utilizing the Vita-Script First Strand cDNA Synthesis Kit (PCCSKU1301). A reaction mixture was prepared in a 1.5 mL Eppendorf tube for cDNA synthesis. This included 4 μL 5× reaction buffer, 1 μL RNA reverse transcriptase (200 U/μL), and 1–6 μL total RNA, adjusted to a final volume of 20 μL with RNAse-free water. For further investigation, the generated cDNA was kept in storage at −80°C. Primers containing base sequences and the NCBI GenBank accession numbers listed in Table 2

were utilized for gene expression studies. As a reference gene, the β-actin gene was employed. For real-time PCR, CFX Connect Real-Time PCR Detection System was utilized. The real-time PCR mixture was made up of 10 μL of 2× Magic SYBR Mix, 1 μL of each of the forward and reverse primers (10 μM), 2 μL of cDNA template, and 6 μL of RNAse-free water. The primers for the relevant genes for RT-PCR are provided in Table 2. RT-PCR was conducted in duplicate for each sample, with at least two independent repetitions in 96-well plates. The following cycling conditions were employed: an initial denaturation at 95°C for 5 min, followed by 40 cycles of 95°C for 15 s, 60°C for 30 s, and 72°C for 30 s. In the course of this study, a melt curve analysis was conducted. The data were analyzed using the Bio-Rad CFX Maestro software. Gene expression differences were analyzed using the $2^{-\Delta\Delta Ct}$ method, as described by Livak and Schmittgen [53].

2.8. Ethics Statement. The Research Ethics Committee of Ferdowsi University of Mashhad approved this research and confirmed that it met the relevant standards in terms of animal experimentation ethics (Decision Number 2023/05-20).

2.9. Statistical Analyses. One-way analysis of variance (ANOVA) was applied to the data obtained from the analyses. Tukey's multiple comparison test was utilized when the data exhibited normal distribution and homogeneity. In cases where the data did not demonstrate normal distribution but showed homogeneity, the Kruskal–Wallis test was applied. For data that lacked homogeneity, the Tamhane test was used. Statistical analyses were performed using SPSS 29 (IBM SPSS Statistics 29) software, with significance evaluated at $p < 0.05$.

3. Results

3.1. Component Profile of Valerian Extract. Component analysis of commercial valerian extract is shown in Table 3. The most abundant compounds identified in the extract, exceeding 5% in relative abundance, were geraniol (19.77%), neryl acetate (16.86%), linalool (16.81%), 6-methyl-5-hepten-2-one (5.54%), and bornyl acetate (5.00%).

3.2. Growth Performance. The growth performance findings of common carp (*C. carpio*) fed with feed supplemented with valerian (*V. officinalis*) extracts and unsupplemented (control) feed are presented in Table 4. After the 30-day feeding trial, there were no statistically significant differences in the growth performance between common carp fed with valerian extract-supplemented feed and those fed unsupplemented (control) feed ($p > 0.05$). Fish survival rates were similar in all groups, and no mortality was reported ($p > 0.05$).

3.3. Mucus Immune Parameters. The effects of different dietary levels of valerian extract on ALP, total Ig, lysozyme, and TP in mucus samples are presented in Table 5. There were no significant differences found among the experimental groups and the control group in terms of ALP, total Ig, lysozyme activity, and TP values ($p > 0.05$).

3.4. Oxidative Biomarkers. The analysis results of serum oxidative parameters at the end of the trial for common carp fed with feed supplemented with valerian extracts are shown in Table 6. TAC and CAT levels of the trial common carp diet containing valerian extract were similar to those of the control group fish ($p > 0.05$). GPX activity was statistically similar to the control group, except for the high-dose valerian extract trial group ($p > 0.05$). However, MDA activity was significantly lower in the V-0.25 group compared to the control group ($p < 0.05$).

3.5. Biochemical Parameters. At the end of the trial, the analysis findings of common carp serum biochemical parameters are presented in Table 7. AST activity in fish fed with feed supplemented with valerian extract was significantly higher only in the high-dose group compared to the control group ($p < 0.05$). LDH and ALP activities were found to be similar in the groups fed with valerian extract-supplemented feed compared to the control group ($p > 0.05$). Fish fed with feed supplemented with 0.25% valerian extract had lower serum ALT activity than the control and other trial groups ($p < 0.05$).

At the end of the trial, the analysis findings of common carp serum biochemical parameters are presented in Table 8. Glucose and triglyceride levels of common carp fed with valerian extract-supplemented diets were similar to those of the control group ($p > 0.05$). ALB levels significantly increased in the group fed with 1% valerian extract compared to the control group ($p < 0.05$). GLB levels were significantly higher in all experimental groups compared to the control group ($p < 0.05$). Cholesterol levels were significantly lower in fish-fed diets containing 0.5% and 1% valerian extract compared to the control group ($p < 0.05$). CRT levels significantly increased in the group fed with 1% valerian extract compared to the control group ($p < 0.05$).

3.6. Gene Expression Analysis. At the end of the trial, findings regarding the gene expression levels of the head kidney in common carp are shown in Figures 1 and 2. The superoxide dismutase (*SOD*) and *CAT* gene expression levels were found to decrease in the groups fed with 0.25% and 0.5% valerian extract compared to the control group ($p < 0.05$). The *GPX* gene expression level was determined to decrease in the experimental groups compared to the control group ($p < 0.05$). The interleukin-1 beta (*IL-1β*) gene expression level increased in V-0.5 group compared to the control group ($p < 0.05$). The *IL-10* and heat shock protein 70 (*HSP70*) gene expression levels were significantly decreased in the group fed with 0.25% valerian extract compared to the control group ($p < 0.05$). Additionally, tumor necrosis factor alpha (*TNF-α*) gene expression levels did not change significantly in the experimental groups compared to the control group ($p > 0.05$).

4. Discussion

The findings of this study suggest that the addition of valerian extract (*V. officinalis*) to the diet has no effect on the growth performance of common carp (*C. carpio*). In a short-term (21-day) feeding trial conducted by Yilmaz et al. [32],

Table 3: SPME–GC/MS analysis of *V. officinalis* extract.

Row	Retention time	Name of the compound	Area	Area %
1	6.093	Isovalerate (ethyl-)	483,310	0.80
2	10.702	6-Methyl-5-hepten-2-one	3,363,315	5.54
3	10.877	β-Myrcene	1,400,195	2.31
4	12.155	*p*-Mentha-1,5,8-triene	172,973	0.28
5	12.365	Limonene	291,605	0.48
6	12.461	Eucalyptol (1,8-Cineole)	203,057	0.33
7	12.693	cis-Ocimene	355,682	0.59
8	13.096	β-Ocimene	587,831	0.97
9	14.763	4-Isopropenyl-1-methylbenzene	217,801	0.36
10	15.236	Linalool	10,206,364	16.81
11	16.598	Trans-3(10)-Caren-2-ol	103,766	0.17
12	16.902	Camphor	72,694	0.12
13	17.006	2- (2-Methyl-1-cyclohexen-1-yl) propanal	294,969	0.49
14	17.778	Geranyl hexanoate	100,187	0.17
15	17.915	Borneol	657,771	1.08
16	18.170	L-Menthol	165,146	0.27
17	18.275	Trans-Carveol	2,419,068	3.98
18	18.809	α-Terpineol	1,067,572	1.76
19	19.248	cis-Carveol	1,237,053	2.04
20	19.659	Pulegone	77,087	0.13
21	19.954	Nerol	1,046,326	1.72
22	20.057	β-Citronellol	2,892,885	4.76
23	20.176	Cymen-8-ol (para-)	960,907	1.58
24	20.601	Carvone	96,797	0.16
25	20.973	Geraniol	12,002,990	19.77
26	21.520	E-Citral	160,819	0.26
27	22.075	Bornyl acetate	3,035,919	5.00
28	23.267	1,4-Dimethyl-3-cyclohexenyl methyl ketone	877,300	1.44
29	23.438	Verbenol	372,336	0.61
30	24.122	Valeric anhydride	78,862	0.13
31	24.274	β-Terpinyl acetate	135,616	0.22
32	24.401	Citronellyl acetate	116,096	0.19
33	25.401	Neryl acetate	10,237,862	16.86
34	26.563	1,4-Dimethoxy-2-tert-butylbenzene	123,664	0.20
35	27.429	Hydroxy-alpha-terpenyl acetate	140,230	0.23
36	28.700	β-Ionone	283,968	0.47
37	28.975	β-Cadinene	199,030	0.33
38	30.302	Epiglobulol	113,478	0.19
39	30.763	Widdrol	216,837	0.36
40	31.803	Spathulenol	601,469	0.99
41	31.944	Caryophyllene oxide	99,265	0.16
42	33.200	10s,11s-Himachala-3(12),4-diene	614,193	1.01
43	34.150	Eudesmol (epi-gamma-)	71,545	0.12
44	34.247	Isozonarol	156,172	0.26
45	35.844	Valerenal	1,148,677	1.89
46	42.108	β-Bisabolene	86,509	0.14
47	43.269	Ethyl palmitate	984,230	1.62
48	47.403	Ethyl oleate	381,575	0.63
			60,713,003	100.00

Table 4: Differences in growth performance parameters among the experimental groups.

Groups	IW (g)	FW (g)	WG (%)	FCR (g feed/g gain)	DFI	SGR (%/day)	SR (%)
V-0	29.78 ± 0.93	54.11 ± 1.48	43.85 ± 3.25	0.94 ± 0.03	9.19 ± 0.34	1.99 ± 0.17	100
V-0.25	29.38 ± 0.85	51.97 ± 4.68	41.67 ± 3.60	0.97 ± 0.03	8.82 ± 0.85	1.88 ± 0.23	100
V-0.5	30.06 ± 0.27	53.79 ± 2.94	42.43 ± 2.17	0.89 ± 0.05	8.53 ± 0.79	1.93 ± 0.17	100
V-1	29.89 ± 0.79	53.78 ± 3.84	43.49 ± 3.51	0.93 ± 0.11	8.75 ± 0.20	1.94 ± 0.20	100
p	n.s. 0.739	n.s. 0.877	n.s. 0.830	n.s. 0.566	n.s. 0.933	n.s. 0.933	

Note: The data are presented as means ± standard deviation (SD) ($n = 3$).
Abbreviations: DFI, daily feed intake; FCR, feed conversion ratio; FW, final weight; IW, initial weight; SGR, specific growth rate; WG, weight gain percentage.

Table 5: Changes in immune parameters in the mucus samples of common carp fed with varying levels of valerian extract over a period of 30 days.

Groups	ALP (U/mg protein)	Total Ig (mg/mg protein)	LZM (U/mg protein)	TP (mg/ml)
V-0	20.00 ± 0.00	2.38 ± 0.12	39.72 ± 2.09	5.00 ± 1.00
V-0.25	22.68 ± 0.46	2.56 ± 0.23	42.78 ± 4.57	4.53 ± 0.56
V-0.5	22.58 ± 4.67	2.40 ± 0.16	40.34 ± 3.51	4.83 ± 1.15
V-1	21.97 ± 2.99	2.42 ± 0.33	41.99 ± 4.21	4.36 ± 0.49
p	n.s. 0.632	n.s. 0.750	n.s. 0.733	n.s. 0.799

Note: The data are presented as means ± standard deviation (SD) ($n = 3$).
Abbreviations: ALP, alkaline phosphatase; LZM, lysozyme; total Ig, total immunoglobulin; TP, total protein.

Table 6: Changes in serum oxidative parameters of common carp fed with varying levels of valerian extract over 30 days.

Groups	TAC (nmol Fe^{2+}/mL)	GPX (mU/ml)	CAT (KU/L)	MDA (mg/ml)
V-0	0.20 ± 0.02	1.62 ± 0.35^{b}	1.29 ± 1.15	0.033 ± 0.00^{a}
V-0.25	0.18 ± 0.00	0.98 ± 0.64^{b}	2.68 ± 0.65	0.022 ± 0.00^{b}
V-0.5	0.20 ± 0.01	1.50 ± 0.51^{b}	0.78 ± 0.36	$0.024 \pm 0.00^{a,b}$
V-1	0.21 ± 0.02	5.00 ± 2.16^{a}	1.67 ± 0.62	$0.024 \pm 0.00^{a,b}$
p	n.s. 0.244	* 0.011	n.s. 0.073	* 0.030

Note: The data are presented as means ± standard deviation (SD) ($n = 9$). Values with different letters in the same row indicate statistical significance within groups ($p < 0.05$). n.s., $p \geq 0.05$ (nonsignificant); $^{*}p < 0.05$.
Abbreviations: CAT, catalase; GPX, glutathione peroxidase; MDA, malondialdehyde; TAC, total antioxidant capacity.

Table 7: Changes in serum enzyme parameters of common carp fed with varying levels of valerian extract over a period of 30 days.

Groups	AST (U/L)	ALT (U/L)	LDH (U/L)	ALP (U/L)
V-0	55.85 ± 7.06^{b}	18.31 ± 1.32^{a}	479.18 ± 22.58	212.40 ± 9.60
V-0.25	$61.20 \pm 5.34^{a,b}$	15.12 ± 0.63^{b}	507.31 ± 45.71	208.55 ± 12.31
V-0.5	$64.06 \pm 3.12^{a,b}$	17.15 ± 1.12^{a}	532.19 ± 90.23	222.42 ± 16.65
V-1	67.68 ± 4.30^{a}	18.84 ± 1.08^{a}	556.85 ± 31.36	219.05 ± 15.97
p	** 0.006	** 0.000	n.s. 0.112	n.s. 0.329

Note: The data are presented as means ± standard deviation (SD) ($n = 9$). Values with different letters in the same row indicate statistical significance within groups ($p < 0.05$). n.s., $p \geq 0.05$ (nonsignificant); $^{**}p < 0.01$.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase.

valerian extracts in the herbal supplement Passif Mood (PM) were shown to effectively increase the growth performance of rainbow trout (*Oncorhynchus mykiss*). These findings highlight that the effects of valerian extract may vary depending on the species and the duration of the experiment and suggest that additional research is needed to better understand the potential benefits and limitations of this herbal supplement.

TABLE 8: Changes in serum biochemical parameters of common carp fed with varying levels of valerian extract over a period of 30 days.

Groups	TP (g/dL)	ALB (g/dL)	GLB (g/dL)	GLU (mg/dL)	CHO (mg/dL)	TG (mg/dL)	CRT (mg/dL)
V-0	$3.11 \pm 0.15^{a,b}$	2.30 ± 0.17^{b}	0.81 ± 0.12^{c}	74.68 ± 4.97	$84.87 \pm 5.59^{a,b}$	213.78 ± 19.83	0.35 ± 0.11^{b}
V-0.25	2.56 ± 0.56^{b}	1.42 ± 0.21^{c}	$1.14 \pm 0.44^{a,b}$	74.77 ± 12.32	92.20 ± 6.38^{a}	199.81 ± 13.86	0.41 ± 0.12^{b}
V-0.5	3.96 ± 0.69^{a}	2.13 ± 0.08^{b}	1.83 ± 0.68^{b}	70.14 ± 6.42	77.70 ± 9.67^{b}	193.59 ± 17.84	$0.45 \pm 0.12^{a,b}$
V-1	4.05 ± 0.73^{a}	2.85 ± 0.52^{a}	$1.20 \pm 0.60^{a,b}$	77.17 ± 11.74	80.26 ± 6.96^{b}	197.02 ± 16.59	0.61 ± 0.11^{a}
p	* 0.001	** 0,00	* 0.020	n.s. 0.634	* 0.014	n.s. 0.222	* 0.004

Note: The data are presented as means ± standard deviation (SD) ($n = 9$). Values with different letters in the same row indicate statistical significance within groups ($p < 0.05$). n.s., $p \geq 0.05$ (nonsignificant); $^{*}p < 0.05$; $^{**}p < 0.01$.
Abbreviations: ALB, albumin; CHO, cholesterol; CRT, creatinine; GLB, globulin; GLU, glucose; TG, triglyceride; TP, total protein.

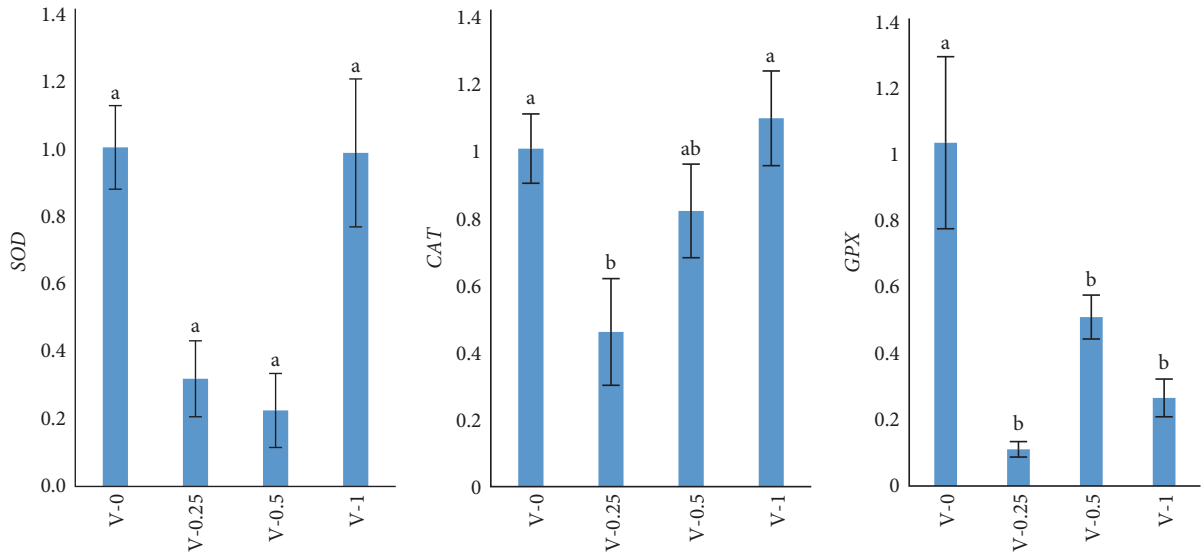

FIGURE 1: Relative gene expressions of *SOD*, *CAT*, and *GPX* in common carp fed diets supplemented with different levels of *Valeriana officinalis* extract for 30 days. Values are presented as means ± standard deviation (SD, $n = 3$). Different superscript letters above bars indicate significant differences among groups ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's post hoc test. Bars sharing the same letter are not significantly different. ANOVA, one-way analysis of variance; *CAT*, catalase; *GPX*, glutathione peroxidase; *SOD*, superoxide dismutase.

Analysis of fish mucus samples provides valuable information to scientists to assess the health status of fish [54, 55]. Studies on the immune components of fish mucus have focused on important elements such as ALP, Igs, lysozyme and TP [56]. In carp, high ALP activity in epidermal cells has been documented during skin wound healing and skin regeneration [57]. Additionally, ALP in the skin mucus of Atlantic salmon (*Salmo salar*) has been suggested as a potential stress indicator [40]. Igs are secreted by lymphocytes and/or epithelial cells; these antibodies recognize antigens like bacteria, and higher basal Ig levels in fish-fed diets supplemented with plant additives have been associated with increased resistance to diseases [58]. Lysozyme (N-acetylmuramidase or muramidase) is a bactericidal enzyme found across a wide range of organisms, including fish [59]. Lysozyme acts directly on gram-positive bacteria, causing lysis of their outer peptidoglycan layer [60]. In gram-negative bacteria, lysozyme targets the peptidoglycan layer, which becomes accessible after the breakdown of the outer membrane by complement proteins and other enzymes [61]. Lysozyme is present in the mucus of freshwater and marine fish, as well as lymphoid tissue, plasma, and other body fluids. Its bacteriolytic activity in fish skin mucus and its presence in other tissues contribute to the host defense mechanism against bacterial infections [62]. In this study, the addition of valerian extract to the diet did not affect ALP, total Ig, lysozyme, and TP activities in common carp. In contrast to our findings, a mixture of three medicinal plant extracts common mallow (*Malva sylvestris*), oregano (*Origanum vulgare*), and Persian shallot (*Allium hirtifolium boiss*) increased the ALP, total Ig, TP, and lysozyme activities of common carp after 30 days of feeding [63]. In addition, previous studies have shown that herbal extracts such as date palm fruit [64], ginger (*Zingiber officinale*) [65], and combined herbs (oak acorn, coriander, and common mallow) [66] increased skin mucosal immune parameters (lysozyme, ALP, and total Ig) in common carp. These findings highlight the diverse effects that different plant extracts can have on mucosal immune parameters in common carp, emphasizing the need for further research to fully understand the potential applications of these herbal supplements in aquaculture practices.

According to Ghiselli et al. [67], TAC is a synthetic index that represents the overall level of antioxidants made up of

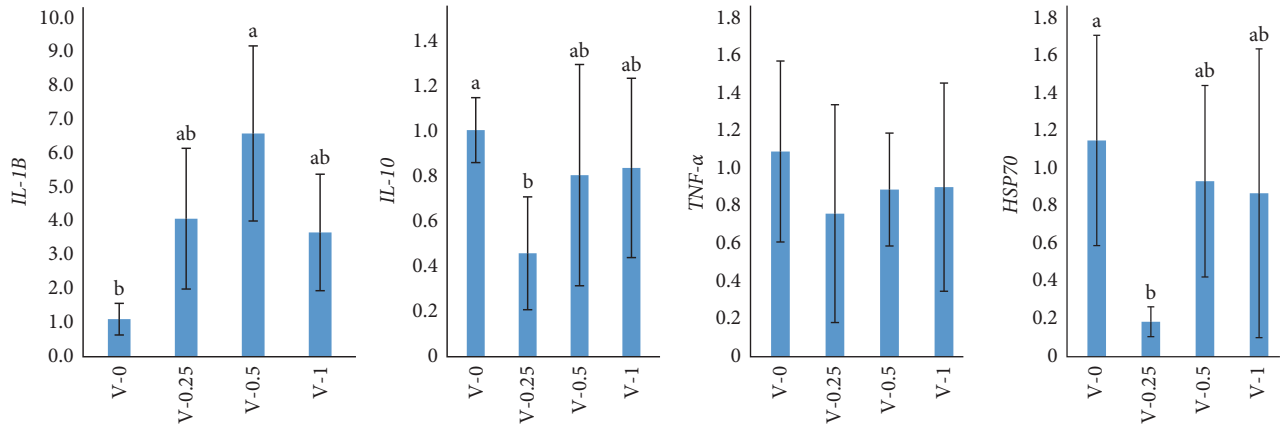

FIGURE 2: Relative gene expressions of *IL-1β*, *IL-10*, *TNF-α*, and *HSP70* in common carp fed diets supplemented with different levels of *Valeriana officinalis* extract for 30 days. Values are presented as means ± standard deviation (SD, $n = 3$). Different superscript letters above bars indicate significant differences among groups ($p < 0.05$), as determined by one-way ANOVA followed by Tukey's post hoc test. Bars sharing the same letter are not significantly different. ANOVA, one-way analysis of variance; *HSP70*, heat shock protein 70; *IL-1β*, interleukin-1 beta; *TNF-α*, tumor necrosis factor alpha.

several antioxidant compounds and antioxidant enzymes. In our investigation, supplementing with valerian extract did not affect TAC levels. In contrast, common carp showed an increase in TAC levels following a 30-day supplementation with silymarin (*Silybum marianum*) extract (1400–2400 mg SIE/kg diet) [68].

The fish antioxidant system includes enzymes like GPX and CAT, which defend against ROS by converting them into inert forms. GPX reduces mitochondrial peroxides to water or alcohol, while CAT breaks down H_2O_2 into water and oxygen [69, 70]. Furthermore, the elevated activity of antioxidant enzymes plays a role in the organism's defense against external factors, including poor water quality, overcrowding, and high environmental temperatures [71]. In our study, GPX activity in the high-dose valerian extract group was similar to that of the control group, as in all other experimental groups. Our results are consistent with Paray et al. [72], which reported no changes in plasma GPX levels in common carp fed 0.5 g kg^{-1} of oak (*Quercus castaneifolia*) leaf extract. However, diets with 1 and 2 g kg^{-1} of the extract increased GPX activity. In contrast, other studies found that *Artemisia annua* [73], *Artemisia absinthium* [74], and combinations of medicinal plants such as common mallow (*M. sylvestris*), oregano (*O. vulgare*), Persian shallot (*Allium hirtifolium*) [63], coriander (*Coriandrum sativum*), oak acorn (*Quercus brantii*), and hawthorn [66, 75] enhanced GPX activity in *C. carpio*.

In the study carried out by Paray et al. [72], an increase in GPX activity was observed in common carp (*C. carpio*) fed with oak leaf (*Q. castaneifolia*) extract for 60 days and subjected to a 6-h crowding stress. Adineh et al. [76] observed that the supplementation of the diet with *Perovskia abrotanoides* extract resulted in an increase in GPX activity in carp, both prior to and following exposure to heat stress. Following heat stress, fish fed with 1% extract exhibited the highest GPX activity, followed by those fed with 0.5% extract. Similarly, GPX activity was observed to increase in gibel carp (*Carassius auratus*) that had been fed a diet supplemented with *Flos populi* extract for a period of 45 days [77]. Assar et al. [78] observed that the administration of 0.2% olive leaf extract (OLE) to common carp for a period of 60 days resulted in an increase in GPX activity both prior to and following infection with *Aeromonas hydrophila*.

The results of our study indicated that there were no statistically significant differences in CAT activity values between the fish that received valerian extract and the control group. Similarly, the investigation carried out by Yousefi et al. [57] revealed no notable discrepancy in CAT activity between the control and treatment groups of common carp fed with marjoram (*Origanum majorana*) extract In contrast to the findings of our study, previous research has demonstrated that incorporating diets enriched with extracts from ginger (*Z. officinale*) [65] and oak leaf (*Q. castaneifolia*) [72].

MDA is acknowledged as a byproduct of lipid peroxidation and is frequently employed as an indicator of oxidative damage [79]. The serum levels of MDA in common carp fed with 0.25% valerian extract-supplemented feed exhibited a consistent decline, irrespective of the level of inclusion. The impact of valerian extract on antioxidant enzymes is likely attributable to the presence of flavonoids, which are renowned for their robust antioxidant capabilities in neutralizing free radicals [80]. Similar results have been documented in studies examining the effects of various extracts on common carp (*C. carpio*). These include Artemisia (*A. annua*) leaf [73, 81], oak (*Q. castaneifolia*) leaf [72], three medicinal plants (common mallow [*Malvae sylvestris*]), oregano (*O. vulgare*), and Persian shallot (*Allium hirtifolium* Boiss) [63], marjoram (*O. majorana*) [57], dandelion [82], and the combination of medicinal herbs (coriander [*C. sativum*], common mallow [*M. sylvestris*], *Pandanus tectorius* [83], and oak acorn [*Q. brantii*]) [66]. Wang et al. [84] found that *Yucca schidigera* extract reduced MDA levels in mirror carp (*C. carpio*). Furthermore, Qin et al. [85] found that in common carp fed with diets supplemented with bitter melon extract (at the highest dose of 1%), serum MDA content decreased. Lin et al. [86] found that after feeding gibel carp (*C. auratus gibelio* var.)

with *Astragalus membranaceus* extract for 8 weeks, the MDA content in the liver decreased. Salih et al. [75] found that common carp (*C.carpio*) fed diets supplemented with 0.5% and 1% hawthorn extract (*Crataegus elbursensis*) for 60 days exhibited decreased MDA levels. In contrast to our study, Hoseini et al. [87] found that common carp fed 1% Russian OLE had the lowest MDA levels, while 3% supplementation resulted in the highest MDA levels. Similarly, Rashidian et al. [88] reported no significant difference in MDA levels between control and nutmeg extract-treated common carp after 4 weeks. Plant extracts vary widely in the bioactive compounds they contain, such as polyphenols, flavonoids, and antioxidants. These compounds can influence antioxidant enzyme activities (GPX and CAT) and lipid peroxidation (MDA) in fish. The concentration of the extract is crucial, with higher doses enhancing enzyme activities and TAC, while lower doses have minimal effects. Extended feeding duration strengthens these effects. Given species and tissue differences, further research is needed to better understand how plant extracts affect antioxidant capacity and enzyme activities in fish.

ALT, AST, ALP, and LDH enzymes are not exclusive to plasma; they are also present in tissues such as the liver, heart, gills, kidneys, muscles, and other organs [89, 90]. Consequently, the concentrations of these enzymes can provide specific insights into organ dysfunction [91]. In this study, common carp fed diets with 0.25% and 0.5% valerian extract showed a dose-dependent increase in AST activity, while ALT activity decreased. LDH and ALP activities remained unchanged across all groups. These findings suggest valerian extract may influence liver function by increasing AST and reducing ALT, potentially indicating regulatory effects. The unchanged LDH and ALP levels call for further research to clarify the extract's impact on these enzymes. Bahrami Babaheydari, Paykan Heyrati, and Dorafshan [92] observed a reduction in LDH and ALT activities in juvenile common carp fed with varying levels of wood betony (*Stachys lavandulifolia Vahl*) extract over a 10-week period. Conversely, ALP and AST activities remained unaltered. Fallahpour, Banaee, and Javadzade [93] observed no changes in plasma ALT and ALP activities in common carp treated with a hydroalcoholic extract of marshmallow (*Althaea officinalis L.*) for a period of 60 days. Nevertheless, the activities of AST and LDH were markedly elevated in fish that had been fed a 10 g/kg dose of *A. officinalis*, in comparison to the control group. Banaee et al. [94] observed an increase in AST, ALT, ALP, and LDH levels in the livers of common carp fed diets supplemented with 1% marshmallow extract (*Althaea officinalis* L.), which was associated with cytotoxicity. Soleimany et al. [95] observed an increase in AST, LDH, and ALP activities in common carp fed with 10 g of *A. officinalis* extract for 45 days. Taher and Al-Niaeem [96] observed that the serum enzyme levels (ALT, AST, ALP, and LDH) decreased in juvenile common carp fed with *Nerium indicum* leaf extract for 14 days, particularly with 0.6% *N. indicum* extract treatment. Al-Shawi et al. [68] found that plasma levels of liver metabolic enzymes (AST, ALT, ALP, and LDH) did not change in all experimental groups after feeding common carp with silymarin extract for 60 days. In a previous study, it was demonstrated that the levels of liver metabolic enzymes (ALP, AST, ALT, and LDH) in common carp fed with *Crataegus elbursensis* extract exhibited a decline over a 60-day period and following exposure to acetamiprid for 14 days [75]. In another study, common carp were provided with OLE for a period of 8 weeks. Prior to the bacterial challenge, serum ALT and AST enzyme activities were comparable between the group fed with 0.1% OLE and the control group. Following the challenge, the fish that had been fed with 0.1% OLE exhibited the lowest values of these biomarkers, indicating liver dysfunction, in comparison to the other groups [78]. Consequently, the impact of plant extracts on liver enzyme activity in common carp is contingent upon the specific type and concentration of the extract employed.

Innate immune biomarkers, including TP, ALB, and GLB, are of significant importance as serum proteins produced and stored by liver tissues. A reduction in the concentration of these biomarkers has been demonstrated to result in a weakening of the immune system [97, 98]. The administration of valerian extract was associated with an increase in serum TP and GLB levels. Similarly, the administration of the highest dose of valerian extract to common carp resulted in an increase in serum ALB levels. Yilmaz et al. [32] reported that the supplementation of PM in feeds resulted in an increase in ALB levels in some groups of rainbow trout. Similarly, the addition of plant extracts to feeds has been demonstrated to elevate serum TP, GLB, and ALB levels in common carp [65, 66, 76, 90]. An elevation in serum TP, ALB, and GLB levels is regarded as a more robust indicator of augmented immunity [99]. An elevation in glucose levels may be indicative of metabolic stress [100]. The results of this study indicate that valerian extract supplementation had no significant effect on glucose levels. The comparable glucose levels observed in the experimental groups indicate that the administration of valerian extract does not induce stress. In contrast, Yilmaz et al. [32] demonstrated that the administration of PM-supplemented feeds resulted in a reduction of serum glucose levels in rainbow trout, as compared to control groups. Similarly, the supplementation of feeds with herbal extracts, including *Althaea officinalis* [93] and *Z. officinale* [57], did not result in a change in serum glucose levels in common carps, as observed in our study. CRT has been demonstrated to prevent nitrogenous waste and renal diseases [101] while simultaneously meeting amino acid requirements and enhancing feed utilization in fish [102]. The present study demonstrated that common carp fed diets supplemented with valerian extract exhibited elevated CRT levels. Similarly, the addition of 5.00 g/kg marshmallow (*Althaea officinalis* L.) extract to the diet of common carp resulted in elevated CRT levels [92].

The utility of cholesterol and triglyceride parameters in evaluating lipid metabolism is well established and plays a pivotal role in the protection of the innate immune system [103]. The findings of this study indicate that the incorporation of valerian extract into the diet of common carp may result in a reduction of cholesterol levels yet no discernible impact on triglyceride levels. Similar results have been found

in other studies that have used extracts from plants such as *Stachys lavandulifolia* [90], a combination of three medicinal plants (*Malvae sylvestris*, *O. vulgare*, and *Allium hirtifolium boiss*) [63], and combined herbal extracts (oak acorn, coriander, and common mallow) [66]. In a similar vein, Mehrinakhi et al. [104] discovered that grape seed extract had no discernible impact on triglyceride levels in common carp. While valerian extract has been demonstrated to be effective in reducing cholesterol levels in common carp, various herbal extracts have been observed to have no impact on triglyceride levels, as evidenced by previous studies.

IL-1β and *TNF-α* are cytokines that play a pivotal role in the regulation of the systemic inflammatory response and immune function. *IL-1β* plays a role in the production of monocytes, leukocytes, lysozyme, and tissue macrophages [105, 106]. The findings of this study indicate an increase in IL-1*β* gene expression levels in common carp fed a diet supplemented with valerian extract compared to the control group. Similarly, significant reductions in IL-1*β* gene expression have been observed in common carp fed with a variety of plant and herbal extracts, including sea buckthorn (*Hippophae rhamnoides* L.), and flavonoids [107]. However, our results contradict those obtained in carp-fed diets supplemented with Moldavian balm (*Dracocephalum moldavia* L.) [108]. The present study did not reveal any alteration in *TNF-α* expression levels in common carp fed with valerian extract. Some researchers have observed an increase in *TNF-α* expression levels in trout fed plant extracts. For instance, the supplementation of common carp with 1% olive leaf (*Olea europea* L.) extract has been observed to result in elevated *TNF-α* expression levels within the spleen tissue [109]. Similarly, Hoseinifar et al. [110] observed a notable elevation in *TNF-α* expression levels among experimental groups of common carp that had been fed a diet incorporating jujube (*Ziziphus jujuba* Mill.) fruit extract, in comparison to the control group. In contrast, Bilen et al. [111] observed that *TNF-α* expression in the kidneys of common carp fed with ethanol extract of *Cistus laurifolius* increased at all sampling times compared to the control group. In contrast, *TNF-α* gene expression in the intestines was not affected in either the treatment or control groups at any sampling time. It is hypothesized that these differences are attributable to the disparate types and doses of plant extracts employed.

IL-10 has the capacity to impede the synthesis of a number of cytokines that are produced as a consequence of antigen or mitogen activation in a variety of cells [112]. The findings of this study indicate that feeding common carp a diet supplemented with a low dose of valerian extract significantly reduced *IL-10* gene expression levels, suggesting an anti-inflammatory response. Similarly, common carp that were supplemented with *Rehmannia glutinosa* extract exhibited a reduction in *IL-10* gene expression levels in the head kidney, spleen, and intestine [113]. In a study conducted by Yilmaz et al. [32], it was demonstrated that all doses of PM resulted in a notable reduction in *IL-10* gene expression levels in treated rainbow trout. In contrast to our findings, Bilen et al. [111] reported an increase in *IL-10* gene expression levels in the kidney and intestine tissues of common carp fed with an ethanolic extract of laurel leaves (*C. laurifolius*) diet. The highest level of *IL-10* gene expression was observed in the intestine, with a 32-fold increase on day 45 of the study. In another study, the expression of the *IL-10* gene was observed to increase in common carp that had been fed with a seed extract of the *Plantago ovata* (psyllium) [114].

HSPs are recruited to facilitate the dissolution or degradation of damaged proteins, which are often associated with stressful situations. *HSP70* is a significant member of this family and is one of the primary proteins activated, particularly under conditions of stress [115, 116]. The present study demonstrated that the expression levels of the *HSP70* gene in the head kidney were markedly diminished in the V-0.25 group that had been administered a valerian extract, thereby indicating a reduction in stress levels in the treated fish. This suggests that valerian extract may possess the capacity to mitigate oxidative stress through its antioxidant effects [117]. Similarly, Rajabiesterabadi et al. [118] observed that the inclusion of olive (*Olea europaea*) leaf extract in the diet of common carp for a period of 8 weeks resulted in a reduction in *HSP70* gene expression within the intestinal tissue. Additionally, Yilmaz [32] discovered that the expression levels of the *HSP70* gene were markedly diminished in the head kidney of all groups of rainbow trout that were fed a diet supplemented with PM. Contrary to our findings, *HSP70* gene expression increased in the livers of *Megalobrama amblycephala* fed anthraquinone extract from *Rheum officinale* Bail [119] and in common carp fed 0.25% and 0.5% *P. ovata* seed extract [114]. However, no significant effect on *HSP70* expression was observed in common carp fed diets containing dandelion (*Taraxacum officinale*) [120] and *P. tectorius* extract [83].

In general, oxidative stress stimulates the expression of genes related to antioxidants in fish in order to counteract free radicals [121]. In particular, valerenic acid has been demonstrated to exert a protective effect against oxidative stress induced by rotenone in SH-SY5Y cells. It plays a pivotal role in the prevention of Parkinson's disease by promoting the expression of genes that encode antioxidant defense mechanisms [122]. Furthermore, it is established that valerenic acid, akin to other nutritional compounds such as whey protein, exerts a beneficial effect on human health as an oxidative stress regulator [123–125]. Furthermore, valenemerin, derived from *V. officinalis*, has been shown to possess neuroprotective effects against oxidative stress [126]. In light of these findings, it can be posited that the administration of *V. officinalis* extract to common carp may result in the downregulation of antioxidant-related gene expression. The present study demonstrated that head kidney *CAT* gene expression levels significantly decreased in the group fed diets supplemented with 0.25% valerian extract compared to the control group, while *SOD* gene expression levels significantly decreased in the groups fed with 0.25% and 0.5% valerian extract. The *GPX* gene expression level has been observed to decline in all experimental groups when compared to the control group. The effects of vValerian extract on antioxidant enzyme activities in common carp have been the subject of several studies. Wang et al. [84] demonstrated that dietary supplementation of *Y. schidigera* extract resulted in elevated relative mRNA levels of CuZn-SOD, CAT, and GPx1a

in mirror carp. Similarly, Taheri Mirghaed, Paknejad, and Mirzargar [73] demonstrated that an *Artemisia* (*A. annua*) leaf extract enhanced the activities of *SOD*, *CAT*, and *GPX* in the liver of common carp. However, following exposure to ammonia, a reduction in gene activity was observed in the liver. In a study conducted by Mahboub et al. [127], it was observed that the common carp exhibited enhanced antioxidant responses when exposed to food-derived zinc oxide nanoparticles (ZnO-NPs) and fed an *Allium hirtifolium* extract. The researchers indicated that as a consequence of the feeding regimen, the expression values of *SOD*, *CAT*, and *GPX* in the liver tissue increased. Cheng, Park, and Gri [83] observed an increase in the expression levels of *SOD* and *CAT* in the head kidneys of common carp fed with *P. tectorius* extract, while the expression level of GPX remained unaltered. In another study, researchers discovered that OLE successfully elevated the transcription levels of liver *SOD*, *Nrf2*, and protein kinase C in common carp, with the objective of reducing oxidative stress and inflammation [78]. Ahmadifar et al. [114] discovered that psyllium (*P. ovata*) seed extract (PSE) did not affect the expression of the CAT gene but did increase the expression of the SOD and GPX genes in the gills of common carp. Lastly, Bin Li et al. [128] observed that feeding yellow river carp with *Humulus scandens* extract for 8 weeks resulted in elevated expression levels of *SOD*, *CAT*, and *GPX* genes in the gills and liver. Given the contradictory findings, further research is needed to elucidate the factors influencing changes in antioxidant gene expression in association with varying dietary levels of plant extracts.

5. Conclusion

In conclusion, the supplementation of common carp (*C. carpio*) diets with valerian extract did not result in a significant impact on growth performance or mucus immune parameters. Nevertheless, it demonstrated potential antioxidant effects, as indicated by reduced MDA levels and altered gene expressions linked to oxidative stress and inflammation. It is noteworthy that the group supplemented with 0.25% valerian extract (V-0.25) exhibited the most pronounced effects. Further studies employing longer periods or varying concentrations of the valerian extract may facilitate a more profound understanding of its physiological effects in carp.

Data Availability Statement

The data supporting the findings of this study are available upon request from the corresponding author.

Conflicts of Interest

The authors declare no conflicts of interest.

Author Contributions

Ebru Yilmaz: project administration, investigation, conceptualization, writing–original draft. **Hamidreza Ahmadniaye Motlagh:** methodology, supervision, writing–original draft. **Sevdan Yilmaz:** writing–review and editing.

Funding

This study was financially supported by Aydin Adnan Menderes University, Collaborative Research Projects (ADU-BAP), with the project number ZRF-23014.

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