

Dietary inclusion of curry plant (*Helichrysum italicum*) extract in rainbow trout (*Oncorhynchus mykiss*) culture: Growth performance and gene expression related to digestion, antioxidant, and immune responses

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Abstract

In this study, a 60-day trial was conducted to determine the effects of curry plant (*Helichrysum italicum*) extract in the diet of rainbow trout (*Oncorhynchus mykiss*) with an initial average live weight of 15.84 ± 0.05 g on growth performance and gene expressions associated with digestion, antioxidant capacity, and immune response. Four experimental diets were formulated as a basal diet (Control; C) and diets supplemented with varying levels of curry plant extract (CP05; 0.5 g kg^{-1} , CP1; 1 g kg^{-1} , and CP2; 2 g kg^{-1}). Growth indices showed significant increases in all curry plant extract groups compared to the C group ($p < 0.05$). Similarly, higher levels of trypsin, lipase, and amylase expressions were observed in the curry plant extract groups compared to the C group ($p < 0.05$). The superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx)

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gene expression levels were elevated in the CP extract groups ($p < 0.05$). The expression levels of interleukin-1 β (IL-1 β), tumor necrosis factor (TNF- α), and interleukin-8 (IL-8) were also influenced by the incorporation of CP extract into the diet, showing significant changes ($p < 0.05$). In conclusion, this study experimentally demonstrates that supplementing the diet of rainbow trout with CP extract can positively influence healthy growth parameters.

KEYWORDS

curry plant, extract, growth, phenolic compound, rainbow trout

1 | INTRODUCTION

As the fastest-growing food production sector, aquaculture has the potential to meet growing food demand (Ahmad et al., 2021; FAO, 2022). Rainbow trout (*Oncorhynchus mykiss*) are one of the species that play a vital role in this growth. Still, challenges such as high stocking rates, disease management issues, and stress-induced disease resistance are increasing (Payne et al., 2022). With antibiotic restrictions, interest in alternative immune-boosting strategies is increasing (Bondad-Reantaso et al., 2023), while limitations in feed ingredients are becoming more apparent (Aas et al., 2022).

The administration of functional ingredients in the diet is a promising option that can improve feed utilization and minimize disease problems (Goda et al., 2018). Eco-friendly, non-toxic, and bioactive functional ingredients have gained popularity in finfish culture over the last decade (Vijayaram et al., 2022). Among these additives, medicinal aromatic plants improve feed utilization, promote healthy growth, support the immune system, and enhance antioxidant properties, thus increasing productivity during the culture period (Awad & Awaad, 2017). The use of flower extracts from medicinal aromatic plants in the diet has been reported to have positive effects on growth, the immune system, and antioxidant activity in various farmed fish (Rashidian et al., 2020; Shekarabi et al., 2021; Sun et al., 2019).

The curry plant (CP) (*Helichrysum italicum*) from the Asteraceae family, a cultivated medicinal aromatic plant, is widely used not only as an ornamental plant but also in alternative medicine and the food industry due to its various pharmacological activities (Kramberger et al., 2021; Viegas et al., 2014). The antioxidant, antimicrobial, and regenerative effects of CP have been investigated in numerous in vitro and in vivo studies. Its internal use has been reported to exhibit anti-inflammatory effects in mice and blood sugar-lowering effects in rats (Kramberger et al., 2021). A systematic review by Šovljanski et al. (2024) indicated that CP essential oil demonstrates strong antimicrobial activity against various bacterial and fungal species, confirmed through in vitro methods such as agar diffusion and microdilution. Gismondi et al. (2020) showed that the essential oil of this plant inhibits cell growth in B16F10 murine melanoma cells. Similarly, Cebi et al. (2024) reported that CP oil suppressed Caco-2 cancer cell growth by 48% while showing no toxic effects on healthy L929 fibroblast cells. Additionally, in vitro studies by Serra et al. (2024) found that the hydrosol form of the plant enhances wound healing by increasing collagen production in human skin stem cells and fibroblasts. Moreover, recent research suggests that CP may also support growth through indirect mechanisms. For instance, Kenig et al. (2021) demonstrated that an infusion of this plant enhances fat metabolism and energy expenditure in liver cells. Another study in diabetic rats found that topically applied essential oil accelerates wound healing and supports tissue regeneration (Andjić et al., 2022). Furlan and Bren (2023) reported that the plant may also contribute to growth indirectly by preserving mitochondrial functions and supporting cellular energy production. Based on such background, using CP as a feed additive in diet may support healthy fish growth. However,

no previous studies have focused on the beneficial effects of dietary CP in aquatic organisms. Therefore, within the scope of this study, which aims to enhance culture efficiency in a healthy way, CP extract was tested for the first time as a functional feed additive in aquaculture during a 60-day feeding trial. At the end of the trial, the growth performance and gene expression related to antioxidant activity, immune parameters, and digestive enzymes of rainbow trout fed with different amounts of CP extract in the diet were determined.

2 | MATERIALS AND METHODS

2.1 | Preparation and phenolic composition of CP extract

The preparation of the extract from the CP (obtained from Van YYÜ Medicinal and Aromatic Plants Garden) was performed with some modifications in the methods described by Karataş et al. (2020) and Ghafarifarsani et al. (2022). Fresh plants (flower parts) were washed with distilled water, dried in the shade at 24°C for 10 days, and then pulverized with a grinder. 100 g of the plant was mixed with 300 mL ethanol (96%) and kept at 200 rpm on an orbital shaker (24 h). The mixture was passed through sterile cheesecloth to separate the solids, centrifuged for 5 min, and filtered through a Whatman filter paper (Whatman Paper, No. 1). The organic solvent was removed by evaporator, and the pure extract was kept at 4°C until use. The extract was freeze-dried before inclusion in the diet using a laboratory-scale lyophilizer (Christ Alpha 2–4 LD Plus) with a standard program.

The phenolic compound concentration of the CP extract was analyzed at Atatürk University (Eastern Anatolia High Technology Application and Research Center, DAYTAM, Erzurum, Turkey). The analysis was performed using an Agilent 6460 Triple Quad LC–MS/MS system paired with a 1290 Infinity UPLC and a reversed-phase C18 column. The column temperature was maintained at 30°C throughout the process. The mobile phases consisted of ultrapure water with formic acid (phase A) and acetonitrile with formic acid (phase B). A consistent solvent flow rate of 0.4 mL min⁻¹ was applied, and the injection volume was set at 5 µL. The CP extract was analyzed according to the quality assurance/quality control (QA/QC) protocol (Can et al., 2024). Phenolic compounds found in CP extract are shown in Table 1.

TABLE 1 The phenolic compound of curry plant extract.

Compound	Final concentration (ng mL ⁻¹)
Chlorogenic acid	49331.11
Quinic acid	12392.32
Quercetin	9039.10
Fumaric acid	2496.05
Caffeic acid	1062.88
Vanillic acid	732.04
4-OH-Benzoic acid	628.18
Syringic acid	537.90
Gallic acid	224.59
Ferulic acid	75.04
Isorhamnetin	72.28
p-Coumaric acid	39.60
Rosmarinic acid	20.52
Peonidin-3-o-glucoside	18.19
Cyanidin-3-o-glucoside	8.18

2.2 | Diets preparation and proximate composition

The ingredients were passed through a mill to make fine flour (<100–150 μm) and mixed with a laboratory-type mixer to form a dough. CP extract was included in the formulated basal diets at 0.5, 1, and 2 g kg^{-1} . Pellets with 2- and 3-mm diameters were obtained with a cold-extruded pellet machine. Then, for gelatinization, the pellets were cooked with a pressurized steamer (1-atmosphere pressure, 20 min) and dried at 40°C to ensure constant moisture content. Crude protein, crude lipid, crude ash, and crude fiber levels for raw materials and diets were determined according to AOAC (2000). Crude protein was analyzed using the Kjeldahl method, and crude lipid levels were determined using the automated extraction method ANKOM XT-15 (Macedon, NY). Crude ash was determined by incineration of the samples in a muffle furnace at 525°C for 12 h. Crude fiber was determined by acid/alkali hydrolysis of the filtered residue and ignition of the sample dried for 3 h. Diet formulations are given in Table 2.

2.3 | Experimental fish, design, and culture conditions

Rainbow trout were obtained from a local fish farm and kept for 15 days at Tokat Gaziosmanpaşa University, Tokat, Türkiye, where the trial was conducted, to acclimatize to the trial conditions. During acclimatization, fish were hand-fed a basal diet thrice daily until satiated. A total of 240 healthy rainbow trout (15.84 \pm 0.05 g) were randomly divided into 12 tanks of 200 L volume, with 20 fish in each tank in triplicate. Formulated diets were prepared to contain 0, 0.5, 1, and 2 g kg^{-1} CP extract and labeled C, CP05, CP1, and CP2, respectively. The feeding protocol was administered ad libitum three times a day (08.00 am, 1.00 and 6.00 pm) with the formulated diets until the fish reached apparent satiation (all feed consumed) for 60 days. A continuous natural spring water flow of 4 L min^{-1} was

TABLE 2 Ingredients and composition of experimental diets.

Ingredients (%)	C	CP05	CP1	CP2
Fish meal	30	30	30	30
Soy protein concentrate	11	11	11	11
Soybean meal	23	23	23	23
Collagen	1.5	1.5	1.5	1.5
Corn gluten	4	4	4	4
Rice protein concentrate	5	5	5	5
Wheat flour	10	9.95	9.9	9.8
Fish oil	14.4	14.4	14.4	14.4
Vitamin-mineral premix	1	1	1	1
Vitamin C	0.1	0.1	0.1	0.1
Curry plant extract	0	0.05	0.1	0.2
Total	100	100	100	100
Crude protein	48.16 \pm 0.01	48.22 \pm 0.02	48.09 \pm 0.02	48.14 \pm 0.01
Crude lipid	18.17 \pm 0.02	18.10 \pm 0.01	18.09 \pm 0.01	18.11 \pm 0.02
Crude ash	7.82 \pm 0.02	7.78 \pm 0.03	7.86 \pm 0.01	7.89 \pm 0.01
Cellulose	2.14 \pm 0.01	2.19 \pm 0.01	2.21 \pm 0.01	2.34 \pm 0.01

Note: Data were presented as means \pm standard error (SE).

Abbreviations: C, basal diet; CP05, a diet supplemented with 0.05% curry plant extract; CP1, a diet supplemented with 0.1% curry plant extract; CP2, a diet supplemented with 0.2% curry plant extract.

provided to each tank throughout the trial. Each day, the water temperature ($^{\circ}\text{C}$), pH, and dissolved oxygen (mg L^{-1}) were recorded, resulting in measurements of $15.4 \pm 0.3^{\circ}\text{C}$, 7.3 ± 0.2 , and $7.4 \pm 0.3 \text{ mg L}^{-1}$, respectively.

2.4 | Growth indices and sampling

At the end of the trial, growth parameters were calculated using the following standard formulas:

$$\text{Weight gain (WG, g)} = \text{Final weight (g)} - \text{Initial weight (g)}.$$

$$\text{Daily weight gain (DWG, g)} = \text{Weight gain (g)} / \text{days}.$$

$$\text{Specific growth rate (SGR, \%day}^{-1}\text{)} = [\text{Ln (final weight)} - \text{Ln (initial weight)}] / \text{days} \times 100.$$

$$\text{Protein efficiency ratio (PER)} = \text{Weight gain (g)} / \text{Protein intake (g)}.$$

$$\text{Feed conversion ratio (FCR)} = \text{Total feed given (g)} / \text{Weight gain (g)}.$$

$$\text{Survival rate (SR, \%)} = [\text{Final number of fish} / \text{Initial number of fish}] \times 100.$$

Fish were fasted for 24 h before sampling. Three fish from each tank, nine per group, were sampled and placed in a eugenol bath (200 mg L^{-1} , 60 s). For gene expression analyses, liver (antioxidant enzymes and immune parameters) and intestinal (digestive enzymes) tissues were immediately removed from sampled fish and stored in RNAlater solution until RNA isolation (stored at 4°C for 24 h and then at -18°C until molecular analysis).

2.5 | RNA isolation

Total RNA was extracted from tissue samples using the DiaRex[®] Total RNA Extraction Kit (TR-0877-100, Diagen, Ankara, Turkey), following the manufacturer's instructions. In summary, $600 \mu\text{L}$ of LBD solution was added to 25 mg of tissue, which was then homogenized using a TissueLyser LT (Qiagen). After homogenization, the mixture was centrifuged twice at 3000 rpm for 30 s each time and allowed to incubate at room temperature for 5 min. The resulting supernatant was transferred to a 2 mL Eppendorf tube, mixed with $600 \mu\text{L}$ of 99% ethanol, and centrifuged at 8000 g (10,000 rpm) for 1 min. The supernatant was then passed through a spin column and centrifuged again at 8000 g (10,000 rpm) for 1 min. Following this, $500 \mu\text{L}$ of WBD-1 and WBD-2 solutions were added sequentially, with centrifugation at 8000 g (10,000 rpm) for 1 min after each addition to complete the washing process. Finally, $30 \mu\text{L}$ of EBD solution was added, and RNA was eluted by centrifugation at 8000 g for 2 min. The quantity and purity of the isolated RNA were assessed using a nanospectrophotometer (QIAxpert), measuring absorbance at 260 and 280 nm. RNA purity was evaluated based on the RNA (260/280) ratio for each sample.

2.6 | cDNA synthesis

All steps of cDNA synthesis were conducted on ice using the SuScript cDNA Synthesis Kit (RT01A026, Sugenomics, Ankara, Turkey). The RNA samples were standardized to a concentration of 100 ng per microliter. For the reaction,

6 μ L of RNA, 4 μ L of 5 \times RT mix, and 10 μ L of DNase/RNase-free water were combined to reach a total volume of 20 μ L. This mixture was then incubated in a thermal cycler (Rotor Gene Q 9000) at 42°C for 60 min, followed by 80°C for 10 min.

2.7 | Gene expression analysis

cDNA products were analyzed using real-time PCR on a RotorGene Q 9000 (Qiagen) with 2 \times SuYBRGreen qPCR Master Mix (PCR01C0253, Sugenumics, Ankara, Turkey). β -actin was used as the reference gene for gene expression analysis, while the target genes included superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), interleukin-1 β (IL-1 β), tumor necrosis factor (TNF- α) and interleukin 8 (IL-8), trypsin, lipase, and amylase. The primers used in the study are listed in Table 3.

The PCR mix consisted of 12 μ L of 2 \times SuYBRGreen qPCR Master Mix, 2.5 μ L each of forward and reverse primers, and 4 μ L of H₂O, bringing the volume to 21 μ L. An additional 4 μ L of cDNA was added to achieve a final volume of 25 μ L. The PCR amplification cycling parameters were set as follows: an initial pre-denaturation at 95°C for 10 min, followed by 45 cycles of 95°C for 45 s and 60°C for 45 s for annealing. To minimize experimental error, PCR reactions were performed in duplicate for all samples. The β -actin reference gene, which showed consistent expression between treatment groups within a given tissue, was used to normalize the Ct values obtained following real-time PCR using the coefficient of variation criteria. Target gene expression levels were quantified using the comparative cycle threshold (Ct) method (2^{- $\Delta\Delta$ Ct}) (Livak & Schmittgen, 2001).

TABLE 3 Primer sequences used to determine the expression of digestive enzyme, antioxidant and immune-related genes in rainbow trout.

Target gene	Primer sequence (5'–3')	Amplicon size (bp)	Annealing T _m (°C)	Accession no.
β -Actin (Beta-Actin)	F: GGAGGCTCCATCTTGCTTC R: GAAGTGGTAGTCGGGTGTGG	158	61	AJ438158.1
Trypsin	F: TATGTGAAGCCCATCCCCTT R: TCTGCCTCCGTCCATGTATC	219	59	XM_021591477
Lipase	F: ATGGCAGCTTTTCCTTCCT R: ATGGTCAGGGTGAGGTTTCAG	197	58	NM_001197210
Amylase	F: ACAAGGAGCATGTGAGGGAA R: CAGGTGGTGTGAGGTTGTG	151	58	XM_036935172
CAT (Catalase)	F: TGATGTACACAGGTGCGTA R: GTGGGCTCAGTGTGTTGAG	195	58	XM_021557350.2
GPx (Glutathione peroxidase)	F: CGAGCTCCATGAACGGTACG R: TGCTTCCCGTTACATCCAC	183	60	HE687022.1
SOD (Superoxide dismutase)	F: TGGTCTGTGAAGCTGATTG R: TTGTCAGCTCCTGCAGTCAC	201	58	AF469663.1
IL-1 β (Interleukin-1 beta)	F: AGCAGGACTACACCAAACCG R: TCCTGATCGTAGAGGCCCAA	184	59	AJ004821.1
TNF- α (Tumor necrosis factor-alpha)	F: GGCTGTGTGGCTTCTCTTA R: AAATGGATGGCTGCTTTCGC	190	58	NM_001124374.1
IL-8 (Interleukin-8)	F: CACAGACAGAGAAGGAAGGAAAG R: TGCTCATCTTGGGGTTACAGA	162	60	NM_001124279.1

2.8 | Statistics

At the end of the trial, the collected data were analyzed using a one-way ANOVA. To identify significant differences between groups, the Tukey test, a post hoc analysis, was employed. Tests were performed with a significance threshold of $\alpha = 0.05$, and results were expressed as mean \pm standard error. Prior to testing, skewness and kurtosis statistics regarding normality assumptions were examined, and the Levene test was used to determine the homogeneity of variances. Statistical analyses were conducted using the SPSS 12 software (Colman & Pulford, 2006). Linear and quadratic polynomial contrasts were applied to assess the linear and quadratic effects of various dietary CP extracts on the growth parameters, with a significance level of $p < 0.10$, using the GLM procedure in JMP software (version 8, SAS Institute Inc., Cary, NC). A segmented regression model with a breakpoint was used to predict the optimum level of dietary CP extract in fish based on FW, SGR, and PER (Oosterbaan, 1994).

3 | RESULTS

The growth parameters of rainbow trout evaluated at the end of the 60-day trial with different levels of dietary CP extract are presented in Table 4. The growth performance metrics, including final weight (FW), weight gain (WG), daily weight gain (DWG), specific growth rate (SGR), and protein efficiency ratio (PER) of the fish, showed a quadratic increase with dietary CP extract doses. Also, FCR levels were quadratically improved with dietary CP extracts. FW values were superior and statistically different ($p < 0.05$) in the CP extract groups compared to the C group. The results obtained for FW in the groups were similar in terms of WG and DWG ($p < 0.05$). The lowest SGR levels were found in the C group ($p < 0.05$), while the CP extract groups were similar among themselves. PER values reached the highest levels in the CP extract groups and differed from the C group ($p < 0.05$). The FCR values in the CP extract groups were the lowest, indicating the best outcomes, and were significantly different from those in the C group ($p < 0.05$). There was no mortality in the tanks during the trial, and the survival rate was recorded at 100% in all groups.

TABLE 4 Growth performance of rainbow trout fed diets supplemented with different levels of CP extract.

Parameters	C	CP05	CP1	CP2	P values	
					Linear	Quadratic
IW	15.82 \pm 0.02	15.83 \pm 0.04	15.90 \pm 0.02	15.84 \pm 0.03	-	-
FW	71.27 \pm 0.70 ^b	89.37 \pm 0.50 ^a	88.58 \pm 0.20 ^a	87.30 \pm 0.27 ^a	0.0001	0.0001
WG	55.46 \pm 0.71 ^b	73.54 \pm 0.53 ^a	72.69 \pm 0.22 ^a	71.47 \pm 0.23 ^a	0.0001	0.0001
DWG	0.93 \pm 0.01 ^b	1.22 \pm 0.01 ^a	1.21 \pm 0.00 ^a	1.19 \pm 0.01 ^a	0.0001	0.0001
SGR	2.51 \pm 0.02 ^b	2.88 \pm 0.02 ^a	2.86 \pm 0.01 ^a	2.85 \pm 0.00 ^a	0.0001	0.0001
PER	2.32 \pm 0.03 ^c	2.72 \pm 0.02 ^a	2.70 \pm 0.01 ^{ab}	2.63 \pm 0.01 ^b	0.0001	0.0001
FCR	0.90 \pm 0.01 ^a	0.76 \pm 0.01 ^b	0.77 \pm 0.00 ^b	0.79 \pm 0.00 ^b	0.0001	0.0002
SR	100	100	100	100	-	-

Note: Data were presented as means \pm standard error (SE). Different lowercase letters in each line showed significant differences ($p < 0.05$).

Abbreviations: C, basal diet; CP05, a diet supplemented with 0.05% curry plant extract; CP1, a diet supplemented with 0.1% curry plant extract; CP2, a diet supplemented with 0.2% CP extract; DWG, average daily gain (g); FCR, feed conversion ratio; FW, final weight (g); IW, initial weight (g); PER, protein efficiency ratio (%); SGR, specific growth rate (% day⁻¹); SR, survival rate (%); WG, weight gain (g).

The broken-line analysis of FW estimated that 0.52 g kg^{-1} of CP extract in the diet had an optimal effect on the final weight of the fish. Additionally, SGR and PER indicated that 0.54 g kg^{-1} of CP extract in the diet provided the best results for the specific growth rate and protein efficiency ratio of the fish (Figure 1). Beyond these thresholds, the yields declined (breakpoint, knot).

Rainbow trout fed diets supplemented with different levels of CP extract were evaluated comparatively among groups regarding gene expression. Gene expression levels related to digestion in the intestine of rainbow trout are presented in Figure 2. The trypsin expression level was upregulated in the CP05, CP1, and CP2 groups compared to the C group ($p < 0.05$). In terms of lipase gene expressions, while upregulation was recorded in all experimental groups compared to the C group, these values were 3.5-fold in the CP05 group, 6.5-fold in the CP1 group, and 7.5-fold in the CP2 group ($p < 0.05$). When amylase gene expression levels were compared, upregulation was realized in the CP05, CP1, and CP2 groups compared to the C group ($p < 0.05$).

The expressions of SOD, CAT, and GPx-related genes in the liver of rainbow trout were compared among the groups, and the differences in expression levels are given in Figure 3. SOD expression levels were upregulated in the CP05 and CP2 groups compared to the C group ($p < 0.05$), while the CP1 group was similar to the C group ($p > 0.05$). CAT levels were upregulated in all experimental groups compared to the C group ($p < 0.05$). In GPx-related gene expression levels, the CP05, CP1, and CP2 groups showed significant differences compared to the C group ($p < 0.05$).

The expression levels of IL-1 β , TNF- α , and IL-8 genes in the liver of rainbow trout are given in Figure 4. IL-1 β gene expression levels were upregulated in the CP05 and CP2 groups compared to the C group, and these levels were 3.8-fold in the CP05 group and 5.5-fold in the CP2 group ($p < 0.05$). In the CP1 group, a significant upregulation was found compared to the C group ($p < 0.05$). Regarding TNF- α gene expression levels, upregulation was observed in the CP1 and CP2 groups compared to the C group ($p < 0.05$), while the CP05 group was similar to

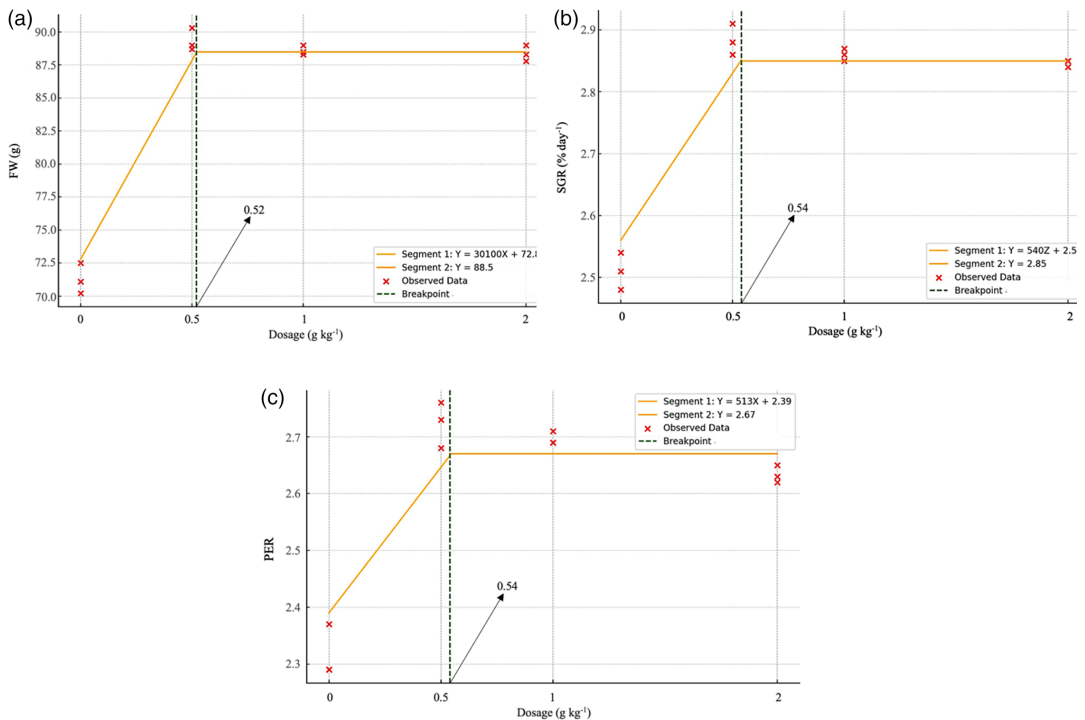


FIGURE 1 Segmented regression with a breakpoint of rainbow trout fed with dietary curry plant extract. (a) Final weight, (b) specific growth rate, and (c) protein efficiency ratio.

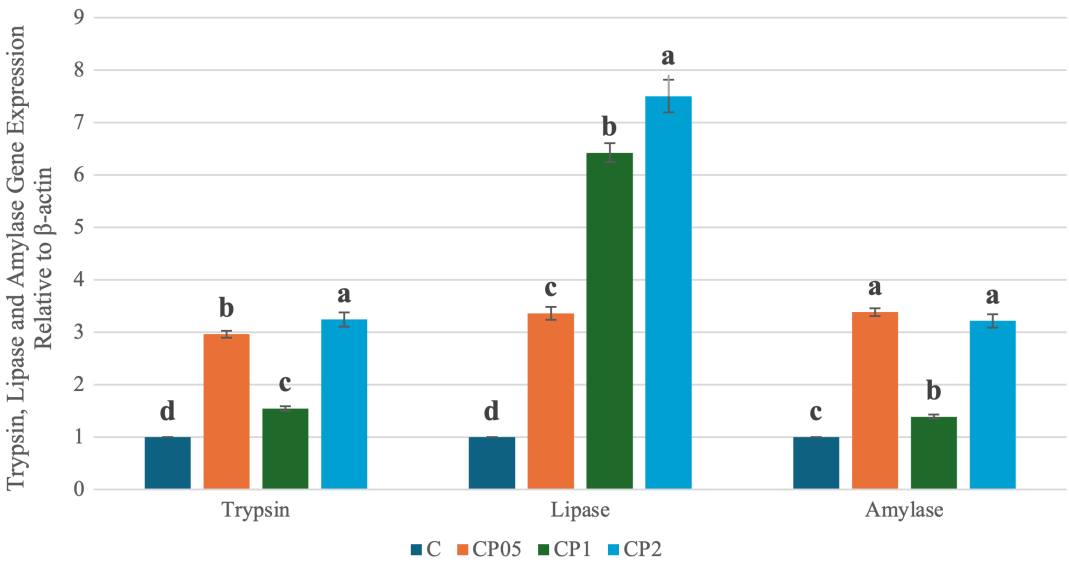


FIGURE 2 Expression levels of digestive enzyme-related genes in the intestine of rainbow trout fed with dietary curry plant extract. The data are the mean ± SE of three fish from each tank. Significance was determined using one-way ANOVA, followed by Tukey's multiple range test. Bars with different letters indicate significant differences ($p < 0.05$).

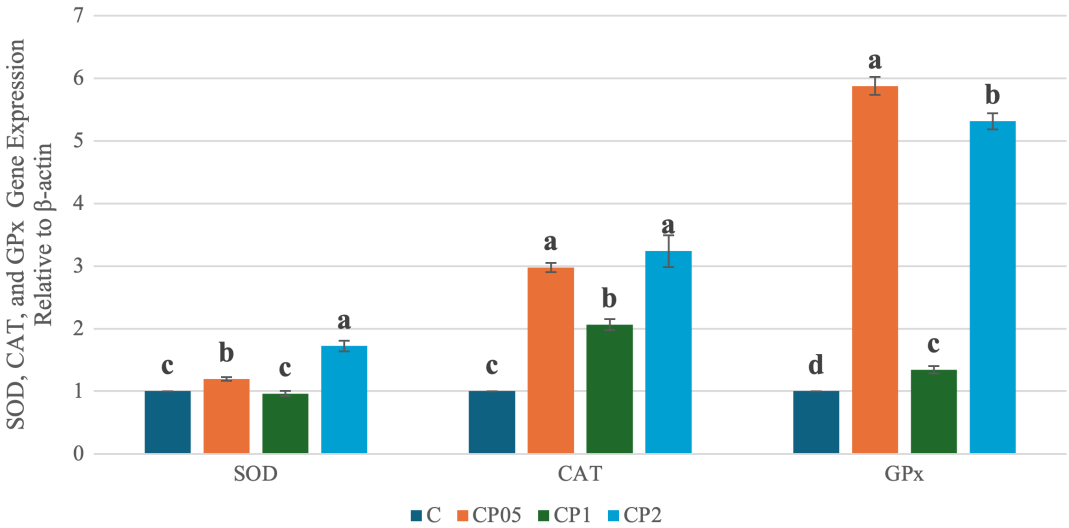


FIGURE 3 Expression levels of antioxidant enzyme-related genes in the liver of rainbow trout fed with dietary curry plant extract. The data are the mean ± SE of three fish from each tank. Significance was determined using one-way ANOVA, followed by Tukey's multiple range test. Bars with different letters indicate significant differences ($p < 0.05$).

the control group ($p > 0.05$). IL-8 gene expression was significantly upregulated in the CP1 and CP2 groups compared to the C group ($p < 0.05$). In the CP05 group, a significant downregulation was detected compared to the C group ($p < 0.05$).

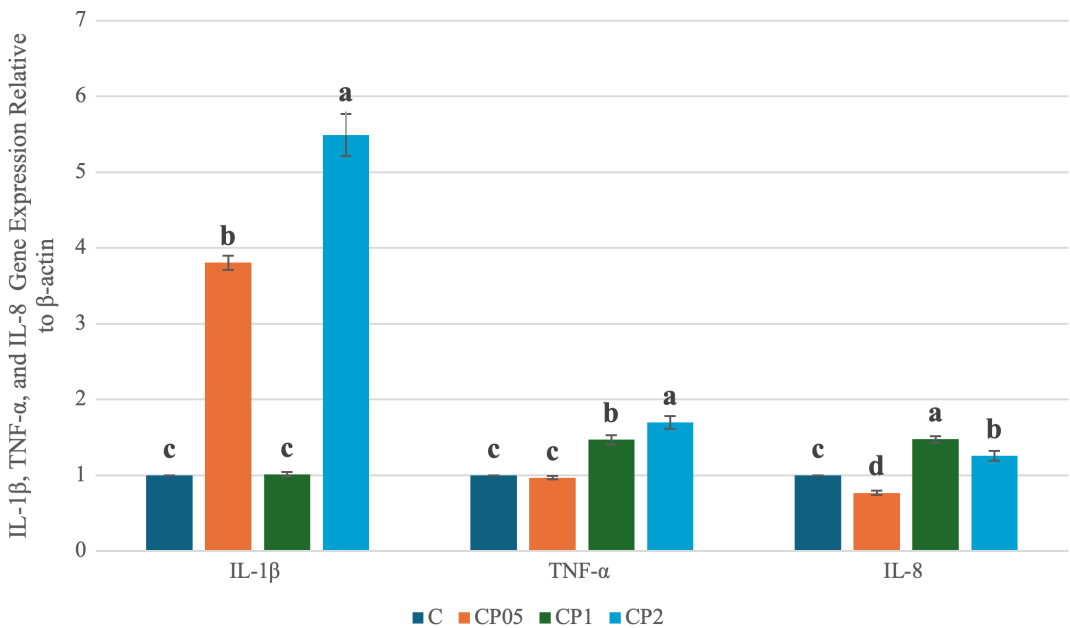


FIGURE 4 Expression levels of immune-related genes in the liver of rainbow trout fed with dietary curry plant extract. The data are the mean \pm SE of three fish from each tank. Significance was determined using one-way ANOVA, followed by Tukey's multiple range test. Bars with different letters indicate significant differences ($p < 0.05$).

4 | DISCUSSION

In this study, we focused on gene expression levels besides growth parameters, as they provide information on the molecular mechanisms underlying the observed physiological responses. Expression levels of genes involved in antioxidant and digestion pathways can indicate how dietary supplementation affects transcriptional regulation (Habte-Tsion et al., 2016). This approach also facilitates understanding the specific genes that respond to dietary intervention, which could contribute to future studies on enzyme activity and functional testing. These data aim to elucidate the mechanisms through which the plant extract exerts its effect.

Although medicinal aromatic plants, which are functional feed additives in fish culture, are not considered primary planning for growth, previous studies have reported significant improvements in the growth of fish fed with these feed ingredients (Gholamhosseini et al., 2021; Salomón et al., 2020; Shalvei et al., 2017). Medicinal aromatic plant extracts are known to increase digestive enzyme secretion and positively affect appetite (Immanuel et al., 2009; Rashidian et al., 2020). In addition, these plant extracts are rich in phenolic compounds that can promote growth and enhance the metabolism of fish (Ghafariarsani et al., 2022; Wangkahart et al., 2022). In the present study, according to the phenolic component analysis performed for the extract of CP extract, the presence of 15 different components was determined, and important phenolics such as chlorogenic acid, quinic acid, and quercetin were represented at high levels. Chlorogenic acid has various biological properties, such as immunogenic, antibacterial, antioxidant, and anti-inflammatory effects (Kabir et al., 2014; Naveed et al., 2018; Xu et al., 2012). Chlorogenic acid, as a feed additive, has been reported to improve growth performance in various animals, including fish (Jin et al., 2023; Liu et al., 2022). It was reported that 600–800 mg kg⁻¹ dietary supplementation of chlorogenic acid in rainbow trout improved FW, WG, SGR, and FCR parameters (Ghafariarsani et al., 2023). Various pharmacological studies have demonstrated quinic acid's antioxidant, antimicrobial, and antiviral properties (Bai et al., 2022; Pero et al., 2009; Zanello et al., 2015). Quercetin is one of the significant phenolics commonly found in plant

extracts. It is known to have various biological effects, such as antioxidative, free radical scavenging, anticancer, and antiviral activities (Ross & Kasum, 2002). Quercetin-supplemented diets improved growth performance in tilapia (Zhai & Liu, 2013) and grass carp (Xu et al., 2019). Previous studies also observed positive results in which different medicinal aromatic plant extracts with known bioactive properties were tested as feed additives. In a study in which 0.1%, 0.5%, and 1% levels of yarrow (*Achillea millefolium* L.) extracts were tested in rainbow trout feed, it was reported that WG and SGR values increased. FCR values improved in fish fed with 1% yarrow extract at the end of the 30-day trial (Bahabadi et al., 2014). In a study in which *Artemisia dracunculus* extract additives at 0 (control), 1%, 2%, and 3% doses in feed were investigated on rainbow trout for 8 weeks, it was suggested that growth parameters and survival rate increased in all additive groups, especially at the 2% level, compared to the control group (Gholamhosseini et al., 2021). The FW, WG, and SGR values of rainbow trout fed with lemongrass (*Melissa officinalis*) extract as a feed additive at 0 (control), 0.1, 0.5, and 1 g kg⁻¹ doses for 75 days increased at 0.5 and 1 g kg⁻¹ additives compared to the control (Bilen et al., 2020). Consistent with the previous data, the present study found that dietary CP extract positively influenced the growth performance of rainbow trout. Fish in the CP extract groups showed improved growth and feed utilization parameters compared to the control group. As can be understood from most studies in which herbal extracts and their phenolic components were tested, the growth-promoting effects of diets supplemented with CP extract in the trial in rainbow trout can be attributed to important functions such as digestion, feed utilization, metabolism, antioxidant capacity, and immune response. The compounds in CP may have enhanced nutrient uptake by regulating digestive gene expression, while the carbohydrate content may have provided prebiotic properties supporting growth. It can be concluded that achieving a balance in the physiological functions of fish positively impacts growth.

In the present study, the gene expression related to digestion revealed that dietary CP extract provided higher levels of trypsin, lipase, and amylase in fish for all CP extract groups. Among the growth parameters, FW, WG, DWG, SGR, and FCR values were similar among the CP extract groups. In the expression of genes regarding digestive enzymes, the highest values for trypsin were recorded in CP2, CP05, and CP1; for lipase in CP2, CP1, and CP05; and for amylase in CP05, CP2, and CP1 groups, respectively. This supports that increased expression of digestion genes in the CP extract groups improved growth performance, but it also suggests that the dose-dependent difference for each expression of digestion genes does not have a direct effect on growth. In a study in which different plant (*Quercus brantii*, *Coriandrum sativum* and *Malva sylvestris*) extracts in feed were investigated in carp (*Cyprinus carpio*) culture, it was reported that digestive enzyme activities were higher in the feed additive groups compared to the control group, but there were differences in growth parameters among experimental groups (Raissy et al., 2022). This difference may be because digestive secretions may vary in response to extracts applied at different doses in the diet. However, in the present study, the significantly improved FCR levels in the CP extract groups also indicate that feed utilization in fish was positively affected by increased expression levels of digestion compared to the C group. Herbal extracts can improve the activity of digestive enzymes by regulating bile secretion, which has important functions in the digestion and absorption of feed (Adel et al., 2021; Bilen et al., 2020; Wangkahart et al., 2022). Increased digestive enzyme activities in fish in this way are related to the presence of phenolics and natural antioxidants in plant extracts that stimulate digestive enzymes. There are studies showing that herbal extracts in the diet increase digestive enzyme activities in rainbow trout with similar action mechanisms (Adel et al., 2021; Bilen et al., 2020; Karataş et al., 2020; Wang et al., 2020). Consistent with the improved digestive enzyme activity data of previous studies, rainbow trout's expression related to digestion can be improved by applying CP extract to the diet. However, elevated gene expression may not inherently indicate improved enzymatic activity, digestibility, or nutrient absorption. This distinction, which can separate transcriptional changes from functional consequences, is particularly important, as gene transcription can be influenced by factors such as post-transcriptional modifications, translational regulation, or protein stability (Vogel & Marcotte, 2012). Thus, although the observed increase in gene expression may suggest a potential trend, future studies should include direct assessments of digestive enzyme activity, feed digestibility, and nutrient absorption to validate these molecular results.

Our results on antioxidant gene expression demonstrated that the use of dietary CP extract increased the levels of CAT and GPx parameters in all groups compared to the C group, except for the SOD levels in the CP1 group. Antioxidant enzymes such as SOD, CAT, and GPx play a role in protecting body tissues from potential damage due to oxidative stress (Ghafarifarsani et al., 2022). Medicinal aromatic plants possess high antioxidant properties due to the phenolic compounds they contain. Węglarz et al. (2022) suggested that the antioxidant activity of CP extract might be attributed to its phenolic content, mainly related to rutoside and chlorogenic acid, known to be potent antioxidants. Sato et al. (2011) identified chlorogenic acid as a polyphenol with high antioxidant properties. Additionally, the importance of quercetin, used as a reference compound to prevent oxidation processes in the food industry, has been emphasized concerning the antioxidant potential of CP extract (Kladar et al., 2015). Analyses conducted within the scope of the current study indicated that the aforementioned phenolic compounds were represented at high levels in the CP extract. This may explain the observed increase in the expression of genes related to antioxidant parameters in the CP extract groups. A similar result was drawn by Elbesthi et al. (2020), suggesting that applying ribwort plantain (*Plantago lanceolata*) extract, which is high in chlorogenic acid content, could increase antioxidant enzymes in rainbow trout. Previous research on plant extracts with high phenolic content used as feed supplements also reported a positive effect on antioxidant enzymes in rainbow trout (Bilen et al., 2020; Elbesthi et al., 2020; Karataş et al., 2020; Salem et al., 2021; Yousefi et al., 2021).

Gene expression levels were higher in the CP05 and CP2 groups for the IL-1 β parameter and in the CP1 and CP2 groups for TNF- α and IL-8, compared to the C group. Growth and disease resistance in fish are known to be directly linked to immune function. Disease prevention can also be achieved by enhancing the immune response. Immunity in fish is closely related to inflammation and is regulated by inflammatory cytokines (Shekarabi et al., 2021). Inflammation is an integral part of the innate immune response, and therefore, inflammatory cytokines are widely known as important biomarkers of immune status (Secombes et al., 2011). As a pro-inflammatory cytokine, IL-1 β is primarily produced by monocytes and macrophages, facilitating the proliferation of leukocytes and the secretion of other cytokines (Low et al., 2003). IL-8 is another potent pro-inflammatory cytokine produced in response to oxidative stress and infections (Seppola et al., 2008). TNF- α , synthesized by various cells, is a multifunctional cytokine involved in apoptosis, phagocytic activity, and the expression of other pro-inflammatory cytokines (Krakauer et al., 1999). Interleukins like IL-1 β and IL-8 regulate the immune response and enhance phagocytosis and lymphocyte proliferation in fish by promoting the release of other cytokines like TNF- α (Baba et al., 2018). Plant extracts can have an immune-stimulatory effect by increasing the expression of pro-inflammatory cytokines (IL-1 β , TNF- α , and IL-8). Several studies have reported that dietary plant extracts can elevate the expression levels of key pro-inflammatory cytokine genes, TNF- α , IL-1 β , and IL-8 in fish (Ahmadifar et al., 2021). In a study where dandelion (*Taraxacum officinale*) flower extract was tested at different doses (1, 2, 3, and 4 g kg⁻¹) as a feed additive for rainbow trout over 56 days, the expression levels of IL-1 β and IL-8 genes were increased in all experimental groups compared to the control group (Shekarabi et al., 2021). Similarly, an increase in IL-1 β and IL-8 gene expression levels was observed in rainbow trout fed a diet supplemented with green tea (*Camellia sinensis*) (Nootash et al., 2013). This effect of plant extracts is suggested to occur through their bioactive components (Reverter et al., 2017). However, previous studies have highlighted that plant extracts can produce different responses on gene expression levels depending on the dose applied in the diet. For instance, although feeding rainbow trout with 0.1 g kg⁻¹ caper bush (*Spinosa Capparis*) extract for 30 days increased the expression of IL-1 β and IL-8 genes compared to the control group, it did not affect TNF- α gene expression (Bilen et al., 2016). Mirghaed et al. (2020) reported that increasing the dose of thyme (*Zataria multiflora*) extract (1, 2, and 3 g kg⁻¹) in the diet over 60 days led to an increase in TNF- α expression levels in rainbow trout. A study on the effects of mint (*Mentha longifolia*) extract (1, 2, and 3 g kg⁻¹) as a feed additive for rainbow trout over 30 days also reported a positive effects of increased doses on TNF- α expression in experimental groups (Heydari et al., 2020). The findings from these studies support the result in our study that there was no difference in TNF- α levels in the CP05 group compared to the control, but higher expression levels were achieved in the CP1 and CP2 groups. Previous findings suggest that plant extracts produce parallel effects on immune gene expressions with increasing doses in the feed. However, it is also reported that

increasing doses of plant extracts in the diet can have a suppressive effect on immune gene expressions. For example, a study on the effects of olive leaf (*Olea europaea* L.) extract as a feed additive for rainbow trout at different rates (0.1%, 0.25%, 0.50%, and 1.0%) found that TNF- α , IL-1 β , and IL-8 gene expressions were higher in the 0.1% group compared to the control, but lower in other experimental groups (Baba et al., 2018). Previous studies indicate that the bioactive components and application dose of plant extracts as feed additives significantly influence the immune responses in fish. It should be noted that our results also showed different responses depending on the dose applied between the CP extract and control diets. However, the generally upregulated expressions of pro-inflammatory cytokines in the feed supplement groups can be considered a result of the immune response mechanism induced by the application of CP extract as an additive. These results likely indicate the stimulatory effect of phenolic compounds in CP extract on the expression of pro-inflammatory cytokine genes in immune cells. Therefore, overall, our results demonstrate that dietary CP extract has improved pro-inflammatory immune gene expression in rainbow trout, contributing to the proliferation of immune-stimulated cells.

5 | CONCLUSION

In conclusion, dietary CP extract has potential effects on growth performance and gene expression related to digestive enzymes, immunity, and antioxidant factors in rainbow trout. Based on the data, rainbow trout diets can be optimized for healthy growth, suggesting the use of dietary CP extract (0.5 g kg⁻¹). However, further research is required on the specific mechanisms by which CP components improve fish culture performance.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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