Aquaculture International (2025) 33:1
https://doi.org/10.1007/s10499-024-01688-w

RESEARCH

Dietary pot marigold (*Calendula officinalis*) extract improved the growth performance, expression of digestive enzymes, antioxidant enzymes and immune-related genes in rainbow trout (*Oncorhynchus mykiss*)

Dogukan Kaya[1] · Boran Karataş[2] · Derya Guroy[3]

Received: 22 May 2024 / Accepted: 21 October 2024 / Published online: 4 November 2024

Abstract

The current study was designed to test the dietary pot marigold (*Calendula officinalis*) extract on growth performance and expression of genes related to digestive enzymes, antioxidant capacity and immune system in rainbow trout (*Oncorhynchus mykiss*) for 60 days. 240 fish (initial weight 15.33 ± 0.26 g) were randomly divided into 12 tanks (volume of 200 L, 20 fish per tank, in triplicates). Experimental diets were supplemented with 0 (C), 0.5 (PM05), 1 (PM1) and 2 (PM2) g kg^{-1} pot marigold extract. Fish were fed ad libitum until the fish reached apparent satiation with nutritionally balanced diets (48% crude protein and 18% crude lipid) three times (08.00 am, 1.00 pm and 6.00 pm) daily. At the end of the study, the fish were individually weighed to measure growth parameters, including final weight (FW), weight gain (WG), specific growth rate (SGR), protein efficiency ratio (PER), and feed conversion ratio (FCR). For gene expression analyses, the liver (antioxidant and non-specific immune parameters) and intestinal tissues (digestion) were sampled from fish (3 from each tank). Results showed that PM treatments had greater growth indices in comparison to the C group. ($P < 0.05$). The highest FW, WG, SGR and PER values were recorded in the PM1 treatment ($P < 0.05$). Similarly, the lowest, i.e. best FCR levels were obtained in the PM1 group ($P < 0.05$). Intestinal trypsin expression levels were significantly higher in the PM05 and PM1 treatments than in the C group, with the highest expression in the PM1 treatment ($P < 0.05$). Lipase expression reached the highest level in the PM1 treatment, while amylase was upregulated in all PM treatments compared to the C group ($P < 0.05$). Hepatic superoxide dismutase (SOD) expression levels were increased in the PM05 group, followed by PM1 and PM2 treatments ($P < 0.05$). Also, catalase (CAT) expression values were higher in the treatment groups than in the C group ($P < 0.05$). The glutathione peroxidase (GPX) expression level in the PM2 treatment was increased compared to the C group, while GPX in the PM05 and PM1 groups was significantly lower than in the C group ($P < 0.05$). Expression levels of interleukin-1β (IL-1β), tumour necrosis factor (TNF-α) and interleukin 8 (IL-8) genes relating to immunity exhibited overexpression in treatment groups compared to the C group. In conclusion, it can be concluded that

Handling Editor: Gavin Burnell

Extended author information available on the last page of the article

diets supplemented with pot marigold extract, especially at 1 g kg^{-1} level, can promote healthy growth for rainbow trout.

Keywords Medicinal plant · Gene expression · Immunity biomarkers · Feed supplement · Growth metrics

Introduction

Rainbow trout (*Oncorhynchus mykiss*), a salmonid with high commercial value, is a species that is farmed in many countries worldwide and is a farmed fish for which there is an increasing demand (FAO 2022). Responses to increasing demand have led to high stocking rates on farms, and these have sometimes given water quality problems, that have resulted in reduced fish welfare, increased stress, and outbreaks of disease (Ahmad et al. 2021). However, in addition to current challenges, the freshwater aquaculture sector, including the rainbow trout farming industry, is expected to face future challenges arising from global climate change (Weatherdon et al. 2016; Vasdravanidis et al. 2022).

Various antibiotics and chemical therapeutics are used to combat the diseases of farmed fish (Bondad-Reantaso et al. 2023), but these chemicals can have negative effects within aquaculture systems and pose a global threat through the emergence of antibiotic-resistant bacteria (Hossain et al. 2022; Adenaya et al. 2023). Many countries have banned the use of these chemicals for treating farmed aquatic animals, so implementing alternative strategies to combating diseases is a priority to ensure the expansion and sustainability of aquaculture. Natural alternatives are encouraged to improve the general health of farmed aquatic animals held in intensive culture (Zhu 2020). Among these, medicinal plants have been shown to improve feed utilization and growth, and act as immuno-stimulants in several species of farmed fish (Awad and Awaad 2017). For this reason, medicinal plants with growth-promoting and immunostimulatory properties as feed supplements have opened a widespread research area in recent years (Mariappan et al. 2023). The extracted forms of these plants can support various activities related to healthy growth in aquaculture due to their advantages in mechanisms of action (Reverter et al. 2014). Medicinal plant extracts with a high content of bioactive molecules and phytochemicals in the diet have shown positive responses in cultured environments (Shaluei et al. 2017; Rashidian et al. 2020; Gholamhosseini et al. 2021).

Pot marigold (PM) (*Calendula officinalis*) is an important medicinal plant from the Asteraceae family. PM is known for its cheap and environmentally friendly cultivation on diverse soils. The pharmaceutical and cosmetic industries' increasing demand for PM has recently attracted attention in the global and local markets (Filipović et al. 2023). Previous studies have revealed that PM contains various phytochemicals (saponins, alkaloids, triterpenoids, flavonoids, coumarins, quinones, etc.) (Jan and John 2017; Dhingra et al. 2022). The pharmacological properties of PM extract include anti-inflammatory, antioxidant, antimicrobial, antibacterial, antifungal, antiviral, antispasmodic, antipyretic, hypoglycemic, immunostimulant, antigenotoxic and antitumor effects (Patil et al. 2022; Varshney et al. 2023). A study conducted by Ghafarifarsani et al. (2023a) revealed that dietary PM powder has beneficial effects on the culture performance of rainbow trout. However, information on the potential of PM extract as a functional feed supplement is limited. We hypothesized that the potential bioactive compounds in this medicinal plant extract would positively affect healthy growth in rainbow trout. Therefore, this study aimed to investigate

the regulation of digestive enzyme activity, antioxidant capacity and immune-related genes and determine PM extract's effects on growth performance in rainbow trout.

Material and methods

Preparation of PM extract

The PM was obtained from Van Yüzüncü Yıl University Medicinal and Aromatic Plants Garden. The PM extract was prepared using the methods described by Karataş et al. (2020) and Ghafarifarsani et al. (2022) with a few adjustments. Concisely, recently harvested PM plants (flower parts) were rinsed with distilled water and air-dried in the shade at room temperature for a period of ten days. After drying, the plants were pulverized with a grinder. Then, 100 g of plant sample was mixed with 300 mL of 96% ethanol and kept at 200 rpm for 24 h on an orbital shaker at room temperature. To separate the solids, the mixture was filtered through sterile cheesecloth, centrifuged at 3000 rpm for 5 min, and filtered through Whatman filter paper (No. 1). The organic solvent was removed with an evaporator to obtain a pure extract, which was stored at 4 °C until use. Before being added to diets, the extract was subjected to freeze-drying. The sample prepared was dried in a laboratory-scale lyophiliser (Christ Alpha 2–4 LD Plus) with a standard program at -60 °C and 10 Pa until the moisture content reached 8–10%. The dried samples were then stored at -20 °C until added to the diets.

Phenolic composition of PM

The PM extract, phenolic compound concentration, was determined at Atatürk University Eastern Anatolia High Technology Application and Research Center Directorate (DAYTAM/Erzurum, Turkey). Chromatographic separation was conducted utilizing an LC–MS/MS system (Agilent 6460 Triple Quad LC–MS/MS with 1290 Infinity UPLC) coupled with a C18 column (Reversed Phase C18 Column). The column temperature was fixed at 30 °C. Mobile phase A (ultrapure water containing formic acid) and mobile phase B (acetonitrile containing formic acid) were used as mobile phases. The solvent flow rate remained constant at 0.4 mL min^{-1}, with an injection volume set at 5 µL. The phenolic compound concentration of the flower extract is presented in Table 1. The results showed that 15 phenolic compounds were found in the plant extract.

Diets preparation and proximate composition

The nutritionally balanced diets were produced at Yalova University with 48% protein and 18% lipid levels that meet the rainbow trout requirements (Hardy 2002; NRC 2011). Fish meal, fish oil, soy protein concentrate, soybean meal flour, collagen, corn gluten, rice protein concentrate and wheat flour were used to prepare the trial diets. PM extract was added to the formulated basal diets at 0.5, 1 and 2 g kg^{-1}. The dry ingredients were passed through a mill and turned into flour at a fine setting. The dietary ingredients were mixed with a laboratory-type mixer to form a dough, and the mixture was prepared into pellets with a 2 mm diameter using a cold extruded pellet machine. After pelletizing, the pellets were cooked with a pressurized steam cooker under 1-atmosphere pressure for 20 min for

Table 1 The phenolic compound concentration of PM extract

Compound	Final Concentration (ng mL^{-1})
Chlorogenic Acid	18,668.15
Syringic Acid	7573.04
Vanillic Acid	5022.84
Quinic Acid	4041.78
Rosmarinic Acid	1469.39
Fumaric Acid	1205.72
Caffeic Acid	811.42
4-OH-Benzoic Acid	787.48
Gallic Acid	329.09
Hesperidin	182.69
Ferulic Acid	118.15
Isorhamnetin	98.08
p-Coumaric Acid	72.68
Quercetin	19.58
Peonidin-3-o-glucoside	13.66

gelatinization. After this process, the diets were dried at 40 °C for constant moisture content. Crude protein, crude lipid and crude ash of raw materials and diets used in the trial were determined according to AOAC (2000) methodology. The Kjeldahl method, based on nitrogen (Nx6.25) measurement, was used for crude protein analysis. Crude lipid levels were determined by an automated extraction method ANKOM XT-15 (Macedon, NY). For crude ash, the process involved burning of the samples in a muffle furnace at 525 °C for 12 h. The cellulose of ingredients and experimental diets were analyzed using a fibre analyzer (Gerhardt FBS6) according to AOAC Official Method 962.09 – fibre in animal feed and pet food (AOAC 2000). The formulation and nutrient composition of the diets are given in Table 2.

Experimental fish, design and culture conditions

The trial was performed at the Fisheries Unit, Agricultural Applications and Research Center, Tokat Gaziosmanpaşa University, Tokat, Turkey. Rainbow trout (*O. mykiss*) were obtained from a local fish farm and kept for 15 days to acclimatize to the experimental conditions. During acclimatization, fish were hand-fed the basal diet daily until satiated. A total of 240 healthy rainbow trout weighing 15.33 ± 0.26 g (each fish weighed individually) were randomly divided into 12 tanks (volume of 200 L, 20 fish per tank, in triplicates). During the trial, fish in the control group (C) were fed the basal diet without PM. In the PM05, PM1 and PM2 groups, fish were given the diets with 0.5, 1 and 2 g kg^{-1} PM, respectively. Fish were fed to satiation three times each day (08.00 am, 1.00 pm and 6.00 pm). The feeding behaviour was monitored, and once the fish stopped consuming the feed or slowed down significantly, it was assumed that they were satiated. Water was continuously supplied to the tanks (4 L min^{-1}) and the tank water was completely renewed

Table 2 Ingredients and proximate chemical composition of diets containing different levels of PM extract

Ingredients (%)	C	PM05	PM1	PM2
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
Pot marigold	0	0.05	0.1	0.2
Total	100	100	100	100
Crude Protein	48.06	48.13	48.04	48.21
Crude Lipid	18.08	18.16	18.06	18.12
Crude ash	7.71	7.75	7.85	7.81
Cellulose	2.05	2.11	2.09	2.18

Per g mixture: vitamin A, 342 IU; vitamin D3, 329 IU; vitamin E, 0.0274 IU; vitamin K3, 5.48 mg; vitamin B1, 2.05 mg; vitamin B2, 3.42 mg; vitamin B3, 20.5 mg; vitamin B5, 5.48 mg; vitamin B6, 2.05 mg; vitamin B12, 2.74 mg; vitamin C, 24.0 mg, Per g mixture: biotin, 0.411 mg; folic acid, 0.685 mg; Zn, 12.3 mg; Mn, 4.80 mg; Cu, 1.64 mg; I, 0.274 mg; Se, 0.0274 mg; Ca, 125 mg; K, 189 mg, Kartal Chemical Incorporated, Kocaeli, Türkiye

daily. Basic water parameters were recorded daily: Temperature, dissolved oxygen and pH were 14.8 ± 0.3 °C, 7.2 ± 0.1, and 7.9 ± 0.2 mg L^{-1}, respectively.

Growth indices

Before the feeding trial and at the end of 60 days, the fish were weighed to measure growth parameters based on the following standard formulae:

$$Weightgain(WG, g) = Finalweight(g) - Initialweight(g).$$

$$Dailyweightgain(DWG, g) = Weightgain(g)/days.$$

$$Specificgrowthrate\left(SGR, \%day^{-1}\right) = [(Ln(finalweight) - Ln(initialweight)/days] \times 100.$$

$$Proteinefficiencyratio(PER) = Weightgain(g)/Proteinintake(g).$$

$$Feedconversionratio(FCR) = Totalfeedgiven(g)/Weightgain(g).$$

Sampling

At the end of the experiment, the fish in the groups were fasted for 24 h before sampling. Nine fish were sampled for each group, three from each replicate. Fish taken from each

tank with a dip net were kept in the eugenol bath (200 mg L^{-1}, 60 s). Liver and intestinal tissues were instantly removed from the sampled fish for gene expression analysis (antioxidant and digestive enzyme activity, non-specific immune parameters). For RNA isolation, 25–50 mg liver and intestinal tissue samples were stored in RNAlater solution until isolation (4 °C for 24 h and then stored at -18 °C until molecular analysis).

RNA isolation

Total RNA was isolated from the tissue samples and extracted with DiaRex® Total RNA Extraction Kit (TR-0877–100, Diagen, Ankara, Turkey) according to the manufacturer's protocol. Briefly, 600 µL LBD solution was added to 25 mg tissue and homogenized in Tissuelyser LT (Qiagen). After homogenization, the tissue was centrifuged twice at 3000 rpm for 30 s and incubated for 5 min at room temperature. The supernatant was then transferred to a 2 mL Eppendorf tube and 600 µL of 99% ethanol was added. Then centrifuged at 8000 g (10,000 rpm) for 1 min and the supernatant was transferred to a spin column and centrifuged again at 8000 g (10,000 rpm) for 1 min. 500 µL of WBD-1 and WBD-2 solutions were added and centrifuged at 8000 g (10,000 rpm) for 1 min and the washing process was completed. After the last wash, 30 µL EBD solution was added and RNA elutions were obtained by centrifugation at 8000 g for 2 min. A nanospectrophotometer (QIAxpert) was used for quantification and purity determination of isolated RNAs. Measurements were performed at 260 nm and 280 nm, and RNA purity was determined using the RNA (260/280) ratio in each sample after the measurement.

cDNA synthesis

All steps of cDNA synthesis were performed on ice using the SuScript cDNA Synthesis Kit (RT01A026, Sugenomics, Ankara, Turkey). All obtained RNA samples were adjusted to 100 nanograms μL^{-1} concentrations. Then, 6 µL RNA, 4 µL 5X RT Mix and 10 µL DNase/RNase free water were added to a total volume of 20 µL. The final volume was incubated in a Thermal Cycler (Rotor Gene Q 9000) at 42 °C for 60 min and then at 80 °C for 10 min.

Gene expression analysis

cDNA products were analyzed by Real-Time PCR using RotorGene Q 9000 (Qiagen) and 2X SuYBRGreen qPCR Master Mix (PCR01C0253, Sugenomics, Ankara, Turkey). In gene expression analysis, β-actin was selected as the standard gene. SOD, CAT, GPX, IL-8, IL-1β, TNF-α, TRP, LPZ, and AML were used as target genes. The primers used in the study are given in Table 3.

PCR mix was 12 uL and 2X SuYBRGreen qPCR Master Mix (PCR01C0253, Sugenomics, Ankara, Turkey), 2.5 uL Forward and Reverse Primer, 4 uL H_2O were added and the total volume was completed to 21 uL. The total volume was increased to 25 µL by adding 4 µL cDNA. The cycling parameters for PCR amplification were as follows: Pre-denaturation at 95 °C for 10 min followed by 45 cycles of 45 s at 95 °C and 45 s at 60 °C for annealing to complete the Real-Time protocol. PCR reactions were performed in duplicate to minimize experimental error in all samples. The beta-actin reference gene was a coefficient change criterion for normalizing the Ct values obtained following Real-Time PCR. The

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´ to 3´)	Annealing Tm (°C)	Reference/ Accession no
β-actin (Beta-actin)	F:GGAGGCTCCATCTTGGCTTC R:GAAGTGGTAGTCGGGTGTGG	61	(Vazirzadeh et al. 2020)
Trypsin	F: TATGTGAAGCCCATCCCGTT R: TCTGCCTCCGTCCATGTATC	59	XM_021591477
Lipase	F: ATGGCAGCTTTCTCCTTCCT R: ATGGTCAGGGTGAGGTTCAG	58	NM_001197210
Amylase	F: ACAAGGAGCATGTGAGGGAA R: CAGGTGGTGTTGAGGTTGTG	58	XM_036935172
CAT (Catalase)	F:TGATGTCACACAGGTGCGTA R: GTGGGCTCAGTGTTGTTGAG	58	(Mousavi et al. 2020),
GPX (Glutathione peroxidase)	F:CGAGCTCCATGAACGGTACG R: TGCTTCCCGTTCACATCCAC	60	(Mousavi et al. 2020),
SOD (Superoxide dismutase)	F: TGGTCCTGTGAAGCTGATTG R: TTGTCAGCTCCTGCAGTCAC	58	(Teimouri et al. 2019)
IL-1β (Interleukin-1 Beta)	F:AGCAGGACTACACCAAACCG R:TCCTGATCGTAGAGGCCCAA	59	(Vazirzadeh et al. 2020)
TNF-α (Tumor Necrosis Factor-Alpha)	F:GGCTGTGTGGCGTTCTCTTA R: AAATGGATGGCTGCTTTCGC	58	(Vazirzadeh et al. 2020)
IL-8 (Interleukin-8)	F: CACAGACAGAGAAGGAAGGAAAG R: TGCTCATCTTGGGGTTACAGA	60	(Salem et al. 2022)

relative expression levels of target gene transcripts were conducted using the comparative cycle threshold (Ct) method (2-ΔΔCt) (Livak and Schmittgen 2001).

Statistics

At the end of the trial, all the data obtained from the study were evaluated by one-way ANOVA. Duncan test, one of the post hoc tests, was performed to determine the difference among the groups. The tests were performed with a significance level (α) of 0.05 (mean ± standard deviation). Assumptions (normal distribution, homogeneity of variances, etc.) were tested before the tests. Statistical data was analysed using the SPSS 12 statistical package program (Colman and Pulford 2006).

Results

Table 4 illustrates the growth parameters of rainbow trout after the trial. All fish survived the growth trial. Supplementing the diet with PM extract significantly increased FW, WG, SGR and PER of fish, compared to the control group ($P < 0.05$). Growth performance showed the highest increase in fish in the PM1 group with an FW of 85.27 ± 0.53 g among PM treatments ($P < 0.05$). The fish in the PM2 and PM05 groups also reached FW values of 83.85 ± 0.22 and 81.85 ± 0.83 g, respectively, which differed from the fish in the control group ($P < 0.05$). The best (i.e. lowest) FCR was found in PM1 and PM2 ($P < 0.05$).

The expression levels of digestive enzyme-related genes in the intestine of rainbow trout are shown in Fig. 1. Trypsin expression levels were significantly higher in the PM05 and PM1 groups than in the C group, with the highest expression recorded in the PM1 group ($P < 0.05$). PM1 group exhibited the highest lipase expression among the treatments ($P < 0.05$). Lipase levels in PM05 and PM2 groups did not differ significantly when compared to the control group ($P > 0.05$). Amylase expression levels were higher in the PM groups compared to the C group ($P < 0.05$).

Expression of antioxidant (SOD, CAT and GPX) genes in rainbow trout liver are shown in Fig. 2. The highest SOD expression was found in the PM05 group, followed by the PM1 and PM2 groups ($P < 0.05$). Similarly, CAT expression was significantly higher

Table 4 Growth performance of rainbow trout fed on diets supplemented with different levels of PM extract

Parameters	C	PM05	PM1	PM2
IW (g)	15.30 ± 0.02	15.35 ± 0.04	15.34 ± 0.02	15.33 ± 0.02
FW (g)	74.42 ± 0.63^{d}	81.85 ± 0.83^{c}	85.27 ± 0.53^{a}	83.85 ± 0.22^{b}
WG (g)	59.12 ± 0.60^{d}	66.50 ± 0.80^{c}	69.93 ± 0.55^{a}	68.52 ± 0.23^{b}
DWG (g)	0.99 ± 0.01^{d}	1.11 ± 0.01^{c}	1.17 ± 0.01^{a}	1.14 ± 0.00^{b}
SGR (% day^{-1})	2.64 ± 0.01^{d}	2.79 ± 0.01^{c}	2.86 ± 0.01^{a}	2.83 ± 0.01^{b}
PER	2.50 ± 0.03^{c}	2.70 ± 0.02^{b}	2.80 ± 0.05^{a}	2.81 ± 0.02^{a}
FCR	0.83 ± 0.01^{a}	0.77 ± 0.01^{b}	0.74 ± 0.01^{c}	0.74 ± 0.00^{c}

Data were shown as means ± SD. Different lowercase letters in each line demonstrated significant differences ($P < 0.05$). PM05, a diet supplemented with 0.05% pot marigold extract; PM1, a diet supplemented with 0.1% pot marigold extract; PM2, a diet supplemented with 0.2% pot marigold extract; IW, initial weight; FW, final weight; WG, weight gain; DWG, average daily gain; SGR, specific growth rate; PER, protein efficiency ratio, FCR, feed conversion ratio

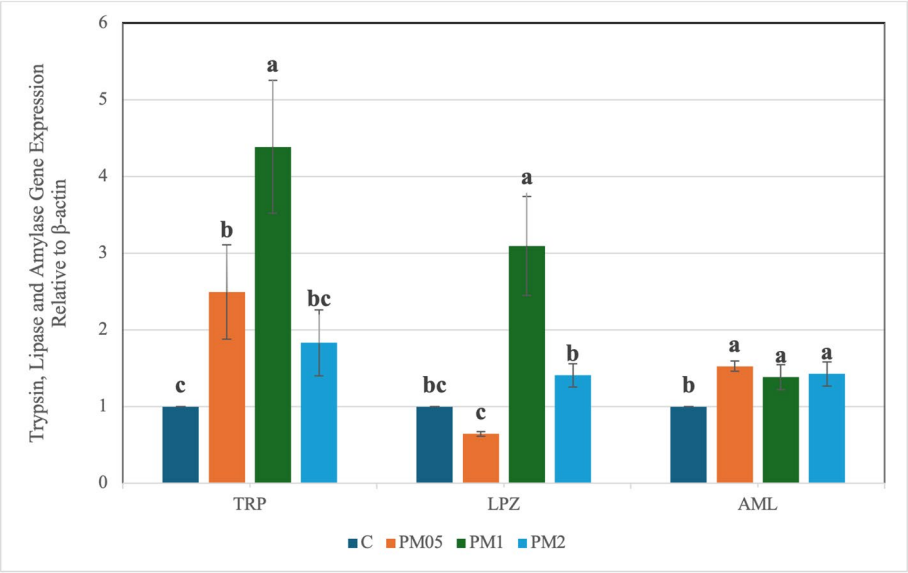

Fig. 1 Expression levels of digestive enzyme-related genes in the intestine of rainbow trout fed with different levels of PM extract

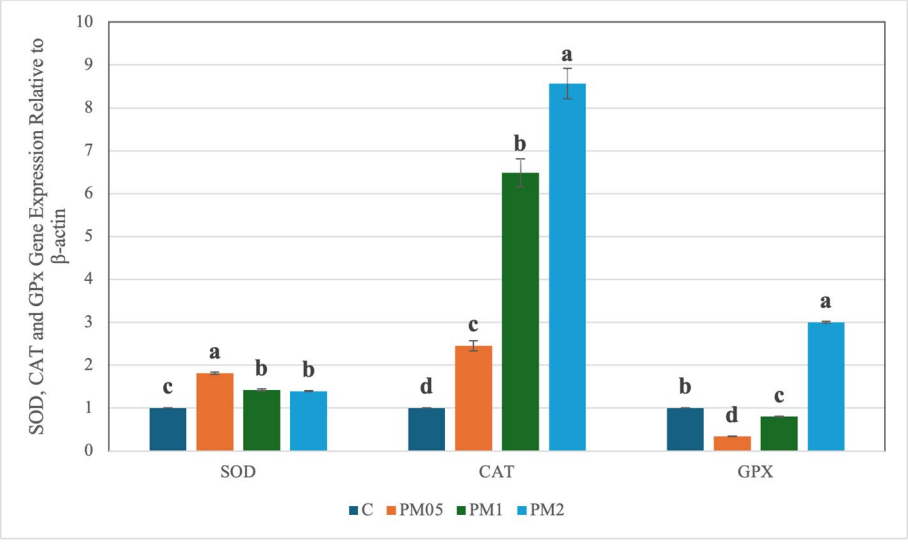

Fig. 2 Expression levels of antioxidant-related genes in the liver of rainbow trout fed with different levels of PM extract

in the PM treatment groups than in the C group ($P < 0.05$). GPX expression in the PM2 group was significantly higher than in the C group, whereas the expression of GPX in the PM05 and PM1 groups was significantly lower than in the C group ($P < 0.05$).

Expression of IL-1β, TNF-α and IL-8 genes in rainbow trout liver are given in Fig. 3. For IL-1β gene expression, overexpression was observed in the PM05, PM1 and PM2 groups compared to the C group ($P < 0.05$). The highest IL-1β expression was observed in the PM2 group, which differed from all other groups ($P < 0.05$). Similarly, TNF-α gene expression was significantly higher in all PM treatments than in the C group, with the highest TNF-α expression observed in the PM2 group ($P < 0.05$). IL-8 gene expression was highest in the PM1 group, followed by PM05 and PM2 groups, respectively. ($P < 0.05$).

Discussion

This study sought to elucidate the impact of PM extract on the growth performance of rainbow trout (*O. mykiss*) by investigating its effects at the molecular level, specifically focusing on digestive enzymes, antioxidant capacity, and immune response. These findings aim to shed light on the underlying mechanisms through which the plant extract exerts its action. This study demonstrated that dietary supplementation of PM extract in rainbow trout (*O. mykiss*) improved growth performance, and the best results were achieved with supplementation of 1 g kg^{-1}. Consistent with this study, dietary inclusion of doses at 0.1, 0.5 and 1 g kg^{-1} of yarrow (*Achillea millefolium*) extract from medicinal plants of the Asteraceae family improved the growth performance of rainbow trout (*O. mykiss*) (Bahabadi et al. 2014). Feeding rainbow trout (*O. mykiss*) with dietary 10, 20 and 30 g kg^{-1} wild tarragon (*Artemisia dracunculus*) extract for 8 weeks showed higher FW, WG and SGR than the control group (Gholamhosseini et al. 2021). Supplementing 0.5 and 1 g kg^{-1} lemon balm (*Melissa officinalis*) extract to the basal diet of rainbow trout increased WG and SGR values after a 75-day trial (Bilen et al. 2020). Only one study on the effects of pot marigold (*C. officinalis*) in rainbow trout (*O. mykiss*) culture was conducted by Ghafarifarsani et al. (2023a). The researchers included pot marigold (*C. officinalis*) powder in the diets at 10, 15 and 20 g kg^{-1} doses and fed rainbow trout (*O. mykiss*) with the diets for 60 days. At

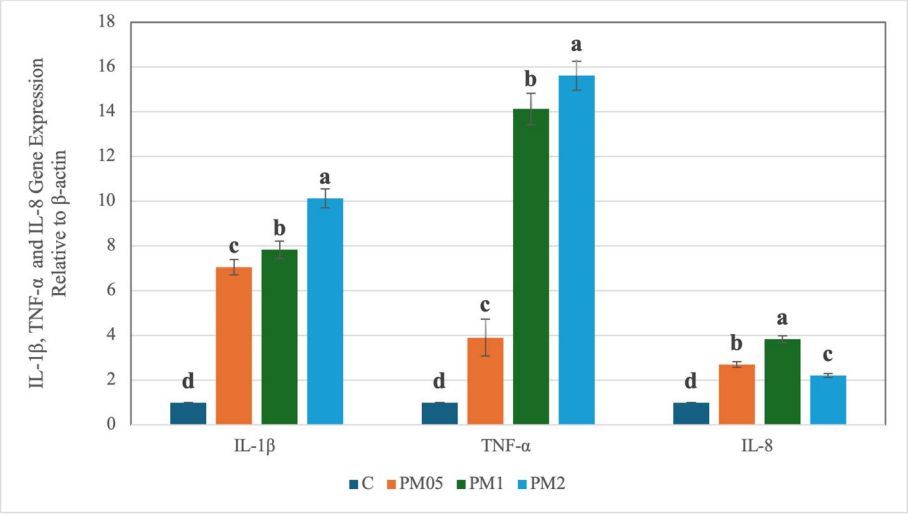

Fig. 3 Expression levels of immune-related genes in the liver of rainbow trout fed with different levels of PM extract

the end of their study, it was noted that FW, WG, SGR, and FCR values improved in the treatment groups and the best growth performance was obtained in the 15 g kg^{-1} pot marigold (*C. officinalis*) powder supplementation group. However, the present study focused on the opportunity to use pot marigold (*C. officinalis*) flower extract in the rainbow trout (*O. mykiss*) diet at 0.5, 1 and 2 g kg^{-1} doses. As a dietary supplement, the extracted form of PM may exhibit similar efficacy at lower doses than its powder substitute. Numerous studies have demonstrated various chemical components of PM, such as terpenoids, flavonoids, coumarins, quinones, essential oils, carotenoids and amino acids (Abdelwahab et al. 2022). In the present study, chlorogenic acid was found to have the highest phenolic compound content. Chlorogenic acid is a bioactive compound known for its immunogenic, antibacterial, antioxidant and anti-inflammatory properties (Xu et al. 2012; Kabir et al. 2014). Ghafarifarsani et al. (2023b) reported that supplementing 600–800 mg chlorogenic acid in rainbow trout (*O. mykiss*) diets improved growth performance. The increased trend in growth performance can be attributed to the bioactive components in PM, which are presented in Table 1. These chemical compounds in the PM can increase nutrient intake by affecting the expression of genes related to digestion. In addition, the presence of carbohydrates may also be a prebiotic property that promotes growth.

In fish, the intake of protein or amino acids from the diet is essential for maintaining optimal growth conditions. The assessment of feed utilization through digestive enzyme activity serves as a valuable tool for evaluating growth performance. Dietary herbal additives positively affect bile secretion, which is essential for the digestion and absorption of nutrients and thus can improve digestive enzyme activity (Wang et al. 2020a; Adel et al. 2021). In our study, fish positively responded to dietary PM supplementation regarding the expression of genes related to digestion. Improving the expression of genes related to digestion could explain the higher growth values in the PM groups. Moreover, enhancement in the expression of digestion genes probably resulted in an improved feed conversion ratio in the treatment groups. Consequently, the increased gene expression in the fish intestine may be related to the phenolic compounds present in the PM extract. Similarly, this was supported in a previous study, where supplementing the diet with rosemary (*Rosmarinus officinalis*) extract increased digestive enzyme activity in rainbow trout (*O. mykiss*) after a 40-day trial intestine of rainbow trout (Karataş et al. 2020). Also, Rashidian et al. (2020) reported that dietary common mallow (*Malvae sylvestris*) extract supplementation boosted digestive enzymes in rainbow trout (*O. mykiss*) after 8 weeks.

The antioxidant system mainly includes the components SOD, CAT and GPX (Fontagné-Dicharry et al. 2014). Monitoring the antioxidant system in fish is essential for understanding the physiological response (Mittler 2002) and may contribute to the efficiency of fish culture (Hoseinifar et al. 2020a). Antioxidant enzyme activities play a vital role in protecting fish health against oxidative processes that cause damage to living cells (Martínez-Álvarez et al. 2005). Flavonoids and phenolics in medicinal plants reveal their antioxidant properties (Li et al. 2013). PM contains high levels of polyphenols that can act as antioxidants, anticancer, antiviral, antimicrobial, anti-inflammatory activity and free radical scavengers (Rojas-Bedoya et al. 2020). Phenolics, especially chlorogenic, syringic, and vanillic acids, are key components of PM extract, contributing to its high antioxidant capacity (Sato et al. 2011; Srinivasulu et al. 2018; Ingole et al. 2021; Węglarz et al. 2022). The antioxidative system in plants, supported by these phenolic compounds, plays a critical role in combating free radicals and promoting healthy growth (Dominic et al. 2022). PM has an antioxidative system that includes antioxidants such as CAT, SOD, GPX, tocopherol, carotenoids and ascorbate (Varshney et al. 2023). According to the present study, SOD and CAT gene expression of rainbow trout (*O. mykiss*) increased in fish-fed diets supplemented with

PM extract, which has a strong antioxidant capacity. Zeilab Sendijani et al. (2020) investigated the effects of dietary dill (*Anethum graveolens*) extract (1, 1.5 and 3 g kg^{-1}) in rainbow trout (*O. mykiss*) and reported that SOD and CAT levels increased at 1 and 1.5 g kg^{-1} doses compared to the control group. Similarly, dietary Persian shallot (*Allium hirtifolium*) (Ghafarifarsani et al. 2022), mistletoe (*Viscum album*) (Yousefi et al. 2021), and rosemary (*R. officinalis*) (Karataş et al. 2020) extract inclusions improved antioxidant activity in rainbow trout (*O. mykiss*). Dietary PM powder increased SOD and CAT levels but did not affect the GPX activity in rainbow trout (*O. mykiss*) (Ghafarifarsani et al. 2023a). This hypothesis was supported in the present study, which revealed that the expression of genes related to SOD and CAT was improved by dietary PM extract inclusion. However, in our study, the GPX expression decreased in the PM05 and PM1 groups compared to the control, while it was higher in the PM2 group. This suggests that the positive response of powdered or extracted forms of PM on GPX activity may be more sensitive than SOD and CAT enzymes in the dose-dependent mechanism of action. Positive expression of the antioxidant genes obtained in general within the scope of the trial may be due to phenolic acids and flavonoid components known to have antioxidant activities in PM extract.

The high density of pathogens and the restricted use of chemical treatments in farmed fish contribute to disease outbreaks and associated mortality, underscoring the importance of maintaining a robust immune system as a key preventive strategy (Mehana et al. 2015). The inclusion of herbal immunostimulants in feed has emerged as a promising approach for enhancing the immune response in cultured fish (Awad and Awaad 2017; Dev et al. 2024). Assessing the expression levels of immune-related genes in fish is a widely utilized and crucial method for monitoring immune responses (Cecchini et al. 2013). Our study, via the application of PM extract as an herbal immunostimulant in the rainbow trout (*O. mykiss*) diet, has focused on the expression levels of key genes involved in immunity. Cytokines are crucial in the innate immune response (Raida and Buchmann 2009). Previous studies have emphasized the importance of cytokines such as TNF-α, IL-8 and IL-1β gene expression levels in predicting effects on immune response (Vazirzadeh et al. 2017; Hoseinifar et al. 2020b). To date, no research has been conducted to investigate the immunostimulatory effects of PM in fish species. Alterations in the expression levels of antioxidant enzyme genes and cytokine-related inflammatory genes may serve as indicators of the immune status in fish. The beneficial effects elicited by medicinal plants are often attributed to their bioactive constituents. Previous data showed that rainbow trout (*O. mykiss*) fed diets supplemented with various medicinal plant extracts exhibited higher cytokine gene expression (Baba et al. 2018; Mirghaed et al. 2020; Adel et al. 2020; Kiadaliri et al. 2020). Our results were consistent with previous studies reporting that dietary herbal supplements can increase the expression of immune genes in rainbow trout (*O. mykiss*). Liu et al. (2021) recommended that vanillic acid may be attractive for treating *Vibrio alginolyticus* related infections in fish farming. Some animal studies have indicated that syringic acid may have neuroprotective effects, potentially benefiting brain health and cognitive function (Dalmagro et al. 2017). Research on animals suggests that chlorogenic acid may have potential benefits for cardiovascular health by helping to reduce inflammatory damage and oxidative stress (Bhandarkar et al. 2019; Wang et al. 2020b). The high amount of chlorogenic acid, syringic acid, vanillic acid, and other elements in this plant extract could explain the increased antioxidant and immune gene expression of fish fed with PM extract. Despite the aforementioned hypothesis, the precise mechanisms by which the components of PM enhance immune responses in fish remain to be fully elucidated and warrant further investigation.

Conclusion

It was concluded that dietary PM extract supplementation promoted growth performance in rainbow trout (*O. mykiss*). The dietary PM extract induced growth in rainbow trout and stimulated the expression of genes associated with digestive and antioxidant enzymes. Moreover, MP extract also improved and up-regulated immune gene expression. The specific mechanisms by which PM components enhance fish production performance require further exploration.

Author contribution Dogukan Kaya: Supervision, Conceptualization, Writing – original draft, Investigation, Methodology, Validation, Formal analysis. Boran Karataş: Data curation, Methodology, Validation, Formal analysis, Writing – review & editing. Derya Guroy: Data curation, Methodology, Investigation, Formal analysis, Writing – review & editing.

Funding This study was part of a project funded by the Scientific and Technological Research Council of Turkey (TUBITAK, Grant No. 123O194, Project proposal: 08/03/2023, Project approval: 10/05/2023).

Data availability Data is provided within the manuscript.

Declarations

Ethics approval This experiment was conducted according to the guidelines of the local ethics committee (EC 2023-Hadyek-03, 51879863–23, 28.04.2023) at Tokat Gaziosmanpaşa University.

Competing interest The authors declare no competing interests.

References

Abdelwahab SI, Taha MME, Taha SME, Alsayegh AA (2022) Fifty-year of global research in *Calendula officinalis L.* (1971–2021): A bibliometric study. Clin Complement Med Pharmacol 2(4):100059. https://doi.org/10.1016/j.ccmp.2022.100059

Adel M, Dawood MA, Shafiei S, Sakhaie F, Shekarabi SPH (2020) Dietary Polygonum minus extract ameliorated the growth performance, humoral immune parameters, immune-related gene expression and resistance against Yersinia ruckeri in rainbow trout (Oncorhynchus mykiss). Aquaculture 519. https://doi.org/10.1016/j.aquaculture.2019.734738

Adel M, Dawood MA, Gholamhosseini A, Sakhaie F, Banaee M (2021) Effect of the extract of lemon verbena (Aloysia citrodora) on the growth performance, digestive enzyme activities, and immune-related genes in Siberian sturgeon (Acipenser baerii). Aquaculture 541:736797. https://doi.org/10.1016/j.aquaculture.2021.736797

Adenaya A, Berger M, Brinkhoff T, Ribas-Ribas M, Wurl O (2023) Usage of antibiotics in aquaculture and the impact on coastal waters. Mar Pollut Bull 188:114645. https://doi.org/10.1016/j.marpolbul.2023.114645

Ahmad A, Abdullah SRS, Hasan HA, Othman AR, Ismail NI (2021) Aquaculture industry: Supply and demand, best practices, effluent and its current issues and treatment technology. J Environ Manage 287:112271. https://doi.org/10.1016/j.jenvman.2021.112271

AOAC (2000) Official methods of analysis, 17th edn. Association of Official Analytical Chemists, Gaithersburg, MD, USA

Awad E, Awaad A (2017) Role of medicinal plants on growth performance and immune status in fish. Fish Shellfish Immunol 67:40–54. https://doi.org/10.1016/j.fsi.2017.05.034

Baba E, Acar Ü, Yılmaz S, Zemheri F, Ergün S (2018) Dietary olive leaf (Olea europea L.) extract alters some immune gene expression levels and disease resistance to Yersinia ruckeri infection in rainbow trout *Oncorhynchus mykiss*. Fish Shellfish Immunol 79:28–33. https://doi.org/10.1016/j.fsi.2018.04.063

Bahabadi MN, Banaee M, Taghiyan M, Haghi BN (2014) Effects of dietary administration of yarrow extract on growth performance and blood biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). International Journal of Aquatic Biology 2(5):275–285. https://doi.org/10.22034/ijab.v2i5.138

Bhandarkar NS, Brown L, Panchal SK (2019) Chlorogenic acid attenuates high-carbohydrate, high-fat diet–induced cardiovascular, liver, and metabolic changes in rats. Nutr Res 62:78–88. https://doi.org/10.1016/j.nutres.2018.11.002

Bilen S, Altief TAS, Özdemir KY, Salem MOA, Terzi E, Güney K (2020) Effect of lemon balm (Melissa officinalis) extract on growth performance, digestive and antioxidant enzyme activities, and immune responses in rainbow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem 46:471–481. https://doi.org/10.1007/s10695-019-00737-z

Bondad-Reantaso MG, MacKinnon B, Karunasagar I, Fridman S, Alday-Sanz V, Brun E, Groumellec ML, Li A, Surachetpong W, Karunasagar I, Hao B, Dall'Occo A, Urbani R, Caputo A (2023) Review of alternatives to antibiotic use in aquaculture. Rev Aquac 15(4):1421–1451. https://doi.org/10.1111/raq.12786

Cecchini S, Paciolla M, Biffali E, Borra M, Ursini MV, Lioi MB (2013) Ontogenetic profile of innate immune related genes and their tissue-specific expression in brown trout, Salmo trutta (Linnaeus, 1758). Fish Shellfish Immunol 35(3):988–992. https://doi.org/10.1016/j.fsi.2013.05.026

Colman AM, Pulford BD (2006) A crash course in SPSS for Windows: updated for versions 10, 11, 12 and 13, Wiley-Blackwell.

Dalmagro AP, Camargo A, Zeni ALB (2017) Morus nigra and its major phenolic, syringic acid, have antidepressant-like and neuroprotective effects in mice. Metab Brain Dis 32:1963–1973. https://doi.org/10.1007/s11011-017-0089-y

Dev AK, Thakur R, Yadav S (2024) Deciphering the importance of herbal immunostimulants in aquaculture, using citation network analysis: A futuristic sustainable approach. Comp Immunol Rep 6:200129. https://doi.org/10.1016/j.cirep.2023.200129

Dhingra G, Dhakad P, Tanwar S (2022) Review on phytochemical constituents and pharmacological activities of plant Calendula officinalis Linn. Biol Sci 2(2):216–228. https://doi.org/10.55006/biolsciences.2022.2205

Dominic S, Hussain AI, Saleem MH, Alshaya H, Jan BL, Ali S, Wang X (2022) Variation in the primary and secondary metabolites, antioxidant and antibacterial potentials of tomatoes, grown in soil blended with different concentration of fly ash. Plants 11(4):551. https://doi.org/10.3390/plants11040551

FAO (2022) The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation, FAO Rome

Filipović V, Ugrenović V, Popović V, Dimitrijević S, Popović S, Aćimović M, Dragumilo A, Pezo L (2023) Productivity and flower quality of different pot marigold (*Calendula officinalis L.*) varieties on the compost produced from medicinal plant waste. Ind Crops Prod 192:116093. https://doi.org/10.1016/j.indcrop.2022.116093

Fontagné-Dicharry S, Lataillade E, Surget A, Larroquet L, Cluzeaud M, Kaushik S (2014) Antioxidant defense system is altered by dietary oxidized lipid in first-feeding rainbow trout (*Oncorhynchus mykiss*). Aquaculture 424:220–227. https://doi.org/10.1016/j.aquaculture.2014.01.009

Ghafarifarsani H, Yousefi M, Hoseinifar SH, Paolucci M, Lumsangkul C, Jaturasitha S, Van Doan H (2022) Beneficial effects of Persian shallot (Allium hirtifolium) extract on growth performance, biochemical, immunological and antioxidant responses of rainbow trout *Oncorhynchus mykiss* fingerlings. Aquaculture 555. https://doi.org/10.1016/j.aquaculture.2022.738162

Ghafarifarsani H, Hoseinifar SH, Molayemraftar T, Raeeszadeh M, Van Doan H (2023) Pot Marigold (Calendula officinalis) Powder in Rainbow Trout (*Oncorhynchus mykiss*) Feed: Effects on Growth, Immunity, and Yersinia ruckeri Resistance. Aquac Nutr 2023:7785722. https://doi.org/10.1155/2023/7785722

Ghafarifarsani H, Nedaei S, Hoseinifar SH, Van Doan H (2023) Effect of Different Levels of Chlorogenic Acid on Growth Performance, Immunological Responses, Antioxidant Defense, and Disease Resistance of Rainbow Trout (*Oncorhynchus mykiss*) Juveniles. Aquac Nutr 2023:3679002. https://doi.org/10.1155/2023/3679002

Gholamhosseini A, Hosseinzadeh S, Soltanian S, Banaee M, Sureda A, Rakhshaninejad M, Heidari AA, Anbazpour H (2021) Effect of dietary supplements of Artemisia dracunculus extract on the haemato-immunological and biochemical response, and growth performance of the rainbow trout (*Oncorhynchus mykiss*). Aquac Res 52(5):2097–2109. https://doi.org/10.1111/are.15062

Hardy RW (2002) Rainbow trout, Oncorhynchus mykiss. C.D. Webster, C. Lim (Eds.), Nutrient Requirements and Feeding of Finfish for Aquaculture, CAB International, Oxon, pp. 184–202

Hoseinifar SH, Yousefi S, Van Doan H, Ashouri G, Gioacchini G, Maradonna F, Carnevali O (2020a) Oxidative stress and antioxidant defense in fish: the implications of probiotic, prebiotic, and synbiotics. Rev Fish Sci Aquac 29(2):198–217. https://doi.org/10.1080/23308249.2020.1795616

Hoseinifar SH, Shakouri M, Van Doan H, Shafiei S, Yousefi M, Raeisi M, Yousefi S, Harikrishnan R, Reverter M (2020b) Dietary supplementation of lemon verbena (Aloysia citrodora) improved immunity, immune-related genes expression and antioxidant enzymes in rainbow trout (Oncorrhyncus mykiss). Fish Shellfish Immunol 99:379–385. https://doi.org/10.1016/j.fsi.2020.02.006

Hossain A, Habibullah-Al-Mamun M, Nagano I, Masunaga S, Kitazawa D, Matsuda H (2022) Antibiotics, antibiotic-resistant bacteria, and resistance genes in aquaculture: risks, current concern, and future thinking. Environ Sci Pollut Res 1–22. https://doi.org/10.1007/s11356-021-17825-4

Ingole A, Kadam MP, Dalu AP, Kute SM, Mange PR, Theng VD, Lahane OR, Nikas AP, Kawal YV, Nagrik SU, Patil PA (2021). A review of the pharmacological characteristics of vanillic acid. J Drug Deliv Therap 11(2-S), 200–204. https://doi.org/10.22270/jddt.v11i2-S.4823

Jan N, John R (2017) Calendula officinalis-an important medicinal plant with potential biological properties. Proc Indian Natl Sci Acad 83(4):769–787. https://doi.org/10.16943/ptinsa/2017/49126

Kabir F, Katayama S, Tanji N, Nakamura S (2014) Antimicrobial effects of chlorogenic acid and related compounds. J Korean Soc Appl Biol Chem 57:359–365. https://doi.org/10.1007/s13765-014-4056-6

Karataş T, Korkmaz F, Karataş A, Yildirim S (2020) Effects of Rosemary (Rosmarinus officinalis) extract on growth, blood biochemistry, immunity, antioxidant, digestive enzymes and liver histopathology of rainbow trout Oncorhynchus Mykiss. Aquac Nutr 26(5):1533–1541. https://doi.org/10.1111/anu.13100

Kiadaliri M, Firouzbakhsh F, Deldar H (2020) Effects of feeding with red algae (Laurencia caspica) hydroalcoholic extract on antioxidant defense, immune responses, and immune gene expression of kidney in rainbow trout (*Oncorhynchus mykiss*) infected with Aeromonas hydrophila. Aquaculture 526. https://doi.org/10.1016/j.aquaculture.2020.735361

Li S, Li SK, Gan RY, Song FL, Kuang L, Li HB (2013) Antioxidant capacities and total phenolic contents of infusions from 223 medicinal plants. Ind Crops Prod 51:289–298. https://doi.org/10.1016/j.indcrop.2013.09.017

Liu H, Xiao M, Zuo J, He X, Lu P, Li Y, Zhao Y, Xia F (2021) Vanillic acid combats Vibrio alginolyticus by cell membrane damage and biofilm reduction. J Fish Dis 44:1799–1809. https://doi.org/10.1111/jfd.13498

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods 25(4):402–408. https://doi.org/10.1006/meth.2001.1262

Mariappan B, Kaliyamurthi V, Binesh A (2023) Medicinal plants or plant derived compounds used in aquaculture. In: Jyothis M, Midhun SJ, Radhakrishnan EK, Ajay K (eds) Recent advances in aquaculture microbial technology. Academic, Cambridge, pp 153–207

Martínez-Álvarez RM, Morales AE, Sanz A (2005) Antioxidant defenses in fish: biotic and abiotic factors. Rev Fish Biol Fisheries 15:75–88. https://doi.org/10.1007/s11160-005-7846-4

Mehana EE, Rahmani AH, Aly SM (2015) Immunostimulants and fish culture: an overview. Annual Research & Review in Biology 5(6):477–489. https://doi.org/10.9734/ARRB/2015/9558

Mirghaed AT, Hoseini SM, Hoseinifar SH, Van Doan H (2020) Effects of dietary thyme (Zataria multiflora) extract on antioxidant and immunological responses and immune-related gene expression of rainbow trout (*Oncorhynchus mykiss*) juveniles. Fish Shellfish Immunol 106:502–509. https://doi.org/10.1016/j.fsi.2020.08.002

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7(9):405–410. https://doi.org/10.1016/S1360-1385(02)02312-9

Mousavi S, Sheikhzadeh N, Tayefi-Nasrabadi H, Alizadeh-Salteh S, Khani Oushani A, Firouzamandi M, Mardani K (2020) Administration of grape (Vitis vinifera) seed extract to rainbow trout (*Oncorhynchus mykiss*) modulates growth performance, some biochemical parameters, and antioxidant-relevant gene expression. Fish Physiol Biochem 46(3):777–786. https://doi.org/10.1007/s10695-019-00716-4

NRC (2011) Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC, p 392 https://doi.org/10.17226/13039

Patil K, Sanjay CJ, DoggALLI N, Devi KR, Harshitha N (2022) A Review of Calendula Officinalis Magic in Science. J Clin Diagn Res 16(2):ZE23–ZE27. https://doi.org/10.7860/JCDR/2022/52195.16024

Raida MK, Buchmann K (2009) Innate immune response in rainbow trout (*Oncorhynchus mykiss*) against primary and secondary infections with Yersinia ruckeri O1. Dev Comp Immunol 33(1):35–45. https://doi.org/10.1016/j.dci.2008.07.001

Rashidian G, Kajbaf K, Prokić MD, Faggio C (2020) Extract of common mallow (Malvae sylvestris) enhances growth, immunity, and resistance of rainbow trout (*Oncorhynchus mykiss*) fingerlings against Yersinia ruckeri infection. Fish Shellfish Immunol 96:254–261. https://doi.org/10.1016/j.fsi.2019.12.018

Reverter M, Bontemps N, Lecchini D, Banaigs B, Sasal P (2014) Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. Aquaculture 433:50–61. https://doi.org/10.1016/j.aquaculture.2014.05.048

Rojas-Bedoya L, Gómez-López C, Marín-Pareja N (2020) Extraction of metabolites from Calendula officinalis and evaluation of their colorant and antibacterial capacity. Rev Colomb Biotecnol 22(1):60–69. https://doi.org/10.15446/rev.colomb.biote.v22n1.79999

Salem MOA, Taştan Y, Bilen S, Terzi E, Sönmez AY (2022) Effects of white mustard (Sinapis alba) oil on growth performance, immune response, blood parameters, digestive and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immunol 131:283–299. https://doi.org/10.1016/j.fsi.2022.10.006

Sato Y, Itagaki S, Kurokawa T, Ogura J, Kobayashi M, Hirano T, Sugawara M, Iseki K (2011) In vitro and in vivo antioxidant properties of chlorogenic acid and caffeic acid. Int J Pharm 403(1–2):136–138. https://doi.org/10.1016/j.ijpharm.2010.09.035

Shaluei F, Nematollahi A, Naderi-Farsani HR, Rahimi R, Kaboutari Katadj J (2017) Effect of ethanolic extract of Zingiber officinale on growth performance and mucosal immune responses in rainbow trout (*Oncorhynchus mykiss*). Aquac Nutr 23(4):814–821. https://doi.org/10.1111/anu.12448

Srinivasulu C, Ramgopal M, Ramanjaneyulu G, Anuradha CM, Kumar CS (2018) Syringic acid (SA)-a review of its occurrence, biosynthesis, pharmacological and industrial importance. Biomed Pharmacother 108:547–557. https://doi.org/10.1016/j.biopha.2018.09.069

Teimouri M, Yeganeh S, Mianji GR, Najafi M, Mahjoub S (2019) The effect of Spirulina platensis meal on antioxidant gene expression, total antioxidant capacity, and lipid peroxidation of rainbow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem 45(3):977–986. https://doi.org/10.1007/s10695-019-0608-3

Varshney A, Dahiya P, Mohan S (2023) Growth, biochemical, and antioxidant response of pot marigold (*Calendula officinalis L.*) grown in fly ash amended soil. Int J Phytoremediation 25(1):115–124. https://doi.org/10.1080/15226514.2022.2063794

Vasdravanidis C, Alvanou MV, Lattos A, Papadopoulos DK, Chatzigeorgiou I, Ravani M, Liantas G, Georgoulis I, Feidantsis K, Ntinas GK, Giantsis IA (2022) Aquaponics as a promising strategy to mitigate impacts of climate change on rainbow trout culture. Animals 12(19):2523. https://doi.org/10.3390/ani12192523

Vazirzadeh A, Dehghan F, Kazemeini R (2017) Changes in growth, blood immune parameters and expression of immune related genes in rainbow trout (*Oncorhynchus mykiss*) in response to diet supplemented with Ducrosia anethifolia essential oil. Fish Shellfish Immunol 69:164–172. https://doi.org/10.1016/j.fsi.2017.08.022

Vazirzadeh A, Marhamati A, Rabiee R, Faggio C (2020) Immunomodulation, antioxidant enhancement and immune genes up-regulation in rainbow trout (*Oncorhynchus mykiss*) fed on seaweeds included diets. Fish Shellfish Immunol 106:852–858. https://doi.org/10.1016/j.fsi.2020.08.048

Wang F, Liu H, Liu F, Chen W (2020) Effects of Chinese yam (*Dioscorea oppositifolia L.*) dietary supplementation on intestinal microflora, digestive enzyme activity and immunity in rainbow trout (Oncorhynchus mykiss). Aquac Res 51(11):4698–4712. https://doi.org/10.1111/are.14815

Wang D, Tian L, Lv H, Pang Z, Li D, Yao Z, Wang S (2020b) Chlorogenic acid prevents acute myocardial infarction in rats by reducing inflammatory damage and oxidative stress. Biomed Pharmacother 132:110773. https://doi.org/10.1016/j.biopha.2020.110773

Weatherdon LV, Magnan AK, Rogers AD, Sumaila UR, Cheung WW (2016) Observed and projected impacts of climate change on marine fisheries, aquaculture, coastal tourism, and human health: an update. Front Mar Sci 3:48. https://doi.org/10.3389/fmars.2016.00048

Węglarz Z, Kosakowska O, Pióro-Jabrucka E, Przybył JL, Gniewosz M, Kraśniewska K, Bączek KB (2022) Antioxidant and antibacterial activity of Helichrysum italicum (Roth) G Don from Central Europe. Pharmaceuticals 15(6):735. https://doi.org/10.3390/ph15060735

Xu JG, Hu QP, Liu Y (2012) Antioxidant and DNA-protective activities of chlorogenic acid isomers. J Agric Food Chem 60(46):11625–11630. https://doi.org/10.1021/jf303771s

Yousefi M, Farsani MN, Ghafarifarsani H, Hoseinifar SH, Van Doan H (2021) The effects of dietary supplementation of mistletoe (*Viscum album*) extract on the growth performance, antioxidant, and innate, immune responses of rainbow trout (*Oncorhynchus mykiss*). Aquaculture 536. https://doi.org/10.1016/j.aquaculture.2021.736385

Zeilab Sendijani R, Abedian Kenari A, Smiley AH, Esmaeili N (2020) The effect of extract from dill *Anethum graveolens* on the growth performance, body composition, immune system, and antioxidant system of rainbow trout. N Am J Aquac 82(2):119–131. https://doi.org/10.1002/naaq.10123

Zhu F (2020) A review on the application of herbal medicines in the disease control of aquatic animals. Aquaculture 526:735422. https://doi.org/10.1016/j.aquaculture.2020.735422

Authors and Affiliations

Dogukan Kaya[1] · Boran Karataş[2] · Derya Guroy[3]

✉ Dogukan Kaya
dogukan.kaya@gop.edu.tr

[1] Faculty of Agriculture, Department of Animal Science, Tokat Gaziosmanpasa University, 60010 TasliciftlikTokat, Turkey

[2] Faculty of Aquaculture, Department of Aquaculture, Van Yüzüncü Yıl University, 65080 Van, Turkey

[3] Department of Aquaculture, Yalova University, Armutlu Vocational College, 77200 Yalova, Turkey