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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

# Effects of *Althaea officinalis* supplementation on growth performance and antioxidant, stress, and immune-related gene expression in rainbow trout (*Oncorhynchus mykiss*)

## *Althaea officinalis* katkısının gökkuşağı alabalığında (*Oncorhynchus mykiss*) büyüme performansı ve antioksidan, stres ve bağışıklık ile ilgili gen ekspresyonu üzerindeki etkileri

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**Abstract** In this study, a 42-day feeding trial was conducted to evaluate the effects of *Althaea officinalis* extract on growth performance, antioxidant defense, stress-associated, and immune-related gene expression in rainbow trout (*Oncorhynchus mykiss*) with an initial average body weight of 2.48 ± 0.01 g. Three experimental diets were formulated: a basal control diet (C) and two diets supplemented with different levels of *A. officinalis* extract (AO1, 1 g $kg^{-1}$ and AO2, 2 g $kg^{-1}$). Growth indices showed significant improvements in both *A. officinalis* supplemented groups compared to the C group ($P < 0.05$). Gene expression levels of antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) were significantly elevated in the supplemented groups ($P < 0.05$). Additionally, the expression of stress, and immune-related genes, including interleukin-1β (IL-1β), tumour necrosis factor-alpha (TNF-α), interleukin-8 (IL-8), and heat shock protein 70 (HSP-70), was also significantly influenced by dietary inclusion of *A. officinalis* extract ($P < 0.05$). Overall, these findings provide experimental evidence that dietary inclusion of *A. officinalis* extract enhances antioxidant capacity, modulates stress and immune-related gene expression, and promotes healthy growth in rainbow trout. These beneficial effects may be attributed to the presence of flavonoids and phenolic compounds in *A. officinalis*, which are known for their antioxidant and immunomodulatory properties.

**Keywords:** Medicinal plant, immunity, gene expression, growth, antioxidant

**Öz:** Bu çalışmada, başlangıç ortalama canlı ağırlığı 2,48 ± 0,01 g olan gökkuşağı alabalıklarında (*Oncorhynchus mykiss*) *Althaea officinalis* ekstraktının büyüme performansı, antioksidan savunma, stres ve bağışıklıkla ilişkili gen ekspresyonu üzerindeki etkilerini değerlendirmek amacıyla 42 günlük bir besleme denemesi gerçekleştirilmiştir. Bu amaçla, biri bazal kontrol diyeti (C) ve diğer ikisi farklı düzeylerde *A. officinalis* ekstraktı içeren (AO1: 1 g $kg^{-1}$, AO2: 2 g $kg^{-1}$) olmak üzere üç deneysel diyet formüle edilmiştir. Büyüme parametreleri, *A. officinalis* takviyesi yapılan gruplarda kontrol grubuna kıyasla anlamlı düzeyde artış göstermiştir ($P < 0,05$). Antioksidan enzimlerden süperoksit dismutaz (SOD) ve katalaz (CAT) genlerinin ekspresyon düzeyleri, takviye edilen gruplarda anlamlı şekilde yükselmiştir ($P < 0,05$). Ayrıca, stres ve bağışıklıkla ilişkili genler olan interlökin-1β (IL-1β), tümör nekroz faktör-alfa (TNF-α), interlökin-8 (IL-8) ve ısı şok proteini 70 (HSP-70) genlerinin ekspresyon düzeyleri de *A. officinalis* ekstraktı ilavesiyle anlamlı şekilde etkilenmiştir ($P < 0,05$). Genel olarak, bu bulgular *A. officinalis* ekstraktı içeren diyetlerin gökkuşağı alabalıklarında antioksidan kapasiteyi artırabileceğini, stres ve bağışıklıkla ilişkili gen ekspresyonunu düzenleyebileceğini ve sağlıklı büyümeyi destekleyebileceğini deneysel olarak ortaya koymaktadır. Bu yararlı etkiler, antioksidan ve immünomodülatör özellikleriyle bilinen *A. officinalis*'te bulunan flavonoidler ve fenolik bileşiklerin varlığına bağlanabilir.

**Anahtar kelimeler:** Tıbbi bitki, bağışıklık, gen ifadesi, büyüme, antioksidan

## INTRODUCTION

Aquaculture plays an important role in providing people with a reliable source of protein. Due to increasing consumer demand, improving fish production in terms of both quality and quantity has become one of the main goals of this sector (Boyd et al., 2022). In aquaculture, feed quality is one of the key factors for increasing productivity (Shekarabi et al., 2022). Therefore, it is important to use feed formulations that meet the specific nutritional needs of aquatic animals (Romano, 2021). Traditional feeds are primarily composed of essential nutrients; however, the use of plant-derived additives in fish nutrition has expanded significantly in recent years (Elumalai et al., 2020; Vijayaram et al., 2024). Such additives may enhance growth performance, boost antioxidant defenses, and improve immune function (Rashidian et al., 2020; Mostafavi et al., 2022).

Medicinal plants have been used for a long time to support human health (Sharma et al., 2024; Monib, 2024). Recently, there has been growing interest in their use in aquaculture (Hu et al., 2024; Ng et al., 2024). Many studies have shown that plant extracts containing bioactive compounds such as flavonoids, phenolic compounds, alkaloids, terpenoids, saponins, glycosides, tannins, steroids, and essential oils can be used as natural supplements without causing side effects in cultured species such as rainbow trout (Van Hai et al., 2015; Rashidian et al., 2020; Mostafavi et al., 2022). *Althaea* *officinalis* L., a member of the Malvaceae family and commonly known as marshmallow, has traditionally been used to relieve dry cough caused by irritation in the throat. Extracts from this plant contain important compounds such as starch (25–35%), pectin (11%), sucrose (10%), and mucilage (5%) (Gudej et al., 1991). In addition, it has a rich content of flavonoids, phenolic acids (like caffeic and p-coumaric acids), isoquercitrin, coumarins, phytosterols, tannins, and various amino acids. Some studies have shown that *A. officinalis* has antimicrobial, anti-inflammatory, and immune-supporting properties (Bonaterra et al., 2020). It also has strong antioxidant activity, which helps to remove harmful free radicals from the body (Elmastas et al., 2004). The diet containing 2.5 and 5 g kg$^{-1}$ of *A. officinalis* extract did not have any adverse effects on liver enzymes in carp (*Cyprinus carpio* L.), and supplements containing the plant extract did not have any side effects (Banaee et al., 2016). However, there is limited information about its effects on fish growth and gene expression. This shows the need for more studies, especially on cultured species.

Rainbow trout (*Oncorhynchus mykiss*) is considered a key species in global aquaculture due to its high economic and nutritional value (FAO, 2022). However, problems such as high stocking density, disease outbreaks, and stress-related health issues are becoming more common. With the reduced use of antibiotics, there is growing interest in alternative methods to support fish immunity, and feed ingredients have become more important (Madhulika et al., 2025). As a result, we hypothesized that adding *A. officinalis* to the diet of rainbow trout may improve their growth and increase the expression of genes related to immunity and antioxidant functions.

## MATERIALS AND METHODS

### Preparation of plant extract

The *A. officinalis* flowers used in this study were collected from the Van Yüzüncü Yıl University campus area. The extraction procedure was carried out with slight modifications, drawing on the methodologies of Karataş (2024) and Banaee et al. (2016; 2017). In summary, fresh flowers were first rinsed with distilled water and then air-dried in a shaded area at ambient temperature for a period of 10 days. Once dried, the flowers were pulverized into a fine powder using a laboratory grinder. This powdered material was then combined with a 1:1 mixture of distilled water and ethanol and subjected to continuous agitation on an orbital shaker at 200 rpm for 24 hours at room temperature. The resulting mixture was initially filtered through sterile cheesecloth and subsequently centrifuged at 3000 rpm for 5 minutes. The supernatant was further clarified by filtration using Whatman No. 1 filter paper. The organic solvent was evaporated using a rotary evaporator to obtain a viscous extract, which was subsequently dried in an oven at 50 °C to yield a powdered form, resulting in 8 g of dried powder (8% of the initial material). The final extract was stored at −20 °C until incorporation into the experimental diets.

No binding agents were added during the diet preparation. The ethanolic extract solution was uniformly sprayed onto the commercial trout feed pellets and then air-dried at room temperature for 24 hours to allow complete solvent evaporation (Bilen et al., 2021 and Sönmez et al., 2022). The sprayed feed was manually mixed to ensure homogeneity and subsequently stored in airtight containers at 4 °C. Fresh batches were prepared weekly to maintain the stability and quality of the supplemented diets.

### Experimental fish and experimental diets

Rainbow trout used in the study were obtained from a local fish farm in Van Province, Türkiye. After transportation to the Aquatic Animals Research Unit at Van Yüzüncü Yıl University, the fish were placed in separate tanks and allowed a 15-day acclimation period to adjust to the experimental environment. Throughout this period, the fish were fed the control diet twice a day.

The experimental diets were formulated by supplementing a commercial trout feed (Skretting, Milas-Muğla, Türkiye) with 0 g kg$^{-1}$ (Control, C), 1 g kg$^{-1}$ (AO1), and 2 g kg$^{-1}$ (AO2) of *A. officinalis* extract. The basal feed composition included 53% crude protein, 18% crude fat, 0.6% crude fiber, 11% ash, 2.6% calcium, 1.7% phosphorus, and 0.7% sodium. All diets were stored in plastic bags at 4 °C until use.

### Experimental design and feeding protocol

A total of 450 healthy rainbow trout with an average initial body weight of 2.48 ± 0.01 g were randomly assigned to 12 tanks (400 L each), with 50 fish per tank and three replicates per dietary treatment. The feeding trial lasted for 6 weeks. Fish were fed twice daily at a feeding rate equivalent to 3% of their body weight. Feed quantities were adjusted every two weeks based on the mean body weight of the fish. Aeration was continuously provided using a centralized air pump system, and 20% of the tank water was renewed daily with dechlorinated freshwater to sustain optimal water quality. Residual feed and fecal matter were removed each morning by siphoning prior to feeding. Throughout the experiment, water quality was monitored, with recorded values of dissolved oxygen at 7.67 ± 0.25 mg/L, temperature at 16.65 ± 0.51 °C, and pH at 8.2 ± 0.07. Lighting was provided with artificial light on a 12-hour light and 12-hour dark cycle.

### Growth indices and sampling

To assess growth performance, individual fish were weighed at both the start and conclusion of the experiment. Growth indices were calculated using commonly accepted formulas as follows (Aydın et al., 2025; Kaya et al., 2025):

Weight Gain (WG; g/fish) = Final weight − Initial weight

Specific Growth Rate (SGR; %/day) = ((ln Final weight − ln Initial weight) / days) × 100

Daily Weight Gain (DWG; g/fish) = (Final weight − Initial weight) / days

Feed Conversion Ratio (FCR) = Total feed given / Weight gain

Survival Rate (SR; %) = (Final number of fish / Initial number of fish) × 100

Protein efficiency ratio (PER) = Weight gain / protein intake

Before sampling, the fish were subjected to a 24-hour fasting period and then anesthetized using clove powder at a concentration of 200 mg $L^{-1}$ (Karataş, 2024). Body weights were measured using a digital scale and recorded for growth performance evaluation.

For statistical analyses, tanks were considered as the experimental units. Growth performance data were calculated based on mean values per tank (three tanks per treatment group). For gene expression analysis, liver tissues were collected from six fish per treatment group, sampling two fish from each tank. Tissue samples (25–50 mg) were preserved in RNAlater solution prior to RNA extraction.

### Gene expression analysis

Total RNA was isolated using the DiaRex® Total RNA Extraction Kit (TR-0877-100, Diagen, Ankara, Türkiye) according to the manufacturer's protocol. The quantity and purity of RNA were measured at 260 and 280 nm using a nanospectrophotometer (QIAxpert). Complementary DNA (cDNA) was synthesized utilizing the Solver ArGe cDNA Synthesis Kit (SLV-M-2021-10-100, Van, Türkiye). Quantitative real-time PCR (qRT-PCR) was carried out using the RotorGene Q 9000 (Qiagen) platform, in conjunction with SuYBRGreen qPCR Master Mix (PCR01C0253, Sugenomics, Ankara, Türkiye), to quantify gene expression levels.

The transcript levels of genes associated with antioxidant activity (SOD, CAT, GPX), immune function (IL-1β, IL-8, TNF-α), and cellular stress (HSP70) was evaluated. Each PCR reaction was prepared in a 25 µL final volume, comprising 4 µL of cDNA, 12 µL of SybrGreen Master Mix, 4 µL of nuclease-free water, and 2.5 µL each of the forward and reverse primers. The amplification protocol included an initial denaturation step at 95 °C for 15 minutes, followed by 45 cycles of denaturation at 95 °C for 30 seconds and annealing at 60 °C for 60 seconds. β-actin was employed as the reference gene for normalization of Ct values. Relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001), and results were expressed as fold changes compared to the control group (Table 1).

**Table 1.** Sequences of forward and reverse primers, accession number, and the conditions of genes selected for real-time PCR

| Target gene | Primer sequence (5´ to 3´) | Amplicon size(bp) | Annealing Tm (°C) | Reference/Accession no. |
|---|---|---|---|---|
| GPx (Glutathione peroxidase) | F: CGAGCTCCATGAACGGTACG<br>R: TGCTTCCCGTTCACATCCAC | 183 | 60 | HE687022.1 |
| CAT (Catalase) | F: TGATGTCACACAGGTGCGTA<br>R: GTGGGCTCAGTGTTGTTGAG | 195 | 58 | XM_021557350.2 |
| SOD (Superoxide dismutase) | F: TGGTCCTGTGAAGCTGATTG<br>R: TTGTCAGCTCCTGCAGTCAC | 201 | 58 | AF469663.1 |
| TNF-α (Tumor Necrosis Factor-Alpha) | F: GGCTGTGTGGCGTTCTCTTA<br>R: AAATGGATGGCTGCTTTCGC | 190 | 58 | NM_001124374.1 |
| IL-1β (Interleukin-1 Beta) | F: AGCAGGACTACACCAAACCG<br>R: TCCTGATCGTAGAGGCCCAA | 184 | 59 | AJ004821.1 |
| IL-8 (Interleukin-8) | F: CACAGACAGAGAAGGAAGGAAAG<br>R: TGCTCATCTTGGGGTTACAGA | 162 | 60 | NM_001124279.1 |
| HSP 70 (Heat Shock Protein 70) | F: CTGCTGCTGCTGGATGTG<br>R: GCTGGTTGTCGGAGTAAGTG | 135 | 59 | AB062281.1 |
| β-actin (Beta-actin) | F: GGAGGCTCCATCTTGGCTTC<br>R: GAAGTGGTAGTCGGGTGTGG | 158 | 61 | AJ438158.1 |

### Statistical analysis

All data were analyzed using SPSS 20 software, and growth performance data were expressed as means ± standard deviation of the mean. One-way analysis of variance (ANOVA) was used to evaluate statistical differences among treatment groups, followed by the Tukey multiple range test. Statistical significance was set at $P < 0.05$.

## RESULTS

Dietary supplementation with *Althaea officinalis* extract significantly affected the growth indices of rainbow trout (Table 2). Final weight, weight gain, daily weight gain, specific growth rate, and protein efficiency ratio were all significantly higher in fish fed diets containing the extract compared to the control group ($P < 0.05$). Both the 0.1% and 0.2% supplementation levels led to improvements in these parameters, with no significant difference between the two supplemented groups.

Feed conversion ratio was significantly lower in the supplemented groups compared to the control, indicating more efficient feed utilization ($P < 0.05$). There were no significant differences in survival rate among the groups, which remained at 100% throughout the trial.

**Table 2.** Growth indices of rainbow trout fed diets supplemented with *Althaea officinalis* extract

| Parameters | C | AO1 | AO2 |
|---|---|---|---|
| IW | 2.48±0.01 | 2.48±0.01 | 2.49±0.01 |
| FW | 8.68±0.09[a] | 9.80±0.40[b] | 9.91±0.15[b] |
| WG | 6.20±0,09[a] | 7.32±0,39[b] | 7.42±0.15[b] |
| DWG | 0.15±0.00[a] | 0.17±0.01[b] | 0.18±0.01[b] |
| SGR | 2.98±0.03[a] | 3.27±0.09[b] | 3.29±0.04[b] |
| PER | 1.98±0.03[a] | 2.34±0.12[b] | 2.38±0.05[b] |
| FCR | 1.05±0.02[b] | 0.89±0.05[a] | 0.88±0.02[a] |
| SR | 100 | 100 | 100 |

*Results are expressed as mean values accompanied by standard deviations (SD). Distinct lowercase letters within the same row indicate statistically significant differences ($P < 0.05$). Abbreviations: C – basal diet; AO1 – diet containing 0.1% Althaea officinalis extract; AO2 – diet containing 0.2% A. officinalis extract; IW – initial weight (g); FW – final weight (g); WG – weight gain (g); DWG – daily weight gain (g); SGR – specific growth rate (% $day^{-1}$); PER – protein efficiency ratio (%); FCR – feed conversion ratio; SR – survival rate (%).

Figure 1 illustrates the hepatic transcript levels of antioxidant-related genes (SOD, CAT, and GPX) in rainbow trout. The expression of SOD was significantly upregulated in both AO1 and AO2 groups compared to the control group ($P < 0.05$). CAT expression showed a dose-dependent increase, being significantly higher in the AO2 group than in both the AO1 and control groups, and also significantly elevated in AO1 compared to the control ($P < 0.05$). In contrast, GPX expression did not differ significantly between the control and AO1 groups, while a significant downregulation was observed in the AO2 group ($P < 0.05$).

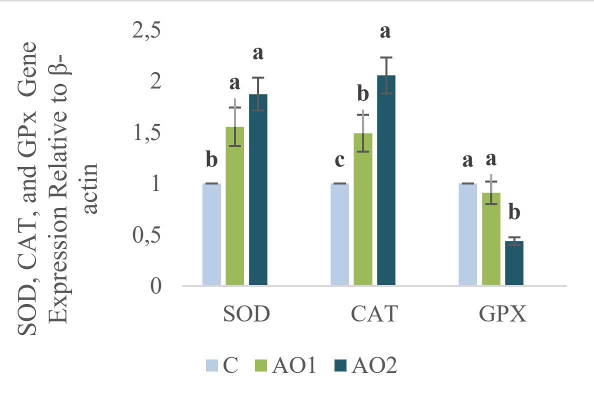


**Figure 1.** Transcript levels of genes associated with antioxidant enzymes in the liver of rainbow trout (n=6)

Figure 2 presents the relative expression levels of stress- and immune-related genes (IL-1β, IL-8, TNF-α, and HSP70). The expression of IL-1β was significantly upregulated in both experimental groups compared to the control, with the highest level observed in the AO2 group ($P < 0.05$). Similarly, TNF-α expression was significantly elevated in the AO2 group relative to the control ($P < 0.05$). The IL-8 transcript level was markedly upregulated in the AO2 group compared to both the control and AO1 groups ($P < 0.05$). A comparable pattern was detected for HSP70, which exhibited a significant increase in expression in the AO2 group ($P < 0.05$).

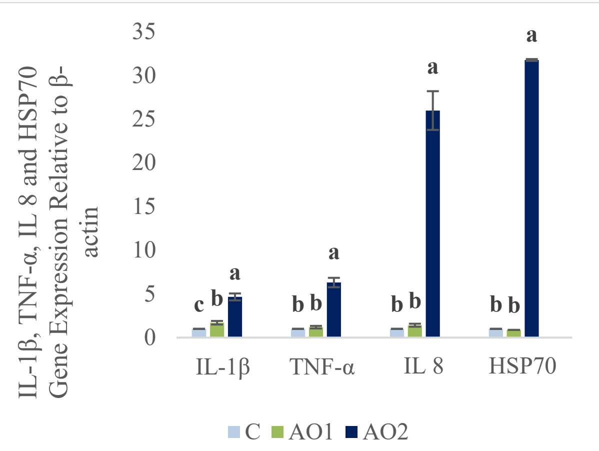


**Figure 2.** Transcript levels of genes associated with stress and immune responses in the liver of rainbow trout (n=6)

## DISCUSSION

The present study focused on antioxidant capacity and immune responses at the molecular level to elucidate the potential effect of dietary *A. officinalis* extract on rainbow trout (*O. mykiss*) culture. The use of medicinal aromatic plant sources in the diet has a significant potential in aquaculture by providing effects on growth indices and improving health parameters in fish. Although various plant extracts have been evaluated as herbal additives in fish feeds, there is limited information on the use of *A. officinalis* extract in fish and no research on its growth-promoting effects on rainbow trout. Our study findings showed that dietary *A. officinalis* extract improved growth performance in rainbow trout. Consistent with our study, various dietary medicinal plant extracts such as yarrow (*Achillea millefolium*) (Bahabadi et al., 2014), wild tarragon (*Artemisia dracunculus*) (Gholamhosseini et al., 2021), pot marigold (*Calendula officinalis*) (Kaya et al., 2025) and curry plant (*Helichrysum italicum*) (Aydın et al., 2025) in the diet improved growth performance in rainbow trout. This can be related to several factors, including improved nutrient utilization, feed conversion rates and optimized digestion, resulting in more efficient feed utilization and growth performance (Barad et al., 2024), because medicinal aromatic plant extracts are rich in phenolic compounds that induce growth and improve fish metabolism (Kaya et al., 2025).

The antioxidant system primarily consists of SOD, CAT, and GPX enzymes (Fontagné-Dicharry et al., 2014). Assessing this system in fish is crucial for interpreting physiological responses (Mittler, 2002) and can enhance the productivity of aquaculture practices (Hoseinifar et al., 2020). The activity of antioxidant enzymes plays a key role in safeguarding fish health by counteracting oxidative stress that harms living cells (Martínez-Álvarez et al., 2005). Flavonoids and phenolic compounds found in medicinal plants exhibit strong antioxidant effects (Li et al., 2013). Previous studies have shown that dietary supplementation with medicinal and aromatic plant extracts, including pot marigold (*Calendula officinalis*) (Kaya et al., 2025), curry plant (*Helichrysum italicum*) (Aydın et al., 2025), and *Cyanus depressus* (Karataş, 2024), enhances the transcript levels of genes related to antioxidant enzymes in rainbow trout. Similarly, this study showed that CAT and SOD gene expression levels in rainbow trout were elevated by dietary *A. officinalis*. Elmastas et al. (2004) clearly demonstrated that *A. officinalis* extract possesses significant antioxidant activity, which can be attributed to its strong hydrogen-bonding capacity, metal-chelating ability, and its effectiveness as a scavenger of superoxide and free radicals. Interestingly, although CAT and SOD antioxidant-related genes were upregulated in response to *A. officinalis* supplementation, a significant downregulation of GPX expression was observed in the AO2 group. This finding may reflect a dose-dependent response, in which the higher inclusion level (2 g $kg^{-1}$) either exceeded the optimal physiological threshold or induced a feedback inhibition mechanism. Similar reductions in GPX expression at 0.5 and 1 g kg$^{-1}$ pot marigold (*Calendula officinalis*) doses have been reported in rainbow trout, potentially due to cellular redox imbalance or adaptive downregulation (Kaya et al., 2025). Also, the supplementation of common carp (*Cyprinus carpio*) diets with valerian extract (*Valeriana officinalis*) at doses of 0.25, 0.5 and 1 g kg$^{-1}$ downregulated GPX gene expression (Yılmaz et al., 2025). These results highlight the importance of identifying optimal dosages to avoid possible inhibitory or counterproductive effects associated with overdosing functional feed additives.

Due to high pathogen loads and limited chemical treatment options in aquaculture, strengthening the fish immune system is essential for disease prevention (Mehana et al., 2015). Dietary herbal immunostimulants have shown potential to enhance immune function in farmed fish (Awad and Awaad 2017; Dev et al., 2024). Monitoring immune-related gene expression is a common and effective method for evaluating immune responses (Cecchini et al., 2013). Our results demonstrated that dietary administration of *A. officinalis*, particularly at 2 g kg$^{-1}$, enhanced the transcript levels of genes associated with immune responses in rainbow trout. The increase in immune-related gene expression may be linked to bioactive compounds in *A. officinalis* extract, such as flavonoids and antioxidants. Several studies have shown that medicinal plants can modulate immune-related gene expression in rainbow trout. For instance, Shekarabi et al. (2021) reported that dietary dandelion (*Taraxacum officinale*) flower extract elevated IL-1β and IL-8 gene expression in all treated rainbow trout groups versus controls. Similarly, Nootash et al. (2013) observed increased IL-1β and IL-8 expression in fish fed green tea (*Camellia sinensis*) supplements. Adel et al. (2020) also reported that dietary supplementation with *Polygonum minus* extract significantly enhanced genes linked to immunity. The immune-stimulating effects of plant extracts are attributed to their bioactive compounds (Reverter et al., 2017). Bioactive compounds have the potential to stimulate the immune system and are thought to improve the defense mechanisms of fish through the production of these cytokines (Sakai et al., 2021). Furthermore, the induction of the immune response through the upregulation of immune-related gene expression can be achieved with these plant-derived bioactive compounds (Awad et al., 2011). These compounds may exert potential effects on signaling pathways that regulate cytokine production (Türker, 2012). The increased immune gene expression observed in groups fed *A. officinalis* extract likely reflects the activation of immune responses, driven by its phenolic constituents. Overall, our findings suggest that dietary *A. officinalis* enhances immune gene expression in rainbow trout, promoting immune cell activity.

Heat shock protein 70 (HSP70) is known to play a critical role in safeguarding cytoplasmic components under various stress conditions (Zhang et al., 2020; Karataş, 2025). In the present study, supplementation with 2 g kg$^{-1}$ of *A. officinalis* extract was found to enhance cellular stress resistance, as evidenced by the upregulation of HSP70 gene expression. Consistent with this finding, previous studies investigating the influence of dietary medicinal plants on HSP70 expression in rainbow trout (*Oncorhynchus mykiss*) have reported similar results. For instance, Sheikhzadeh et al. (2019) demonstrated that dietary supplementation with green tea (*Camellia sinensis*) induced HSP70 expression in rainbow trout. Wang et al. (2024) reported that the supplementation of Chinese herbal medicines to the diet of rainbow trout upregulates HSP70 gene expression. Likewise, Karataş (2024) observed an upregulation of HSP70 expression in trout administered a diet supplemented with *Cyanus depressus* extract. Despite these promising findings, further research is required to comprehensively understand the regulatory mechanisms by which dietary herbal compounds modulate HSP70 expression in fish. While increased expression of pro-inflammatory cytokines (IL-1β, TNF-α, IL-8) and stress protein HSP70 may suggest enhanced immune responses, it is important to consider that such upregulation can also indicate subclinical inflammation or cellular stress. The pronounced elevation of these markers in the AO2 group might reflect a mild stress response triggered by the higher extract dose. Correspondingly, the downregulation of the antioxidant enzyme gene GPX in this group may signify an imbalance in oxidative status, potentially due to excessive phytogenic compound exposure. These findings highlight the need for careful dose optimization to balance immune stimulation and stress avoidance.

In conclusion, although both AO1 and AO2 groups showed improvements in growth performance and gene expression compared to the control, the difference between the two experimental groups was not statistically significant in most measured parameters. Notably, the 2 g kg$^{-1}$ dose led to a marked increase in HSP70 and IL-8 expression, which may indicate cellular stress or immune overstimulation. Therefore, from a practical and economic perspective, the 1 g kg$^{-1}$ dose may already be sufficient to achieve the desired biological effects without triggering potential stress-related responses or incurring unnecessary feed costs. Further dose-optimization studies could help confirm the most effective and safe inclusion level for *A. officinalis* extract in rainbow trout diets.

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## AUTHORSHIP CONTRIBUTIONS

All authors contributed to the idea and design of the study. Material preparation and investigation were performed by Boran Karataş and Doğukan Kaya. The writing/editing was carried out by Boran Karataş and Doğukan Kaya, and all authors have read and approved the article.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ETHICAL APPROVAL

The experiment was approved by the Local Ethics Committee for Aquatic Vertebrates at Van Yüzüncü Yıl University (Protocol No: 2025/08) and was conducted in accordance with standard ethical guidelines.

## DECLARATION OF AI USE

We have not used AI-assisted technologies in creating this article.

## DATA AVAILABILITY

All relevant data is in the article.

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