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RESEARCH ARTICLE ARAŞTIRMA MAKALESİ

Impact of dietary gallic acid on growth indices and the expression of antioxidant, stress, and immunity-related genes in rainbow trout (*Oncorhynchus mykiss*)

Gallik asidinin gökkuşağı alabalığında *(Oncorhynchus mykiss*) büyüme endeksleri ve antioksidan, stres ve bağışıklık ilgili genlerin ekspresyonu üzerindeki etkisi

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Abstract: This study investigates the effects of dietary gallic acid (GA) supplementation on growth performance and the expression of genes linked to antioxidant, stress, and immune functions in rainbow trout (*Oncorhynchus mykiss*). Fish with an average body weight of 2.84 ± 0.25 g were fed diets containing 0 mg/kg (control), 300 mg/kg (G300), 450 mg/kg (G450), and 600 mg/kg (G600) of GA over 60 days. The results revealed significant improvements in growth indices, including weight gain, specific growth rate, and feed conversion ratio, in GA-supplemented groups compared to the control ($P<0.05$). At the molecular level, GA supplementation significantly upregulated the expression of antioxidant-related genes (SOD, CAT, GPX), stress-related genes (HSP70), and immune-related genes (TNF-α, IL-1β). The G300 group consistently exhibited the most pronounced transcriptional responses, while higher doses (G450 and G600) showed diminished or inconsistent effects. These findings suggest that a dietary inclusion of 300 mg/kg GA optimally enhances fish health and productivity by modulating key molecular pathways. This dosage is recommended as an effective feed additive for improving the performance and resilience of rainbow trout in aquaculture.

Keywords: Feed supplement, organic acid, growth, gene expression

Öz: Bu çalışmada, gökkuşağı alabalığında (*Oncorhynchus mykiss*) diyet gallik asit (GA) takviyesinin büyüme performansı ve antioksidan, stres ve bağışıklık fonksiyonlarıyla bağlantılı genlerin ifadesi üzerindeki etkileri araştırılmıştır. Ortalama vücut ağırlığı 2,84 ± 0,25 g olan balıklar, 60 gün boyunca 0 mg/kg (kontrol), 300 mg/kg (G300), 450 mg/kg (G450) ve 600 mg/kg (G600) GA içeren diyetlerle beslenmiştir. Sonuçlar, GA takviyeli gruplarda kontrole kıyasla kilo alımı, özgül büyüme oranı ve yem dönüşüm oranı da dahil olmak üzere büyüme endekslerinde önemli iyileşmeler olduğunu ortaya koymuştur ($P<0,05$). Moleküler düzeyde, GA takviyesi antioksidanla ilişkili genlerin (SOD, CAT, GPX), stresle ilişkili genlerin (HSP70) ve bağışıklık ile ilişkili genlerin (TNF-α, IL-1β) ifadesini önemli ölçüde artırmıştır. G300 grubu sürekli olarak en belirgin transkripsiyonel tepkileri sergilerken, daha yüksek dozlar (G450 ve G600) azalan veya tutarsız etkiler gösterdi. Bu bulgular, 300 mg/kg GA'nın diyete dahil edilmesinin, temel moleküler yolları düzenleyerek balık sağlığını ve üretkenliğini en iyi şekilde artırdığını göstermektedir. Bu dozaj, su ürünleri yetiştiriciliğinde gökkuşağı alabalığının performansını ve dayanıklılığını iyileştirmek için etkili bir yem katkı maddesi olarak önerilmektedir.

Anahtar Kelimeler: Yem takviyesi, organik asit, büyüme, gen ifadesi

INTRODUCTION

Aquaculture is one of the fastest-growing industries globally, playing a critical role in addressing the increasing demand for food (Verdegem et al., 2023; Obirikorang et al., 2024). Among farmed fish species, rainbow trout (*Oncorhynchus mykiss*) stands out as a key species due to its rapid growth, adaptability to diverse environmental conditions, and high economic value (Vasdravanidis et al., 2022; Alkan et al., 2025). However, achieving optimal growth performance and resilience in rainbow trout requires precise nutritional strategies to support health and counteract environmental stressors (Ciji and Akhtar, 2021; Toomey et al., 2024). Enhancing growth and health in rainbow trout is not only vital for the sustainability and profitability of aquaculture but also aligns with the broader goals of efficient and sustainable food production.

In recent years, organic acids have garnered considerable attention as feed additives in aquaculture due to their potential to improve growth, enhance nutrient utilization, and modulate immune responses in fish. Research indicates that organic acids can promote fish health by increasing nutrient absorption, regulating intestinal microbiota, and stimulating digestive enzyme activity (das Neves et al., 2021; Ghafarifarsani et al., 2023; Yousefi et al., 2023). Moreover, these compounds have been shown to boost antioxidant capacity and enhance immune function in fish (Duan et al., 2018; Yilmaz, 2019; Zhang et al., 2020; Jin et al., 2023; Zhang et al., 2023).

Gallic acid (GA), a naturally occurring phenolic acid found abundantly in plants and fruits (Sousa et al., 2024; Xiang et al., 2024), has gained attention as a promising dietary supplement due to its potent anti-inflammatory, antimicrobial, and

antioxidant properties (Hadidi et al., 2024; Zhao et al., 2024). Research conducted on terrestrial animal species has shown that the inclusion of dietary GA supplementation leads to notable enhancements in growth performance and overall health status (Wei et al., 2016; Samuel et al., 2017; Zhao et al., 2021; Xu et al., 2022). However, despite its proven efficacy in terrestrial species, the application of GA in aquaculture remains underexplored.

Emerging research has begun to highlight the potential of GA in aquaculture species. For instance, Ghafarifarsani et al. (2023) reported significant enhancements in growth indices, antioxidant status, and immune function in common carp (*Cyprinus carpio*) subjected to crowding stress when supplemented with dietary GA. Similarly, Zhao et al. (2024) demonstrated that GA alleviates fish enteritis by suppressing immune cell activation, cytokine release, apoptosis, and oxidative stress while promoting anti-inflammatory intestinal metabolites with immunomodulatory and antioxidant effects. Despite these promising findings, the specific effects of GA on rainbow trout a globally significant aquaculture species remain largely unexplored.

This research seeks to fill the existing knowledge gap by examining how dietary supplementation with GA influences growth indices, feed efficiency, and the expression of critical antioxidant genes (SOD, CAT, GPX), immune-related genes (IL-1β, TNF-α, and IL-8), and stress-related gene (HSP70), in rainbow trout. The study also aims to identify the optimal dosage of GA that maximizes its beneficial effects by evaluating various levels of incorporation. Ultimately, this investigation explores the potential of GA as a feed additive to enhance growth performance and promote the expression of antioxidant, immune, and stress-related genes in rainbow trout. By providing critical insights into nutritional strategies that foster healthier and more resilient fish populations, this research aspires to contribute to the advancement of sustainable aquaculture practices and improve production outcomes.

MATERIALS AND METHODS

Experimental fish

Rainbow trout were obtained from a local aquaculture facility located in Van Province, Turkey. Following their collection, the fish were transported to the Aquatic Animals Experiment Unit at Van Yüzüncü Yıl University in Van, Turkey, where they were placed in individual tanks. Before the commencement of the experiment, the fish underwent a 15-day acclimatization period to adapt to the experimental conditions and were fed a standard control diet twice each day.

Experimental diets

Experimental diets were prepared by incorporating gallic acid (Sigma-Aldrich, Germany) into a basal diet (composition: 54% crude protein, 15% crude fat, 1% crude fiber, 8.9% crude ash, 1.3% phosphorus, 1.5% calcium, and 0.3% sodium; Skretting, Milas-Muğla, Turkey) at concentrations of 0 (Control), 300, 450, and 600 mg/kg. The inclusion levels were based on the study by Ghafarifarsani et al. (2023). To prepare the diets, 300 mL of water was added per kilogram of the basal diet to form a smooth dough. The required amount of gallic acid was thoroughly mixed into the dough, which was then shaped into sticks using a mincer and dried by fan air (22°C). The dried sticks were pelleted (2 mm diameter) and stored in airtight plastic bags at 4°C until use (Hoseinifar et al., 2017; Ghafarifarsani et al., 2021).

Experimental design and feeding protocols

Four experimental diets containing varying levels of gallic acid (0 mg/kg as Control, 300 mg/kg as G300, 450 mg/kg as G450, and 600 mg/kg as G600) were tested. A total of 360 healthy fish with an initial average weight of 2.84 ± 0.25 g were randomly assigned to four dietary treatments, with three replicates per group (30 fish per tank). The feeding trial lasted 60 days, during which fish were fed 3% of their body weight three times daily (Karataş, 2025). Feed amounts were adjusted biweekly based on average fish weight. The 12 tanks (400 L each) were aerated using a central air pump, and 20% of the water in each tank was exchanged daily with dechlorinated water to maintain optimal water quality. Uneaten feed and feces were removed from the tanks each morning before feeding. Key water quality parameters were monitored and maintained at 7.64 ± 0.39 mg/L dissolved oxygen, 15.35 ± 0.19°C temperature, and pH 8.2 ± 0.05. Artificial lighting provided a 12-hour light and 12-hour dark photoperiod.

Growth indices and sampling

At the start and end of the trial, fish were individually weighed to calculate growth indices. Growth parameters were determined using standard formulas.

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

Daily weight gain (DWG; g/fish) = (Final weight (g) − Initial weight (g)) / days,

Specific growth rate (SGR; % / day) = ((ln (final weight) – ln (initial weight)) / days) x 100,

Thermal growth coefficient (TGC) = ((final weight)1/3 – (initial weight)1/3) / temperature in ^{o}C x time in days) x 1000,

Feed conversion ratio (FCR) = Total feed given (g) / Weight gain (g),

Survival rate (SR; %) = (Final number of fish / Initial number of fish) x 100.

Before sampling, the fish were subjected to a fasting period of 24 hours and were anesthetized using clove powder at a concentration of 200 mg/L (Karataş, 2024). Body weight was recorded using a digital scale (Sartorius/0.01 g). For the purpose of gene expression analysis, liver tissues were extracted from six randomly chosen fish within each treatment group, with two fish selected from each replicate tank. Liver

samples (25–50 mg) were collected and preserved in RNAlater solution until the RNA extraction process.

Gene expression analysis

Total RNA was isolated using the DiaRex® Total RNA Isolation Kit (TR-0877-100, Diagen, Ankara, Turkey) in accordance with the manufacturer's guidelines. The concentration and purity of the isolated RNA were evaluated with a QIAxpert nanospectrophotometer (Qiagen) at a wavelength of 260/280 nm. cDNA synthesis was performed using the Solver ArGe cDNA Synthesis Kit (SLV-M-2021-10-100, Van, Turkey), following the methodology outlined by Önalan (2019). Quantitative real-time PCR (qRT-PCR) was conducted using the Solver ArGe qPCR Master Mix (SLV-M-2021-01-0.5ML, Van, Turkey) on a Rotor-Gene Q 9000 thermal cycler (Qiagen). A total of eight genes were analyzed, including seven target genes and one reference gene. Each 25 µL PCR reaction contained 12 µL SybrGreen qPCR Master Mix, 4 µL cDNA, 4 µL H_2O, and 2.5 µL of forward and reverse primers (Table 1). The PCR protocol included an initial denaturation at 95°C for 15 minutes, followed by 45 cycles of 95°C for 30 seconds and 60°C for 60 seconds. Beta-actin served as the reference gene for normalization purposes. Gene expression data were analyzed using the ΔΔCt method (Livak and Schmittgen, 2001), with results presented as fold changes relative to the control group.

Statistical analysis

Data analysis was conducted using SPSS v20 (IBM, Chicago, IL, 2011), with results expressed as mean ± standard error of the mean (SEM). A one-way analysis of variance (ANOVA) was utilized to assess differences between treatment groups, followed by Duncan's multiple range test for subsequent pairwise comparisons. A significance level of $P < 0.05$ was established for statistical relevance.

Table 1. Details regarding the sequences of forward and reverse primers, accession numbers, and the conditions for the genes chosen for real-time PCR

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

RESULTS

Table 2 displays the growth performance metrics for rainbow trout fed the experimental diets. Throughout the 60-day feeding trial, no mortality occurred in either the control or gallic acid (GA) groups. The inclusion of gallic acid in the diet significantly enhanced final weight (FW), weight gain (WG), specific growth rate (SGR), daily weight gain (DWG), and thermal growth coefficient (TGC) compared to the control group ($P<0.05$). Among the treatment groups, the G450 diet yielded the highest growth indices, while the most efficient feed conversion ratio (FCR) was observed in the G300 group ($P<0.05$).

Table 2. Effects of dietary gallic acid on growth performance in rainbow trout

Growth performance	Experimental diets			
	Control	G300	G450	G600
Initial weight (g)	2.84 ± 0.03	2.84 ± 0.02	2.84 ± 0.04	2.84 ± 0.04
Final weight (g)	9.82 ± 0.07^{c}	10.68 ± 0.12^{ab}	10.79 ± 0.05^{a}	10.41 ± 0.06^{b}
WG (g)	6.97 ± 0.07^{c}	7.83 ± 0.12^{ab}	7.93 ± 0.05^{a}	7.57 ± 0.06^{b}
DWG (g)	0.12 ± 0.00^{c}	0.13 ± 0.00^{ab}	0.13 ± 0.00^{a}	0.13 ± 0.00^{b}
SGR (%/day)	2.06 ± 0.01^{c}	2.20 ± 0.02^{ab}	2.21 ± 0.00^{a}	2.16 ± 0.00^{b}
TGC	0.79 ± 0.00^{c}	0.85 ± 0.00^{ab}	0.86 ± 0.00^{a}	0.83 ± 0.00^{b}
FCR	1.04 ± 0.02^{d}	0.89 ± 0.00^{s}	0.94 ± 0.00^{b}	0.98 ± 0.01^{c}
SR (%)	100	100	100	100

Data are presented as means ± SEM (n = 3). Different lowercase letters in each row indicate significant differences among groups ($P<0.05$). G300, diet supplemented with 300 mg/kg gallic acid; G450, diet supplemented with 450 mg/kg gallic acid; G600, diet supplemented with 600 mg/kg gallic acid; WG, weight gain; DWG, daily weight gain; SGR, specific growth rate; TGC, thermal growth coefficient; FCR, feed conversion ratio; SR, survival rate.

The expression levels of SOD, CAT, and GPX, are shown in Figure 1. CAT gene expression was significantly upregulated in the G300 and G450 groups compared to the control, whereas it was significantly downregulated in the G600 group (P<0.05). For the SOD gene, expression was upregulated in the G300 group but downregulated in both the G450 and G600 groups relative to the control (P<0.05). Likewise, GPX gene expression was significantly upregulated in the G300 group, while downregulation was observed in the G450 group (P<0.05). No notable difference in GPX expression was detected between the G600 group and the control group (P>0.05).

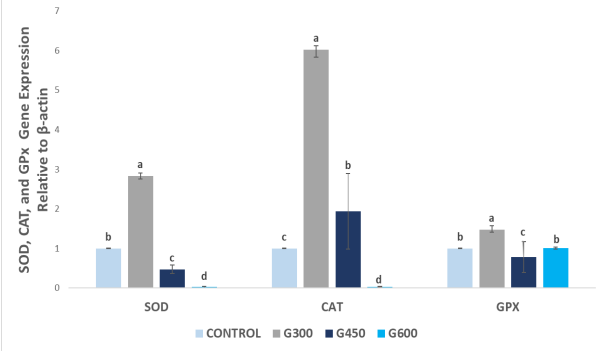

Figure 1. Effect of dietary gallic acid on the expression of antioxidant-related genes (SOD, CAT, GPX) in rainbow trout (n = 6)

The expression levels of IL-1β, TNF-α, IL-8, and HSP70, are depicted in Figure 2. The IL-1β gene showed increased expression in the G300 group compared to the control, although this difference was not statistically significant (P>0.05). Downregulation of IL-1β was observed in both the G450 and G600 groups relative to the control (P<0.05). For TNF-α, expression was elevated in the G300 group and decreased in the G600 group compared to the control (P<0.05), with no significant difference between the G450 and control groups (P> 0.05). The expression of IL-8 was significantly downregulated in all gallic acid-treated groups compared to the control (P<0.05). The stress-related HSP70 gene was significantly upregulated in the G300 group and downregulated in the G600 group compared to the control (P<0.05). No notable difference was detected between the G450 and control groups (P> 0.05).

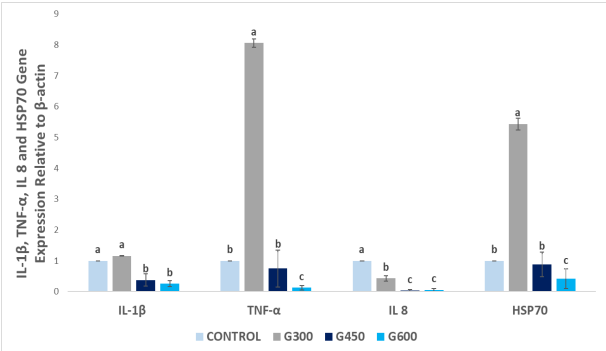

Figure 2. Effect of dietary gallic acid on immune response-related genes (IL-1β, TNF-α, IL-8) and stress-related HSP70 gene expression in rainbow trout (n = 6)

DISCUSSION

The use of organic acids as feed additives to enhance fish growth and health has gained considerable attention in recent years (Ghafarifarsani et al., 2023; Yousefi et al., 2023). The performance of growth is a vital aspect influencing the economic viability of aquaculture production (das Neves et al., 2021). This study found that diets enriched with gallic acid (GA) led to significant enhancements in growth metrics, including weight gain (WG), specific growth rate (SGR), and daily weight gain (DWG) in rainbow trout, when compared to the control group, regardless of the level of supplementation. Additionally, feed conversion ratio (FCR) was significantly enhanced across all GA-treated groups, reflecting more efficient feed utilization. These results align with previous findings demonstrating the positive effects of dietary organic acids on the growth indices of different fish species (Yilmaz, 2019; Zhang et al., 2020; Ghafarifarsani et al., 2023; Zhang et al., 2023).

The observed improvements in growth indices may be attributed to enhanced nutrient utilization facilitated by gallic acid (Ghafarifarsani et al., 2023). Organic acids are recognized for their ability to enhance the secretion and function of digestive enzymes, which in turn facilitates more efficient digestion, absorption, and utilization of vital nutrients (das Neves et al., 2021; Hassaan et al., 2018; Huan et al., 2018). Furthermore, the antimicrobial properties of organic acids and their protective effects on the intestinal epithelium contribute to improved gut health, which may further enhance growth performance (Shah et al., 2015; das Neves et al., 2021).

The antioxidant capacity of aquaculture species is influenced by various factors, including dietary practices and environmental conditions (Zhang et al., 2020; Karataş, 2024). In this research, the dietary addition of 300 mg/kg GA (G300 group) significantly upregulated the expression of antioxidant enzyme-related genes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), compared to the control and other GA-treated groups. These findings suggest that GA supplementation enhances the antioxidant defense system, protecting fish from oxidative damage. The results are consistent with the reported antioxidant properties of gallic acid and its ability to prevent oxidative DNA damage in both animals and humans (Ferk et al., 2011). Similar effects of other organic acids, such as succinic acid, chlorogenic acid, and malic acid, on the antioxidant capacity of aquatic organisms have been reported (Duan et al., 2018; Safari et al., 2021; Ghafarifarsani et al., 2023; Jin et al., 2023). The effectiveness of GA supplementation, particularly at 300 mg/kg, underscores the importance of optimizing dosage levels to achieve the desired antioxidant effects. While gene expression analysis provides valuable insights, further studies are needed to confirm these findings through enzymatic activity assays and direct assessments of oxidative stress markers.

Immune-related genes, including TNF-α, IL-1β, and IL-8, are essential components of the innate immune response in fish and are frequently influenced by dietary modifications

(Safari et al., 2021; Karataş, 2025; Kaya et al., 2025). In this study, supplementation with 300 mg/kg GA significantly upregulated TNF-α and IL-1β expression levels, suggesting immunostimulatory effects. However, no significant changes were observed in IL-8 expression across the treatment groups. These findings are consistent with studies reporting that organic acid-enriched diets enhance the expression of immune-related genes (Yilmaz, 2019; Zhang et al., 2020; Safari et al., 2021; Yousefi et al., 2023). Nevertheless, it is important to note that gene expression does not necessarily translate to functional immune enhancement due to potential post-transcriptional regulatory mechanisms (Vogel and Marcotte, 2012; Karataş, 2025). Future studies should include functional assays, such as assessments of lysozyme activity, phagocytic activity, and tests for disease resistance, to confirm these molecular findings.

Heat shock proteins (HSPs), such as HSP70, serve as biomarkers of biological stress and play a protective role under stressful conditions (Zhang et al., 2020; Jiang et al., 2016). In this study, dietary GA at 300 mg/kg increased HSP70 expression, suggesting enhanced cellular stress resilience. Similar effects of organic acids on HSP70 expression have been documented in previous research (Duan et al., 2018; Yilmaz, 2019; Yousefi et al., 2023). However, the specific role of GA in modulating stress and HSP70 pathways in rainbow trout remains unclear, necessitating further investigation.

CONCLUSION

This research indicates that the addition of gallic acid to the diet notably improves growth performance, boosts antioxidant capacity, and enhances immune responses in rainbow trout. Specifically, 300 mg/kg GA proved to be the optimal dose, leading to improved growth indices, feed conversion efficiency, and upregulation of key antioxidant and immune-related genes. These findings highlight the potential of gallic acid as an effective feed additive in aquaculture. However, further research is warranted to confirm these molecular observations through functional assays and to explore the long-term impacts of GA supplementation on fish health and productivity.

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ETHICAL APPROVAL

The experiment was approved by the Van Yuzuncu Yil University Aquatic Vertebrates Local Ethics Committee (protocol no: 2024/04-05) and conducted in accordance with standard ethical guidelines.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY

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

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