Original Article

Effects of *Panax ginseng* Essential Oil on Immunological and Antioxidant Parameters and Disease Resistance in Rainbow Trout

Morteza Yousefi[1*], Olesya Anatolyevna Petrukhina[1], Natalia Yurievna Rodionova[1], Nadezhda Sachivkina[1], Arfenya Karamyan[1], Artyom Brigida[2], Hossein Adineh[3]

1. Department of Veterinary Medicine, RUDN University, Moscow, Russia.
2. All-Russian Research Institute of Integrated Fish Farming - a Branch of the Federal State Budgetary Scientific Institution "Federal Research Center for Animal Husbandry Named After Academy Member L. K. Ernst" (L. K. Ernst Federal Research Center for Animal Husbandry), Vorovsky, Moscow, Russia.
3. Department of Fisheries, Faculty of Agriculture and Natural Resources, Gonbad Kavous University, Gonbad Kavous, Iran.

Use your device to scan and read the article online

How to Cite This Article Yousefi, M., PetrukhinaM O. A., Rodionova, N. Y., Sachivkina, N., Karamyan, A., & Brigida, A., et al. (2026). Effects of *Panax ginseng* Essential Oil on Immunological and Antioxidant Parameters and Disease Resistance in Rainbow Trout. *Iranian Journal of Veterinary Medicine, 20*(2), 403-412. http://dx.doi.org/10.32598/ijvm.20.2.1005676

http://dx.doi.org/10.32598/ijvm.20.2.1005676

ABSTRACT

Background: Disease outbreaks pose a significant threat to the aquaculture industry. The use of supplementary feeds is particularly popular among fish farmers to enhance the fish overall health and combat disease.

Objectives: This study aimed to evaluate the effects of dietary ginseng, *Panax ginseng*, essential oil (GEO), on immunological and antioxidant parameters, as well as disease resistance, in rainbow trout, *Oncorhynchus mykiss*.

Methods: Fish were fed diets containing 0 mL/kg (control; CTL), 0.5 mL/kg (GEO0.5), 1.0 mL/kg (GEO1), 1.5 mL/kg (GEO1.5), 2 mL/kg (GEO2), 2.5 mL/kg (GEO2.5), 3. mL/kg (GEO3), and 3.5 mL/kg (GEO3.5) GEO for 8 weeks, in triplicate. Then, they were experimentally infected with *Aeromonas hydrophila*.

Results: Results indicated that the GEO3 treatment demonstrated the highest levels of serum superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), while the lowest serum malondialdehyde (MDA) levels were recorded in the GEO2 and GEO2.5 groups. Furthermore, the GEO3 treatment resulted in the lowest serum levels of alanine aminotransferase (ALT) and lactate dehydrogenase (LDH), whereas the GEO2 treatment showed reduced serum levels of alkaline phosphatase (ALP) and aspartate aminotransferase (AST). Immunological assessments revealed that the GEO3 treatment had the highest serum and mucus lysozyme and immunoglobulin (Ig) levels, along with elevated serum alternative complement, myeloperoxidase (MPO), and mucus protease activity. Blood respiratory burst activity and mucus ALP were also highest in the GEO2.5 and GEO3 treatments. Notably, the lowest post-infection mortality rate was observed in the GEO3 group.

Conclusion: Based on these findings, it is recommended to include 2-3 mL/kg of GEO in trout feed to enhance fish health and disease resistance.

Keywords: Antioxidant, Biochemistry, Disease, Immunological, Phytogenic

Article info:
Received: 16 Oct 2024
Accepted: 07 Dec 2024
Publish: 01 Mar 2026

*** Corresponding Author:**
Morteza Yousefi, Associate Professor.
Address: *Department of Veterinary Medicine, RUDN University, Moscow, Russia.*
Phone: *+7 (495) 434-70-07*
E-mail: *myousefi81@gmail.com*

Copyright © 2026 The Author(s);
This is an open access article distributed under the terms of the Creative Commons Attribution License (CC-By-NC: https://creativecommons.org/licenses/by-nc/4.0/legalcode.en), which permits use, distribution, and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Introduction

Disease outbreaks pose a significant threat to the aquaculture industry, resulting in global annual losses of approximately 6 billion USD due to these incidents (Stentiford et al., 2017; Combe et al., 2023). Most fish diseases are manageable through effective management strategies, as opportunistic pathogens often trigger them. For instance, aeromonad septicemia is caused by *Aeromonas hydrophila*, a bacterium commonly found in aquatic environments (Lenchenko et al., 2022). This pathogen affects various aquaculture species, including the rainbow trout, *Oncorhynchus mykiss*, an important cold-water fish with an annual global production exceeding 900000 tons (FAO, 2022).

Among the management strategies employed in aquaculture, the use of supplemented feeds is particularly popular among fish culturists. A diverse array of feed supplements is available in the industry, tailored to specific objectives (Lee et al., 2015). These supplements primarily target the innate immune system, which plays a crucial role in reducing pathogen localization and entry into fish bodies (Mokhtar et al., 2023). Two key components of the innate immune response are humoral factors and skin mucus. Humoral immunity provides systemic protection against pathogens, while skin mucus serves as a physical barrier to prevent pathogen entry (Bedekar & KV, 2022; Salinas et al., 2022).

In addition to enhancing the immune system, these supplements can also increase the antioxidant capacity of fish, supporting tissue health and strengthening immune responses against pathogens (Biller & Takahashi, 2018; Abasubong et al., 2023). Medicinal herbs represent a significant category of feed additives known for their diverse advantages, particularly in enhancing immune and antioxidant systems. Ginseng, *Panax ginseng*, is a traditional medicinal herb. Research has shown that ginseng extract can be beneficial when included in the diets of Nile tilapia, *Oreochromis niloticus* (Goda 2008), and African catfish, *Clarias gariepinus* (Mehrim et al., 2022), with notable improvements in growth performance, immune parameters, and antioxidative status at a dosage of 200 mg/kg. Additionally, administering 2 mL/kg of Ginseng essential oil (GEO) in the diets of Nile tilapia also yielded similar benefits (Ahmed et al., 2022).

However, a study involving rainbow trout found that doses of 100-300 mL/kg of ginseng extract did not significantly affect growth performance, innate immune parameters, or disease resistance (Bulfon et al., 2017). Notably, there is currently no data on the effects of ginseng essential oil on rainbow trout. Consequently, this study aims to evaluate the effects of administering 0.5-3.5 mL/kg of GEO on antioxidant and immunological parameters, as well as resistance to *A. hydrophila* in rainbow trout.

Materials and Methods

Experimental protocol

GEO was incorporated into the fish diet at varying concentrations, as per previous research by Ahmed et al. (2022). The concentrations included: 0 mL/kg (control; CTL), 0.5 mL/kg (GEO0.5), 1.0 mL/kg (GEO1), 1.5 mL/kg (GEO1.5), 2 mL/kg (GEO2), 2.5 mL/kg (GEO2.5), 3 mL/kg (GEO3), and 3.5 mL/kg (GEO3.5), as outlined in Table 1. All animal experiments were conducted at the Research Institute of Integrated Fish Farming, 24 Sergeeva Street, Moscow region, Noginsk district, 142460, Russian Federation, and were approved by the Ethics Committee of Peoples' Friendship University of Russia, Moscow, Russia. Rainbow trout fingerlings (mean weight: 16.7±0.64) were obtained from a local farm and transported to the laboratory, where they were acclimated in a 2000-L tank and fed the CTL diet for two weeks. Following the acclimation period, the fish were randomly distributed into 24 tanks, each containing 90 L of water, with 30 fish per tank. The various diets were administered to the fish over a period of two months, with feeding occurring twice daily until the fish appeared to be satiated. A consistent water flow rate of 0.5 L/min was maintained throughout the experiment, and fish waste was siphoned off daily.

Water quality parameters, including temperature (14.1±0.65 ˚C), dissolved oxygen (7.65±0.88 mg/L), pH (7.6±0.32), and total ammonia (0.44±0.1 mg/L), were monitored using digital probes (Hach multi-parameter meter, USA) and a photometer (Palintest Co., 7100, UK).

Sampling

At the end of the feeding trial, 5 fish from each tank were anesthetized using a clove extract bath (2 g/L) for blood sampling via the caudal vein with 2-mL syringes. Additionally, a set of 5 fish per tank was anesthetized as previously described and then placed in polyethylene bags containing 5 mL of physiological saline for mucus collection. After gently rubbing the fish for 1 minute,

the collected mucus was transferred into plastic tubes for analysis.

Blood respiratory burst activity

Fresh blood (100 µL) was poured into a microtiter plate well and mixed with an equal volume of nitro blue tetrazolium. The mixture was incubated for 30 min; then, 100 µL of the mixture was added to a glass tube containing 2 mL of N, N-dimethyl formamide and centrifuged (300 rpm, 5 min). The supernatant was harvested, and its optical density was read at 620 nm (Mohammadian et al., 2016).

Serum analysis

Blood serum was obtained by centrifuging (7000 rpm, 10 min), after the whole blood was left at room temperature for 4 h to clot. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were determined using commercial kits provided by Zist Chem Co. (Tehran, Iran). Serum myeloperoxidase (MPO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA) were measured by available commercial kits (Zellbio Co., Germany) on a microplate reader.

Serum lysozyme activity was determined according to the method described by Ellis (1990). Briefly, 25 µL of serum sample was mixed with 1 mL of bacterial suspension (*Micrococcus luteus* in phosphate buffer, pH 6.2) and placed in a spectrophotometer. The optical density (OD) of the mixture was read at 500 nm for 5 min, and each 0.001 decrease in OD per minute was considered as one unit of lysozyme.

Serum alternative complement (ACH50) activity was determined as described by Yano (1992). Serum samples were serially diluted in a veronal buffer containing EGTA, magnesium, and gelatin. To each dilution, sheep erythrocytes were added, suspended in the same buffer, and incubated for 90 min. The hemolytic reaction was stopped by adding Ethylenediaminetetraacetic acid (EDTA) to each tube. The tubes were centrifuged (5000 rpm, 10 min) and the OD of the supernatant was read at 412 nm. The dilution factor that gave 50% hemolysis was considered the ACH50 activity.

Serum immunoglobulin (Ig) was measured according to Siwicki and Anderson (1993). Equal volumes of serum and polyethylene glycol (12%) were mixed and shaken for 2 h. Then, the mixture was centrifuged (5000 rpm, 15 min) and its soluble protein level was measured using a commercial kit (Zist Chem Co., Tehran, Iran). The difference between the serum soluble protein levels before and after precipitation was equal to the serum Ig level.

Skin mucus analysis

The collected skin mucus was first centrifuged for 15 min (13000 rpm), and the supernatant was used for immunological assessments. Mucus lysozyme activity and Ig level were measured as described above for the serum samples. Mucus ALP activity was measured by a commercial kit (Zist Chem Co., Tehran, Iran). Mucus protease activity was measured using the AZO-casein method, as described previously (Iversen & Jørgensen, 1995).

Bacterial challenge

After 2 months of feeding, the fish were experimentally infected with *A. hydrophila*, as described by Yarahmadi et al. (2014). The bacterium was cultured on nutrient agar medium. A suspension of the bacterium (1.8×10^7 cells/mL) was prepared in physiological saline before injection into the fish. Fifteen fish from each tank were caught, anesthetized in a clove extract bath (2 g/L), and intraperitoneally injected with 0.1 mL of the bacterium suspension. The fish were returned to their tanks and monitored for mortality over 10 days (Yarahmadi et al., 2014).

Statistical analysis

The collected data were analyzed using regression to identify relationships between dietary GEO levels and the tested parameters. As there were no strong relationships, the data were subjected to one-way ANOVA. Serum ALT, AST, ALP, LDH, Ig, NBT, SOD, and mucus immunological parameters did not meet the ANOVA assumptions; therefore, they were log-transformed before analysis. Percentile data were arcsine-transformed before analysis. Significant differences ($P<0.05$) among the treatments were determined using the Duncan multiple range test. SPSS software, version 22 was used for analysis.

Results

Dietary GEO had a significant impact on various antioxidant parameters. Specifically, SOD activity was notably elevated, with the highest levels recorded in the GEO3 treatment. Similarly, CAT activity increased significantly across the GEO1 to GEO3.5 treatments, peaking in the GEO3 group. GPx activity also showed a sig-

Table 1. Composition of the experimental diets

Ingredients (g/kg)		CTR	GEO0.5	GEO1	GEO1.5	GEO2	GEO2.5	GEO3	GEO3.5
Wheat meal		190	190	190	190	190	190	190	190
Soybean meal		190	190	190	190	190	190	190	190
Soybean oil		80	80	80	80	80	80	80	80
Fish canning byproduct[1]		200	200	200	200	200	200	200	200
Poultry byproduct[2]		290	290	290	290	290	290	290	290
Vitamin premix[3]		5	5	5	5	5	5	5	5
Mineral premix[3]		10	10	10	10	10	10	10	10
Methionine		2	2	2	2	2	2	2	2
Lysine		3	3	3	3	3	3	3	3
Cellulose		30	25.8	21.6	17.4	13.2	9	4.8	0.6
GE		0	4.2	8.4	12.6	16.8	21	25.2	29.4
Proximate composition	Moisture	91	91.8	90.8	91.3	90.6	91.4	90.3	90
	Crude protein	400	399	403	398	399	404	402	400
	Crude fat	176	172	175	173	177	179	176	174
	Crude ash	90.2	90.8	91.3	90.2	90.9	91.6	91	90.6
	Gross energy (kJ/g)	19	18.8	19.1	18.8	19	19.2	19.1	19.0

[1]Crude protein 63%; crude fat 14%, [2]Crude protein 54%; crude fat 22%, [3] The premix provided following amounts per kg of feed: A: 1000 IU; D3: 5000 IU; E: 20 mg; B5: 100 mg; B2: 20 mg; B6: 20 mg; B1: 20 mg; H: 1 mg; B9: 6 mg; B12: 1 mg; B4: 600 mg; C: 50 mg; Mg: 350 mg; Fe: 13 mg; Co: 2.5 mg; Cu: 3 mg; Zn: 60 mg; Se: 0.3 mg; I: 1.5 mg; Mn: 10 mg.

nificant rise, with maximum levels found in the GEO2.5 and GEO3 treatments. Furthermore, MDA levels significantly decreased in the GEO2 to GEO3 treatments when compared to the CTL group (Table 2).

Increasing dietary GEO levels resulted in significant decreases in serum ALT and LDH activities, with the lowest activity observed in the GEO3 treatment. An increase from 3 to 3.5 mL/kg of dietary GEO resulted in a significant rise in serum ALT and LDH activities; however, these levels remained significantly lower than those of the control group. Additionally, serum AST activity decreased significantly with higher dietary GEO levels, reaching its lowest point at the GEO 2 treatment. While enzyme activity remained stable at 2.5 and 3 mL/kg of GEO, a significant increase was observed at the 3.5 mL/kg level, although it was still lower than the control treatment. Serum ALP activity significantly decreased in the GEO2 treatment compared to the control group. Still, it increased significantly when dietary GEO levels were raised from 2 to 3.5 ml/kg compared to the control treatment (Table 3).

At dietary GEO levels exceeding 0.5 mL/kg, serum lysozyme and ACH50 activities significantly increased, peaking at a GEO level of 3 mL/kg. Serum Ig levels also rose significantly in fish fed diets containing more than 1.5 mL/kg of GEO, with the highest Ig concentration associated with the GEO3 treatment. Additionally, serum MPO activity significantly increased in the GEO2, GEO2.5, and GEO3 treatments compared to the control group, with the highest activity noted in the GEO3 treatment. RB activity also significantly increased in fish on GEO-supplemented diets, peaking at the GEO2.5 treatment (Table 4).

Mucus lysozyme activity significantly increased at GEO concentrations above 1.5 mL/kg, while mucus Ig and protease levels rose across all dietary GEO levels. The highest concentrations of lysozyme, Ig, and prote-

Table 2. Antioxidant parameters of rainbow trout fed diets containing graded levels of *P. ginseng* essential oil for eight weeks

Treatments	Antioxidant Parameters			
	SOD (U/mL)	CAT (U/mL)	GPx (U/mL)	MDA (nM/L)
CTL	2.1±0.12^{a}	4.3±0.25^{a}	1.1±0.05^{a}	21.2±1.43^{c}
GEO0.5	2.4±0.18^{b}	4.7±0.82^{ab}	1.5±0.21^{b}	21.2±1.37^{c}
GEO1	2.6±0.16^{b}	5.1±0.24^{b}	2.2±0.07^{c}	22.5±1.23^{c}
GEO1.5	3.3±0.03^{c}	6.7±0.2^{c}	2.7±0.03^{d}	20.1±1.5^{bc}
GEO2	3.5±0.18^{cd}	8.5±0.42^{d}	3.5±0.2^{f}	17.5±1.02^{a}
GEO2.5	3.9±0.31^{de}	9.2±0.31d^{e}	3.9±0.27^{g}	17.6±2.06^{a}
GEO3	4.3±0.38^{e}	9.7±0.36^{e}	4.1±0.37^{g}	17.8±0.8^{ab}
GEO3.5	2.4±0.21^{b}	5.4±0.42^{b}	3.1±0.09^{e}	20.5±1.15^{c}
Sig.	<0.001	<0.001	<0.001	0.001

Abbreviations: CTL: Control; GE: *Panax ginseng*, Essential oil; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; MDA: Malondialdehyde.

Note: Different letters within a column show significant differences among the treatments (n=3; Duncan multiple range test).

ase were observed in the GEO3 treatment. Additionally, mucus ALP activity significantly increased in the GEO2, GEO2.5, and GEO3 treatments, with the greatest activity noted in the GEO2.5 and GEO3 groups (Table 4).

Post-challenge mortality significantly decreased when fish were fed a diet containing >1.5 mL/kg GEO. The lowest mortality was observed in the GEO3 treatment (Table 5).

Table 3. Metabolic enzyme activities of rainbow trout fed diets containing graded levels of *P. ginseng* essential oil for eight weeks

Treatments	Metabolic Enzymes			
	ALT (U/L)	AST (U/L)	ALP (U/L)	LDH (U/L)
CTL	41.2±1.39^{f}	30.4±4.17^{f}	19.4±0.99^{ab}	55.4±2.65^{e}
GEO0.5	39.1±2.55^{ef}	28.7±0.74^{ef}	21.7±2.32^{abc}	48.5±2.06^{e}
GEO1	33.3±1.28^{de}	23.1±0.53^{bc}	24.5±3.3^{bcd}	41.6±1.07^{d}
GEO1.5	29.5±6.69^{cd}	25.4±2.09^{cd}	20.6±2.72^{ab}	38.5±3.88^{cd}
GEO2	26.3±4.27^{bc}	19.2±2.02^{a}	18.4±4^{a}	25.6±4.27^{ab}
GEO2.5	22.5±0.38^{ab}	20.4±1.32^{ab}	26.4±1.32^{cd}	27.4±2.22^{b}
GEO3	19.6±1.06^{a}	21.3±0.77^{ab}	24.3±2.04^{bcd}	22.6±0.67^{a}
GEO3.5	28.4±1.23^{cd}	26.1±0.53^{cd}	27.4±1.99^{d}	34.5±0.43^{c}
Sig.	<0.001	<0.001	0.006	<0.001

Abbreviations: CTL: Control; GE: *Panax ginseng*, essential oil; ALT: Serum alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; LDH: Lactate dehydrogenase.

Note: Different letters within a column show significant differences among the treatments (n=3; Duncan multiple range test).

Table 4. Immune responses of rainbow trout fed diets containing graded levels of *P. ginseng* essential oil for eight weeks

Sample Type	Immunological Assays	CTL	GEO0.5	GEO1	GEO1.5	GEO2	GEO2.5	GEO3	GEO3.5	Sig.
Humoral	lysozyme (U/mL)	18.4±1.59^{a}	21.3±2.14^{ab}	24.4±1.27^{b}	29.6±3.15^{c}	38.5±3.17^{d}	42.4±1.64^{d}	51.5±4.58^{e}	32.3±2.04^{c}	<0.001
	ACH50 (U/mL)	14.3±2.6^{a}	13.5±2.24^{ab}	18.2±1.19^{b}	23.8±0.51^{c}	31.5±1.77^{d}	34.3±1.87^{d}	38.4±1.06^{e}	31.3±1^{d}	<0.001
	Ig (mg/mL)	1.05±0.02^{a}	1.08±0.07^{a}	1.11±0.06^{a}	1.24±0.29^{ab}	2.12±0.06^{c}	2.45±0.14^{cd}	2.86±0.11^{d}	1.41±0.22^{b}	<0.001
	MPO (U/mL)	1.04±0.04^{a}	1.08±0.12^{a}	1.02±0.11^{a}	1.12±0.11^{a}	1.54±0.08^{b}	1.73±0.02^{c}	1.96±0.06^{d}	1.15±0.07^{a}	<0.001
	RB (OD)	0.33±0.002^{a}	0.39±0.03^{b}	0.41±0.01^{c}	0.47±0.005^{d}	0.72±0.01^{f}	0.76±0.01^{g}	0.74±0.002^{fg}	0.51±0.008^{e}	<0.001
Mucosal	lysozyme (U/mL)	12.1±2.33^{a}	11.5±1.03^{a}	14.2±0.86^{ab}	14.4±1.81^{ab}	18.5±2.52^{bc}	22.8±4.71^{cd}	26.7±3.53^{d}	20.5±1.65^{cd}	<0.001
	Ig (mg/dL)	0.71±0.07^{a}	0.91±0.03^{b}	0.95±0.01^{bc}	1.08±0.05^{c}	1.31±0.07^{d}	1.45±0.24^{d}	1.73±0.1^{e}	1.38±0.13^{d}	<0.001
	Protease (U/mL)	4.31±0.22^{a}	4.51±0.04^{b}	4.6±0.07^{b}	5.11±0.04^{c}	7.7±0.16^{e}	8.4±0.06^{f}	9.31±0.22^{g}	6.9±0.15^{d}	<0.001
	ALP (U/mL)	0.97±0.02^{a}	1.1±0.03^{a}	1.06±0.09^{a}	1.12±0.1^{a}	1.35±0.2^{b}	1.69±0.09^{c}	1.85±0.15^{c}	1.12±0.09^{a}	<0.001

Abbreviations: CTL: Control; GE, *Panax ginseng*, essential oil; RB: Respiratory burst activity.

Note: Different letters within a row show significant differences among the treatments (n=3; Duncan multiple range test).

Discussion

Research has demonstrated that GEO possesses significant radical-scavenging properties. According to Chung IlMin et al. (2011), GEO contains 639 mg of total phenolic compounds (expressed as gallic acid) per 100 g, along with a remarkable 79% radical scavenging activity. However, identifying the specific components of GEO responsible for this antioxidant effect remains challenging, as previous studies have primarily focused on β-caryophyllene (Dahham et al., 2015; Gushiken et al., 2022), with other key constituents yet to be thoroughly investigated. In the current study, GEO notably enhanced the activities of SOD, CAT, and GPx, while simultaneously reducing MDA levels. SOD, CAT, and GPx are critical components of the antioxidant defense system, effectively neutralizing superoxide ions, hydrogen peroxide at varying concentrations, and hydroperoxides (Ebrahimzadeh Mousavi et al., 2023). These enhancements in enzyme activity may contribute to im-

Table 5. Post-challenge mortality of rainbow trout fed diets containing graded levels of *P. ginseng* essential oil for eight weeks

Post-challenge Mortality (%)	
CTL	53.3±6.67^{d}
GEO0.5	44.4±7.7^{cd}
GEO1	48.9±3.85^{d}
GEO1.5	42.2±3.85^{bcd}
GEO2	37.8±3.85^{abc}
GEO2.5	31.1±10.2^{ab}
GEO3	28.9±3.85^{a}
GEO3.5	31.1±7.7^{ab}
Sig.	0.001

Abbreviations: CTLL: Control; GE, *Panax ginseng*, essential oil.

Note: Different letters within a column show significant differences among the treatments (n=3; Duncan multiple range test).

proved performance in fish when exposed to oxidative stress.

Additionally, MDA is a byproduct of lipid peroxidation and a highly toxic compound that can oxidize other biological molecules (Hoseini et al., 2023). The observed decrease in MDA levels in GEO-treated fish may result from the increased activity of antioxidant enzymes and/or the direct radical scavenging effects of GEO. These findings align with previous research on Nile tilapia, which indicated that dietary GEO could enhance the activities of SOD, CAT, and GPx while reducing MDA levels under both normal and stressful conditions (Ahmed et al., 2022). Moreover, ginseng extract has been shown to boost SOD activity in whiteleg shrimp, *Penaeus vannamei* (Kim et al., 2024).

While cytosolic enzymes are not functional in serum, they serve as valuable indicators of cellular health and enzyme leakage (Hoseini et al., 2022). Enzymes such as ALT and AST are predominantly found in fish liver, whereas ALP and LDH are mainly concentrated in erythrocytes and skeletal muscle (Hoseini et al., 2020). The results of this study indicate that dietary GEO administration promotes healthier fish tissues. In contrast, the addition of 100-300 mg/kg ginseng extract to the diet of rainbow trout did not produce significant changes in plasma ALT, AST, and ALP activities (Bulfon et al., 2017), possibly due to variations in dosage and the form of the additive used. The observed improvements in tissue health among GEO-treated fish may be linked to enhanced antioxidant conditions, resulting in fewer prooxidants available to damage living cells.

The innate immune system plays a crucial role in the early recognition and elimination of pathogens. In fish, this system comprises various components found in blood and skin mucus, which collectively work to reduce pathogen load within the body. Igs present in fish blood and mucosal surfaces have been shown to enhance disease resistance when their basal levels are elevated (Cuesta et al., 2004; Salinas et al., 2011). Lysozyme, also found in fish blood and mucosal surfaces, effectively targets and kills gram-positive bacteria (Song et al., 2021). Complement proteins secreted by the liver play a crucial role in germ killing, opsonization, and inflammation (Bavia et al., 2022). MPO from neutrophils plays a significant role in generating antimicrobial compounds such as hypochlorous acid (Chen et al., 2019).

Additionally, neutrophil function can be assessed using the RB test, which indicates superoxide anion production (Biller & Takahashi, 2018). These systemic immune components are vital for monitoring the body for pathogen recognition and elimination. Notably, fish have demonstrated improved resistance to pathogen injections when these immune parameters are enhanced through dietary interventions (Semwal et al., 2023). In line with these findings, fish treated with GEO exhibited lower mortality rates after being injected with *A. hydrophila* in this study. In contrast, previous research on rainbow trout fed diets containing 100-300 mg/kg of ginseng extract showed no significant changes in plasma levels of lysozyme, Ig, MPO, or resistance to *Yersinia ruckeri* injection (Bulfon et al., 2017). These discrepancies may arise from variations in dosage and the formulation of the additive used. Conversely, higher doses of GEO (1-5 g/kg) are effective in enhancing various immunological parameters in Nile tilapia (Ahmed et al., 2022).

Fish skin mucus plays a vital role in immune function by serving as a physical barrier (Salinas et al., 2022). Components such as skin lysozyme and Ig contribute to the immune response, mirroring their functions in fish blood. Additionally, the activities of skin mucus proteases and ALP facilitate the physical removal of surface pathogens, helping to suppress inflammation caused by these pathogens (Lallès, 2019; Abbasi et al., 2023). Furthermore, the immune components in skin mucus are particularly effective in preventing the spread of diseases transmitted through water. These mechanisms significantly reduce the likelihood of pathogen colonization on fish skin and subsequent penetration into the fish body, which is crucial in aquaculture settings where the surrounding water is the primary pathway for pathogen transmission (Hoseini et al., 2024).

In conclusion, this study demonstrates that GEO can be utilized as a feed additive for rainbow trout fingerlings. It boosts antioxidant and immunological parameters, increasing disease resistance. The findings suggest that a dosage of 2-3 mL/kg of GEO is sufficient to provide these benefits in rainbow trout.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Ethics Committee of RUDN University, Moscow, Russia (Protocol No.: 9b, 2024).

Funding

The study was supported by a grant from the Russian Science Foundation (RSF), Moscow, Russia (Code: 24-26-00212).

Authors' contributions

Conceptualization, supervision, data curation, funding acquisition, formal analysis, and review & editing: Morteza Yousefi; Project administration, methodology, and investigation: Morteza Yousefi, and Olesya Anatolyevna Petrukhina; Writing the original draft: All authors.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors gratefully acknowledge the support provided by the Agrarian and Technological Institute of RUDN University, Moscow, Russia, for the completion of this grant.

References

Abasubong, K. P., Gabriel, N. N., & Adjoumani, J. J. Y. (2023). Ferulic acid as feed additives in aquaculture: A review on growth, immune response, and antioxidant status of finfish. In N.N. Gabriel, E. Omoregie & K. P. Abasubong (Eds.), *Emerging sustainable aquaculture innovations in Africa* (pp. 251-272), Singapore: Springer. [DOI:10.1007/978-981-19-7451-9_10]

Abbasi, M., Taheri Mirghaed, A., Hoseini, S. M., Rajabiesterabadi, H., Hoseinifar, S. H., & Van Doan, H. (2023). Effects of Dietary Glycine Supplementation on Growth Performance, Immunological, and Erythrocyte Antioxidant Parameters in Common Carp, Cyprinus carpio. *Animals : An Open Access Journal from MDPI, 13*(3), 412. [DOI:10.3390/ani13030412] [PMID]

Ahmed, M. M., Mohammed, A. T., Farag, M. R., Hassan, M. A., Mawed, S. A., & Alagawany, M., et al. (2022). Dietary supplementation of Nile tilapia (Oreochromis niloticus) with Panax ginseng essential oil: Positive impact on animal health and productive performance, and mitigating effects on atrazine-induced toxicity. *Frontiers in Marine Science, 9,* 920057. [DOI:10.3389/fmars.2022.920057]

Bavia, L., Santiesteban-Lores, L. E., Carneiro, M. C., & Prodocimo, M. M. (2022). Advances in the complement system of a teleost fish, Oreochromisniloticus. *Fish & Shellfish Immunology, 123,* 61–74. [DOI:10.1016/j.fsi.2022.02.013] [PMID]

Biller, J. D., & Takahashi, L. S. (2018). Oxidative stress and fish immune system: Phagocytosis and leukocyte respiratory burst activity. *Anais da Academia Brasileira de Ciencias, 90*(4), 3403–3414. [DOI:10.1590/0001-3765201820170730] [PMID]

Bulfon, C., Bongiorno, T., Messina, M., Volpatti, D., Tibaldi, E., & Tulli, F. (2017). Effects of Panax ginseng extract in practical diets for rainbow trout (Oncorhynchus mykiss) on growth performance, immune response and resistance to Yersinia ruckeri. *Aquaculture Research, 48,* 2369-2379. [DOI:10.1111/are.13072]

Chen, C., Yang, B., Abbas Raza, S. H., Zhang, D., Wu, T., & Zhang, Z., et al. (2019). Role of Myeloperoxidase of northern snakehead (Channa argus) in Aeromonas veronii infection. *Microbial Pathogenesis, 135,* 103622. [DOI:10.1016/j.micpath.2019.103622] [PMID]

Chung, I.M., Praveen, N., & Ahmad, A. (2011). Essential oil composition and antioxidant activity of Panax ginseng of different regions in Korea. *Asian Journal of Chemistry, 23(8),* 3699-3702. [Link]

Combe, M., Reverter, M., Caruso, D., Pepey, E., & Gozlan, R. E. (2023). Impact of Global Warming on the Severity of Viral Diseases: A Potentially Alarming Threat to Sustainable Aquaculture Worldwide. *Microorganisms, 11*(4), 1049. [DOI:10.3390/microorganisms11041049] [PMID]

Cuesta, A., Meseguer, J., & Esteban, M. A. (2004). Total serum immunoglobulin M levels are affected by immunomodulators in seabream (Sparus aurata L.). *Veterinary Immunology and Immunopathology, 101*(3-4), 203–210. [DOI:10.1016/j.vetimm.2004.04.021] [PMID]

Dahham, S. S., Tabana, Y. M., Iqbal, M. A., Ahamed, M. B., Ezzat, M. O., & Majid, A. S., et al. (2015). The Anticancer, Antioxidant and Antimicrobial Properties of the Sesquiterpene β-Caryophyllene from the Essential Oil of Aquilaria crassna. *Molecules (Basel, Switzerland), 20*(7), 11808–11829. [DOI:10.3390/molecules200711808] [PMID]

Ebrahimzadeh Mousavi, H., Taheri Mirghaed, A., Hoseini, S. M., Ghelichpour, M., Aghaei Moghaddam, A., & Gharavi, B., et al. (2023). Myrcene as water conditioner, stress-reducing and antioxidant agent in transportation of common carp, Cyprinus carpio, with plastic bags. *Aquaculture Reports, 28,* 101458. [DOI:10.1016/j.aqrep.2022.101458]

Ellis, A. E. (1990). Lysozyme assays. In J. S. Stolen, T. C. Fletcher, D. P. Anderson, B. S. Roberson & W. B. Van Muiswinkel (Eds.), *Techniques in fish immunology* (pp. 101-103). Fair Haven: SOS publication. [Link]

FAO. (2022). *The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation.* Rome: FAO. [Link]

Goda, A. M. A. S. (2008). Effect of dietary ginseng herb (Ginsana® G115) supplementation on growth, feed utilization, and hematological indices of Nile tilapia, Oreochromis niloticus (L.), fingerlings. *Journal of the World Aquaculture Society, 39*(2), 205-214. [DOI:10.1111/j.1749-7345.2008.00153.x]

Gushiken, L. F. S., Beserra, F. P., Hussni, M. F., Gonzaga, M. T., Ribeiro, V. P., & de Souza, P. F., et al. (2022). Beta-caryophyllene as an antioxidant, anti-inflammatory and re-epithelialization activities in a rat skin wound excision model. *Oxidative Medicine and Cellular Longevity, 2022,* 9004014. [DOI:10.1155/2022/9004014] [PMID]

Hoseini, S. M., Ghelichpour, M., Hoseinifar, S. H., & Van Doan, H. (2024). Sodium butyrate suppresses stress, immunosuppression and oxidative stress in common carp, Cyprinus carpio, reared at high stocking density. *Aquaculture International, 32*(7), 10009-10028. [DOI:10.1007/s10499-024-01647-5]

Hoseini, S. M., Mirghaed, A. T., Paray, B. A., Hoseinifar, S. H., & Van Doan, H. (2020). Effects of dietary menthol on growth performance and antioxidant, immunological and biochemical responses of rainbow trout (Oncorhynchus mykiss). *Aquaculture (Amsterdam, Netherlands), 524,* 735260. [DOI:10.1016/j.aquaculture.2020.735260]

Hoseini, S. M., Yousefi, M., Afzali-Kordmahalleh, A., Pagheh, E., & Taheri Mirghaed, A. (2023). Effects of dietary lactic acid supplementation on the activity of digestive and antioxidant enzymes, gene expressions, and bacterial communities in the intestine of common carp, cyprinus carpio. *Animals: An Open Access Journal from MDPI, 13*(12), 1934. [DOI:10.3390/ani13121934] [PMID]

Hoseini, S. M., Paolucci, M., Arghideh, M., Hosseinpour Delavar, F., Zavvar, F., & Hoseinifar, S. H., et al. (2022). Effects of dietary glycine administration on biochemical responses to ammonia toxicity in common carp, Cyprinus carpio. *Aquaculture Research, 53,* 2185-2194. [DOI:10.1111/are.15737]

Iversen, S. L., & Jørgensen, M. H. (1995). Azocasein assay for alkaline protease in complex fermentation broth. *Biotechnology Techniques, 9,* 573-576. [DOI:10.1007/BF00152446]

Kim, B., Jeon, H. J., Rhee, M. H., Kim, J. H., & Han, J. E. (2024). The effects of Panax ginseng on growth enhancement, innate immunity, and microbiome profiling in Penaeus vannamei. *Journal of Ginseng Research, 48*(6), 552–558. [DOI:10.1016/j.jgr.2024.06.002] [PMID]

Lallès, J. P. (2019). Biology, environmental and nutritional modulation of skin mucus alkaline phosphatase in fish: A review. *Fish & Shellfish Immunology, 89,* 179-186. [DOI:10.1016/j.fsi.2019.053] [PMID]

Lee, C. S., Lim, C., & Webster, C. D. (2015). *Dietary nutrients, additives, and fish health.* NJ, USA: Wiley-Blackwell. [DOI:10.1002/9781119005568]

Lenchenko, E., Lenchenko, S., Sachivkina, N., Kuznetsova, O., & Ibragimova, A. (2022). Interaction of Cyprinus carpio Linnaeus with the biofilm-forming Aeromonas hydrophila. *Veterinary World, 15*(10), 2458–2465. [DOI:10.14202/vetworld.2022.2458-2465] [PMID]

Makesh, M., Bedekar, M. K., & Rajendran, K. V. (2022). Overview of fish immune system. In: M. Makesh & K. V. Rajendran (Eds), *Fish immune system and vaccines*. Singapore: Springer. [DOI:10.1007/978-981-19-1268-9_1]

Mehrim, A. I., Refaey, M. M., Hassan, M. A. E., Zaki, M. A., & Zenhom, O. A. (2022). Ginseng® as a reproductive enhancer agent for African catfish, Clarias gariepinus (Burchell, 1822). *Fish Physiology and Biochemistry, 48*(1), 15–32. [DOI:10.1007/s10695-021-00969-y] [PMID]

Mohammadian, T., Alishahi, M., Tabandeh, M. R., Ghorbanpoor, M., Gharibi, D., & Tollabi, M., et al. (2016). Probiotic effects of Lactobacillus plantarum and L. delbrueckii ssp. bulguricus on some immune-related parameters in Barbus grypus. *Aquaculture International, 24,* 225-242. [DOI:10.1007/s10499-015-9921-8]

Mokhtar, D. M., Zaccone, G., Alesci, A., Kuciel, M., Hussein, M. T., & Sayed, R. K. (2023). Main components of fish immunity: An overview of the fish immune system. *Fishes, 8*(2), 93. [DOI:10.3390/fishes8020093]

Salinas, I., Zhang, Y. A., & Sunyer, J. O. (2011). Mucosal immunoglobulins and B cells of teleost fish. *Developmental and Comparative Immunology, 35*(12), 1346–1365. [DOI:10.1016/j.dci.2011.11.009] [PMID]

Salinas, I., Ding, Y., Fernández-Montero, Á., & Sunyer, J. O. (2022). Mucosal immunity in fish. In K. Buchmann & C. J. Secombes (Eds), *Principles of Fish Immunology*. Cham: Springer. [DOI:10.1007/978-3-030-85420-1_12]

Semwal, A., Kumar, A., & Kumar, N. (2023). A review on pathogenicity of Aeromonas hydrophila and their mitigation through medicinal herbs in aquaculture. *Heliyon, 9*(3), e14088. [DOI:10.1016/j.heliyon.2023.e14088] [PMID]

Siwicki, A. K., & Anderson, D. (1993). *Nonspecific defense mechanisms assay in fish: II. Potential killing activity of neutrophils and macrophages, lysozyme activity in serum and organs and total immunoglobulin level in serum*. Olsztyn, Poland: Wydawnictwo Instytutu Rybactwa Srodladowego. [Link]

Song, Q., Xiao, Y., Xiao, Z., Liu, T., Li, J., & Li, P., et al. (2021). Lysozymes in Fish. *Journal of Agricultural and Food Chemistry, 69*(50), 15039–15051. [DOI:10.1021/acs.jafc.1c06676] [PMID]

Stentiford, G. D., Sritunyalucksana, K., Flegel, T. W., Williams, B. A., Withyachumnarnkul, B., & Itsathitphaisarn, O., et al. (2017). New Paradigms to Help Solve the Global Aquaculture Disease Crisis. *PLoS Pathogens, 13*(2), e1006160. [DOI:10.1371/journal.ppat.1006160] [PMID]

Yano, T. (1992). Assays of hemolytic complement activity. In: J. S. Stolen (Ed.), *Techniques in fish immunology* (pp. 131-141). Texas: SOS Publication. [Link]

Yarahmadi, P., Kolangi Miandare, H., Farahmand, H., Mirvaghefi, A., & Hoseinifar, S. H. (2014). Dietary fermentable fiber upregulated immune related genes expression, increased innate immune response and resistance of rainbow trout (Oncorhynchus mykiss) against Aeromonas hydrophila. *Fish & Shellfish Immunology, 41*(2), 326–331. [DOI:10.1016/j.fsi.2014.09.007] [PMID]

This Page Intentionally Left Blank