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Panax ginseng essential oil as a dietary additive: growth and digestive enzyme benefits for rainbow trout

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Abstract. This study aimed to evaluate the effects of dietary ginseng, *Panax ginseng*, essential oil (GE) on the growth performance and digestive enzyme activities in rainbow trout, *Oncorhynchus mykiss*. Fish were fed diets containing 0 ml/kg (control; CTL), 0.5 ml/kg (GE0.5), 1.0 ml/kg (GE1.0), 1.5 ml/kg (GE1.5), 2.0 ml/kg (GE2.0), 2.5 ml/kg (GE2.5), 3.0 ml/kg (GE3.0), and 3.5 ml/kg (GE3.5) GE for 8 weeks. Results indicated that the highest growth performance and feed efficiency were achieved with the GE2.0 and GE2.5 treatments. The GE2.5 and GE3.0 treatments exhibited the highest activities of gut trypsin, chymotrypsin, and lipase. But there were no significant differences in the gut amylase activity among the treatments. In conclusion, dietary GE at 2.5 ml/kg is recommended for trout feed supplementation to reach the highest production.

Keywords: feed additive, aquaculture, gut, digestion, phytobiotic

Authors' contribution: Yousefi M. — investigation, methodology, data curation, formal analysis, writing — original draft; Brigida A.V. — investigation, data curation, methodology; Hoseini S.M. — data curation, methodology, formal analysis, writing — editing. The final draft manuscript was revised by all authors. All authors read and approved the final manuscript.

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Introduction

Dietary formulation is vital for fish growth in aquaculture, influenced by the balance of proteins, fats, carbohydrates, vitamins, and minerals. High-quality proteins support muscle development, while fats supply energy. Additionally, the digestibility of ingredients is crucial; easily digestible diets enhance nutrient absorption and promote faster growth [1].

Feed additives are used in aquafeed to improve fish production and welfare. A diverse array of feed supplements is available in the industry, tailored to specific objectives (see [2]). Incorporating targeted feed additives that enhance the gut digestive process is essential for optimizing aquaculture practices [3]. These additives not only improve growth performance and feed efficiency but also contribute to the overall health and sustainability of aquaculture operations [4]. As research continues to evolve, the development of more specialized additives tailored to specific species and dietary needs will further enhance the benefits of these approaches in aquaculture.

Medicinal herbs serve as valuable feed additives known for their numerous benefits, particularly in enhancing immune and antioxidant functions. One prominent example is ginseng, specifically *Panax ginseng*, which is a widely recognized traditional medicinal herb. Research has shown that adding ginseng extract to the diets of Nile tilapia, *Oreochromis niloticus*, and African catfish, *Clarias gariepinus*, can significantly improve growth performance, immune responses, and antioxidant levels when administered at a dosage of 200 mg/kg [5, 6]. Additionally, incorporating 2 ml/kg of Ginseng essential oil (GE) into Nile tilapia diets yielded similar beneficial effects [7].

In contrast, studies on rainbow trout have found that administering ginseng extract at doses ranging from 100 to 300 ml/kg did not produce significant changes in growth performance, innate immune responses, or disease resistance [8]. Furthermore, there is currently no data available on the effects of GE on rainbow trout. Thus, **this study aims** to explore how the administration of 0.5…3.5 ml/kg of GE affects growth performance and digestive enzyme activity in rainbow trout.

Materials and methods

Experimental protocol. GE was prepared and added to the fish diet at different concentrations: 0 ml/kg (control; CTL), 0.5 ml/kg (GE0.5), 1.0 ml/kg (GE1.0), 1.5 ml/kg

(GE1.5), 2.0 ml/kg (GE2.0), 2.5 ml/kg (GE2.5), 3.0 ml/kg (GE3.0), and 3.5 ml/kg (GE3.5), as detailed in Table 1. All animal experiments were conducted in Research Institute of Integrated Fish Farming, 24 Sergeeva street, Noginsk district, Moscow region, 142460, Russian Federation and were approved by the Ethics Committee Peoples' Friendship University of Russia, Moscow, Russia. Rainbow trout fingerlings were sourced from a nearby farm and brought to the laboratory, where they were acclimatized in a 2000-L tank and fed the CTL diet for one week. After acclimation, the fish were randomly assigned to 24 tanks, each with 90 L of water, housing 30 fish per tank. The different diets were given to the fish over a two-month duration, with feeding occurring twice daily until they showed signs of fullness. A steady water flow rate of 0.5 L/min was maintained throughout the experiment, and fish waste was removed daily through siphoning. Water temperature, dissolved oxygen, pH and total ammonia were 14.1 ± 0.65 °C, 7.65 ± 0.88 mg/L, 7.60 ± 0.32 and (0.44 ± 0.10 mg/L), respectively. At the conclusion of the feeding period, all fish in each tank were bulk-weighed to assess growth performance, following the methodology described by Abbasi et al. [9].

Table 1

Composition of the experimental diets

Ingredients, g/kg	Treatments							
	CTR	GE0.5	GE1.0	GE1.5	GE2.0	GE2.5	GE3.0	GE3.5
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 by-product[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	91.0	91.8	90.8	91.3	90.6	91.4	90.3	90.0
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	91.0	90.6

Note. 1 — Crude protein 63 %; crude fat 14 %; 2 — Crude protein 54 %; crude fat 22 %; 3 — Amineh Gostar Co. (Tehran, Iran).

Source: completed by M. Yousefi , A.V. Brigida, S.M. Hoseini.

Sampling and analysis. At the end of the feeding trial, five fish from each tank were anesthetized using a clove extract bath (2 g/l). The abdominal cavity was then opened,

and the midgut was dissected for immediate freezing in liquid nitrogen for subsequent digestive enzyme assays.

The gut samples were homogenized in an equal volume of phosphate buffer pH 7.0. The homogenates were then centrifuged at 13000 rpm (15 min) and the supernatants were used for enzymatic assays. Amylase activity was determined based on the degradation of starch as a substrate [10]. Lipase activity was determined using p-nitrophenyl myristate as substrate, as described before [11]. Trypsin and chymotrypsin activities were measured using DL-arginine-p-nitroanilide [12] and N-benzoyl-L-tyrosine ethyl ester [13], respectively, as substrates.

Statistical analysis. The data were subjected to one-way ANOVA. Final weight, FCR, amylase, chymotrypsin and lipase did not meet the ANOVA assumptions; hence log-transformed before analysis. Significant ($P < 0.05$) differences among the treatments were determined by the Duncan multiple range test. SPSS v.22 was used for analysis.

Results and Discussion

The final weight, specific growth rate (SGR), and weight gain showed significant increases in the GE1.0 to GE3.5 treatments, with the highest values recorded in the GE2.5 treatment. Conversely, feed conversion ratio (FCR) significantly decreased in the GE2.0 to GE3.0 treatments, with the lowest FCR observed in the GE2.0 and GE2.5 treatments (Table 2).

While dietary GE did not significantly impact gut amylase activity, it did have a notable effect on gut lipase, trypsin, and chymotrypsin activities. All GE treatments resulted in significant increases in gut trypsin, chymotrypsin, and lipase activities, with the highest levels found in the GE2.5 and GE3.0 treatments (Table 3).

Table 2

Growth performance and survival of rainbow trout fed diets containing graded levels of *P. ginseng* essential oil for eight weeks

Parameters	Treatments								
	CTL	GE0.5	GE1.0	GE1.5	GE2.0	GE2.5	GE3.0	GE3.5	Sig.
Initial weight, g	16.6 ± 0.37	16.6±0.17	16.2±0.54	16.7±0.17	16.8±1.02	15.5±1.03	16.6±0.49	18.8±0.77	0.291
Final weight, g	27.2±1.66a	29.0±0.89a	31.3±2.59b	33.5±0.75bc	42.4±1.95de	45.4±0.35e	39.6±0.51d	35.5±0.69c	<0.001
Weight gain, %	64.3±7.31a	74.4±3.58ab	93.0±11.7bc	100±6.45bc	154±25.7d	194±22.6e	138±9.83d	112±13.6c	<0.001
Specific growth rate, %/d	0.89±0.08a	0.99±0.04ab	1.17±0.11bc	1.24±0.06c	1.66±0.18d	1.92±0.13e	1.55±0.07d	1.34±0.11c	<0.001
Feed conversion ratio	1.86±0.15c	1.83±0.06bc	1.87±0.15c	1.75±0.13bc	1.30±0.15a	1.24±0.13a	1.67±0.09bc	1.58±0.12b	<0.001
Survival, %	100	100	100	100	100	100	100	100	-

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

Source: completed by M. Yousefi , A.V. Brigida, S.M. Hoseini.

Table 3

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

Treatments	Parameters			
	Amylase, U/mg protein	Trypsin, U/mg protein	Chymotrypsin, U/mg protein	Lipase, U/mg protein
CTL	0.15 ± 0.01	1.42 ± 0.04a	2.30 ± 0.08a	1.30 ± 0.10a
GE0.5	0.18 ± 0.02	1.54 ± 0.06b	2.50 ± 0.05b	1.26 ± 0.05a
GE1.0	0.14 ± 0.01	1.62 ± 0.01bc	2.40 ± 0.12b	1.40 ± 0.006b
GE1.5	0.16 ± 0.03	1.69 ± 0.03c	2.80 ± 0.04c	1.45 ± 0.04b
GE2.0	0.17 ± 0.02	2.30 ± 0.03d	3.70 ± 0.05e	1.92 ± 0.02d
GE2.5	0.16 ± 0.01	2.60 ± 0.12e	3.90 ± 0.01f	1.80 ± 0.06d
GE3.0	0.15 ± 0.001	2.50 ± 0.06e	4.20 ± 0.02g	2.10 ± 0.04e
GE3.5	0.18 ± 0.04	1.71 ± 0.04c	3.10 ± 0.02d	1.55 ± 0.04c
Sig.	0.300	< 0.001	< 0.001	< 0.001

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

Source: completed by M. Yousefi , A.V. Brigida, S.M. Hoseini.

Essential oils may promote growth, possess antibacterial properties, and enhance immune responses in various fish species. Additionally, they can boost the antioxidant capacity and improve the resilience of aquatic animals against infectious diseases. Therefore, application of essential oils as feed supplements has recently increased in the aquaculture industry [14].

A prior study examining the supplementation of trout feed with ginseng ethanolic extract found that doses between 100…300 mg/kg did not significantly impact fish growth performance [8]. In contrast, our research indicates that a GE concentration exceeding 1.5 ml/kg acts as a growth promoter for rainbow trout, likely due to enhanced feed efficiency. These findings align partially with those observed in Nile tilapia, where doses of 1—2 ml/kg GE significantly improved both growth performance and feed efficiency [7]. The discrepancies between our results and those of the earlier trout study may stem from the lower concentrations of ginseng extract used previously or variations in the key compounds present in GE.

Enhancements in digestive processes can lead to improved nutrient absorption and subsequently better fish growth performance. Research has demonstrated that essential oils can boost gut digestive enzyme activity in fish [14]. However, data on the impact of ginseng products on fish digestive enzymes remain limited. Notably, Ahmed et al. [7] reported that while GE did not significantly affect digestive enzymes in Nile tilapia under normal conditions, it did enhance amylase activity when the fish were exposed to a pesticide. Based on our findings, the improved growth performance observed in GE-treated fish may be partially attributed to increased nutrient digestion. Similarly, enhancements in growth performance and feed efficiency have been linked to elevated digestive enzyme activities in fish fed diets supplemented with essential oils [15].

In conclusion, this study demonstrates that GE can be utilized as a feed additive for rainbow trout fingerlings. It improves digestive enzymes and growth performance, when administered at a dose of 2.5 ml/kg.

Conclusion

This study shows that ginseng essential oil (GE) can be effectively used as a feed additive to enhance growth performance and digestive enzyme activities in rainbow trout fry. Specifically, the optimal concentration was found to be 2.5 ml/kg, which resulted in the highest growth rate and increased enzyme activities of trypsin, chymotrypsin and lipase. These results suggest that GE can serve as a useful additive in aquaculture by promoting better nutrient absorption and feed efficiency in trout. Future studies may further explore species-specific responses to GE and its role in enhancing the resilience of aquaculture species to various environmental stressors.

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Эфирное масло женьшеня обыкновенного как пищевая добавка: польза для роста и пищеварительных ферментов радужной форели

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Аннотация. Цель исследования — оценка влияния кормовой добавки эфирного масла женьшеня *Panax ginseng* (GE) на показатели роста и активность пищеварительных ферментов радужной форели *Oncorhynchus mykiss*. Рыбы получали рационы, содержащие 0 мл/кг (контроль; CTL), 0,5 мл/кг (GE0,5), 1,0 мл/кг (GE1,0), 1,5 мл/кг (GE1,5), 2,0 мл/кг (GE2,0), 2,5 мл/кг (GE2,5), 3,0 мл/кг (GE3,0) и 3,5 мл/кг (GE3,5) эфирного масла женьшеня, в течение 8 недель. Результаты показали, что самые высокие показатели роста и эффективности корма были достигнуты в группах GE2,0 и GE2,5. Группы GE2,5 и GE3,0 показали самую высокую активность трипсина кишечника, химотрипсина и липазы. Но не было никаких

существенных различий в активности кишечной амилазы между группами. В заключение, для достижения наивысшей производительности в качестве кормовой добавки для форели рекомендуется добавка, содержащая эфирное масло женьшеня в дозировке 2,5 мл/кг.

Ключевые слова: кормовая добавка, аквакультура, кишечник, пищеварение, фитобиотик

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