Original Article

Hematological and Biochemical Responses of Rainbow Trout to Dietary Essential Oil of *Abies sibirica* Under Heat Stress

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ABSTRACT

Background: Heat stress is a major challenge in aquaculture, often impairing fish growth, health, and productivity, necessitating the exploration of natural feed additives to enhance resilience.

Objectives: This study aimed to evaluate the effects of dietary Siberian fir essential oil (SFE) on feed efficiency, production efficiency, and hematobiochemical responses to heat stress in rainbow trout.

Methods: SFE was incorporated into the diets at four different concentrations: 0 mg/kg (control; CT), 100 mg/kg (100MG), 200 mg/kg (200MG), and 400 mg/kg (400MG). The fish were fed these diets for 60 days, followed by a 24-hour exposure to heat stress.

Results: Dietary SFE significantly enhanced both feed efficiency and production efficiency, particularly at the 100 mg/kg level. Furthermore, SFE supplementation led to a marked reduction in the activities of plasma alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, effectively mitigating the elevations of these enzymes following heat stress. Fish treated with SFE exhibited higher levels of red blood cells, hematocrit, hemoglobin, white blood cells, and neutrophil percentages. In contrast, the CT group experienced an increase in mean corpuscular volume and mean corpuscular hemoglobin after stress, suggesting that this group endured a greater level of stress.

Conclusion: Dietary supplementation of 100 mg/kg SFE can be regarded as an effective feed additive for trout culture, as it enhances economic viability and strengthens the fish's resistance to heat stress.

Keywords: Essential oil, Feed additive, Hematology, Biochemistry, Stress

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Introduction

Rainbow trout, *Oncorhynchus mykiss*, represents a key cold-water aquaculture species, with global production exceeding 900,000 tons annually (FAO, 2024). While relatively robust, this species faces significant threats from elevated water temperatures—a growing concern as climate change intensifies (Yousefi et al., 2024). Rising global temperatures directly impact ectothermic aquatic organisms, reducing dissolved oxygen availability while increasing metabolic rates (Missaghi et al., 2017; Little et al., 2020). These physiological disruptions elevate oxidative stress, impair growth, and compromise health (Islam et al., 2022; Yousefi et al., 2024).

Mitigating these climate-driven challenges requires innovative strategies, among which phytogenic feed additives show exceptional promise (Onomu & Okuthe 2024). Herbal supplements, in particular, exhibit dual benefits—enhancing antioxidant defenses while modulating immune function—thereby improving stress resilience, disease resistance, and growth performance in fish (Tafti et al., 2020; Tour SavadKouhi et al., 2021; Kamble et al., 2024). Plant essential oils contain volatile compounds with a variety of biological effects, including antioxidant, antimicrobial, and anti-inflammatory effects. Therefore, they have been extensively used in aquaculture to enhance growth rate, stress resistance, and immunocompetence (Sutili et al., 2018; Souza et al., 2019; Dawood et al., 2022).

Siberian fir, *Abies sibirica*, a keystone species of Russia's boreal forests, produces essential oils with documented radical-scavenging and antimicrobial properties (Polyakov et al., 2014; Burkova et al., 2023). Despite this pharmacological potential, effects of Siberian fir essential oil (SFE) have remained unexplored in aquaculture. This study represents the first investigation into the effects of dietary SFE on rainbow trout, evaluating its capacity to enhance fish production, economic feasibility, and hematological/biochemical responses to a heat stress.

Materials and Methods

Origin and composition of SFE

SFE was sourced from Mirrolla Co. in Moscow, Russia. Its composition was analyzed using gas chromatography-mass spectrometry (GC–MS), revealing that the primary components included 14-β-H-Pregna (39.68%), 2-octadecoxyethanol (12.36%), α-pinene (10.36%), and eicosane (1.05%) (Table 1).

Diet formulation and feeding trial

SFE was incorporated into the diets at four different concentrations: 0 mg/kg (control; CT), 100 mg/kg (100MG), 200 mg/kg (200MG), and 400 mg/kg (400MG). The diet was formulated by mixing the feed ingredients listed in Table 2 with 30% water to create a uniform paste, to which the essential oil was added. This mixture was then pelletized using a meat grinder and dried with a fan. Prior to the feeding trial, the proximate composition of the diets was analyzed using standard methods (Horwitz& AOAC, 2005). The produced pellets were stored at 4 ˚C until they were needed for the experiment.

All animal experiments were approved by the Ethics Committee of RUDN University, Moscow, Russia (Protocol No. 4, dated April 8, 2025). The study utilized rainbow trout acquired from a commercial farm and transported under controlled conditions to our laboratory. The fish (4.13±0.11 g) were acclimatized to laboratory conditions for 10 days, during which they were fed the CT diet. Following this acclimatization period, 240 fish were distributed into 12 tanks, each with a capacity of 60 liters, at a density of 20 fish per tank. The fish were fed the designated diets for 60 days, with a daily feeding rate set at 3% of their biomass, which was calculated and adjusted biweekly. Throughout the experiment, all tanks were continuously aerated, and fish waste was siphoned daily. Additionally, 30% of the tank water was replaced with clean, dechlorinated tap water. Water quality parameters, including temperature (17.0±0.86 ˚C), dissolved oxygen (8.51±0.33 mg/L), pH (7.70±0.11), and total ammonia (0.54±0.03 mg/L), were monitored during the study.

Calculation of production and economic efficacies

At the end of the feeding trial, the following parameters were calculated (Equations 1, 2, 3, and 4):

1. Biomass gain (g/tank) = Final biomass – Initial biomass

2. Feed efficiency = $\frac{\text{Tank's biomass gain (g)}}{\text{Total feed offered to tank (g)}}$

3. Feed cost (USD/kg fish produced) = $\frac{\text{Feed initak per tank (g)} \times \text{feed price (USD/g)}}{\text{Final biomass (g)} - \text{initial biomass (g)}}$

4. Production efficiency (kg fish/m³) = $\frac{\text{final biomass (kg)}}{\text{tank's volume (m}^3\text{)}}$

Table 1. Composition of scots pine essential oil determined by GC-MS

Detected Compounds	Area%	Retention Time (min)
α-pinene	10.36	5.042
Camphene	2.19	5.327
Δ-3-carene	2.13	6.624
l-bornyl acetate	8.85	12.99
Trans-caryophyllene	0.19	15.984
α-Humulene	0.12	16.695
β-bisabolene	0.07	17.806
14-β-h-pregna	39.68	27.233
Methyldiethylborane	8.04	29.459
2-octadecoxyethanol	12.36	30.243
Hexacosane	2.25	34.928
Octacosane	3.06	37.320
Eicosane	10.05	37.331
2-(4-methylphenyl)indolizine	0.3	44.802
Total	99.67	

Heat stress induction

At the end of the feeding trial, ten fish from each tank underwent a short-term heat stress protocol. The water temperature was gradually increased from 17 ˚C to 25 ˚C over a 12-hour period, at a rate of 1 ˚C every 1.5 hours, using submersible heaters. This target temperature was chosen based on previous studies conducted on the same species (Yousefi et al., 2024). The fish were maintained at 25 ˚C for 96 hours to assess their heat resistance. To evaluate the physiological responses to heat stress, blood samples were collected after 24 hours of exposure to the stress, as outlined in the subsequent sections.

Blood sampling and analysis

Blood samples were taken from all treatments, before and 24 hours after the heat stress. Fish were caught by a dip-net and anesthetized in 100 ppm eugenol. Blood was withdrawn from the caudal vessel using heparinized syringes. Whole blood was freshly used to determine the red blood cell count (RBC), white blood cell count (WBC), hematocrit (HCT), hemoglobin (HB), mean corpuscular volume (MCV), mean corpuscular HB (MCH), MCH concentration (MCHC), and differential leukocyte count, according to standard protocols (Blaxhall, 1972). A portion of each blood sample was centrifuged for 10 min at 5,000 rpm and 4 ˚C to separate the plasma. The plasma specimens were stored at −70 ˚C until analysis. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were measured using commercial kits (Zist Chem Co., Tehran, Iran) according to the manufacturer's protocols.

Statistical analysis

The Shapiro-Wilk's test was used to confirm normal distribution of the data. Then, the data of fish growth, production, and economic efficiency were analyzed by one-way ANOVA and Tukey's tests. Hematological data were analyzed by two-way ANOVA (diet × stress). Where significant interactions were found, pair-comparisons among the treatment combinations were made using Tukey's test. All analyses were conducted using SPSS software, version 22 at $P<0.05$.

Table 2. Ingredients and chemical composition of the basal diet (g/kg)

Ingredients (g/kg)		CT	100MG	200MG	400MG
Fishmeal[1]		210	210	210	210
Poultry slaughterhouse by product		300	300	300	300
Soybean meal		160	160	160	160
Wheat meal		200	200	200	200
Sunflower oil		15	15	15	15
Soybean oil		20	20	20	20
Corn meal		80	80	80	80
Methionine		3	3	3	3
Lysine		2	2	2	2
Vitamin premix[2]		5	5	5	5
Mineral premix[3]		5	5	5	5
Proximate composition (%)	Moisture	9.12	ND	ND	ND
	Crude protein	42.5	ND	ND	ND
	Crude lipid	14.8	ND	ND	ND
	Crude ash	7.58	ND	ND	ND
Feed price (USD/kg)		0.95	0.97	0.99	1.03

ND: Not determined.

[1] Kilka powder analysis: Protein: 70-72%: fat: 8-11%: ash: 11.6%: moisture: 7-9%. [2] Vitamin premix (per kg of diet): vitamin A: 2000 IU; vitamin B1 (thiamin): 5 mg; vitamin B2 (riboflavin): 5 mg; vitamin B6: 5 mg; vitamin B12: 0.025 mg; vitamin D3: 1200 IU; vitamin E: 63 mg; vitamin K3: 2.5 mg; folic acid: 1.3 mg; biotin: 0.05 mg; pantothenic acid calcium: 20 mg; inositol: 60 mg; ascorbic acid (35%): 110 mg; niacinamide: 25 mg. [3] Mineral premix (per kg of diet): $MnSO_4$: 10 mg; $MgSO_4$: 10 mg; KCl: 95 mg; NaCl: 165 mg; $ZnSO_4$: 20 mg; KI: 1 mg; $CuSO_4$: 12.5 mg; $FeSO_4$: 105 mg; Co: 1.5 mg.

Results

The fish production indices are summarized in Table 3. The dietary intervention had a significant impact on both final biomass and biomass gain. Notably, these parameters were significantly higher in the 100MG and 200MG treatments compared to the CT group, with the 100MG treatment yielding the highest values.

Economic efficiency indices are illustrated in Figure 1. The dietary SFE significantly influenced feed efficiency, feed cost, and fish production efficiency. Specifically, feed efficiency increased significantly, while feed cost decreased markedly in the 100MG treatment compared to the CT group. Additionally, fish production efficiency was significantly enhanced in both the 100MG and 200MG treatments relative to the CT group, with the 100MG treatment demonstrating the highest efficiency.

Interaction effects between stress and diet were observed in RBC, MCV, and MCH. RBC levels significantly increased in the 200MG and 400MG treatments compared to the CT group prior to stress; however, no significant differences in RBC were noted among the

Table 3. Production indices of rainbow trout in the CT, 100MG, 200MG, and 400MG treatments

Variables	Mean±SEM				P
	CT	100MG	200MG	400MG	
Initial biomass (g/tank)	81.9±1.24^{a}	83±1.36^{a}	81.9±2.22^{a}	83.5±0.6^{a}	0.7836
Final biomass (g/tank)	367±4.86^{c}	462±14^{a}	429±6.21^{ab}	402±10.5^{bc}	0.0006
Biomass gain (g/tank)	284±5.8^{c}	379±13.2^{a}	347±6.4^{ab}	319±10.7^{bc}	0.0007

Note: Different letters within a row indicate significant differences among the treatments (n=3, Tukey's test).

treatments after stress. MCV and MCH remained unchanged across treatments both before and after stress, although stress significantly elevated MCV and MCH in the CT treatment (Table 4).

While stress and diet did not significantly affect MCHC, they had independent effects on blood HCT and HB levels. Both parameters showed a significant decrease following stress. HCT levels were significantly higher in SFE-treated fish compared to the CT group, with the 200MG treatment exhibiting the highest value. Furthermore, both the 100MG and 200MG treatments had significantly elevated blood HB levels compared to the other treatments (Table 4).

The effects of stress and diet were also evident in the WBC, lymphocyte percentages, and neutrophil percentages. Prior to stress, SFE-treated fish exhibited significantly higher WBC than the CT group. After stress, the 100MG and 200MG treatments showed significantly elevated WBC compared to the CT group, with both treatments demonstrating a significant increase in WBC after stress compared to before. Before stress, the 100MG and 200MG treatments had significantly higher blood lymphocyte percentages than the other treatments; however, no significant differences were observed among treatments after stress. Neutrophil percentages did not differ significantly among treatments before stress, but the 100MG and 200MG treatments had significantly higher neutrophil percentages after stress. Blood monocyte percentages significantly increased after stress, although dietary intervention did not significantly affect this parameter. Additionally, neither diet nor stress had significant effects on blood eosinophil levels (Table 5).

Stress and diet independently influenced plasma ALT activity (Figure 2). Heat stress significantly elevated plasma ALT activity, while the 100MG and 200MG treatments exhibited significantly lower plasma ALT activities compared to the CT group.

Interaction effects of stress and diet were also observed in plasma AST and ALP activities (Figure 2). SFE-treated fish had significantly lower plasma AST

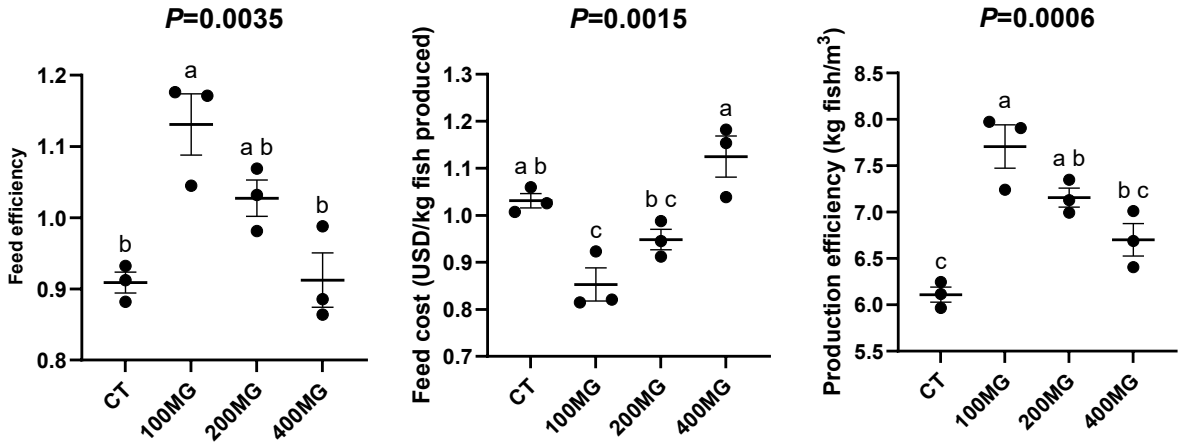


Figure 1. Economic efficiency indices of rainbow trout (Mean±SEM) in the CT, 100MG, 200MG, and 400MG treatments

Note: Different letters above the points indicate significant differences among the treatments (n=3, Tukey's test).

Table 4. Erythrogram of rainbow trout in the CT, 100MG, 200MG, and 400MG treatments before and after heat stress

Variables	Stress	Treatments Mean±SEM				ANOVA		
		CT	100MG	200MG	400MG	Stress	Diet	Stress × Diet
RBC (10^6/µL)	Before	1.07±0.02^{b}	1.11±0^{b}	1.17±0.02^{a}	1.17±0.00^{a}	0.0021	0.7123	0.0009
	After	1.12±0.02^{ab}	1.10±0.02^{ab}	1.06±0.04^{a}	1.03±0.02^{a}			
HCT (%)	Before	33.2±0.49	37.0±0.26	37.5±0.5	35.4±0.32	0.0009	<0.0001	0.7351
	After	32.1±0.38	34.6±0.81	35.6±0.67	33.9±0.98	Before>after	CT^{c}, 100MG^{ab}, 200MG^{a}, 400MG^{b}	
HB (g/dL)	Before	6.61±0.11	7.39±0.04	7.47±0.12	7.02±0.05	<0.0001	0.0002	0.1310
	After	6.7±0.19	7.07±0.12	6.69±0.23	6.30±0.13	Before>after	CT^{b}, 100MG^{a}, 200MG^{a}, 400MG^{b}	
MCV (fL)	Before	309±0.58^{b}	319±1.00^{ab}	318±1.15^{ab}	317±0.58^{ab}	0.3449	0.0686	0.0095
	After	320±1.53^{a}	318±0.58^{ab}	320±2.08^{a}	311±5.29^{ab}			
MCH (pg)	Before	61.7±0.17^{b}	63.2±0.06^{ab}	63.1±0.21^{ab}	63.1±0.06^{ab}	1.000	0.5098	0.0061
	After	63.7±0.53^{a}	62.6±0.64^{ab}	63±0.06^{ab}	61.8±0.78^{ab}			
MCHC (mg/dL)	Before	19.9±0.02	19.8±0.08	19.9±0.05	19.9±0.03	0.2098	0.1148	0.7750
	After	19.9±0.06	19.6±0.22	19.7±0.06	19.8±0.07			

Note: Different letters within a row indicate significant differences among the treatments (n = 3, Tukey's test).

activities than the CT group before stress. After stress, the CT, 200MG, and 400MG treatments showed significant increases in plasma AST activity, with the lowest plasma AST activity recorded in the 100MG treatment. Prior to stress, the 100MG treatment had significantly lower plasma ALP activity than the CT group. Stress significantly increased plasma ALP activity across all treatments, but SFE-treated fish maintained significantly lower plasma ALP activities compared to the CT group after stress (Figure 2).

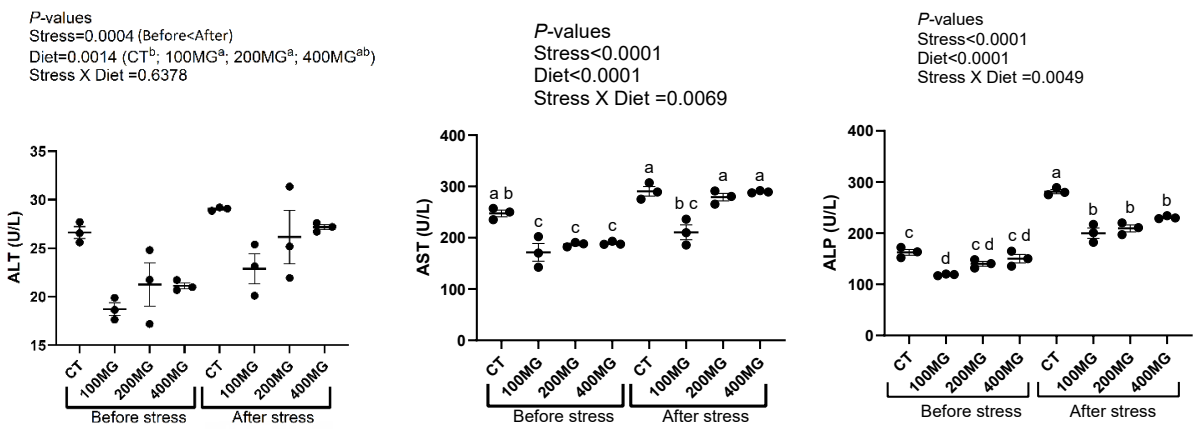


Figure 2. Plasma enzyme levels in rainbow trout (Mean±SEM) in the CTL, CT, 100MG, 200MG, and 400MG treatments before and after heat stress

Note: Different letters above the points indicate significant differences among the treatments (n=3, Tukey's test).

Table 5. Leukogram of rainbow trout in the CT, 100MG, 200MG, and 400MG treatments before and after heat stress

Variables	Stress	Treatments Mean±SEM				ANOVA		
		CT	100MG	200MG	400MG	Stress	Diet	Stress × Diet
WBC (10^3/µL)	Before	18.2±0.61^{c}	22.4±0.15^{b}	22.0±0.17^{b}	21.8±0.42^{b}	<0.0001	<0.0001	<0.0001
	After	17.8±0.38^{c}	34.3±0.52^{a}	32.0±1.21^{a}	20.1±0.15^{bc}			
Lymphocyte (%)	Before	81.7±0.75^{a}	70.1±0.70^{b}	71.7±1.85^{b}	80.2±0.42^{a}	<0.0001	<0.0001	0.0016
	After	82.6±0.55^{a}	78.8±0.78^{a}	79.6±1.59^{a}	81.7±0.45^{a}			
Neutrophil (%)	Before	12.7±0.36^{b}	14.9±0.50^{b}	14.9±0.43^{b}	13.7±0.21^{b}	<0.0001	<0.0001	<0.0001
	After	12.7±0.46^{b}	21.3±1.23^{a}	20.4±0.93^{a}	13.8±0.29^{b}			
Monocyte (%)	Before	6.20±0.96	5.40±0.31	5.00±0.12	5.70±0.32	<0.0001	0.0657	0.8582
	After	7.80±0.26	6.60±0.23	6.80±0.44	7.60±0.25	Before<after		
Eosinophil (%)	Before	1.33±0.33	1.00±0.00	1.00±0.00	1.33±0.33	0.1984	0.3193	0.5472
	After	1.67±0.33	1.67±0.33	1.00±0.00	1.33±0.33			

Note: Different letters within a row indicate significant differences among the treatments (n=3, Tukey's test).

Discussion

This study demonstrated that the supplementation of dietary SFE can enhance both feed and production efficiencies, thereby improving the economic viability of trout farming. This is the first report highlighting the positive effects of dietary SFE on the economic aspects of fish farming. The observed improvement in feed efficiency may be attributed to enhanced nutrient digestion and absorption within the fish gut (Farag et al., 2022; Ghafarifarsani et al., 2023) and/or the anti-stress effects of SFE, which could lower the basal metabolic rate (Korni et al., 2023; Yousefi et al., 2025), thus making more energy available for nutrient retention.

Dietary SFE was found to increase RBC, HCT, and HB values in the fish. The elevation of these parameters enhances the gas exchange capacity of the fish, contributing positively to their overall welfare (Hoseini et al., 2022). This increase may result from enhanced hematopoiesis and hemoglobin synthesis. During periods of stress, fish require a greater capacity for gas mobilization due to an elevated basal metabolic rate (Barton, 2002). However, in the present study, heat stress significantly reduced hematological parameters, likely due to hemodilution. As stress increases the respiration rate (Barton 2002), the balance of hydromineral content may be disrupted in freshwater fish, leading to hemodilution. The rise in MCV and MCH in the control group may indicate the higher stress levels experienced by these fish, resulting in the release of younger RBCs into circulation (Clauss et al., 2008). These younger cells are larger and contain more hemoglobin than older RBCs, which explains the observed increases in MCV and MCH (Clauss et al., 2008).

Leukocytes play a crucial role in the humoral immune system, participating in various functions, such as phagocytosis, cytotoxicity, and antibody production (Maisey & Imarai 2011). The present findings align with previous research indicating that dietary supplementation with herbal additives can increase WBC in fish (Chelemal Dezfoulnejad & Molayemraftar 2022; Yousefi et al., 2022b). In the SFE-treated groups, a decrease in lymphocyte percentage was observed alongside an increase in neutrophil percentage. Neutrophils are essential for the early recognition and elimination of pathogens, while lymphocytes are responsible for adaptive immunity and antibody production, which occurs later (Yousefi et al., 2022a). Therefore, the results suggest that dietary SFE alters the leukogram in a manner that prepares the fish for potential pathogenic challenges (Yousefi et al., 2022a). Similar outcomes have been reported in studies involving fish fed diets supplemented with other herbal additives (Yousefi et al., 2022a).

The enzymes ALT, AST, and ALP are located in the cytosol of cells and are not functional in the bloodstream; rather, they serve as indicators of tissue health (Ghelichpour et al., 2017). When tissue cells are damaged, these enzymes are released into circulation, making their elevated levels indicative of tissue health. ALT is predominantly found in hepatocytes; thus, its activity in the blood serves as a marker for hepatic health. ALP indicates the status of the hepatobiliary system, while AST is a non-specific marker of tissue health (Hoseini et al., 2018). Previous studies have shown that thermal stress leads to increased activity of these enzymes in fish circulation (Dagoudo et al., 2023; Khieokhajonkhet et al., 2023), which supports the findings of the current study. However, the dietary SFE supplementation resulted in decreased levels of these enzymes, suggesting that this feed additive possesses tissue-protective properties. This effect may be attributed to antioxidant effects of its main components (Srivastava et al., 2021; Onanuga & Okpala 2022; Balachandran et al., 2023), which help safeguard living cells from free radical damage.

Conclusion

In conclusion, the inclusion of 100 mg/kg SFE in the diet of rainbow trout is recommended, as it enhances both economic viability and fish welfare under conditions of heat stress.

Ethical Considerations

Compliance with ethical guidelines

The ethical principles observed in the article.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors reported no conflict of interest.

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