

Research Article

Tarragon (*Artemisia dracunculus*) Essential Oil at Optimized Dietary Levels Prompted Growth, Immunity, and Resistance to Enteric Red-Mouth Disease in the Rainbow Trout (*Oncorhynchus mykiss*)

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Fingerlings of the rainbow trout, *Oncorhynchus mykiss* ($n = 300$, 10.63 ± 0.6 g), were fed tarragon (*Artemisia dracunculus*) essential oil (TGO) for 2 months to examine its effects on growth properties, immunity, and resistance to *Yersinia ruckeri* infection. The treatments were control or TG1, TG2 (fed 0.5% TGO), TG3 (1% TGO), and TG4 (2% TGO). According to the results, an improvement was observed in growth parameters in all TGO-treated groups compared to the control ($P < 0.05$). The digestive enzyme activities (protease and lipase) were significantly elevated in response to dietary TGO ($P < 0.05$). The immune system of the fish was enhanced by TGO, as it stimulated the immune parameters in serum (lysozyme, myeloperoxidase (MPO), alternative complement (ACH₅₀), Ig) and mucus (lysozyme, protease, ACH₅₀, Ig) ($P < 0.05$). The treatments, TG3 and TG4, showed more immune performance in response to TGO ($P < 0.05$). The fish in TG2 treatment had a higher levels of serum total protein than other groups ($P < 0.05$). The concentration of triglycerides (TRIG) and cholesterol (CHOL) in serum significantly decreased ($P < 0.05$) in response to TGO, as the lowest levels were observed in the treatment, TG3. The antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)) of serum elevated in TGO-treated fish, with the maximum values for the TG4 group ($P < 0.05$). TGO reduced ($P < 0.05$) alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels in serum. After bacterial challenge, the TGO-treated fish showed lower mortality compared to the control, where the lowest mortality was observed in TG4 ($P < 0.05$). In conclusion, TGO improved growth, immunity, and survival after bacterial challenge in the rainbow trout, with more performance in fish fed 1%–2% TGO.

1. Introduction

Undoubtedly, aquaculture is a reliable way to provide protein resources for humans. Owing to the ever-increasing human demands for aquatic products, improving fish production in terms of quality and quantity is one of the priorities of aquaculture [1]. The use of food supplements, such as immunostimulants, probiotics, and antibiotics, is one of the most important methods in aquaculture to improve growth, immunity, nutritional value, and disease resistance [2–5]. Treatment of bacterial diseases by antibiotics is very common in aquaculture, but some restrictions regarding environmental

and human health-related problems have severely limited their use [6]. Creating antibiotic-resistant strains, changing the flora of natural ecosystems, and the accumulation of antibiotics or their derivatives in aquatic meat are among the most important effects of antibiotics [7–9]. Therefore, researchers have focused on identifying safe alternatives to antibiotics. Phytobiotics are a group of plant-based compounds including essential oils and plant extracts, whose benefits in stimulating fish growth, immune system, and resistance to pathogens [10–14]. Compared to chemicals, medicinal plants and their derivatives have more stable therapeutic effects and the pathogenic resistance to them is relatively less. In addition, no

negative impacts on the environment and organisms and low-cost production are other advantages of using this group of materials [15]. Plant-based materials have shown antimicrobial and antioxidant activities in fish [16, 17].

Plant essential oils (PEO) are a group of volatile aromatic compounds and oily liquids that are produced during the process of secondary metabolism in plants [18–20]. Since the essential oils have had positive effects on the growth, immunity, and resistance of fish against pathogens, these compounds have been seriously considered as an alternative to antibiotics [21–23]. Tarragon (*Artemisia dracunculus*) belongs to the Asteraceae family and is used in traditional medicine [24–26].

The anti-inflammatory and antimicrobial properties of tarragon have been reported in many researches [27–31]. Biochemical composition of tarragon, especially in leaves includes the phenolic and flavonoid compounds, which have strong antioxidant and antimicrobial effects [30, 32–34].

Rainbow trout, *Oncorhynchus mykiss* (salmonidae), is an important commercial fish in the aquaculture industry due to its high ability to adapt to the culture environment, easily reproduction, high meat quality, and marketability [35, 36].

One of the main problems related to raising of rainbow trout is the occurrence and spread of diseases, especially in high fish densities. Therefore, the use of safe and environmentally favorite alternatives to antibiotics can enhance the resistance of fish against diseases and consequently fish production [37, 38].

Yersiniosis is a bacterial disease in salmonidae caused by *Yersinia ruckeri* [39]. Surface and internal hemorrhages and high mortality (>70%) can be symptoms of this disease in salmonidae [40, 41]. This study tried to investigate the effect of TGO on growth, immunity, and resistance of rainbow trout to the *Y. ruckeri* infection. The results of the present research can enhance rainbow trout aquaculture.

2. Materials and Methods

2.1. Biochemicals in Tarragon Essential Oil. The tarragon (*A. dracunculus*) essential oil was provided by Nikoshimi Co., Iran. The chemicals in the TGO composition were analyzed by GC–mass spectrometry (Agilent Technologies, 6890 N, 5,975 C, USA). The composition of TGO included mainly the compounds, chavicol (82.2%), trans-ocimene (4.2%), limonene (1.6%), and *z*- β -ocimene (3.1%), and α -pinene (0.7%).

2.2. Antioxidant Potentials of Tarragon Essential Oil. TGO antioxidant capacities were evaluated based on four methods including (a) DPPH (2,2-diphenyl-1-picrylhydrazyl) method, (b) evaluation of total phenol, (c) evaluation of total flavonoid content, and (d) ABTS⁺ radical scavenging assay (Table 1). In the DPPH method, the ability of TGO in removing free radicals was measured at 517 nm upon action of DPPH with TGO according to Ahmad et al. [42]. Folin–Ciocalteu method was used to assay total phenolic compounds. The assay was conducted at 720 nm upon the reaction of Folin–Ciocalteu with sodium carbonate according to Mhadhebi et al. [43]. Total flavonoid content was measured at 415 nm upon reaction of the flavonoid compounds in the sample with sodium nitrate

TABLE 1: The antioxidant potentials of TGO by different methods.

Assay methods	
Total phenolics (mg GAE/g)	7.12 ± 0.71
Total flavonoids (mg rutin/g)	11.22 ± 1.51
DPPH (inhibition percentage (%))	72.73 ± 2.02
ABTS (μ g/mL)	9.88 ± 1.2

and aluminum chloride according to Pandey et al. [44]. ABTS⁺ radical scavenging activity was determined at 734 nm upon neutralization of ABTS radical cations by the antioxidant [45].

2.3. Experimental Design. Three hundred rainbow trout (10.63 ± 0.6 g) fingerlings were prepared from a trout farm in Karaj (Iran) and carried to Alborz Caspian Cold Water Fish Breeding Complex (ACFC), Karaj, Iran, for further testing. The fish were transported to the ACFC under continuous aeration and distributed into 12 tanks (300 L, 25 fish per tank) with a stable water flow and fed commercial trout diet (46% protein, 12% crude lipid, 11% crude fiber, 9% ash, and 5% moisture) for 7 days. After 7 days adaptation period, fish were fed test diets containing TGO for 60 days, as four experimental groups in three replicates as follows: TG1 (control or untreated fish), TG2 (0.5% TGO), TG3 (1% TGO), and TG4 (2% TGO). The experimental diets were prepared by mixing TGO with a commercial basal diet (Faradaneh Co., Iran) according to Hajirezaee and Khanjani [46]. The composition of basal diet included 46% protein, 12% crude lipid, 11% crude fiber, 9% ash, and 5% moisture.

Feeding of the fish with test diets conducted at 3% of biomass four times a day at 8:00 AM, 12:00 noon, 4:00 PM, and 8:00 PM. The water quality parameters were checked daily, which were in optimum range for temperature (15–16°C), pH (6.9–7.3) (Model AP110), and oxygen (7.15–8.5 mg/L) (oxygen meter, model: 607132 Pro20i).

2.4. Sampling Process. After the feeding period, the fish of each treatment were weighed to determine growth parameters. Then, fish were anesthetized (200 mg/L clove powder) and the blood samples of five fish per tank were obtained from the caudal vein by 2 mL syringes to assay biochemical and immune parameters. The blood samples were transferred into nonheparinized tubes, centrifuged (13,000x g for 10 min) to separate serum, and the serum samples stored at –20°C. Skin mucus was by placing the fish in plastic bags containing 50 mmol sodium chloride for 2 min.

2.5. Evaluation of Growth Performance. After the 60-day experiment, the growth parameters were determined according to the following formulas:

$$\text{Weight gain (WG) (g)} = \frac{\text{Fish final weight (g)} - \text{fish initial weight (g)}}{\text{fish initial weight (g)}}, \quad (1)$$

$$\text{Feed conservation ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}}, \quad (2)$$

$$\begin{aligned} \text{Specific growth rate (SGR)}(\%/ \text{day}) \\ = ((\text{Ln fish final weight} \div \text{Ln fish initial weight}) / \\ \text{duration of rearing (day)}) \times 100, \end{aligned} \quad (3)$$

$$\text{Survival rate (SR)}(\%) = \frac{\text{Number of alive fish}}{\text{Number of dead fish}} \times 100. \quad (4)$$

2.6. Serum Biochemicals. The biochemicals (cortisol (CORT), glucose (GLU), cholesterol (CHOL), triglyceride (TRIG), albumin (ALB), and total protein (TP)) in blood were assayed using assay kits (Pars Azmoon, Co., Iran); according to manufacturer's instructions, GLU was determined at 546 nm upon oxidation of glucose by glucose oxidase. TP was measured at 545 nm upon reaction of the protein with copper ions in an alkaline solution. ALB was estimated at 600 nm after reaction of ALB with bromocresol green. CHOL was determined after an enzymatic hydrolysis and oxidation reaction modulated by cholesterol esterase and cholesterol oxidase. TRIG was determined at 546 nm upon the action of glycerol-3-phosphate-oxidase on the TRIG.

2.7. Serum Biochemicals. All enzymes and biochemicals were assayed using assay kits (Pars Azmoon, Co., Iran); according to manufacturer's instructions, SOD (superoxide dismutase) activity was assayed at 440 nm by estimating the reductions in cytochrome c by superoxide radicals generated by xanthine oxidase. GPX (glutathione peroxidase) activity was measured at 340 nm by estimating the oxidation rate of oxidized glutathione upon action of glutathione reductase followed by oxidation of nicotinamide adenine dinucleotide phosphate (NADPH). CAT (catalase) was assayed at 240 nm by estimating the decomposition rate of hydrogen peroxide (H₂O₂) to oxygen and water.

Thiobarbituric acid (TBA) assay was used to measure the MDA (malondialdehyde) content [47]. AST (aspartate aminotransferase) activity was assayed at 410 nm by estimating the production of oxaloacetate from L-aspartate and malate from oxaloacetate upon the action of malate dehydrogenase. ALP (alkaline phosphatase) was measured at 410 nm upon decomposition of p-nitrophenylphosphate as substrate to phosphate and p-nitrophenol. ALT (alanine aminotransferase) activity was assayed at 410 nm by estimating the production of pyruvate from L-alanine and D-lactate from pyruvate upon the action of alanine aminotransaminase.

2.8. Immune Parameters. The lysozyme activity was determined at 450 nm by estimating the lysis rate of a lysozyme-sensitive bacteria, *Micrococcus luteus* [48]. The alternative complement activity (ACH₅₀) was determined by estimating the lysis rate of sheep red blood cells as substrates by the complement system [49]. The differences in protein content before and after precipitating total immunoglobulin (Ig) by polyethylene glycol solution was measured as Ig content [50]. MPO activity in serum was assayed at 450 nm upon the reaction of myeloperoxidase with 3,3',5,5'-tetramethylbenzidine hydrochloride (TMB) as substrate according to Quade

and Roth, [51]. Protease activity in mucus was estimated at 440 nm upon hydrolysis of azocasein as substrate [52].

2.9. Digestive Enzyme Activities. To assay digestive enzyme activities (i.e., amylase, lipase, and protease), the intestine tissue of the fish (five fish/tank) was sampled after anesthesia with 200 mg/L clove powder. After mechanically homogenization of tissues in Tris buffer [53], the samples were centrifuged (6,500xg, at 4°C, for 10 min), the supernatant separated and stored at -80°C. Protease activity was measured after the action of the enzyme with 1% casein as substrate and the absorbance read at 280 nm [54]. For determination of lipase (at 405 nm) and amylase (at 540 nm) activities [55, 56], p-nitrophenyl myristate and 1% starch were used as substrates, respectively.

2.10. Bacterial Challenge. The resistance of the TGO-supplemented fish to bacterial infection was evaluated by challenging them with *Y. ruckeri* bacteria (KC291153) for 14 days. *Y. ruckeri* was first inoculated in broth culture medium for 48 at 26°C and then centrifuged (6,000xg for 10 min) and the deposit washed twice with phosphate buffer. The bacterial suspension (BS) at dosage of 10⁷ cells/mL was prepared according to Naderi Farsani et al. [57]. Fish (*n* = 15/tank) were injected with 0.1 mL of BS, and the cumulative mortality (%) was estimated over the challenge.

2.11. Statistical Analysis. SPSS software (version 20) was used for data analysis. After the evaluation of data distribution and homogeneity by Shapiro-Wilk and Levene's tests, respectively, one-way analysis of variance (one-way-ANOVA) was used to investigate the differences at *P* < 0.05. After that, Tukey's test was used to compare the means.

3. Results

3.1. Growth and Survival. The performance of TGO on the fish growth was demonstrated in this research. The FW and WG significantly increased in all TGO-supplemented fish compared to the control group (Table 2, *P* < 0.05). The FCR improved by TGO showed lower values in TGO groups compared to the control group (*P* < 0.05). The differences (*P* > 0.05) in FCR values of TGO groups were statistically similar (Table 2). SGR values were statistically similar (*P* > 0.05) between all groups. Over the feeding period, all groups showed a SR (*P* > 0.05) of 100% (Table 2).

3.2. Digestive Enzymes. The activity of digestive enzymes (protease, amylase, and lipase) elevated (*P* < 0.05) in TGO groups compared to the control (Table 3). The highest activity of the enzymes was observed in TG3 treatment (Table 3, *P* < 0.05).

3.3. Blood Biochemical Parameters. The ALB and GLO concentrations had no differences (*P* > 0.05) in all treatments (Table 4). CHOL, TRIG, CORT, and GLU levels declined (*P* < 0.05) in all TGO treatments (Table 4). TG2 group had the higher TP content than control, and all groups showed no difference (*P* < 0.05) with the control (Table 4).

3.4. Antioxidant Enzymes. SOD and CAT elevated (*P* < 0.05) in response to TGO, with the maximum activity in the TG3

TABLE 2: Growth parameters of rainbow trout (*O. mykiss*) over 60 days feeding with TGO.

Parameters	TG1	TG2	TG3	TG4
IW (g)	9.50 ± 1.11 ^a	11.71 ± 1.16 ^a	9.35 ± 1.40 ^a	11.67 ± 1.17 ^a
FW (g)	49.02 ± 1.53 ^b	53.88 ± 1.62 ^a	54.43 ± 1.49 ^a	52.75 ± 1.54 ^a
WG (g)	38.29 ± 0.42 ^b	42.16 ± 0.78 ^a	42.85 ± 0.53 ^a	43.08 ± 0.60 ^a
SGR (%/day)	2.11 ± 0.003 ^a	2.44 ± 0.04 ^a	2.31 ± 0.02 ^a	2.54 ± 0.03 ^a
FCR	1.12 ± 0.029 ^a	1.12 ± 0.016 ^b	1.14 ± 0.013 ^b	1.15 ± 0.014 ^b
SR (%)	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a	100.00 ± 0.00 ^a

Control or TG1 (untreated), TG2 (0.5% TGO), TG3 (1% TGO), TG4 (2% TGO). IW, initial weight; FW, final weight; WG, weight gain; SGR, specific growth rate; FCR, feed conversion ratio; and SR, survival rate. Data are represented as mean ± SE. Different letters (a and b) in the same row indicate significant differences ($P < 0.05$).

TABLE 3: Digestive enzymes of rainbow trout (*O. mykiss*) over 60 days feeding with TGO.

Parameters	TG1	TG2	TG3	TG4
Amylase (U/mg prot)	1.55 ± 0.12 ^b	2.16 ± 0.067 ^a	2.32 ± 0.1 ^a	2.21 ± 0.05 ^a
Protease (U/mg prot)	8.83 ± 0.27 ^b	11.36 ± 0.25 ^a	11.16 ± 0.97 ^{ab}	10.74 ± 0.31 ^{ab}
Lipase (U/mg prot)	6.04 ± 0.08 ^c	7.10 ± 0.06 ^b	8.03 ± 0.10 ^a	7.31 ± 0.21 ^b

Control or TG1 (untreated), TG2 (0.5% TGO), TG3 (1% TGO), and TG4 (2% TGO). Different letters (a–c) in the same row indicate significant differences ($P < 0.05$).

TABLE 4: Biochemical parameters of rainbow trout (*O. mykiss*) over 60 days feeding with TGO.

Parameters	TG1	TG2	TG3	TG4
TP (g/dL)	3.65 ± 0.07 ^b	4.07 ± 0.05 ^a	4.05 ± 0.04 ^{ab}	4.03 ± 0.08 ^{ab}
ALB (g/dL)	2.42 ± 0.04 ^a	2.55 ± 0.02 ^a	2.59 ± 0.03 ^a	2.57 ± 0.04 ^a
GLO (g/dL)	2.35 ± 0.12 ^a	2.35 ± 0.05 ^a	2.43 ± 0.03 ^a	2.43 ± 0.05 ^a
TRIG (mg/dL)	158.75 ± 2.41 ^a	133.77 ± 1.87 ^c	135.37 ± 2.21 ^c	146.66 ± 1.37 ^b
CHOL (mg/dL)	190.3 ± 1.58 ^a	150.08 ± 3.13 ^b	149.52 ± 1.42 ^b	152.77 ± 3.8 ^b
GLU (mg/dL)	79.71 ± 1.13 ^a	59.81 ± 1.21 ^b	55.21 ± 0.79 ^c	60.55 ± 1.19 ^{ab}
CORT (nmol/L)	55.4 ± 1.03 ^a	45.5 ± 0.73 ^b	46.2 ± 0.87 ^b	45.44 ± 1.31 ^b

Control or TG1 (untreated), TG2 (0.5% TGO), TG3 (1% TGO), and TG4 (2% TGO). TP, total protein; ALB, albumin; GLO, globulin; TRIG, triglycerides; CHOL, cholesterol; GLU, glucose; and CORT, cortisol. Different letters (a–c) in the same row indicate significant differences ($P < 0.05$).

TABLE 5: Antioxidant enzyme and MDA content of rainbow trout (*O. mykiss*) over 60 days feeding with TGO.

Parameters	TG1	TG2	TG3	TG4
SOD (U/mL)	11.54 ± 0.66	16.2 ± 0.99 ^{ab}	15.03 ± 1.12 ^a	16.65 ± 1.22 ^a
CAT (U/mL)	40.77 ± 1.22 ^b	52.44 ± 1.22 ^a	54.32 ± 1.34 ^a	52.56 ± 1.02 ^a
GPx (U/mL)	49.65 ± 1.19 ^b	57.61 ± 1.28 ^a	54.89 ± 1.2 ^{ab}	53.32 ± 1.15 ^{ab}
MDA (nmol/mL)	2.5 ± 0.1 ^a	1.32 ± 0.08 ^b	1.3 ± 0.07 ^b	1.41 ± 0.04 ^b

Control or TG1 (untreated), TG2 (0.5% TGO), TG3 (1% TGO), and TG4 (2% TGO). SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; and MDA, malondialdehyde. Different letters (a–b) in the same row indicate significant differences ($P < 0.05$).

group (Table 5). TGO groups had no differences ($P > 0.05$) in CAT activity with the non-TGO treated group (Table 5). GPx values increased ($P < 0.05$) in TGO-treatments (Table 6), while other treatment the values similar ($P > 0.05$) to the non-TGO-treated group and TG2 (Table 5). TGO groups showed lower ($P < 0.05$) MDA levels compared to non-TGO-treated group (Table 6).

3.5. Liver Enzymes. ALP and ALT activities significantly decreased ($P < 0.05$) in response to TGO (Table 6), while their levels were similar ($P > 0.05$) in the TGO groups (Table 6). AST activity was reduced in TG3 and TG4 groups compared to the control (Table 6, $P < 0.05$).

3.6. Serum and Mucus Immune Parameters. Dietary TGO improved the immune performance of the fish, which is presented in Figures 1 and 2. The lysozyme activity, Ig levels, and MPO activity in serum were higher in TGO groups compared to control (Figure 1, $P < 0.05$); however, there were no differences ($P > 0.05$) in MPO values of the TGO treatments (Figure 1). Serum ACH₅₀ activity elevated in TG3 and TG4 treatments compared to the control (Figure 1, $P < 0.05$). The lysozyme activity, Ig levels, and MPO activity in mucus showed significant elevations ($P < 0.05$) in TGO groups compared to the control (Figure 2). The mucus ACH₅₀ activity elevated ($P < 0.05$) in TG3 and TG4 compared to non-TGO supplemented fish (Figure 2). The TG4

TABLE 6: Liver enzymes of rainbow trout (*O. mykiss*) over 60 days feeding with TGO.

Parameters	TG1	TG2	TG3	TG4
ALT (U/mL)	19.22 ± 0.70 ^a	13.05 ± 0.62 ^b	14.53 ± 0.81 ^b	15.63 ± 0.63 ^b
AST (U/mL)	11.55 ± 0.76 ^a	10.42 ± 0.63 ^{ab}	8.47 ± 0.34 ^b	9.19 ± 0.24 ^b
ALP (U/mL)	25.2 ± 0.70 ^a	19.9 ± 0.65 ^b	16.7 ± 0.72 ^b	17.5 ± 0.63 ^b

Control or TG1 (untreated), TG2 (0.5% TGO), TG3 (1% TGO), and TG4 (2% TGO). ALT, alanine aminotransferase; AST, aspartate aminotransferase; and ALP, alkaline phosphatase. Different letters (a–b) in the same row indicate significant differences ($P < 0.05$).

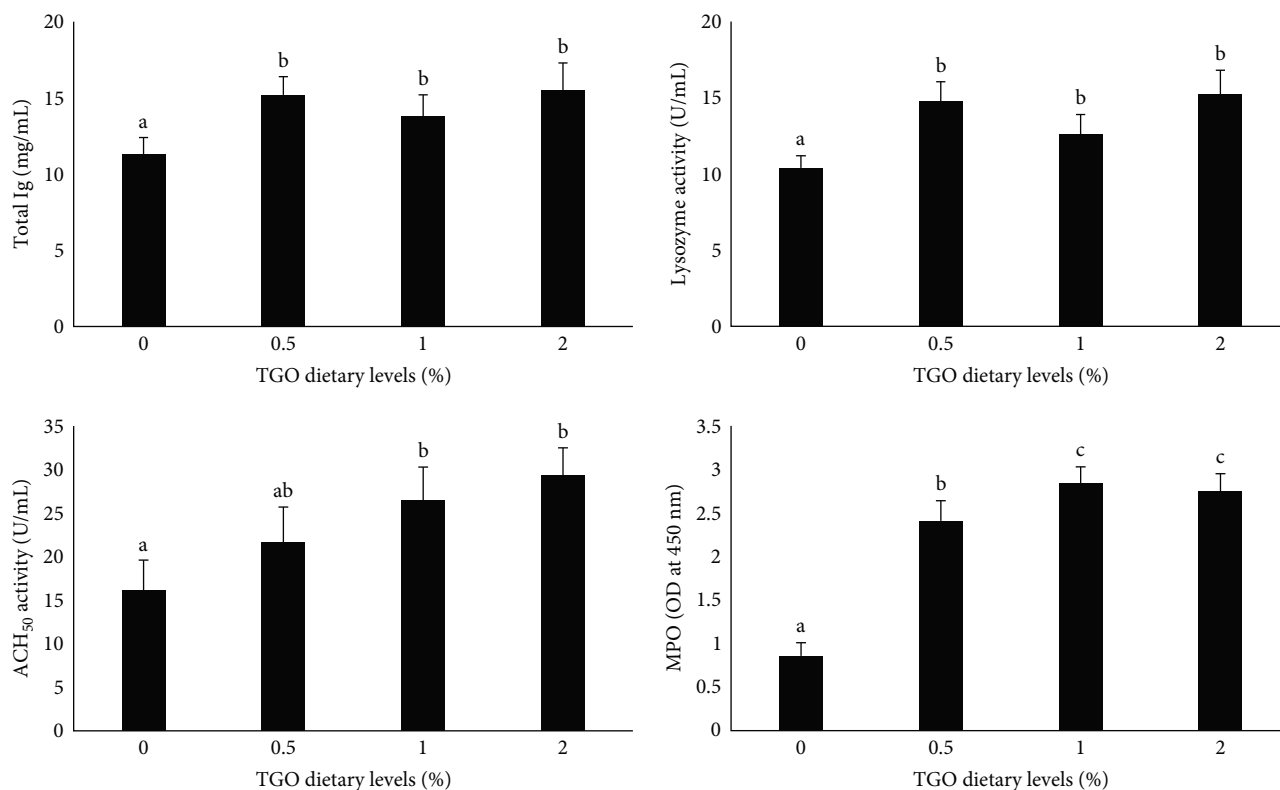


FIGURE 1: The serum immune component changes in rainbow trout (*O. mykiss*) over 60 days feeding with TGO (TG1 (untreated), TG2 (0.5% TGO), TG3 (1% TGO), and TG4 (2% TGO)). Different letters above the bars show significant differences among the experimental groups ($P < 0.05$).

group showed more immune performance ($P < 0.05$) of serum (Figure 1) and mucus (Figure 2).

3.7. Bacterial Challenge. The cumulative mortality (%) was lower in TGO treated fish compared to the untreated ones after the bacterial challenge (Figure 3, $P < 0.05$). TG3 and TG4 showed lowest mortality (Figure 3, $P < 0.05$). The highest mortality ($P < 0.05$) was observed in the control (Figure 3).

4. Discussion

Phytogetic bioactive components have showed a large spectrum of antioxidant and antimicrobial properties in fish [58, 59]. This study evaluated the growth and immune boosting effects TGO in the rainbow trout. Numerous studies have investigated the growth and immune enhancing properties of plant-based substances, such as extracts and essential oils in fish [21, 60–63]. In this study, FW and WG improved in TGO groups and TGO also improved FCR of the fish. In agreement

with our results, Gholamhosseini et al. [64] observed a significant improvement in the rainbow trout growth after 60 days of supplementation with 1%–3% tarragon, which was attributed to the effects of tarragon on appetite, function of pancreas [65, 66], and also to phenolic and aromatic compounds in this plant [66]. The supplementation of common carp with 0.5%–1% *Artemisia absinthium* extract also increased growth performance in the fish [67]. However, Zare et al. [68] observed no improvement in growth performance of the rainbow trout supplemented with 30 g tarragon powder, which may be due to the differences in size, physiology, dietary level of tarragon, and culture condition. The improving effects of TGO may be due to the increases in the amylase, protease, and lipase activities, as their levels were higher in TGO groups [69]. Although there were many studies regarding the inducing impacts of essential oils on digestive enzyme activity in fish [70–73], we found no literature with TGO. Generally, it seems that essential oils induce digestive enzymes by affecting the pancreas, where they are produced. However, the mechanism of essential

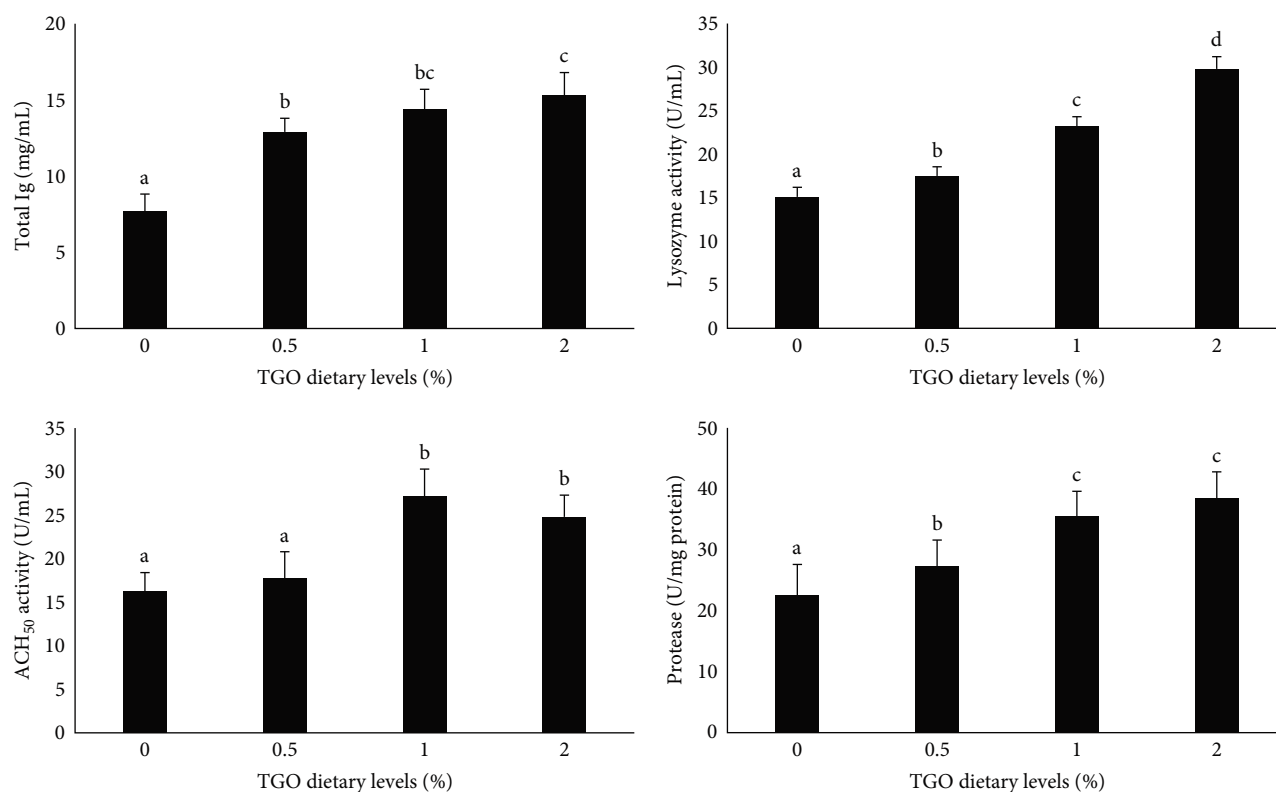


FIGURE 2: The mucus immune component changes in rainbow trout (*O. mykiss*) over 60 days feeding with TGO (TG1 (untreated), TG2 (0.5% TGO), TG3 (1% TGO), and TG4 (2% TGO)). Different letters above the bars show significant differences among the experimental groups ($P < 0.05$).

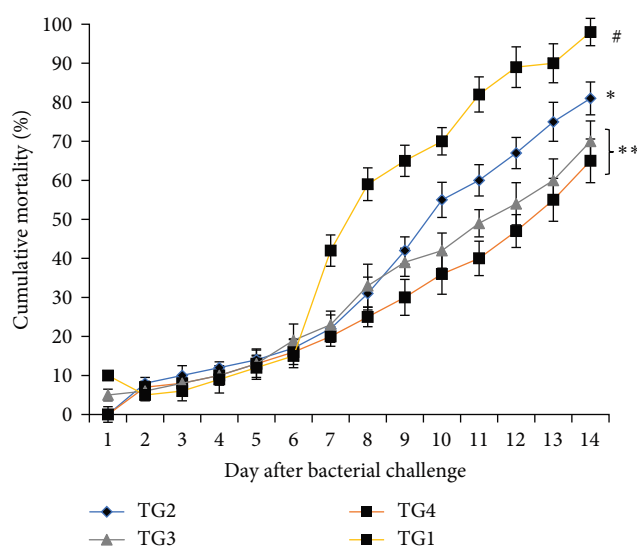


FIGURE 3: Cumulative mortality (mean \pm SE) of rainbow trout (*O. mykiss*) over 14 days challenge with *Yersinia ruckeri* (TG1 (untreated), TG2 (0.5% TGO), TG3 (1% TGO), and TG4 (2% TGO)). Significant differences are shown with different symbols ($P < 0.05$).

oils on fish digestive enzymes is yet unknown. The prompting impacts of plant oils on fish growth may be due to the presence of some antioxidant, antibacterial, anti-inflammatory, and immunogenic components in TGO composition [59, 74–77].

For example, the presence of eugenol as a growth and immune prompting compound [59, 74–78].

Blood biochemicals and their changes strongly affect the fish health [79]. The TP levels increased in response to TGO, while CHOL, TRIG, GLU, and CORT levels reduced in TGO-treated fish. Increases in TP after essential oil supplementation have been reported by some studies, which was in agreement with our results [80–82]. TP concentrations in fish blood can be changed in response to nutritional and immune conditions. TP includes globulins, antibodies, and albumin, and thus any increases in its concentrations may indicate an improved immunity for fish [83].

Globulins act as precursors of immunoglobulins [84]. The increased levels of TP in the TGO-supplemented fish may indicate the immunogenic effect of TGO. In the present study, the levels of GLU and CORT were reduced in the TGO-supplemented fish, suggesting a stress-ameliorating function for TGO. Although the stress-ameliorating effects of tarragon have been reported in a study by Kaya et al. [85] in laying hens, we could not find any data in fish. However, the stress-ameliorating effects of essential oils have been demonstrated in fish [86–88]. The stress-ameliorating effects of PEO could be attributed to phenolic compounds in their composition and their role against oxidative stress. The feeding of the fish with diet containing TGO significantly reduced CHOL and TRIG levels. Similar results have been also reported the reduced in CHOL and TRIG levels in fish blood after PEO supplementation [81, 82, 89, 90]. It seems that PEO such as

TGO prevents hyperlipidemia and fat accumulation by affecting the biosynthesis and metabolism of lipids, although the exact mechanism is not yet understood [89, 91]. The reducing effects of PEO on TRIG and CHOL levels can be beneficial for the general health of fish.

The antioxidant properties of phytobiotics are usually attributed to the phenolic compounds in biochemical composition. In the present study, decreases in MDA in the treated may be due to the phenolic compounds of TGO [32, 33]. Similarly, decreases in MDA levels have been reported in fish supplemented with essential oils, which indicates their protective impacts against oxidative stress [70, 92].

Antioxidant structures such as terpenes and phenols in PEO may be responsible for the protective effects of PEO against oxidative stress [93]. TGO enhanced antioxidant capacity, as stimulated the antioxidant enzymes, SOD, CAT, and GPx. Similarly, the stimulating action of PEO on antioxidant enzymes is also reported in fish [94–96].

The release of liver metabolic enzymes (LMEs) into the blood and increases in their blood levels could be an indicator of liver disorders and damages [97–100]. LMEs are released into the blood due to the liver damage.

In this research, serum ALT, ALP, and AST levels declined following TGO supplementation, suggesting a protective function for TGO on liver. This protective function has been also proposed for other essential oils in fish. For example, menthol essential oil decreased ALT, AST, and ALP in the Nile tilapia, *Oreochromis niloticus* [101]. The metabolic enzymes decreased in rainbow trout fed *Mentha spicata*, *Thymus vulgaris*, and *Salvia officinalis* essential oils [102]. Furthermore, the concentrations of AST, ALT, ALP, and LDH in blood of common carp decreased in response to geranium, *Pelargonium graveolens* essential oil [103].

TGO improved the fish immunity, as the immune components of serum and mucus including MPO, lysozyme, Ig, ACH₅₀, and protease increased in the supplemented fish. The elevated activity of lysozyme usually indicates an elevation in phagocytosis [104]. The complement system does immunogenic functions through modulating inflammatory pathways, lysis activity, and opsonization [105]. The immunogenic properties of PEO are widely demonstrated in fish. For example, use of 2%–3% black cumin, *Bunium persicum* essential oil [106], 3.0 mL/kg *Origanum onites* essential oil [107] in rainbow trout, origanum, *Origanum vulgare* essential oil in Redbelly tilapia, *Tilapia zillii* [108] significantly enhanced innate immune components.

Although the mechanisms for the immune-inducing effects of essential oils on fish are still a little unknown, their immunogenic properties may be due to the presence of vitamins, phenolic, and terpenic structures in their composition [21, 59, 109]. In the present study, we observed an significant increase in resistance of TGO-supplemented fish to *Y. ruckeri* infection, especially for fish of TG4 treatment, which showed the lowest mortality after the bacterial challenge. The existence of some antimicrobial compounds in the biochemical content of essential oils has been demonstrated in some researches [110–112]. The biochemical content of TGO includes some antibacterial compounds such as methyl

chavicol, as the GC–MS results also showed 82.2% chavicol [32]. The antibacterial properties of TGO have been also demonstrated in some studies [30, 32, 113].

5. Conclusion

Dietary TGO could be used as food additive for the rainbow trout, as it showed growth and immune prompting effects. The best performance was observed in fish-fed diet containing 2% TGO. However, further study is necessary to identify the mechanisms and mode of action of TGO with fish growth and immunity.

Data Availability

The datasets in this study are available from the corresponding author upon reasonable request. All data and materials are available for publication.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed in this study.

Conflicts of Interest

Authors have no conflicts of interest to declare for the publication of the present work.

Authors' Contributions

All the authors have participated sufficiently in conducting the present research, including testing (Dr. Saeed Hajirezaee), data analysis (Dr. Mohammad Hossein Khanjani), writing the article in English (Dr. Saeed Hajirezaee and Dr. Saman Ahani), and editing the article in the revision stage (Dr. Zahra Ghiasvand).

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