Journal of Agricultural Sciences
(Tarim Bilimleri Dergisi)

J Agr Sci-Tarim Bili
e-ISSN: 2148-9297
jas.ankara.edu.tr

Growth, Blood Parameters, Immune Response and Antioxidant Enzyme Activities in Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792) Fed Diets Supplemented with Fumitory (*Fumaria officinalis*)

Abdelsalam FILOGH[a], Soner BİLEN[b], Adem Yavuz SÖNMEZ[b*], Mahmut ELP[c]

[a]*Benghazi University, Faculty of Science, Department of Zoology, Benghazi, LIBYA*

[b]*Kastamonu University, Faculty of Fisheries, Department of Basic Sciences, Kastamonu, TURKEY*

[c]*Kastamonu University, Faculty of Fisheries, Department of Aquaculture, Kastamonu, TURKEY*

ARTICLE INFO

Research Article
Corresponding Author: Adem Yavuz SÖNMEZ, E-mail: aysonmez@kastamonu.edu.tr
Received: 12 August 2021 / Revised: 03 January 2022 / Accepted: 02 February 2022 / Online: 18 January 2023

Cite this article

FILOGH A, BİLEN S, SÖNMEZ A Y, ELP M (2023). Growth, Blood Parameters, Immune Response and Antioxidant Enzyme Activities in Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792) Fed Diets Supplemented with Fumitory (*Fumaria officinalis*). *Journal of Agricultural Sciences (Tarim Bilimleri Dergisi),* 29(1):47-59. DOI: 10.15832/ankutbd.982032

ABSTRACT

In a feeding experiment for 75 days, the effects of fumitory (*Fumaria officinalis*) extract on growth, haematology, immune response and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*) were evaluated. The aqueous methanolic extract of the plant was mixed with feeds at three different levels, 0.1% (FO1), 0.2% (FO2) and 0.3% (FO3), and feed with no plant extract donated as the control. All four groups (mean initial weight: 13.02 ± 0.02 g) were fed with the prepared diets twice daily *ad libitum* in a triplicate experiment. Blood and liver samples were taken from the fish on 15, 30, 45, 60 and 75 days. Also, overall growth parameters were determined based on body weight data recorded from all fish before and at the end of feeding trial. Results indicated that there were variations in nonspecific immune parameters (lysozyme, myeloperoxidase and oxidative radical production), but supplementation of the plant extract did not affect the immune response of the fish significantly. On the other hand, there was a general increase in the antioxidant enzyme activities (superoxide dismutase, catalase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase) evaluated in this study. Furthermore, possibly the most remarkable finding of the study is that the growth performance in the FO3 group was roughly 30% higher than that of the control group. Considering all findings in the present study, we conclude that 0.3% supplementation of fumitory extract would be beneficial for rainbow trout farming with respect to elevated growth and antioxidant status.

Keywords: Growth promoter, Supplement, Organic, Medicinal plant, Feed additive

1. Introduction

Increasing world population brings along a huge demand of food worldwide (Salem et al. 2021). Undoubtedly, one of the most popular sources to meet up the food demand is seafood. In this context, the fact is that approximately 60% of animal protein requirement will be fulfilled by seafoods, especially after 2050s, which indicates the worth of aquatic food production systems. The limited or static production trend of fish products from capture fisheries stresses that production must be enhanced from aquaculture. In recent years, with the advancement in technology and application of technologies, there has been enhancement in seafood production through aquaculture (Bilen et al. 2015). However, in intensive aquaculture systems, there are emergence of several subsequent factors, such as impaired animal welfare and physiological functions, stress on species under culture as well as environmental degradation. Today, fish producers use vaccines, synthetic drugs and chemicals in order to prevent such losses and make production system efficient (Lalumera et al. 2004; Ji et al. 2007; Larragoite et al. 2016; Karga et al. 2020). However, there exist several adverse effects of these synthetic and chemical products on farmed animals (Serrano 2005; Cabello 2006; Defoirdt et al. 2011). In addition, similar negative impacts have been indicated on aquatic organisms (Defoirdt et al. 2007; Baquero et al. 2008). To address these issues, in recent years, a great number of studies have intensively focused on application of organic-based feed additives that have stress relieving, anti-inflammatory, immunostimulant, antifungal, and antimicrobial properties (Asadi et al. 2012; Balamurugan et al. 2016; Hernandez et al. 2016; Kirubakaran et al. 2016; Mohamed et al. 2018; Taştan & Salem 2021; Sönmez et al. 2022). Among various organic-origin products, microorganisms, plants, plant extracts and essential oils have been tested in several recent studies as alternatives to drugs, chemicals and antibiotics (Bilen et al. 2019, 2020; Arslan et al. 2018; Sönmez et al. 2015a, 2021; Elbesthi et al. 2020; Yilmaz et al. 2019, 2020; Amoush et al. 2021; Lakwani et al. 2021).

Fumitory (*Fumaria officinalis*) is one of the 55 plant species in Fuminaria family (Mitich, 1997). These plants are distributed in West and Middle Europe with 17 species found in Turkey (Rehman et al. 2013). Fumitory, known as a medicinal plant, is used as a digestive stimulant and diuretic (Sajjad et al. 2015), with antimicrobial properties (Bisset & Wichtl 2001). Fumitory contains isoquinolin as the dominant alkaloid (Sajjad et al. 2015), covering a total phenolic content of 10.5 mgGAE g^{-1} (dry weight) with 78.9% antioxidant activity (Şengül et al. 2009). It is speculated that with all these properties, this medicinal plant may have immune stimulating and antioxidant effects.

In this context, effects of aqueous methanolic extract of fumitory (*F. officinalis*) on growth performance, haematology, immune responses and antioxidant activities on rainbow trout (*Oncorhynchus mykiss*) have been evaluated in this study. The main purpose of this study therefore was to assess a novel immune stimulating and antioxidant product that is easy to procure and prepare, and has potential application in aquaculture industry.

2. Material and Methods

2.1. Experimental design

The study was conducted at Kastamonu University Inland and Marine Fish Production, Application and Research Centre using 12 net cages (1.5×1.5×1.5 m) designated as 4 treatments with 3 replicates. A total of 480 rainbow trout juveniles (mean initial weight: 13.02±0.02 g) were stocked into cages as 40 fish per cage. Aqueous methanolic extract of *Fumaria officinalis* was added to feeds by spraying at three levels, 0.1% (FO1), 0.2% (FO2) and 0.3% (FO3). The fish were fed *ad libitum* twice a day for 75 days. Liver tissue and blood (using heparinized syringes) were sampled from 3 fish per cage every 15 days. Immunological and haematological changes were evaluated in blood samples, whereas antioxidant activities were determined using liver samples.

2.2. Extraction method

Leaves of the plant were powdered subsequent to drying under shade. Powdered leaves (100 g) were mixed with 1 L of 40% methanol. Then the mixture was stored in a dark place for 72 days and mixed by inverting twice a day during this period. The mixture was filtered to remove particulates and the solvent was evaporated completely using an evaporator at 75 °C. To determine the exact amount of extract, 50 mL of distilled water was heated at 50 °C, added to the extract present in the evaporator flask, and the total amount of extract was determined by weighing the sample. Finally, extract was prepared in phosphate buffered saline (PBS) as per the dose quantity for this study, prepared solutions were kept at -20 °C until use and at +4 °C during the study period (Bilen et al. 2016). Analysis of composition of *F. officinalis* extract was carried out according to Özkan et al. (2017) using GC-MS QP2010 Ultra (Shimadzu) and results are presented in Table 1.

2.3. Calculation of growth parameters

The fish were weighed prior to the feeding trial and at the end of the study. Growth performances of the fish were computed as per the formulas given below:

$$Weight\ gain\ (WG) = 100\ x\ \frac{Wf-Wi}{W_i} \quad (1)$$

$$Specific\ growth\ rate\ (SGR) = 100\ \times\ \frac{\ln Wf - \ln Wi}{days} \quad (2)$$

$$Food\ conversion\ rate\ (FCR) = \frac{Feed\ intake}{Weight\ gain} \quad (3)$$

Where; Wf: Final weight, Wi: Initial weight.

2.4. Determination of haematological indices

Total erythrocyte count, haematocrit levels and haemoglobin amount were determined according to Blaxhall & Daisley (1973). Mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration values were computed by the formulas described by Lewis et al. (2006).

Table 1- Composition of the plant (*F. officinalis*) extract used in this study

Peak#	*Area%*	*Name*
1	1.15	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-
2	0.92	Ethyl n-propyl ketone
3	4.18	Hydroperoxide, 1-ethylbutyl (CAS)
4	4.74	Ethanol, 2-(hexyloxy)-
5	0.54	3-HEXEN-2-ONE
6	0.18	Hexanoic acid, 2-ethyl- (CAS)
7	0.76	2,4-Decadienal, (E,Z)- (CAS)
8	1.22	Deca-2(E),4(E)-dienal
9	1.94	Tetradecane
10	0.20	Heptadecane, 2,6,10,14-tetramethyl-
11	0.29	Cyclohexanone, 2-(hydroxymethyl)-
12	0.55	Dodecanoic acid (CAS)
13	0.60	1-Tetradecanol
14	1.08	Hexadecane
15	1.22	Dodecanoic acid, 1-methylethyl ester
16	0.25	E-14-Hexadecenal
17	0.33	Heptadecane
18	2.16	Tetradecanoic acid
19	0.34	Octadecane (CAS)
20	0.99	Pentadecanoic acid
21	0.83	8-(2-Acetyloxiran-2-yl)-6,6-dimethylocta-3,4-dien-2-one
22	35.99	Oleic Acid
23	15.21	Palmitic acid
24	9.09	Octadecanoic acid (CAS)
25	0.50	11,14-Eicosadienoic acid, methyl ester
26	0.34	10-Octadecenoic acid, methyl ester
27	8.60	9,12-Octadecadienoic acid (Z,Z)-
28	4.50	Propiolic acid
29	0.71	Hexadecanoic acid, butyl ester
30	0.59	Bicyclo[4.3.0]nonan-1-ol, 7,9-bis(methylene)-2,2,6-trimethyl-
TOTAL	100	

2.5. Determination of non-specific immune parameters

Oxidative radical production was analyzed as described by Siwicki & Anderson (1993). Briefly, 0.1 mL of blood was mixed with 0.1 mL solution containing 0.2% nitroblue tetrazolium (NBT) and incubated for 30 min at 25 °C. Fifty microliter suspension was taken from the mixture and 1 mL of N, N-dimethyl formamide was added on top of it. The mixture was centrifuged at 3000 g for 5 min. Finally, the mixture was transferred to a clean tube and absorbance was recorded against N, N-dimethyl formamide blank at 540 nm. Results were calculated by multiplying with 4.

Lysozyme activity was determined by slightly modifying the method of Ellis (1990). Accordingly, 100 µl *Micrococcus lysodeikticus* suspension (prepared by dissolving 0.02 g *M. lysodeikticus* bacterial cells, Sigma-Aldrich in 100 mL phosphate buffered saline solution) was mixed with 10 µl of fish plasma in 2 replicates. Changes in absorbance values at 530 nm were recorded at 0 and 4 min.

The method described by Sahoo et al. (2005) was modified in order to determine myeloperoxidase level. Briefly, 10 µl of serum was taken from the samples and transferred to the wells with 2 replicates. Ca^{+2} and Mg^{+2} free Hank's Balanced Salt Solution (HBSS) at 125 µl was added to wells. Then 35 µl of TMB substrate solution (Sigma-Aldrich) and 35 µl of 5 mM fresh hydrogen peroxide (Sigma-Aldrich) were added. Finally, changes in absorbance values were recorded at 450 nm at 0 and 4.5 min.

2.6. Analysis of antioxidant enzyme activity

Antioxidant enzyme activities of fish were determined in liver tissues on 15, 30, 45, 60 and 75th day using commercial kits according to instructions of the manufacturers: Superoxide dismutase (SIGMA SOD Assay Kit, Item no: 19160-1KT-F); Catalase (CAYMAN Catalase Assay Kit, Item no: 707002); Glutathione peroxidase (CAYMAN Glutathione Peroxidase Assay Kit, Item no: 703102); Glucose-6-phosphate dehydrogenase (SPI-BIO G6PDH Assay Kit, Item no:0112); Lipid peroxidation (CAYMAN TBARS Assay Kit, Item no: 10009055).

2.7. Statistical analysis

Variances in obtained data for different parameters were determined using one-way ANOVA. In order to find out any difference present between groups, Duncan's multiple range test was employed. The level of significance was set at 95%. All analyses were conducted using SPSS for Windows (Version 22, IBM).

3. Results

The changes in growth performance of fish are summarized in Table 2. From the growth performance data obtained by the end of the feeding trial (day-75), the highest weight increase was observed in rainbow trout fed with 0.3% *F. officinalis* extract.

Table 2- Growth performances of rainbow trout (*Oncorhynchus mykiss*) fed with *Fumaria officinalis* supplemented feed in different doses

Groups	*Initial Weight (g)*	*Final Weight (g)*	*Weight Gain (%)*	*SGR*	*FCR*
Control	12.45±0.2	69.14±0.3^{b}	454.94±7.86^{b}	2.28±0.01^{b}	1.4±0.007
FO1	12.34±0.16	65.10±0.5^{b}	427.99±8.96^{b}	2.22±0.02^{b}	1.4±0.01
FO2	12.10±0.04	68.78±0.32^{b}	468.89±38.42^{b}	2.31±0.09^{b}	1.43±0.03
FO3	12.23±0.07	87.88±0.2^{a}	618.18±44.62^{a}	2.62±0.08^{a}	1.35±0.3

SGR: Specific growth rate; FCR: Food conversion ratio. Different superscript letters in the same column show significant differences between groups (P<0.05). Data are expressed as mean±SE.

As evident from Table 2, the highest final weight was determined in the FO3, followed by FO2, control and FO1 groups, respectively (P<0.05). Although, there was no significant difference between control, FO1 and FO2 groups, the final weight of fish in the FO3 group was significantly higher than other groups (P<0.05). Similarly, weight gain (%) and SGR of the FO3 group were significantly higher compared to other groups (P<0.05). Regarding FCR, it was observed that although the FO3 group had lower FCR in comparison with control and other treatment groups, this difference was statistically insignificant (P>0.05).

Changes in the oxidative radical production (ORP) were determined from blood samples collected on 15, 30, 45, 60 and 75th days. Results obtained are provided in Figure 1. On the 15, 30 and 45th day of the study, although there were variations in the ORP of rainbow trout fed with FO extract, these differences were not statistically significant (P>0.05). Results of day 60 revealed that control group had the highest ORP level (1.03±0.22 mg/mL), whereas, ORP levels in treatment groups did not differ. At the last sampling day of the study, the highest value was recorded in the control group (4.63±0.23 mg/mL), followed by the FO1 (4.31±0.29 mg/mL), FO2 (3.7±0.3 mg/mL) and FO3 (3.63±0.24 mg/mL) groups, respectively. In regards to the statistical analyses, FO1 was found to be similar to the control group (P>0.05), while FO2 and FO3 treatment groups exhibited lower ORP (P>0.05).

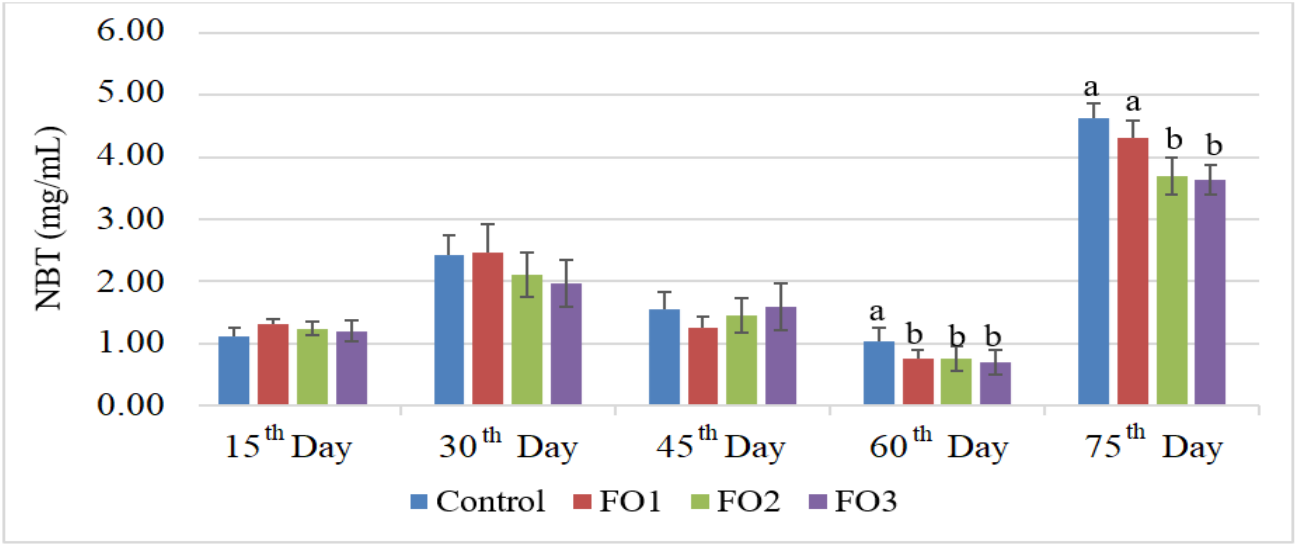

Figure 1 - Changes in oxidative radical production of rainbow trout (*Oncorhynchus mykiss*) fed with *Fumaria officinalis* supplemented feed in different doses. Different lowercase letters on the bars show significant differences between groups at a particular sampling time (n=3).

Changes in the lysozyme activity of rainbow trout are presented in Figure 2. It was observed that although lysozyme activity varied between groups, these differences were not significant (P>0.05) except for the FO1 group. On the 60th day, lysozyme activity in FO1 group increased significantly (P<0.05) compared to the control and other experimental groups.

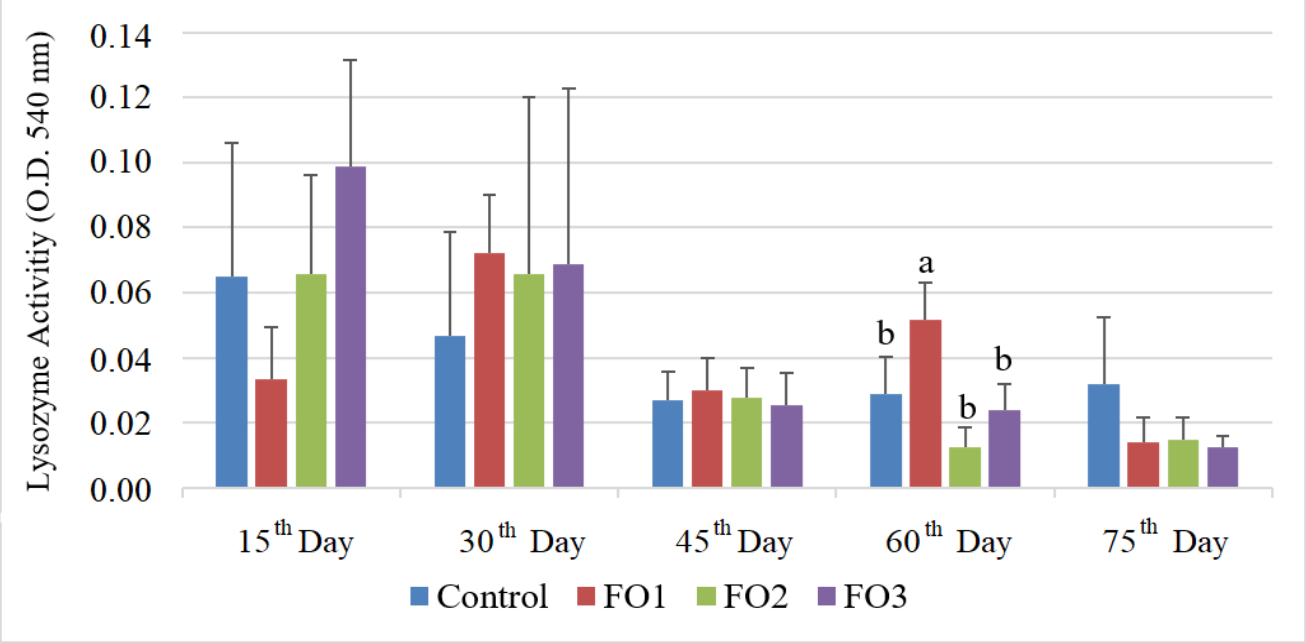

Figure 2- Changes in lysozyme activity of rainbow trout (*Oncorhynchus mykiss*) fed with *Fumaria officinalis* supplemented feed in different doses. Different lowercase letters on the bars show significant differences between groups at a particular sampling time (n=3)

Myeloperoxidase (MPO) activity was determined from the serum of fish every 15 days throughout the study and the results are presented in Figure 3. On the 15th day of the study, MPO was found as 22.53±6.77 in FO3, 18.09±10.71 in FO1, 10.63±4.06 in FO2 and 10.56±1.83 in the control group. The highest MPO activity was recorded in FO3 group followed by the FO1 group (P<0.05). In addition, although the lowest MPO activity was detected in the control group, no difference was found between control and FO2 groups (P>0.05). On the 30th day, MPO activities of the experimental groups were 50.47±23.01, 45.04±13.66, 36.80±12.27 and 19.45±13 in the FO1, control, FO2 and FO3 groups, respectively. Although, the highest myeloperoxidase value was recorded in FO1 group, it was not significantly different from that of the control group (P>0.05). Moreover, MPO values did not differ among the experimental groups on 45, 60 and 75th days of the study.

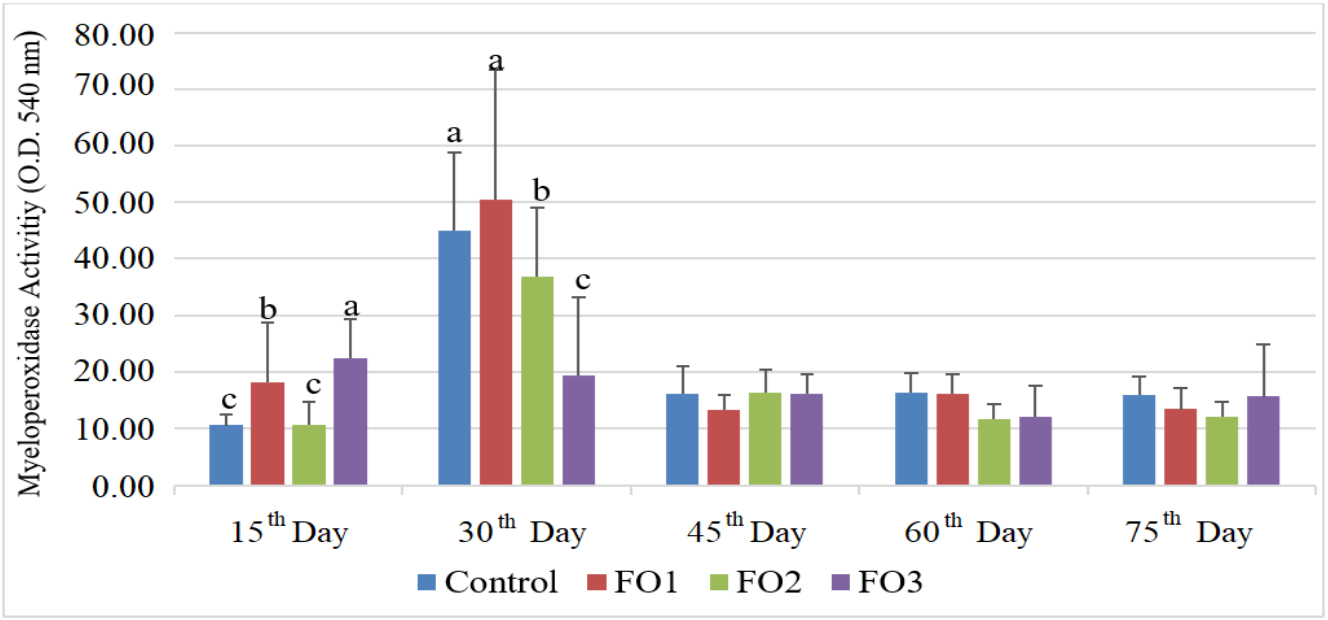

Figure 3- Changes in myeloperoxidase activity of rainbow trout (*Oncorhynchus mykiss*) fed with *Fumaria officinalis* supplemented feed in different doses. Different lowercase letters on the bars show significant differences between groups at a particular sampling time (n=3)

During the study, changes in haematological parameters were also determined from blood samples collected from fish at 15-day intervals (Table 3). Mean corpuscular haemoglobin concentration (MCHC) was the only parameter that did not differ between groups at any sampling time. On 15th day of the study, red blood cell (RBC) value of FO1 was similar to control ($P>0.05$), whereas, FO2 and FO3 groups presented decreased RBC counts ($P<0.05$). While, no change was observed in the haemoglobin (HGB) content between groups, haematocrit (HCT) levels of FO2 and FO3 groups decreased. None of the other parameters were affected on day 15.

Table 3- Blood parameters of rainbow trout (*Oncorhynchus mykiss*) fed with *Fumaria officinalis* supplemented feed in different doses

Day	*Groups*	*RBC*	*HGB*	*HCT*	*MCV*	*MCH*	*MCHC*
15	**Control**	1.19±0.03^{a}	7.63±0.45	26±0.81^{a}	217.82±3.29	67.93±1.50	67.93±0.36
	FO1	1.23±0.03^{a}	7.93±0.29	26.12±0.46^{a}	215.32±2.05	68.48±1.74	68.48±0.90
	FO2	1.01±0.04^{b}	7.70±0.37	22.50±0.83^{b}	222.26±1.72	73.01±1.28	73.01±0.37
	FO3	0.94±0.03^{b}	7.20±0.28	21.22±0.84^{b}	226.49±1.86	79.17±1.39	79.17±0.56
30	**Control**	0.37±0.04^{b}	7.27±0.02^{a}	16.75±3.08^{b}	229.22±1.92^{b}	230.19±1.03^{a}	31.88±0.00
	FO1	1.03±0.04^{a}	7.92±0.22^{a}	23.12±0.86^{a}	241.97±0.74^{a}	77.43±1.34^{b}	34.34±0.48
	FO2	0.96±0.04^{a}	5.67±0.36^{b}	20.47±1.23^{a}	248.95±4.36^{a}	62.26±0.00^{b}	29.46±0.00
	FO3	1.04±0.04^{a}	7.72±0.37^{a}	23.52±0.81^{a}	245.38±1.57^{a}	73.92±1.73^{b}	32.78±0.83
45	**Control**	1.23±0.03^{a}	8.10±0.45	28.15±0.81^{a}	229.22±1.92^{a}	65.87±2.82^{c}	28.71±1.06
	FO1	1.01±0.03^{b}	7.52±0.23	24.44±0.81^{b}	241.97±0.74^{b}	74.72±1.14^{b}	30.88±0.46
	FO2	0.96±0.04^{b}	7.58±0.47	25.20±1.63^{b}	248.95±4.36^{b}	74.65±2.31^{b}	29.56±1.01
	FO3	0.88±0.04^{c}	7.53±0.14	22.47±0.59^{b}	245.38±1.57^{b}	86.45±4.40^{a}	32.64±0.94
60	**Control**	1.11±0.08^{b}	8.10±0.15^{a}	26.1±1.81^{a}	225.19±6.28^{b}	53.15±15.70^{b}	31.57±1.98
	FO1	0.90±0.02^{b}	6.36±0.36^{b}	22.1±1.81^{b}	225.97±7.52^{b}	67.09±4.01^{a}	29.12±1.06
	FO2	0.96±0.06^{b}	5.27±0.64^{c}	23.13±1.57^{b}	240.06±2.06^{a}	63.75±1.15^{a}	26.54±0.27
	FO3	1.28±0.03^{a}	7.80±0.35^{a}	29±1.12^{a}	226.09±4.28^{b}	60.81±1.76^{a}	26.87±0.38
75	**Control**	1.45±0.10	8.95±0.15^{a}	34.83±2.06^{a}	240.88±2.60	62.69±3.49	25.98±1.18
	FO1	1.31±0.04	7.66±0.23^{b}	28.97±2.07^{b}	237.71±3.37	58.76±1.26	24.73±0.49
	FO2	1.28±0.06	6.85±0.20^{b}	30.57±1.34^{b}	238.83±1.71	54.02±2.39	22.58±0.85
	FO3	1.24±0.05	6.82±0.11^{b}	29.28±1.20^{b}	235.68±1.18	55.33±2.05	23.47±0.84

RBC: Red blood cell, HGB: Haemoglobin, HCT: Haematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration. Different superscript letters in the same column show significant differences between groups at a particular sampling time. Data are expressed as mean±SE.

On day 30, RBC count in all treatment groups was higher than the control ($P<0.05$). HGB value of FO2 was lower than that of control, while HGB values of FO1 and FO3 groups were not different. In contrast, all treatment groups exhibited an increase in HCT compared to the control group. Similarly, MCV values of fish in all treatment groups were higher than that of the control. In addition, all experimental groups presented decreased MCH values.

RBC, HCT and MCV values of all the treated groups were lower than that of control on 45th day of the study ($P<0.05$). HGB values did not differ among groups ($P>0.05$). In contrast, all treated groups exhibited increased MCH values.

When the haematological results of the 60th day were examined, it was noticed that FO3 group had the highest RBC count ($P<0.05$), while other treatment groups had similar counts compared to the control ($P>0.05$). HGB contents of FO1 and FO2 groups were lower than that of the control, whereas HGB of FO3 group was not different. Similarly, FO1 and FO2 groups presented decreased HCT values. In case of MCV, an increase was found only in the FO2 group, compared to the control. MCH values of all treatment groups were higher than that of the control group ($P<0.05$).

On the 75th day, the highest HGB value was recorded in the control group ($P<0.05$). While similar results were observed for HCT levels, no differences were found in other parameters examined in the study ($P>0.05$).

Changes in superoxide dismutase (SOD) activity determined at the end of the study are presented in Figure 4. It was observed that SOD activity did not differ among the groups at any sampling time ($P>0.05$).

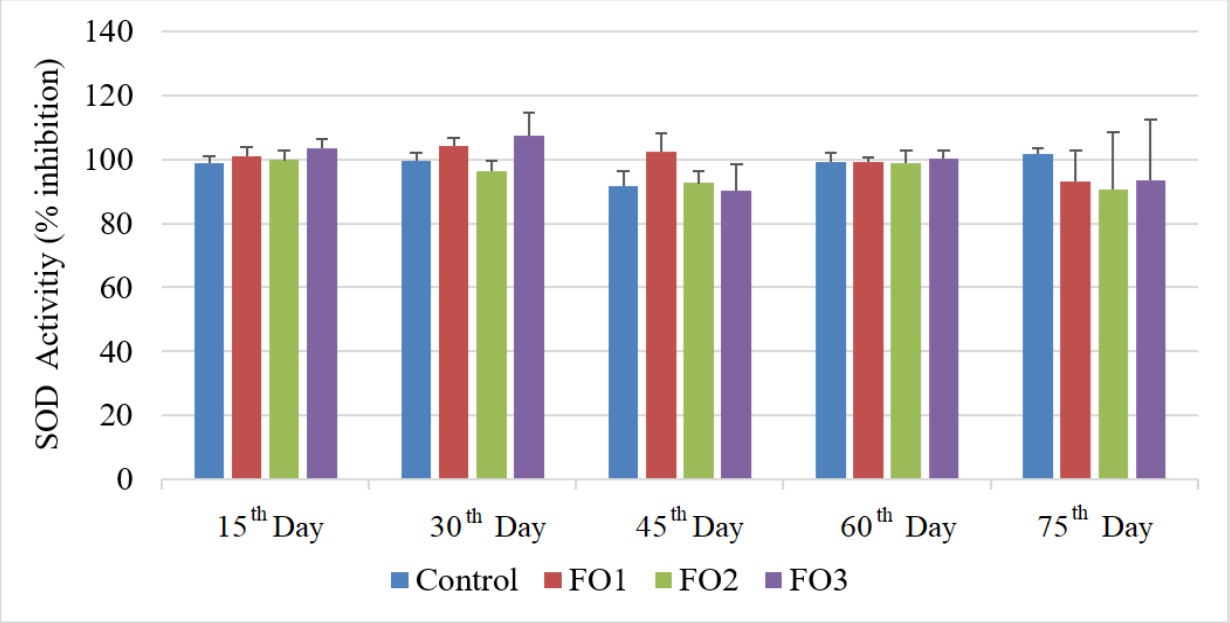

Figure 4- Changes in liver superoxide dismutase activity of rainbow trout (*Oncorhynchus mykiss*) fed with *Fumaria officinalis* supplemented feed in different doses (n=3)

Results of catalase (CAT) activity are presented in Figure 5. On 15th day of the study, no difference in CAT was observed among treated and control groups (P>0.05). On 30th day, the highest CAT value was observed in FO2 group (P<0.05). CAT activity of FO3 group did not differ in comparison to control, and the lowest CAT activity was found in the FO1 group. Results of day 45 exhibited that all treated groups had lower CAT activity than that of the control. On the 60th day, highest CAT activity was observed in FO2 group, followed by FO1, control and FO3 groups, respectively. Finally, at the last sampling time, all treated groups had decreased CAT activities in comparison to control group.

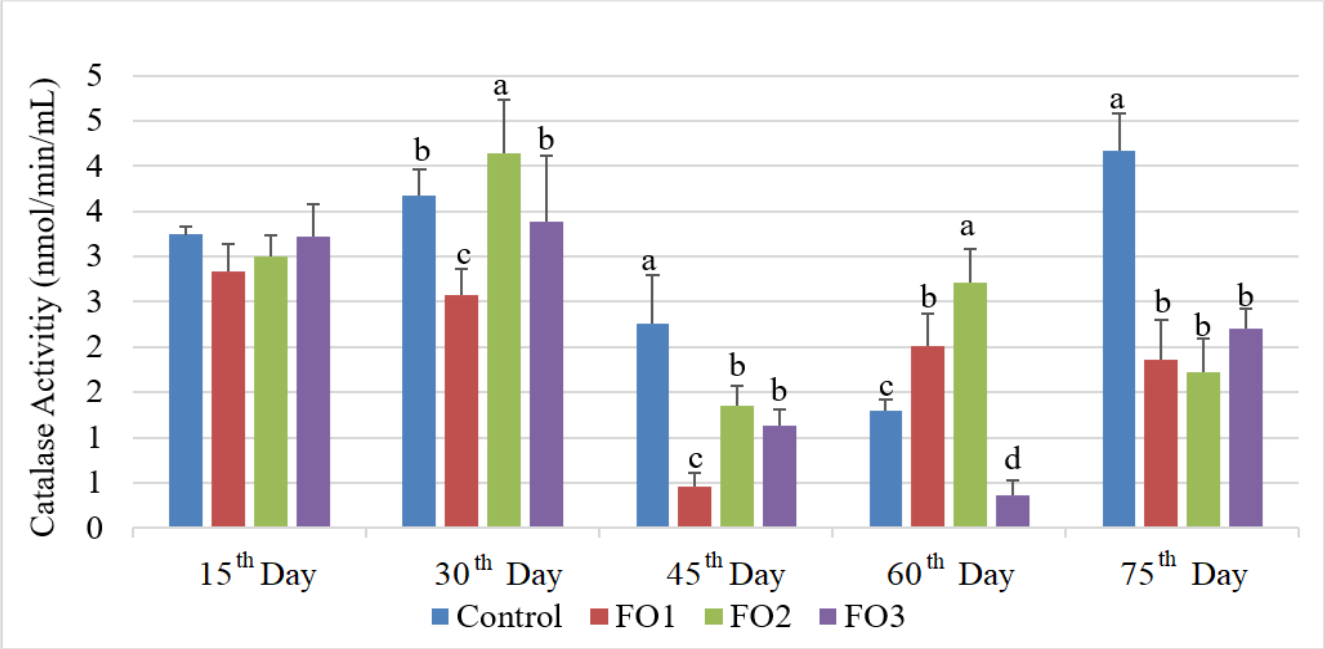

Figure 5- Changes in liver catalase activity of rainbow trout (*Oncorhynchus mykiss*) fed with *Fumaria officinalis* supplemented feed in different doses. Different lowercase letters on the bars show significant differences between groups at a particular sampling time (n=3)

Results of glutathione peroxidase (GPx) activity obtained from liver samples from fish on day 15, 30, 45, 60 and 75 are provided in Figure 6. There were no differences in GPx activities among groups on the 15, 30 and 45th days of the study (P> 0.05). On day 60, the highest GPx activity was observed in the control group followed by FO1, FO2 and FO3 groups, respectively (P<0.05). Furthermore, at the last sampling time, all treated groups had increased GPx activities compared to the control, where the highest value was in FO3 group.

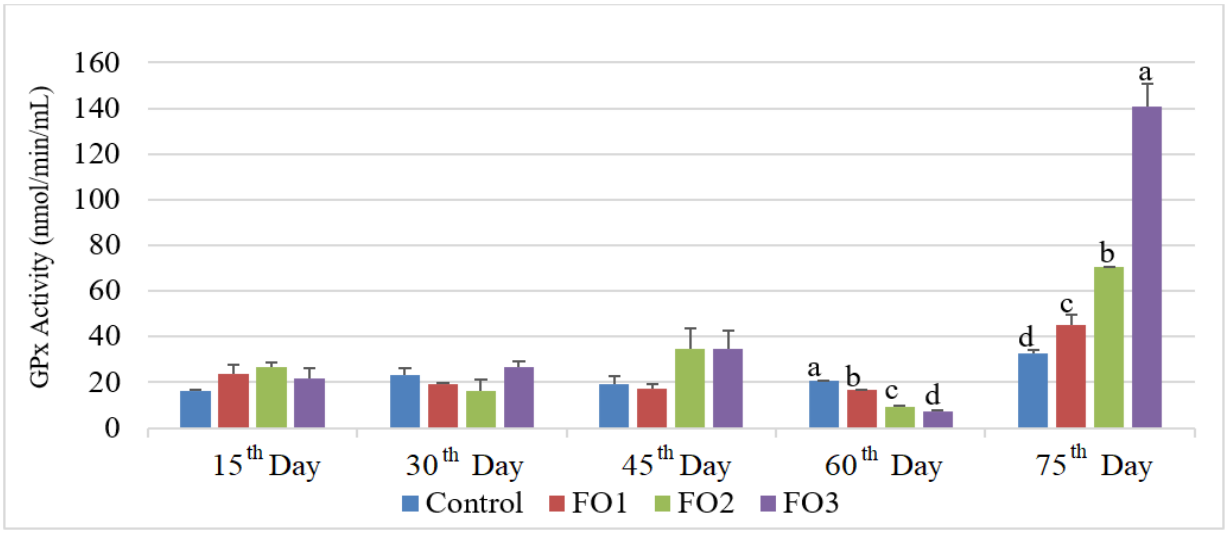

Figure 6- Changes in liver glutathione peroxidase activity of rainbow trout (*Oncorhynchus mykiss*) fed with *Fumaria officinalis* supplemented feed in different doses. Different lowercase letters on the bars show significant differences between groups at a particular sampling time (n=3)

Results of glucose-6-phosphate dehydrogenase (G6PDH) activities determined from liver samples collected from fish on day 15, 30, 45, 60 and 75 are presented in Figure 7. Regarding G6PDH activity, there was no difference observed between groups on 15, 30 and 60th days (P>0.05). On the contrary, control group showed the highest G6PDH activity on day 45 in comparison to all treated groups (P<0.05). In contrast, all the treated groups had increased G6PDH activities on the 75th day with significantly higher levels found in FO2 and FO3 groups compared to the control.

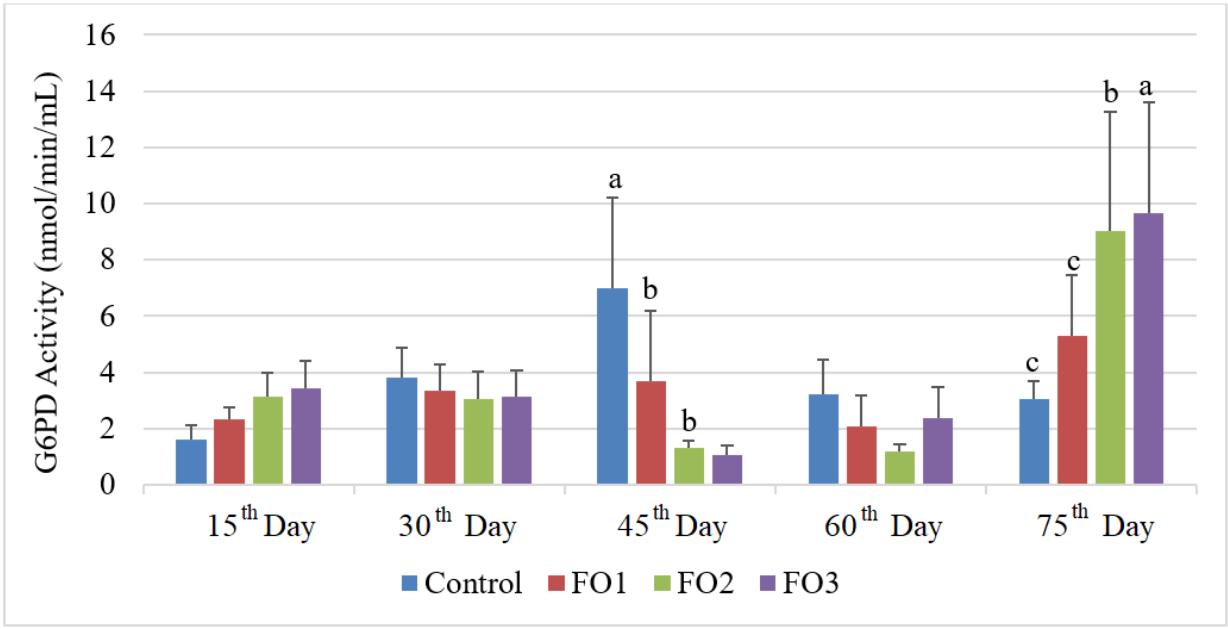

Figure 7- Changes in liver glucose-6-phosphate dehydrogenase activity of rainbow trout (*Oncorhynchus mykiss*) fed with *Fumaria officinalis* supplemented feed in different doses. Different lowercase letters on the bars show significant differences between groups at a particular sampling time (n=3)

Changes in lipid peroxidation (LPO) determined using liver samples collected from fish at 5 sampling times are presented in Figure 8. Although, there were slight variations in LPO values among groups, no difference was observed at any sampling time (P>0.05).

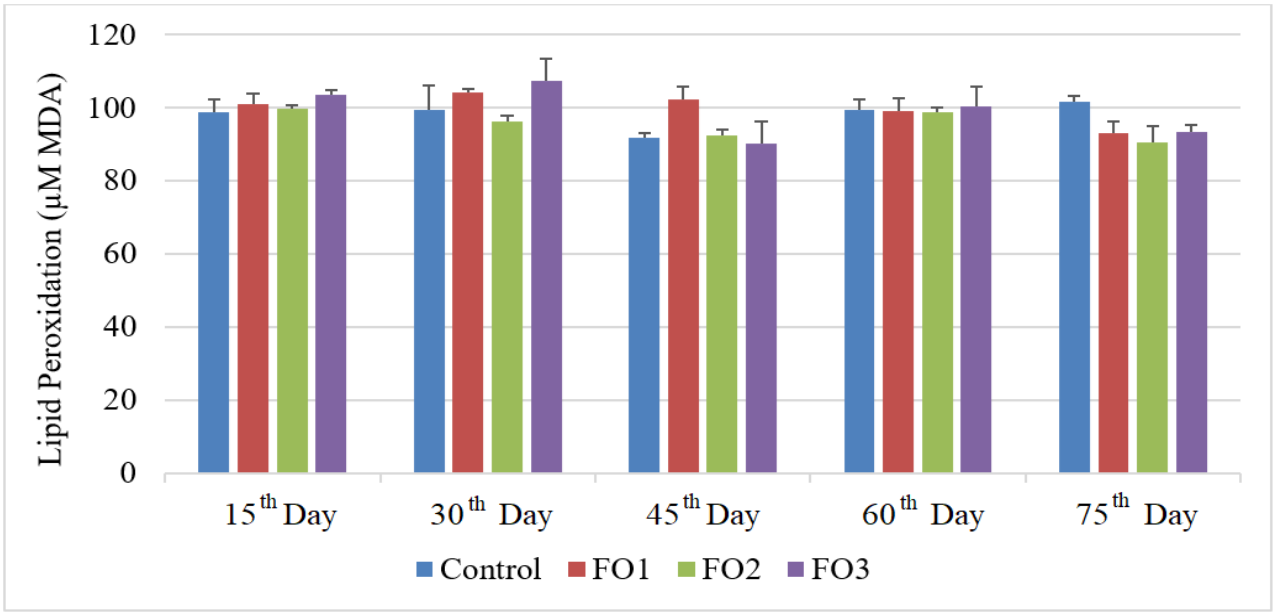

Figure 8- Changes in liver lipid peroxidation of rainbow trout (*Oncorhynchus mykiss*) fed with *Fumaria officinalis* supplemented feed in different doses (n=3)

4. Discussion

In the present work, it was observed that humoral immune responses did not change at early stages but decreased in long term, whereas antioxidant parameters were elevated at the later part of the experiment. It was noticed that control group exhibited more effective results on blood parameters. Furthermore, a significantly increased growth performance was recorded in the FO3 group. Therefore, it was inferred that the use of *F. officinalis* at 0.3% could substantially increase growth of rainbow trout.

Growth performance results of the study indicated that the best performance was obtained by the FO3 group, presenting a mean body weight of 87.88±0.2 g with an elevated growth performance around 30%. Another important point here is that the FCR decreased but not significantly ($P>0.05$), with the increase of fish growth. Several studies reported that the use of plant extracts affects growth of fish (Awad & Awad 2017). Moreover, this effect may depend on dose, feeding duration, species and physiological conditions (Harikrishnan et al. 2011, Gannam & Schrock, 1999). Elevated digestive enzyme activities can also significantly enhance the growth performance of fish (Awad et al. 2012). In this context, it can be deciphered that the phenolic compounds or other functional ingredients contained in the aqueous methanolic extract of *F. officinalis* are effective on growth hormones that promotes a faster growth in fish. It can also be stated that components of the plant might have influenced utilization of feed by activating digestive enzymes. Similar to these results, Mahdavi et al. (2013) found that *Aloe vera* extract increased growth, weight gain and SGR of carp when administered at 0.1, 0.5 and 2.5% rates. In another study, Awad et al. (2012) reported that lupine, mango and nettle supplemented into feed increased weight gain, SGR and digestive enzyme activities in rainbow trout fed for two months. In contrast, kefir administration had no effects on growth performance of Çoruh trout, *Salmo coruhensis* (Can et al. 2012).

Reactive oxygen species (ROS) such as superoxide radicals emerge as a result of immune response and they play a vital role in elimination of pathogens. Removal of ROS is extremely important for the continuation of crucial physiological and metabolic activities. SOD enzyme performs the task of removing these anions and plays a significant role in the destruction of superoxide free radicals. Increase in SOD activity in the present study may indicate elevated superoxide radical production within the cells. In this mechanism, ORP activity gets involved. Moreover, increase in ORP indicates enhanced superoxide radical production by the cells, whereas, increase in SOD indicates enhanced catabolism of superoxide radicals and the cell is induced accordingly for this. Therefore, SOD and ORP should not be evaluated independently. Theoretically, when ORP increases, an increase in SOD activity is also expected.

ORP is one of the most important cellular activities that prevent growth and proliferation of pathogens in the body (Divyagnaneswari et al. 2007). In our study, ORP activity did not change or decrease at the end of the study. This could be attributed to the increased antioxidant responses. Previously, tetra, oyster mushroom and common nettle supplementation increased ORP in rainbow trout (Bilen et al. 2011; Bilen et al. 2016). Likewise, Nya & Austin (2009) demonstrated that ginger administration increased ORP in rainbow trout. Contrary to these results, Bilen & Bulut (2010) reported that rainbow trout fed with laurel leaf extract did not exhibit any change in intracellular ORP.

The innate immune system contains antiprotease, protease, lysozyme, antibodies, complement and lytic factors that are present in the serum to prevent growth of microorganisms and prohibit their adhesion to tissues (Alexander & Ingram 1992). From our lysozyme activity results, an increase was observed initially, but it decreased at the end of the study. In this context, it can be opined that *F. officinalis* exhibited lytic feature. It is to mention that lysozyme activity in generally enhanced in fish fed with medicinal plants (Abarike et al. 2019, Almabrok et al. 2018). This fact was demonstrated earlier in different fish species using various plants, such as *Astragalus membranaceus, Angelica sinensis* and *Crataegus hupehensis* in Nile tilapia (Abarike et al. 2019); *Achyranthes aspera* in rohu (Rao et al. 2006); *Solanum trilobatum* in Mozambique tilapia (Divyagnaneswari et al. 2007); *Astragalus radix* & *Ganoderma lucidum* in carp (Yin et al. 2009).

Myeleperoxidase (MPO) activity increased on day 15 and varied thereafter. This situation may be attributed to the antioxidant property of the *F. officinalis*. MPO has a significant role in killing microorganisms. Thus, increase in MPO indicates that the immune response is mounted. Similar to this study, Christybapita et al. (2007) did not obtain any change in myeloperoxidase activity in the Mozambique tilapia fed with *Eclipta alba* extract.

SOD activity did not vary during sampling times of the study. This might have caused due to ORP effects. In a previous study, carbamazepine administration significantly reduced SOD activity in rainbow trout (Li et al. 2010). It has been demonstrated that treatment with extract obtained from *Suaeda maritime* caused decreased SOD activity (Thirunavukkarasu et al. 2010). Moreover, Sönmez et al. (2015b) observed a reduction in SOD activity in rainbow trout fed with peppermint oil. In contrast, some studies described an increased SOD activity in fish fed with different plant extract supplementations (Thirunavukkarasu et al. 2010; Keleştemur & Özdemir 2013; Sönmez et al. 2015b).

CAT is an antioxidant enzyme that performs the task of catalysing the dismutation of superoxide radicals eliminated by SOD to H_2O_2 and it is found in intra-cellular peroxisomes. CAT activity is expected to elevate when SOD activity increases. In our study, CAT activity varied independently to SOD activity and increased in treated groups at different sampling times. Gülçin et al. (2009) observed an elevation in CAT activity of rainbow trout fed with melatonin. Similarly, kefir supplementation caused an increase in CAT activity of Çoruh trout (Can et al. 2012). Contrarily, Sönmez et al. (2015b) described that mint administration did not affect CAT activity, or it decreased depending on the time.

GPx catalyses the formation of NADPH and GSSG, which are essential for glutathione reductase enzyme to function. An increase in GPx activity of rainbow trout was observed at the end of the study. Based on this, it is inferred that GPx activity is positively influenced by the long-term use of *F. officinalis* extract. Similar to our results, Zhang et al. (2015) also demonstrated an increased GPx activity of Japanese seabass (*Lateolabrax japonicus*) fed with magnesium and vitamin E supplemented feeds. Moreover, thyme and sage supplementation caused an elevation in GPx activity in rainbow trout (Sönmez et al. 2015b). Contrary to these results, Li et al. (2010) reported a decreased GPX activity in rainbow trout due to carbamazepine administration.

G6PDH catalyses the pentose phosphate pathway by producing NADPH. Produced NADPH is essential for the glutathione reductase and CAT enzymes. Therefore, G6PDH enzyme is important for decomposition of H_2O_2. G6PDH activity generally increased only at the end of the study in all treated groups. These data support the elevation in GPx activity at the end of the study and agree with the work conducted by Sönmez et al. (2015b).

LPO is determined by estimating the amount of malondialdehyde (MDA) formed by the release of free oxygen radicals and is a highly important factor in determining oxidative stress. Basically, an increase in MDA level indicates elevated oxidative stress and LPO (Yagi 1984). In this study, *F. officinalis* administration did not affect MDA level in rainbow trout. Contrary to our results, MDA levels were found to be elevated in tilapia fed with selenium and alpha-tocopherol (Keleştemur & Özdemir (2013). Şahan et al. (2017) demonstrated a decrease in MDA level in rainbow trout fed with rosehip. Similarly, significantly decreased levels of MDA were detected in Mozambique tilapia (Amer 2016) and rainbow trout (Gülçin et al. 2009) fed with spirulina and melatonin supplemented diets, respectively.

5. Conclusions

Based on the findings of the present study, it is suggested that it is pertinent to feed rainbow trout with *Fumaria officinalis* in order to strengthen its antioxidant system. Dietary supplementation of *F. officinalis* at 0.3% rate for 90 days elevated growth performance by around 30%, which is the most notable outcome obtained in this study. In addition, use of this plant extract did not have any negative effect on fish survival rate or other immune and antioxidant responses. However, this work was limited to three different doses of the plant (0.1, 0.2, and 0.3%) and further studies should investigate higher doses.

Acknowledgements

This study was presented as the doctoral thesis of the first author at Kastamonu University, Institute of Science.

Ethics Approval Statement

Study protocol was approved in advance by Kastamonu University Local Ethics Committee of Animal Trials with the approval number of 2017.01.

References

Abarike E D, Jian J, Tang J, Cai J, Yu H & Chen L (2019). Traditional Chinese medicine enhances growth, immune response, and resistance to *Streptococcus agalactiae* in Nile Tilapia. *Journal of Aquatic Animal Health* 31(1): 46-55. https://doi.org/10.1002/aah.10049

Alexander J B & Ingram G A (1992). Noncellular nonspecific defence mechanisms of fish. Annual Review of Fish Diseases 2: 249-279. https://doi.org/10.1016/0959-8030(92)90066-7

Almabrok A A, Amhamed İ D, Mohamed G A, Bilen S & Altief T A S (2018). Effect of *Tilia tomentosa* methanolic extract on growth performance, digestive enzyme activity, immune response and haematological indices of common carp (*Cyprinus carpio*). Marine Science and Technology Bulletin 7(1): 12-20. https://doi.org/10.33714/masteb.421047

Amer S A (2016). Effect of *Spirulina platensis* as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile Tilapia (*Oreochromis niloticus*). Benha Veterinary Medical Journal 30(1): 1-10. https://doi.org/10.21608/bvmj.2016.31332

Amoush O A A, Bilen S, Sönmez A Y & Elp M (2021). Antioxidant and immunostimulant responses in rainbow trout (*Oncorhynchus mykiss*) fed with cherry stem extract. Aquaculture Research https://doi.org/10.1111/are.15594

Arslan G, Sönmez A Y & Yanık T (2018). Effects of grape *Vitis vinifera* seed oil supplementation on growth, survival, fatty acid profiles, antioxidant contents and blood parameters in rainbow trout *Oncorhynchus mykiss.* Aquaculture Research 49(6): 2256–2266. https://doi.org/10.1111/are.13686

Asadi M, Mirvaghefei A, Nematollahi M, Banaee M & Ahmadi K (2012). Effects of Watercress (*Nasturtium nasturtium*) extract on selected immunological parameters of rainbow trout (*Oncorhynchus mykiss*). Open Veterinary Journal 2(1): 32-39

Awad E & Awad A (2017). Role of medicinal plants on growth performance and immune status in fish. Fish & Shellfish Immunology 67: 40-54. https://doi.org/10.1016/j.fsi.2017.05.034

Awad E, Austin B & Lyndon A (2012). Effect of dietary supplements on digestive enzymes and growth performance of rainbow trout (*Oncorhynchus mykiss*, Walbaum). Journal of American Science 8(12): 858-864

Balamurugan J, Kumar T T A, Prakash S, Meenakumari B, Balasundaram C & Harikrishnan R (2016). Clove extract: A potential source for stress free transport of fish. Aquaculture 454: 171–175. https://doi.org/10.1016/j.aquaculture.2015.12.020

Baquero F, Martinez J L & Canton R (2008). Antibiotics and antibiotic resistance in water environments. Current Opinion in Biotechnology 19: 260–265. https://doi.org/10.1016/j.copbio.2008.05.006

Bilen S & Bulut M (2010). Effects of laurel (*Laurus nobilis*) on the non-specific immune responses of rainbow trout (*Oncorhynchus mykiss*, Walbaum). Journal of Veterinary and Animal Advances 9(8): 1275-1279. https://doi.org/10.3923/javaa.2010.1275.1279

Bilen S, Bilen A M & Önal U (2015). The effects of oxygen supplementation on growth and survival of rainbow trout (*Oncorhynchus mykiss*) in different stocking densities. Iranian Journal of Fisheries Sciences 14(3): 538-545

Bilen S, Bulut M & Bilen A M (2011). Immunostimulant effects of *Cotinus coggyria* on rainbow trout (*Oncorhynchus mykiss*). Fish & Shellfish Immunology 30(2): 451-455. https://doi.org/10.1016/j.fsi.2010.12.013

Bilen S, Kenanoglu O N, Terzi E, Ozdemir R C & Sonmez A Y (2019). Effects of tetra *(Cotinus coggygria)* and common mallow *(Malva sylvestris*) plant extracts on growth performance and immune response in Gilthead Sea bream *(Sparus aurata)* and European Sea bass *(Dicentrarchus labrax).* Aquaculture 512: 734251. https://doi.org/10.1016/j.aquaculture.2019.734251

Bilen S, Ünal S & Güvensoy H. (2016). Effects of oyster mushroom (*Pleurotus ostreatus)* and nettle (*Urtica dioica*) methanolic extracts on immune responses and resistance to *Aeromonas hydrophila* in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 454: 90-94. https://doi.org/10.1016/j.aquaculture.2015.12.010

Bilen S, Ispir S, Kenanoglu O N, Taştan Y, Güney K & Terzi E (2020). Effects of Greek juniper (*Juniperus excelsa*) extract on immune responses and disease resistance against *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*). *Journal of Fish Diseases* 44(6): 729-738. https://doi.org/10.1111/jfd.13293

Bisset N G & Wichtl M (2001). Herbal Drugs and Phytopharmaceuticals (2nd ed.). CRC Press, London.

Blaxhall P C & Daisley K W (1973). Routine hematological methods for use with fish blood. *Journal of Fish Biology* 5: 771–781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Can E, Kurtoğlu İ Z, Benzer F, Erişir M, Kocabaş M, Kızak V, Kayım M & Çelik H T (2012). The effects of different dosage of kefir with different durations on growth performances and antioxidant system in the blood and liver tissues of Çoruh trout (*Salmo coruhensis*). *Turkish Journal of Fisheries and Aquatic Sciences* 12: 277-283. https://doi.org/10.4194/1303-2712-v12_2_12

Cabello F C (2006). Heavy use of prophylactic antibiotics in aquaculture: A growing problem for human and animal health and for the environment. Environmental Microbiology 8(7): 1137-1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Christybapita D, Divyagnaneswari M & Michael R D (2007). Oral administration of *Eclipta alba* leaf aqueous extract enhances the nonspecific immune responses and disease resistance of *Oreochromis mossambicus.* Fish & Shellfish Immunology 23(4): 840-852. https://doi.org/10.1016/j.fsi.2007.03.010

Defoirdt T, Boon N, Sorgeloos P, Verstraete W & Bossier P (2007). Alternatives to antibiotics to control bacterial infections: Luminescent vibriosis in aquaculture as an example. Trends in Biotechnology 25: 472–479. https://doi.org/10.1016/j.tibtech.2007.08.001

Defoirdt T, Sorgeloos P & Bossier P (2011). Alternatives to antibiotics for the control of bacterial disease in aquaculture. Current Opinion in Microbiology 14: 251–258. https://doi.org/10.1016/j.mib.2011.03.004

Divyagnaneswari M, Christybapita D & Michael R D (2007). Enhancement of nonspecific immunity and disease resistance in *Oreochromis mossambicus* by *Solanum trilobatum* leaf fractions. Fish & Shellfish Immunology 23: 249–259. https://doi.org/10.1016/j.fsi.2006.09.015

Elbesthi R T A, Yürüten Özdemir K, Taştan Y, Bilen S & Sönmez A Y (2020). Effects of ribwort plantain (*Plantago lanceolata*) extract on blood parameters, immune response, antioxidant enzyme activities, and growth performance in rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry 46: 1295-1307. https://doi.org/10.1007/s10695-020-00790-z

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, SOS Publications, Fair Haven, pp 101-103.

Gannam A L & Schrock R M (1999). Immunostimulants in fish diets. Journal of Applied Aquaculture 9(4): 53-89. https://doi.org/10.1300/J028v09n04_06

Gülçin İ, Beydemir Ş, Hisar O, Köksal E & Reiter R J (2009). Melatonin administration increases antioxidant enzymes activities and reduces lipid peroxidation in the rainbow trout (*Oncorhynchus mykiss*, Walbaum) erythrocytes. *Turkish Journal of Veterinary and Animal Sciences* 33(3): 241-245. https://doi.org/10.3906/vet-0803-1

Harikrishnan R, Balasundaram C & Heo M S (2011). Impact of plant products on innate and adaptive immune system of cultured finfish and shellfish. Aquaculture 317: 1-15. https://doi.org/10.1016/j.aquaculture.2011.03.039

Hernandez A J, Romero A, Gonzalez-Stegmaier R & Dantagnan P (2016). The effects of supplemented diets with a phytopharmaceutical preparation from herbal and macroalgal origin on disease resistance in rainbow trout against *Piscirickettsia salmonis*. Aquaculture 454: 109–117. https://doi.org/10.1016/j.aquaculture.2015.12.016

Ji S C, Jeong G S, Im G S, Lee S W, Yoo J H & Takii K (2007). Dietary medicinal herbs improve growth performance, fatty acid utilization, and stress recovery of Japanese flounder. Fisheries Science 73: 70–76. https://doi.org/10.1111/j.1444-2906.2007.01303.x

Karga M, Kenanoğlu O N & Bilen S (2020). Investigation of antibacterial activity of two different medicinal plants extracts against fish pathogens. *Journal of Agricultural Production* 1(1): 5-7. https://doi.org/10.29329/agripro.2020.341.2

Keleştemur G T & Özdemir Y (2013). Effects of dietary viamin A and E on growth performance and antioxidant status in blood of juvenile rainbow trout (*Oncorhynchus mykiss*, W. 1792) exposed to flow rate stress. *Journal of Animal and Plant Sciences* 23(3): 821-827

Kirubakaran C J W, Subramani P A & Michael R D (2016). Methanol extract of *Nyctanthes arbortristis* seeds enhances non-specific immune responses and protects *Oreochromis mossambicus* (Peters) against *Aeromonas hydrophila* infection. Research in Veterinary Science 105: 243–248. https://doi.org/10.1016/j.rvsc.2016.02.013

Lakwani M A S, Kenanoğlu O N, Taştan Y & Bilen S (2021). Effects of black mustard (*Brassica nigra*) seed oil on growth performance, digestive enzyme activities and immune responses in rainbow trout (*Oncorhynchus mykiss*). Aquaculture Research 53(1): 300-313. https://doi.org/10.1111/are.15577

Lalumera G M, Calamari D, Galli P, Castiglioni S, Crosa G & Fanelli R (2004). Preliminary investigation on the environmental occurrence and effects of antibiotics used in aquaculture in Italy. Chemosphere 54(5): 661–668. https://doi.org/10.1016/j.chemosphere.2003.08.001

Larragoite E T, Tacchi L, LaPatra S E & Salinas I (2016). An attenuated virus vaccine appears safe to the central nervous system of rainbow trout (*Oncorhynchus mykiss*) after intranasal delivery. Fish & Shellfish Immunology 49: 351–354. https://doi.org/10.1016/j.fsi.2016.01.006

Lewis S M, Bain B J & Bates I (2006). Dacie and Lewis Practical Haematology. Churchill Livingstone Elsevier, Philadelphia

Li Z H, Li P & Randak T (2010). Effect of a human pharmaceutical carbamazepine on antioxidant responses in brain of a model teleost in vitro: An efficient approach to biomonitoring. Journal of Applied Toxicology 30(7): 644-648. https://doi.org/10.1002/jat.1534

Mahdavi M, Hajimoradloo A & Ghorbani R (2013). Effect of *Aloe vera* extract on growth parameters of common carp (*Cyprinus carpio*). *World Journal of Medical Sciences* 9: 55-60

Mohamed G A, Amhamed İ D, Almabrok A A, Barka A B A, Bilen S & Elbeshti R T (2018). Effect of celery (*Apium graveolens*) extract on the growth, haematology, immune response and digestive enzyme activity of common carp (*Cyprinus carpio*). Marine Science and Technology Bulletin 7(2): 51-59. https://doi.org/10.33714/masteb.457721

Mitich L W (1997). Fumitory (*Fumaria officinalis* L.). Weed Technology 11(4): 843-845. https://doi.org/10.1017/S0890037X00043542

Nya E J & Austin B (2009). Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases* 32(11): 971-977. https://doi.org/10.1111/j.1365-2761.2009.01101.x

Özkan O E, Güney K, Gür M, Pattabanoğlu E S, Babat E & Khalifa M M (2017) Essential oil of oregano and savory: Chemical composition and antimicrobial activity. Indian Journal of Pharmaceutical Education and Research 51(3): S205–S208. https://doi.org/10.5530/ijper.51.3s.13

Rao Y V, Das B K, Jyotyrmayee P & Chakrabarti R (2006). Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish & Shellfish Immunology 20(3): 263–273. https://doi.org/10.1016/j.fsi.2005.04.006

Rehman S, Latif A, Ahmad S & Khan A U (2013). Antibacterial screening of *Fumaria officinales* Linn. A drug of choice in complementary and alternative medicine. Retrieved in April, 15, 2021 from https://www.academia.edu/7487284/Antibacterial_Screening_of_Fumaria_officinales_Linn._A_Drug_of_choice_in_Complementary_and_Alternative_Medicine

Sahoo P K, Kumari J & Mishra B K (2005). Non- specific immune responses in juveniles of Indian major carp. *Journal of Applied Ichthyology* 21(2):151-155. https://doi.org/10.1111/j.1439-0426.2004.00606.x

Sajjad S, Aghajanshakeri S, Anousheh D & Mikaili P (2015). Ethno-botanical, bioactivities and medicinal mysteries of *Fumaria officinalis* (Common Fumitory). *Journal of Pharmaceutical and Biomedical Sciences* 5(11): 857-862

Salem M O A, Salem T A, Yürüten Özdemir K, Sönmez A Y, Bilen S & Güney K (2021). Antioxidant enzyme activities and immune responses in rainbow trout (*Onchorhynchus mykiss*) juveniles fed diets supplemented with dandelion (*Taraxacum ofcinalis*) and lichen (*Usnea barbata*) extracts. Fish Physiology and Biochemistry 47: 1053-1062. https://doi.org/10.1007/s10695-021-00962-5

Serrano P H (2005). Responsible use of antibiotics in aquaculture (No.469). Food & Agriculture Organization

Siwicki A K & Anderson D P (1993). Immunostimulation in fish: Measuring the effects of stimulants by serological and immunological methods. The Nordic Symposium on Fish Immunology, Lysekil, Sweden

Sönmez A Y, Bilen S, Albayrak M, Yılmaz S, Biswas G, Hisar O & Yanık T (2015a). Effects of dietary supplementation of herbal oils containing 1,8-cineole, carvacrol or pulegone on growth performance, survival, fatty acid composition, and liver and kidney histology of rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Turkish Journal of Fisheries and Aquatic Sciences* 15(4):813-819. https://doi.org/10.4194/1303-2712-v15_4_04

Sönmez A Y, Bilen S, Alak G, Hisar O, Yanık T & Biswas G (2015b). Growth performance and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*) juveniles fed diets supplemented with sage, mint and thyme oils. Fish Physiology and Biochemistry 41: 165–175. https://doi.org/10.1007/s10695-014-0014-9

Sönmez A Y, Bilen S, Taştan Y, Serag K J B, Toring C C, Romero J B, Kenanoğlu O N & Terzi E (2021). Oral administration of *Sargassum polycystum* extracts stimulates immune response and increases survival against *Aeromonas hydrophila* infection in *Oncorhynchus mykiss*. Fish & Shellfish Immunology 117: 291-298. https://doi.org/10.1016/j.fsi.2021.08.020

Sönmez A Y, Bilen S, Yürüten Özdemir K, Alagöz K, Özçelik H (2022). Effect of aqueous methanolic extract of pomegranate peel (*Punica granatum*) and veratrum (*Veratrum album*) on oxidative status, immunity and digestive enzyme activity in rainbow trout (*Oncorhynchus mykiss*). *Journal of Agricultural Sciences* https://doi.org/10.15832/ankutbd.870923

Şahan A, Duman S, Çolak S Ö, Çinar E & Bilgin R (2017). Determination of some hematological and non-specific immune defences, oxidative stress and histopathological status in rainbow trout (*Oncorhynchus mykiss*) fed rosehip (*Rosa canina*) to *Yersinia ruckeri*. *Turkish Journal of Fisheries and Aquatic Sciences* 17: 91-100. https://doi.org/10.4194/1303-2712-v17_1_11

Şengül M, Yıldız H, Güngör N, Çetin B, Eser Z & Ercişli S (2009). Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. *Pakistan Journal of Pharmaceutical Sciences* 22(1): 102-106

Taştan Y & Salem M O A (2021). Use of phytochemicals as feed supplements in aquaculture: A review on their effects on growth, immune response, and antioxidant status of finfish. *Journal of Agricultural Production* 2(1): 32-43. https://doi.org/10.29329/agripro.2021.344.5

Thirunavukkarasu P, Ramkumar L, Ramanathan T & Silambarasan D (2010). Anti oxidant activity of selected coastal medicinal plants. *World Journal of Fish and Marine Sciences* 2(2): 134-137

Yagi K (1984). Assay for plasma lipid peroxidase. Methods in Enzymology 109: 328-331. https://doi.org/10.1016/S0076-6879(84)05042-4

Yilmaz S, Ergun S, Çelik E Ş, Yigit M & Bayizit C (2019). Dietary trans-cinnamic acid application for rainbow trout (*Oncorhynchus mykiss*): II. Effect on antioxidant status, digestive enzyme, blood biochemistry and liver antioxidant gene expression responses. Aquaculture Nutrition 25(6): 1207-1217. https://doi.org/10.1111/anu.12935

Yilmaz S, Ergün S, Yigit M, Yilmaz E & Ahmadifar E (2020). Dietary supplementation of black mulberry (*Morus nigra*) syrup improves the growth performance, innate immune response, antioxidant status, gene expression responses, and disease resistance of Nile tilapia (*Oreochromis niloticus*). Fish & Shelfish Immunology 107(A): 211-217. https://doi.org/10.1016/j.fsi.2020.09.041

Yin G, Ardo L, Thompson K D, Adams A, Jeney Z & Jeney G (2009). Chinese herbs (*Astragalus radix* and *Ganoderma lucidum*) enhance immune response of carp, *Cyprinus carpio*, and protection against *Aeromonas hydrophila*. Fish & Shellfish Immunology 26(1): 140-145. https://doi.org/10.1016/j.fsi.2008.08.015

Zhang C X, Huang F, Li J, Wang L, Song K & Mai K S (2015). Interactive effects of dietary magnesium and vitamin E on growth performance, body composition, blood parameters and antioxidant status in Japanese seabass (*Lateolabrax japonicus*) fed oxidized oil. Aquaculture Nutrition 22(3): 708-722. https://doi.org/10.1111/anu.12393