Fish Physiol Biochem (2021) 47:1053–1062
https://doi.org/10.1007/s10695-021-00962-5

Antioxidant enzyme activities and immune responses in rainbow trout (*Onchorhynchus mykiss*) juveniles fed diets supplemented with dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) extracts

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Received: 25 October 2020 / Accepted: 4 May 2021 / Published online: 17 May 2021

Abstract In the present study, antioxidant activity, immune responses, and growth performance of rainbow trout (*Onchorhynchus mykiss*) juveniles fed with diets supplemented with dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) extracts were assessed. Four different concentrations of aqueous methanolic extract of the plants (0% (control), 0.1%, 0.5%, and 1% (D, dandelion; L, lichen) were added to the diets, and fish were fed for 75 days. On the 15th, 45th, and 75th day of the study, liver antioxidant enzyme activities were determined, and immune responses were determined every 15th day. The results showed that SOD activity increased in the fish group of 0.1% D on the 15th and 45th day compared to control; however, it was lower in all the lichen extract-treated groups than in control at almost all sampling times, except on the 15th day in the 0.1% L group. CAT activity showed an increased value ($P<0.05$) in 0.5% L and 1% L treated fish groups on the 15th day, in fish of 1% D and 1% L groups on 45th and on 75th day in 0.1% D group. GPX activity increased on the 15th day of the study in fish of 0.1% D group, on the 45th day in 1% D and 1% L groups and on the 75th day in fish of 0.5% D, 0.1% D, and 0.5% L groups ($P<0.05$). G6PDH enhanced in all treatment groups compared to control on the 15th day, except in 0.1% L and 0.5% L groups. An elevated G6PDH activity was also observed on the 75th day of the study in 0.5% D, 1% D, and 0.5% L fish groups. An increase on lipid peroxidation (LP) was observed in all L groups on the 45th day of the study. Lysozyme activity was determined to be the highest in 0.5% and 1% L on the 45th day, in 0.1% L on the 60th day and in the 0.5% L fish group on the 75th day compared to control ($P<0.05$). Myeloperoxidase was found to be the highest at the end of the study in 1% L fish group compared to the control ($P<0.05$). In conclusion, we suggest the use of dandelion to combat oxidative stress and to lower FCR and the use of lichen to modulate the immune response in rainbow trout. The use of such products will be economical for aquaculture and harmless for the environment.

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Keywords Rainbow trout · Medicinal plant · Antioxidant activity · Immune response · *Taraxacum officinalis* · *Usnea barbata*

Introduction

Increasing human population has heightened the demand for animal-derived protein. According to FAO (2018), it is estimated that animal-derived protein consumption per capita will shoot 40 kg in 2030 compared to a present day need of 20 kg per capita.

Aquaculture production takes a major place in meeting up the increasing demand for animal-derived protein. Therefore, intensification of production systems has taken a key role in boosting the aquaculture production. Though such intensification with the use of state of art technologies has increased yield per unit area (Bilen et al. 2013), the environmental consequences affect fish health, leading to damage to the environment and thus it leads to poor production (Sönmez et al. 2015a).

Immune system weakens as a result of metabolic disturbances in the antioxidant system or stress caused by production processes or environmental consequences, such as subjecting fish to handling stress and vaccination, abrupt changes in water temperature at different time periods (Elbesthi et al. 2020). These events jeopardize the fish health. Use of both immunostimulants and antioxidant additives in fish feeds has already shown promising prospects (Sönmez et al. 2015b). The use of antioxidant additives in fish feed has become the need of the hour to lower stress and overcoming health issues (Amhamed et al. 2018; Arslan et al. 2018).

Usnea barbata is a lichen of the Usneaceae family that grows epiphytically on trunks and branches of trees and has antimicrobial and antifungal activity (Madamombe and Afolayan 2003). The main ingredients of the plant are dibenzofuran derivative usnic acid and this plant is an important role against inflammation and shows anti-proliferative properties (Engel et al 2007). *Taraxacum officinale* has been used in folk medicine in the treatment of breast and uterus cancers, hepatic disorders, and inflammation (Mir et al. 2013).

In this study, changes in antioxidant enzyme status of rainbow trout juveniles (*Onchorhynchus mykiss*) were investigated after administering aqueous methanolic extracts of dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*). The primary objective of the study was to assess the antioxidant and immune enhancing properties of the herbs, dandelion, and lichen, which are plentiful in Turkey.

Materials and methods

Experimental protocol and fish

The study was carried out at Kastamonu University Inland Waters and Saltwater Fish Production, Application and Research Institute. In the experiment, 1050 number of rainbow trout (*Oncorhynchus mykiss*) juveniles (avg. wt. 12.14 ± 0.04 g) were randomly stocked in 21 net cages (1.5×1.5 m $\times 1.5$ m) in three replicates for a 75-day trial. During study periods, water temperature ranged between 16 and 10 °C. Water pH was 7.0 ± 0.2, whereas dissolved oxygen was 8.0 ± 0.9 mg/l. The fish were acclimatized for a week and fed with control pelleted feed without any additives.

Experimental design

In the study, dandelion (*T. officinalis*) and lichen (*U. barbata*) were used as feed supplements. These plants were collected from rural areas around Kastamonu province (41° 25′ 50 N, 33° 45′ 19 E) and all body parts of the plants dried under a shade. The dried plants were powdered and the aqueous methanolic extract was prepared as per Bilen et al. (2016). Briefly, dried 50 g of the plants were percolated with 1 L of 40% methanol. After solvent evaporated 50 mL of deionized water added to the mix and the weight of the extract was determined.

The dandelion (D) and lichen (L) methanolic extracts were added to the feeds in the ratios of 0.1, 0.5, and 1%. Throughout the study, the fish were fed ad libitum twice a day. Liver and white muscle samples were collected on the 15^{th}, 45^{th}, and 75^{th} days of the study in order to determine the antioxidant enzyme activities. For determination of immune responses, blood samples (200 µL) were collected from caudal vein every 15^{th} day in heparinized syringes. The samples were stored in sterilized vials at − 80 °C until further analysis.

Determination of enzyme activities

The liver tissues were soaked in distilled water to remove the blood and directly dropped into a liquid nitrogen tank within micro-tubes after drying. They were maintained at − 80 °C before analyses. Liver tissues were cut into 0.1-g pieces and homogenized in phosphate buffer containing 1 mL EDTA. Thoroughly homogenized tissue samples were centrifuged at 10,000 g for 45 min and the supernatants were used in the analyses (Vaglio and Landriscina 1999).

SOD activity was determined using SIGMA 19160-1KT-F SOD Assay Kit, (SIGMA, USA). For catalase activity, Cayman 707002 Catalase Assay Kit (Cayman, USA) was used. Similarly, for glutathione peroxidase activity, Cayman 703102 Glutathione Peroxidase Assay Kit (Cayman, USA), SPI-BIO 0112 G6PDH activity Assay Kit for glucose-6-phosphate dehydrogenase activity, Cayman 10009055 TBARS Assay Kit (Cayman, USA) was used to determine lipid peroxidation activity.

Immune status

Oxidative anion production was determined according to Siwicki et al. (1994). Lysozyme activity was determined according to Ellis (1990) and myeloperoxidase was determined according to Sahoo et al. (2005).

Growth performance

All fish were weighed at the beginning and end of the study. Growth performance was calculated according to the following formulas of Ricker (1979):

$$\mathrm{WG}(\%) = 100 \times \frac{\mathrm{Wf}-\mathrm{Wi}}{\mathrm{Wi}}$$

$$\mathrm{FCR} = \frac{\text{feed intake (g)}}{\text{weight gain (g)}}$$

$$\mathrm{SGR}\left(\frac{\%}{\text{day}}\right) = 100 \times \frac{\ln\mathrm{Wf}-\ln\mathrm{Wi}}{\text{days fed}}$$

where WG, weight gain; Wf, final weight; Wi, initial weight; FCR, feed conversion ratio; SGR, specific growth rate.

Ethical guideline

All experimental fish were handled according to the relevant international guidelines. The study protocol was approved in advance by the local Ethics Committee for Animal Research Studies at the Kastamonu University (KUHADYEK-2016.10).

Statistical analyses

The data were checked for normality before analysis. One-way analysis of variance (ANOVA) and LSD multiple range tests were performed to ascertain any difference in enzyme activity values among different treatment groups at any sampling time using SPSS for Windows v. 22.0 program (SPSS Inc., Chicago, IL, USA). Statistical analysis was performed in the same way for all parameters tested. The accepted level of significance was set at $P<0.05$.

Results

Trends in SOD activity were as seen during the study are summarized in Table 1.

The highest SOD activity was determined in the 0.1% D and 0.1% L fish groups and it was significantly lower ($P<0.05$) in fish groups of 0.5% L and 1% L compared to control during initial period of the study (15th and 45th day). Interestingly, at the end of the study, the highest SOD activity was observed in control group ($P<0.05$).

The results of the CAT activity are presented in Table 2.

Table 1 Effect of dietary supplementation of dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) aqueous methanolic extracts on rainbow trout (*Onchorhynchus mykiss*) liver superoxide dismutase activity throughout the experiment (U/ml)

Groups	Day 15	Day 45	Day 75
Control	79.57 ± 10.04^{c}	75.43 ± 12.20^{b}	126.92 ± 18.79^{a}
0.1% D	102.43 ± 9.59^{a}	105.66 ± 12.27^{a}	118.00 ± 20.84^{b}
0.5% D	80.39 ± 10.37^{c}	103.07 ± 11.27^{a}	93.63 ± 10.27^{dc}
1% D	78.21 ± 3.03^{c}	64.00 ± 14.29^{c}	57.42 ± 6.79^{e}
0.1% L	91.36 ± 1.32^{b}	31.62 ± 7.83^{d}	84.50 ± 8.28^{d}
0.5% L	13.08 ± 8.19^{e}	21.84 ± 8.81^{e}	100.72 ± 8.45^{c}
1% L	46.50 ± 9.33^{d}	11.41 ± 4.32^{e}	109.58 ± 13.35^{c}

All data are expressed as mean and standard deviation ($n=3$). *D* dandelion, *L* lichen. Different superscript letters show differences between groups at the same sampling time ($P<0.05$)

Table 2 Effect of dietary supplementation of dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) aqueous methanolic extracts on rainbow trout (*Onchorhynchus mykiss*) liver catalase (CAT) activity throughout the experiment (nmol/min/ml)

Groups	Day 15	Day 45	Day 75
Control	0.79 ± 0.16^{b}	1.19 ± 0.12^{b}	0.99 ± 0.11^{b}
0.1% D	0.76 ± 0.07^{b}	1.08 ± 0.17^{b}	1.33 ± 0.31^{a}
0.5% D	0.71 ± 0.15^{b}	1.12 ± 0.17^{b}	1.05 ± 0.13^{b}
1% D	0.83 ± 0.11^{b}	1.36 ± 0.18^{a}	0.87 ± 0.15^{cb}
0.1% L	0.76 ± 0.04^{b}	1.00 ± 0.18^{b}	1.03 ± 0.16^{b}
0.5% L	0.97 ± 0.17^{a}	0.88 ± 0.18^{c}	0.82 ± 0.06^{c}
1% L	0.92 ± 0.24^{a}	1.35 ± 0.08^{a}	0.79 ± 0.19^{c}

All data are expressed as mean and standard deviation ($n=3$). *D* dandelion, *L* lichen. Different superscript letters show differences between groups at the same sampling time ($P<0.05$)

An elevated CAT activity was noticed in 0.5 and 1% L fish groups on 15th day of the study compared to that of other groups ($P<0.05$). The highest CAT level was determined on 45th day of the study, in 1% D and 1% L fed fish groups ($P<0.05$). Following that, a significant decrease in CAT activity was observed in 0.1% D fish group on 75th day compared to all other groups. Changes in glutathione peroxidase (GPX) activities are presented in Table 3.

On 15th day, GPX activity was significantly increased in 0.1% D fish group, whereas all other experimental groups' GPX activity decreased compared to control ($P<0.05$). At day 45, GPX activity showed an increase in the 0.1% D and 1% L groups compared to control group ($P<0.05$). On the last sampling day, the highest increase was observed in the group that was fed with diet supplemented with 0.5% D ($P<0.05$). Changes in glucose-6-phosphatase dehydrogenase (G6PDH) activity in all studied groups are summarized in Table 4.

At day 15, G6PDH activity of all groups, except for 0.1% L group, showed an increase compared to the control group ($P<0.5$). At day 45, G6PDH activity in all treated fish groups decreased significantly compared to the control group ($P<0.05$). At day 75, G6PDH activity of 0.5% D, 1% D, and 0.5% L fish groups was significantly elevated compared to control. The lipid peroxidation results are presented in Table 5.

Lipid peroxidation decreased significantly in all treated fish groups compared to control on 15th day of the study ($P<0.05$). On 45th day, an elevation was observed in 0.5% L fish group compared to control ($P<0.05$). At day 75, no differences were determined between control and 0.1% D groups, whereas all the other treatment groups' malondialdehyde values increased ($P<0.05$).

Oxidative radical production (ORP) was not affected during the sampling days of the study in any treated fish group compared to control ($P>0.05$) (Fig. 1).

On 30th day, fish of D groups had no significant differences compared to control, although in all L fish groups, ORP was decreased ($P<0.05$). Lysozyme activity was

Table 3 Effect of dietary supplementation of dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) aqueous methanolic extracts on rainbow trout (*Onchorhynchus mykiss*) liver glutathione peroxidase (GPX) activity throughout the experiment (nmol/min/ml)

Groups	Day 15	Day 45	Day 75
Control	16.16 ± 1.19^{b}	19.56 ± 1.72^{b}	15.25 ± 1.19^{c}
0.1% D	21.60 ± 6.92^{a}	22.45 ± 5.47^{b}	22.01 ± 0.53^{b}
0.5% D	11.15 ± 1.46^{c}	15.54 ± 2.07^{c}	30.50 ± 1.46^{a}
1% D	12.39 ± 1.98^{c}	28.96 ± 1.94^{a}	18.62 ± 2.60^{c}
0.1% L	11.95 ± 1.41^{c}	12.13 ± 1.61^{c}	14.70 ± 0.84^{c}
0.5% L	15.12 ± 2.32^{b}	14.94 ± 1.53^{c}	24.30 ± 0.74^{b}
1% L	13.48 ± 3.07^{bc}	26.36 ± 0.39^{a}	14.91 ± 1.09^{c}

All data are expressed as mean and standard deviation ($n=3$). *D* dandelion, *L* lichen. Different superscript letters show differences between groups at the same sampling time ($P<0.05$)

Table 4 Effect of dietary supplementation of dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) methanolic extracts on rainbow trout (*Onchorhynchus mykiss*) liver glucose-6-phosphatase dehydrogenase (G6PDH) activity throughout the experiment (nmol/min/ml)

Groups	Day 15	Day 45	Day 75
Control	65.58 ± 0.65^{c}	160.34 ± 14.51^{a}	101.03 ± 3.32^{c}
0.1% D	80.18 ± 0.86^{b}	126.98 ± 1.35^{b}	99.75 ± 5.38^{c}
0.5% D	80.65 ± 1.58^{b}	129.71 ± 11.66^{b}	130.63 ± 0.42^{a}
1% D	80.16 ± 1.60^{b}	131.99 ± 4.58^{b}	113.14 ± 6.78^{b}
0.1% L	49.38 ± 2.15^{d}	21.03 ± 8.23^{c}	97.23 ± 2.62^{c}
0.5% L	65.01 ± 2.20^{c}	72.87 ± 5.56^{d}	111.68 ± 3.44^{b}
1% L	87.32 ± 2.50^{a}	97.50 ± 11.51^{d}	104.57 ± 9.67^{c}

All data are expressed as mean and standard deviation ($n=3$). *D* dandelion, *L* lichen. Different superscript letters show differences between groups at the same sampling time ($P<0.05$)

Table 5 Effect of dietary supplementation of dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) aqueous methanolic extracts on rainbow trout (*Onchorhynchus mykiss*) white muscle lipid peroxidation throughout the experiment (μM MDA)

Groups	Day 15	Day 45	Day 75
Control	115.29 ± 8.47^{a}	68.33 ± 7.91^{c}	74.47 ± 6.81^{c}
0.1% D	85.41 ± 8.61^{c}	64.94 ± 8.93^{c}	72.73 ± 16.09^{c}
0.5% D	94.38 ± 3.37^{b}	54.32 ± 3.13^{d}	82.75 ± 3.24^{b}
1% D	98.28 ± 5.07^{b}	60.85 ± 6.08^{c}	96.65 ± 3.97^{a}
0.1% L	97.02 ± 2.24^{b}	77.22 ± 7.63^{b}	87.67 ± 4.02^{b}
0.5% L	82.75 ± 8.13^{c}	89.75 ± 10.34^{a}	86.12 ± 5.40^{b}
1% L	85.17 ± 12.12^{c}	76.82 ± 5.27^{b}	83.63 ± 0.82^{b}

All data are expressed as mean and standard deviation ($n = 3$). *D* dandelion, *L* lichen. Different superscript letters show differences between groups at the same sampling time ($P < 0.05$)

significantly increased almost in all L groups at any sampling time compared to control (Fig. 2).

Myeloperoxidase activity increased in all treatment groups, except in 0.1% D and 0.5% L fish groups compared to control on 15th day of the study ($P < 0.05$) (Fig. 3).

In the study, none of the experimental groups had a difference on growth performance (Table 6). The highest final weight was observed in fish of 0.5% D group. Only 0.5% D fish group showed a decrease in FCR value compared to all other groups ($P < 0.05$).

Discussion

In the present study, changes in antioxidant enzyme status (SOD, CAT, GPX, G6PDH, and lipid peroxidation), and immune responses, such as oxidative radical production, lysozyme, and myeloperoxidase activities of rainbow trout fed diet containing dandelion and lichen aqueous methanolic extract at different doses, were observed at different sampling times.

Reactive oxygen species must be eliminated from the cells and organs rapidly, and effectively in order to maintain vital metabolic activities of organisms. SOD is the most important and well-known enzyme among enzymatic antioxidants in eukaryotic organisms (Lushchak 2014). As our results revealed an increasing trend in SOD activity, such an increase was also observed in the control group. It is therefore hypothesized that the most appropriate plant species to enhance the efficacy of SOD activity was by 0.1% D. It can be said that the lichen did not show antioxidant feature in terms of SOD activity. Based on the findings from the 0.1% L fish group, it can also be concluded that 15 days of use has a substantial impact on oxidative stress in terms of SOD activity. Similar to the groups that received dandelion supplementation in our study, Gülçin et al. (2009) observed that SOD activity increased in trout fed with the diets supplemented with melatonin. Li et al. (2010) observed that carbamazepine administration considerably decreased SOD activity in trout. These results are consistent with those obtained in fish groups fed with lichen supplemented diet. Furthermore, Thirunavukkarasu et al. (2010) revealed that the extract obtained from *Citrullus colcynthis* species increased SOD activity, whereas the extract of *Suaeda maritime* decreased SOD activity similar to that in rainbow trout groups fed with lichen supplements. In agreement with the findings of our study,

Table 6 Growth performance of rainbow trout (*Onchorhynchus mykiss*) fed with different doses of dandelion (*Taraxacum officinalis*) and lichen (*Usnea barbata*) aqueous methanolic extracts

Groups	Initial weight	Final weight	Weight gain (%)	SGR	FCR
Control	12.26 ± 0.06	52.01 ± 0.05	325.17 ± 25.39	1.93 ± 0.08	1.24 ± 0.10^{b}
0.1% D	12.15 ± 0.06	53.71 ± 0.04	344.09 ± 38.61	1.98 ± 0.12	1.15 ± 0.13^{b}
0.5% D	12.32 ± 0.05	57.89 ± 0.05	371.18 ± 36.33	2.06 ± 0.10	1.06 ± 0.10^{a}
1% D	12.34 ± 0.06	51.79 ± 0.06	320.91 ± 31.43	1.91 ± 0.10	1.12 ± 0.11^{b}
0.1% L	12.03 ± 0.02	53.59 ± 0.07	340.56 ± 26.10	1.97 ± 0.11	1.18 ± 0.16^{b}
0.5% L	12.18 ± 0.02	56.40 ± 0.09	359.77 ± 27.80	2.03 ± 0.17	1.24 ± 0.17^{b}
1% L	12.38 ± 0.02	51.35 ± 0.08	311.95 ± 28.74	1.89 ± 0.11	1.16 ± 0.15^{b}

All data are expressed as mean and standard error. *D* dandelion, *L* lichen. Different superscript letters show differences between groups ($P < 0.05$). *SGR* specific growth rate, *FCR* feed conversion ratio

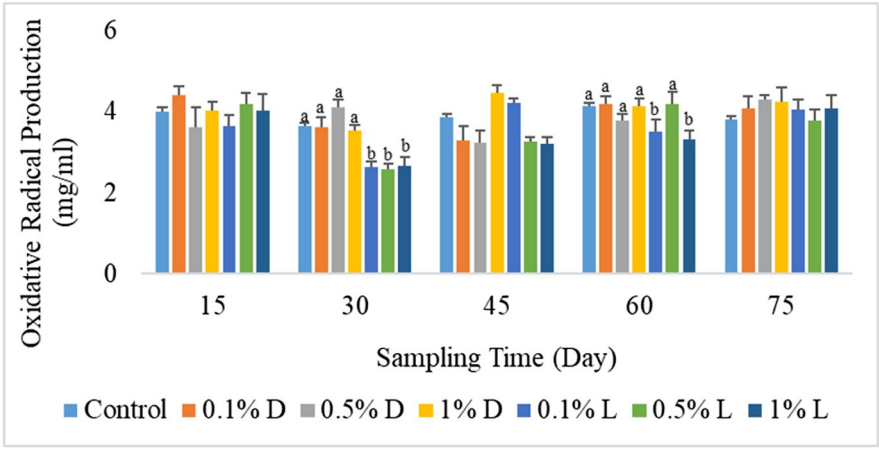

Fig. 1 Effect of dietary supplementation of dandelion and lichen on oxidative radical production in blood of rainbow trout after feeding for 75 days. Different letters show differences between groups at the same sampling time

considerable increases were observed in SOD activities of rainbow trout fed with vitamin A and E supplements (Keleştemur and Özdemir 2013). In another research, SOD activity increased in trout juveniles fed with sage and thyme diets similar to the results obtained with dandelion supplement in our study, whereas SOD activity decreased in trout juveniles fed with the peppermint similar to that observed with lichen supplements (Sönmez et al. 2015b). In their study, Elgaml et al. (2015) fed tilapia (*Oreochromis niloticus*) with the diets supplemented with selenium and alpha-tocopherol and observed a decrease in SOD activity. In several studies, SOD activity was observed to be increased in the fish provided with different diets (Yonar et al. 2015; Zhang et al. 2016; Zhao et al. 2017).

CAT locates in the peroxisomes of the cell and it catalyzes dismutation of superoxide radicals to H_2O_2 that are eliminated by SOD. Although CAT activities determined at different sampling times did not show any change in rainbow trout fed with dandelion extract, there was an increase of its level in fish of 0.5% and 1% L groups. However, our overall observation is that the plants used in this study did not exert any significant effect on CAT activity except in fish of 1% D and 1% L on 45th day, and 0.1% D on 75th day. Similarly, Gülçin et al. (2009) observed that CAT activity increased in the trout fed with feeds supplemented with melatonin. An increase in CAT activity

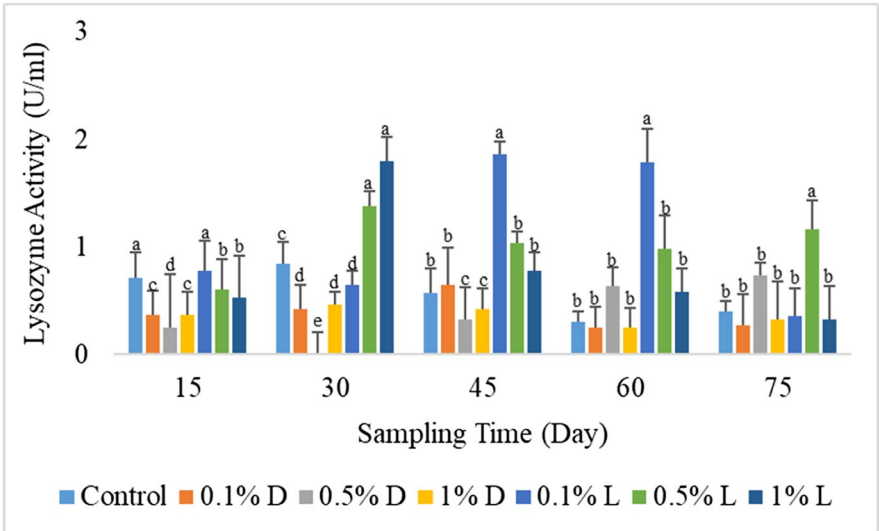

Fig. 2 Effect of dietary supplementation of dandelion and lichen on lysozyme activity in blood serum of rainbow trout after feeding for 75 days. Different letters show differences between groups at the same sampling time

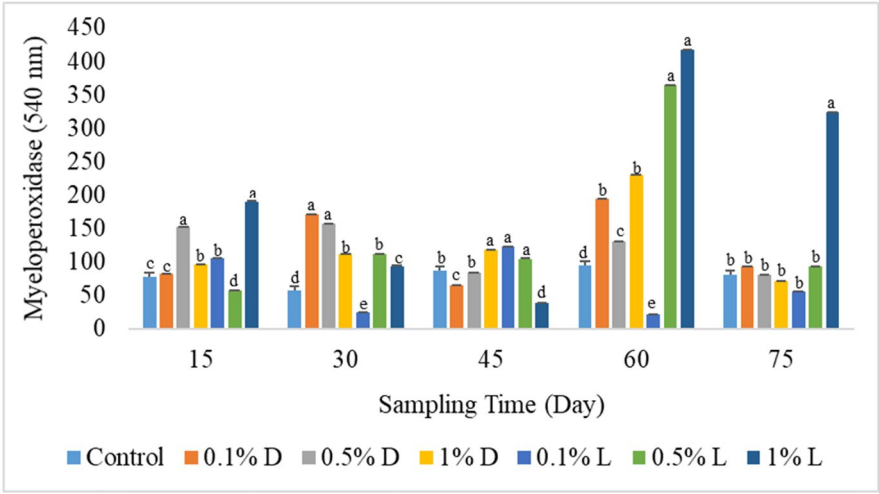

Fig. 3 Effect of dietary supplementation of dandelion and lichen on myeloperoxidase activity in blood serum of rainbow trout after feeding for 75 days. Different letters show differences between groups at the same sampling time

was observed in Coruh trout (*Salmo coruhensis*) fed with kefir (Kızak and Celik 2012). Moreover, Zhang et al. (2016) observed an increase in CAT activity in Japanese seabass (*Lateolabrax japonicus*) fed with the feed enriched with magnesium and vitamin E; Şahan et al. (2017) observed an increased level in tilapia fed with ginger; and Amer (2016) observed an increased CAT level in tilapia fed with *Spirulina platensis*. On the contrary, Sönmez et al. (2015b) demonstrated a decrease in CAT activity in rainbow trout administered with thyme, sage, and peppermint oil, and Özlüer-Hunt et al. (2016) did not find a change in CAT activity in the trout fed with yeast nucleotide protein.

GPX catalyzes the synthesis of NADPH and GSSG, which are necessary for the functioning of glutathione reductase. In this study, GPX activity showed a time-dependent change with the most consistent values observed in the 0.5% D fish group. An elevated GPX activity was also observed in 1% D and 0.5% L fish groups at the end of the study. Li et al. (2010) observed that the use of carbamazepine caused a considerable decrease in GPX activity of trout. Moreover, magnesium and vitamin E supplementation caused an increase in GPX activity in Japanese seabass (Zhang et al. 2016). Similarly, the use of trichlorfon caused an increase in GPX activities in tilapia (Yonar et al. 2015), and the use of thyme, sage, and peppermint oil resulted in an elevation in GPX activity in trout (Sönmez et al. 2015b).

G6PDH catalyzes the pentose phosphate pathway by producing NADPH. Therefore, the NADPH produced is essential for reductase and CAT. In this regard, G6PDH has a vital function in H_2O_2 breakdown. G6PDH activity decreased in all treatment fish groups on the 45th day of the study. The highest value was obtained from fish of the 1% D group at day 75. The increased G6PDH activity in rainbow trout fed with dandelion is consistent with the results of the study conducted by Sönmez et al. (2015b) on the same fish species.

The malondialdehyde (MDA) is a product of lipid peroxidation caused by free oxygen radicals and it is an extremely important indicator of oxidative stress. Increased MDA level indicates increased lipid peroxidation and it is regarded as the marker of cell damage (Yagi 1984). In our study, only 0.1% D fish group had a decrease in MDA level at all sampling times compared to control. Similar to results in rainbow trout supplemented with lichen in our study, Keleştemur and Özdemir (2013) observed that using selenium and alpha-tocopherol increased MDA levels in tilapia. On the other hand, Şahan et al. (2017) observed a decrease in MDA levels in trout fed with rose hip; moreover, Amer (2016) found a decrease in MDA levels in tilapia fed with spirulina. Studies generally found a decrease in MDA levels based on the product type used (Kızak and Celik, 2012; Özlüer-Hunt et al. 2016; Sönmez et al. 2015b; Yonar et al. 2015).

Non-specific immune responses, such as ORP, MPO, and LYS activities, were examined in rainbow

trout treated with dandelion and lichen extracts. A decreased ORP was recorded in lichen-treated groups when compared to control. No differences were observed in dandelion-treated fish groups compared with the control. Similarly, Bilen and Bulut (2010) found no differences in oxidative radical production in rainbow trout administered with laurel extract. On the contrary, an increase in oxidative radical production was observed in rainbow trout treated with melissa (Bilen et al. 2020a, b) and Tilia tomentosa in common carp (Almabrok et al. 2018) aquaeus methanolic extracts. Lysozyme, which is an enzyme that provides non-specific immune response to pathogens during infection, was affected by lichen extract administration in rainbow trout. MPO activity was also induced in treatment groups compared to control. Similarly, *Prunus domestica* (Terzi et al 2021) and tetra (Bilen and Elbeshti 2019) extracts caused an elevation of these two variables in rainbow trout. Differently, Bilen et al. found no differences on LA levels after Malva sylvestris application (Bilen et al. 2020a, b).

In the present study, the highest final weight was attained by fish in 0.5% D group, but it was not significantly different. Moreover, FCR value of the same treatment group was significantly decreased. In line with this study, Bilen and Bilen (2012) observed no differences in growth of rainbow trout fed diet containing with tetra and laurel. Intestinal microbiota is the first key to the growth promotion and improving animal performance. In this regard, some ingredients of the medicinal plant can affect the fish microbiota and cause increase or decrease in fish growth, or digestive enzyme activity. In our study, because of no differences observed in growth performance, it could be opined that the plants used do not affect the digestive system of the fish in terms of growth performance. However, the elevated digestive enzyme activity could be the reason of affected FCR in 0.5% D group.

Conclusion

The usage of medicinal extracts as feed supplements has potential as an alternative to therapeutics in aquaculture. The aim of the current study was to determine the potential effects of dietary supplementation of dandelion and lichen aqueous methanolic extracts on liver antioxidant and humoral immune status. The result revealed that antioxidant status of the treated fish changed at variable time points. Overall good results were obtained in fish of 0.1% D group in terms of antioxidant. Considering the low FCR obtained from the 0.5% D fish group, it is suggested that use of dandelion at a rate of 0.5% in trout cultivation will be beneficial in preventing oxidative stress and positively affecting the feed conversion in fish. Moreover, in general, lichen could be suggested as an immunostimulant. We encourage further investigations on such products, that are easily accessible and natural, for possible use in fish farming.

Acknowledgements The study was presented as a Master's thesis of Mohamed Omar Abdalla SALEM at Kastamonu University, Institute of Science.

Author contribution Mohamed Omar Abdalla Salem, Tarek A. Salem, and Keriman Yürüten Özdemir performed the work. Adem Yavuz Sönmez analyzed data and interpreted the results. Soner Bilen designed the study and interpreted the results. Kerim Güney provided the plants and created the conception.

Data availability Data are available from corresponding author upon reasonable request.

Declarations

Ethics approval Study protocol was approved in advance by the local Ethics Committee for Animal Research Studies at the Kastamonu University (KUHADYEK-2016.10).

Consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

References

Almabrok AA, Amhamed ID, Mohamed GA, Bilen S, Altief TAS (2018) Effect of Tilia tomentosa methanolic extract on growth performance, digestive enzyme activity, immune system and haematological indices of common carp (Cyprinus carpio). Mar Sci Tech Bull 7(1):12–20

Amhamed ID, Mohamed GA, Almabrok AA, Altief TAS, Bilen S (2018) Efficacy of dietary Chenopodium album extract on some health parameters, digestive enzymes and growth performance in juvenile Cyprinus carpio. Alınteri Zirai Bilimler Dergisi 33(2):165–176

Amer SA (2016) Effect of *Spirulina platensis* as feed supplement on growth performance, immune response and antioxidant status of mono-sex Nile tilapia (*Oreochromis niloticus*). Benha Vet Med J. 30:1–10. https://doi.org/10.21608/bvmj.2016.31332

Arslan G, Sönmez AY, 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*. Aquac Res 49(6):2256–2266. https://doi.org/10.1111/are.13686

Bilen S, Bulut M (2010) Effects of laurel (*Laurus nobilis*) on the non-specific immune responses of rainbow trout (*Oncorhynchus mykiss*, Walbaum). J Anim Vet Adv 9(8):1275–1279. https://doi.org/10.3923/javaa.2010.1275.1279

Bilen S, Bilen MA (2012) Tetra (*Cotinus coggygria*) ve defne (*Laurus nobilis*) bitkilerinin alabalıklarda (*Oncorhynchus mykiss*) büyümeyi teşvik edici etkileri. Alinteri J Agr Sci 22(B):26–33

Bilen S, Kızak V, Gezen AM (2013) Floating fish farm unit (3FU) Is it an appropriate method for Salmonid production. Mar Sci Tech Bull 2(1):9–13

Bilen S, Altunoğlu ÇY, Ulu F, Biswas G (2016) Innate immune and growth promoting responses to caper (*Capparis spinosa*) extract in rainbow trout (*Oncorhynchus mykiss*). Fish Shellfish Immun 57:206–212. https://doi.org/10.1016/j.fsi.2016.08.040

Bilen S, Elbeshti HTAG (2019) A new potential therapeutic remedy against Aeromonas hydrophila infection in rainbow trout (Oncorhynchus mykiss) using tetra, Cotinus coggygria. J Fish Dis 42(10):1369-1381. https://doi.org/10.1111/jfd.13061

Bilen S, Altief TAS, Özdemir KY, Salem MOA, Terzi E, Güney K (2020a) Effect of lemon balm (Melissa officinalis) extract on growth performance, digestive and antioxidant enzyme activities, and immune responses in rainbow trout (Oncorhynchus mykiss). Fish Physiol Biochem 46(1):471–481

Bilen S, Karga MK, Altunoğlu YÇ, Ferhat ULU, Biswas G (2020b) Immune responses and growth performance of the aqueous methanolic extract of malva sylvestris in Oncorhynchus mykiss. Mar Sci Tech Bull 9(2):159–167

Elbesthi RTA, Özdemir KY, Taştan Y, Bilen S, Sönmez AY (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 Physiol Biochem 46(4):1295–1307. https://doi.org/10.1007/s10695-020-00790-z

Elgaml SA, Khalil R, Hashish EA, El-Murr A (2015) Protective effects of selenium and alpha-tocopherol against lead-induced hepatic and renal toxicity in *Oreochromis niloticus*. J Aquacult Res Dev 6:1. https://doi.org/10.4172/2155-9546.1000299

Ellis AE (1990) Lysozyme assays. In: Stolen JS, Fletcher TC, Anderson DP, Roberson BS, Van Muiswinkel WB (eds) Techniques in fish immunology, 1st edn. SOS Publications, New Jersey, pp 101–103

Engel K, Schmidt U, Reuter J, Weckesser S, Simon-Haarhaus B, Schempp CM (2007) *Usnea barbata* extract prevents ultraviolet-B induced prostaglandin E2 synthesis and COX-2 expression in HaCaT keratinocytes. J Photochem Photobiol, B 89(1):9–14

FAO (2018) Aquaculture Department. The state of world fisheries and aquaculture, Rome

Gülçin O, Beydemir S, Hisar O, Köksal E, Reiter RJ (2009) Melatonin administration increases antioxidant enzymes activities and reduces lipid peroxidation in the rainbow trout (*Oncorhynchus mykiss*, Walbaum) erythrocytes. Turk J Vet Anim Sci 33:241–245

Keleştemur GT, Özdemir Y (2013) Effects of dietary vitamin a and e on growth performance and antioxidant status in blood of juvenile rainbow trout (Oncorhynchus mykiss, w. 1792) exposed to flow rate stress. J Anim Plant Sci 23:821–827

Kızak MK, Celik HT (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*). Turk J Fish Aquat Sc 12:1–2. https://doi.org/10.4194/1303-2712-v12_2_12

Li ZH, 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. J Appl Toxicol 30:644–648. https://doi.org/10.1002/jat.1534

Lushchak VI (2014) Free radicals, reactive oxygen species, oxidative stress and its classification. Chem Biol Interact 224:164–175

Madamombe IT, Afolayan AJ (2003) Evaluation of antimicrobial activity of extracts from South African Usnea barbata. Pharm Biol 41(3):199–202

Mir MA, Sawhney SS, Jassal MMS (2013) Qualitative and quantitative analysis of phytochemicals of Taraxacum officinale. Wudpecker J Pharm Pharmocol 2(1):1–5

Özlüer-Hunt A, Özkan-Yılmaz F, Berköz M, Engin K, Gündüz SG, Yalın S (2016) Effects of dietary nucleotide yeast on immune responses and antioxidant enzyme activities of rainbow trout juveniles (*Oncorhynchus mykiss*). Isr J Aquacult Bamidgeh 68:1264–1276

Ricker WE (1979) Growth rates and models. In: Hoar WS, Randall DJ, Brett JR (eds) Fish Physiology. Academic Press, New York, pp 677–743

Şahan A, Duman S, Çolak SÖ, Çınar 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*. Turk J Fish Aquat Sc 17:91–100. https://doi.org/10.4194/1303-2712-v17_1_11

Sahoo PK, Kumari J, Mishra BK (2005) Non-specific immune responses in juveniles of Indian major carps. J Appl Ichthyol 21:151–155. https://doi.org/10.1111/j.1439-0426.2004.00606.x

Siwicki AK, Anderson DP, Rumsey GL (1994) Dietary intake of immunostimulants by rainbow trout effects non-specific immunity and protection against furuncluosis. Vet Immunol Immunopathol 41:125–139

Sönmez AY, 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. Turk J Fish Aquat Sc 15:813–819. https://doi.org/10.4194/1303-2712-v15_4_04

Sönmez AY, 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 Physiol Biochem 41:165–175. https://doi.org/10.1007/s10695-014-0014-9

Terzi E, Kucukkosker B, Bilen S, Kenanoglu ON, Corum O, Özbek M, Parug SS (2021) A novel herbal immunostimulant for rainbow trout (Oncorhynchus mykiss) against Yersinia ruckeri. Fish Shellfish Immunol 110:55–66

Thirunavukkarasu P, Ramkumar L, Ramanathan T, Silambarasan D (2010) Anti oxidant activity of selected coastal medicinal plants. World J Fish Mar Sci 2:134–137

Yagi K (1984) Assay for plasma lipid peroxidase. Methods Enzymol 109:328–331

Yonar ME, Yonar SM, Pala A, Silici S, Sağlam N (2015) Trichlorfon-induced haematological and biochemical changes in *Cyprinus carpio*: ameliorative effect of propolis. Dis Aquat Organ 114:209–216. https://doi.org/10.3354/dao02866

Vaglio A, Landriscina C (1999) Changes in liver enzyme activity in the Teleost *Sparus aurata* in response to cadmium intoxication. Ecotoxicol Environ Saf 43(1):111–116. https://doi.org/10.1006/eesa.1999.1778

Zhang C-x, Huang F, Li J, Wang L, Song K, Mai K-s (2016) Interactive effects of dietary magnesium and vitamin E on growth performance, body composition, blood parameters and antioxidant status in Japanese sea bass (*Lateolabrax japonicus*) fed oxidized oil. Aquacult Nutr 22:708–722. https://doi.org/10.1111/anu.12393

Zhao Y, Jiang X, Kong X, Di G, Nie G, Li X (2017) Effects of hypoxia on lysozyme activity and antioxidant defences in the kidney and spleen of *Carassius auratus*. Aquac Res 48:223–235. https://doi.org/10.1111/are.12876