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Effect of lemon balm (*Melissa officinalis)* extract on growth performance, digestive and antioxidant enzyme activities, and immune responses in rainbow trout (*Oncorhynchus mykiss*)

Soner Bilen · Tarek Abdalsalam Salem Altief · Keriman Yürüten Özdemir · Mohamed Omar Abdalla Salem · Ertugrul Terzi · Kerim Güney

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Abstract This study was conducted to determine the effects of dietary supplementation of lemon balm (*Melissa officinalis*) aqueous methanolic extract on growth performance, blood parameters, digestive and antioxidant enzyme activities, and non-specific immune responses in rainbow trout (*Oncorhynchus mykiss*). Fish with an average weight of 23.03 ± 0.07 g were fed a diet supplemented with an aqueous methanolic extract of lemon balm at a dose of 0 (control), 0.1 (LB0.1), 0.5 (LB0.5), and 1 g kg^{-1} (LB1) for 75 days. The final weight, weight gain, and specific growth rate were observed to be significantly increased in LB0.5 and LB1 groups compared with that of the control. No differences were observed in feed conversion ratio values. WBC increased at the 45th day of the study in LB0.1 group. No differences were determined in RBC ($P > 0.05$). At the end of the study, lipase increased significantly in all experimental groups compared with the control. Pepsin was also elevated in LB0.5 and LB1 groups compared with the control. Increased trypsin was determined in LB1 group ($P < 0.05$). SOD activity increased at the end of the study in LB1 group ($P < 0.05$). CAT values had no differences compared with control. GR activity increased in all experimental groups compared with control. GPx improved in LB0.5 and LB1 groups significantly ($P < 0.05$). Lipid peroxidation was decreased in LB1 group compared with that of control, but this decrease was not significant ($P < 0.05$). Oxidative radical production and lysozyme activity significantly increased in LB1 group ($P < 0.05$). The highest MPO was determined in control group ($P < 0.05$). Current results suggest that lemon balm extract stimulates growth promoting antioxidant and immune responses in rainbow trout.

Keywords Lemon balm · *Melissa officinalis* · Growth · Hematology · Digestive enzyme · Antioxidant · Immune responses

S. Bilen (✉) · K. Y. Özdemir · E. Terzi
Faculty of Fisheries, Department of Aquaculture, Kastamonu University, Kastamonu, Turkey
e-mail: sbilen@kastamonu.edu.tr

T. A. S. Altief
Department of Basic Veterinary Medical Sciences, Omar Al-Mukhtar University, Al Bayda, Libya

M. O. A. Salem
Institute of Science, Department of Aquaculture, Kastamonu University, Kastamonu, Turkey

K. Güney
Faculty of Forestry, Department of Forest Engineering, Kastamonu University, Kastamonu, Turkey

Introduction

Aquaculture is one of the fastest growing industries and contributes more than half of the total fish production in the world (FAO 2018). However, when the industry grows through its technology and production rate gradually, losses caused by diseases seem to be the main problem. A wide range of practices and innovations focus on reducing infection pressure to the environment and optimizing disease resistance and resilience of fish.

To prevent fish losses due to stress-related non-infectious diseases or infectious diseases, the scientists mainly focus on application of immunostimulants. The use of immunostimulants in aquaculture is considered as an advanced and suitable alternative to antibiotics and other chemicals (Bilen et al. 2011). Medicinal plants and herbs are assuring to be indispensable wellspring of curatives in the fish environment as these substances provide a less costly therapeutic measure and more remarkable precision without exposure to risk with their immunostimulant effects (Madhuri et al. 2012). These products added to feeds cure many diseases (Shakya 2017), promote growth (Altunoglu et al. 2017), reduce stress (Sonmez et al. 2015b), improve immunity (Bilen et al. 2016a), and prevent infections in fish under culture (Shakya 2017).

Many studies suggested that herbs in fish diets have positive effects on growth and disease resistance in fishes. The herbs being cheaper, eco-friendly with minimum side effects are used as the substitutes to the costly antibiotics in fish health management. World Health Organization (WHO) encourages the use of supplementary diets combined with medicinal herbs or plants, which minimize the application of chemicals in fish diet (Dada 2015). In this context, herbs can be used in fish diet to increase feed consumption in fish under culture (Lević et al. 2008). Various plants are used in animal nutrition containing bioactive compounds which stimulate feed intake, improve secretion of digestive enzymes, and activate immune responses (Citarasu 2010). These plants are also known to possess antibacterial, antiviral, and antioxidant properties. It is also well known that herbs and herbal products added to fish diets changed the consumption patterns, enhanced feed consumption, and stimulated digestion by elevating the secretion of different digestive enzymes, bile, pancreatic enzyme activities, and mucus in fishes (Lee and Gao 2012; Platel et al. 2002).

Lemon balm (*Melissa officinalis*) from Lamiaceae family that includes 200 genera is an enduring herbaceous plant which grows boundlessly from the Central and Southern Europe to Iran and Central Asia. It is, likewise, well-known worldwide for its edible properties (Ghayour et al. 2010). The leaves of lemon balm are used for the symptomatic treatment of gastrointestinal disturbances, neurological diseases associated with oxidative stress, and adjuvant therapy for pain associated to functional dyspepsia (Amooaghaie 2011; Hohmann et al. 1999; Mazzanti et al. 2008; Oniga et al. 2010).

Considering the above mentioned, the objective of this study was to evaluate the effect of dietary lemon balm supplementation on growth promotion, hematology, antioxidant (SOD, CAT, GR, GPx, and lipid peroxidation) and non-specific immune responses, such as oxidative radical production (ORP), myeloperoxidase (MPO) activities, and lysozyme activities (LA) in rainbow trout (*Oncorhynchus mykiss*).

Materials and methods

Experimental design and fish

This study was conducted in triplicate over 75 days in 12 mesh 1.5 × 1.5 × 1.5 m cages capacity in Kastamonu University Inland and Marine Fish Research and Application Center, Turkey. Experimental rainbow trout, with an average body weight of 23.03 ± 0.07 g, were obtained from the same fish farm. A total of 100 fish were stocked in cages in 3 replicates. Aqueous methanolic extracts of lemon balm (LB) were added to the fish's basal diet at the rate of 0 (control), 0.1 (LB0.1), 0.5 (LB0.5) and 1 (LB1) g kg^{-1} by spraying. During the study, all groups were fed with the diets twice a day ad libitum by hand during the study. Before starting the experiment, fish were acclimatized to the experimental feeding regimen using a commercial diet for 2 weeks (trout commercial pellet). Bulk fish live-weight increments were measured every 2 weeks, and feed intake was recorded daily throughout the study. At the end of the study, fish were individually taken weight and length for determining growth performance parameters. In addition, 5 fish per tank were collected for chemical analyses. Samples were kept at − 80 °C until antioxidant and digestive enzymes analysis.

Preparation of aqueous methanolic extract of lemon balm and diets

Lemon balm (*Melissa officinalis L.*) was purchased from local area sedate store (Kastamonu, Turkey). The plant extract was prepared according to the procedure described previously by Bilen et al. (2016b). In brief, 50 g lemon balm was percolated with 1 L methanol (40%) for 3 days and then filtered. The solvent was evaporated, and lastly concentrate was dissolved in 50 mL deionized water (50 °C). Exact amount of the extract solution was mixed with experimental diets

according to the doses by spraying. All feeds were then coated with fish oil at the same volume by spraying again. The experimental diets were kept at − 20 °C until use.

Growth parameters

Growth performance parameters of weight gain ((WG) %), specific growth rate (SGR) and feed conversion ratio (FCR) were calculated according to the method described by Ricker (1979).

$$\text{Weight gain (\%)} = [(\text{final weight} - \text{initial weight})/\text{initial weight}] \times 100;$$
$$\text{Specific growth rate (SGR)} = [(\ln \text{final weight} - \ln \text{initial weight})/\text{days}] \times 100;$$
$$\text{Feed conversion ratio (FCR)} = \text{Feed Offered}/\text{Weight Gain (g)}$$

Hematology

White blood cell counts ((WBC) × 10^4/mm^3), red blood cell counts ((RBC) × 10^6/mm^3), hemoglobin ((Hb) g/dl) levels, and hematocrit ((HCT) %) measurements were assessed according to the methods described by Blaxhall and Daisley (1973). Blood indices included the mean cell volume ((MCV) fl), mean cell Hb ((MCH) pg/cell), and mean cell Hb concentration ((MCHC) %), which were calculated according to the formulae of Lewis et al. (2001).

Digestive enzymes

Digestive enzyme (amylase, lipase, pepsin, and trypsin) activities were assayed at the end of the study. The whole digestive tract and hepatopancreas were thoroughly homogenized in ice-cold sterilized distilled water and centrifuged at 15,000×*g* for 45 min at 4 °C. The supernatant was used as a crude enzyme source. Amylase activity was analyzed by the starch-hydrolysis method described by Bernfeld (1955). Lipase activity was determined using the method delineated by Furne et al. (2005). Pepsin was assayed using the method of Worthington (1988). Trypsin was determined using Na-Benzoyl-DL-arginine 4-nitroanilide hydrochloride as a substrate according to Erlanger et al. (1961). Specific activities of all digestive enzymes were calculated as milligrams of protein. All enzyme activity units were calculated using the following equations:

$$\text{Amylase} = [(\text{Sample}-\text{Blank})2 \times 7712] - [1.082 \times (\text{Sample}-\text{Blank})] + 0.082 = \text{Result/mg protein}$$

$$\text{Lipase} = [(\text{Sample}-\text{Blank}) \times (0.2359 + 0.0153)2]/\text{mg protein}$$

$$\text{Trypsin} = [(\text{Last Result}-\text{First Result})/10\ \text{min}] = \text{Absorption Result}$$
$$[(\text{Absorption Result} \times 1\ \text{million})/8.800]/2 = \text{Result/mg protein}$$

Antioxidant enzyme activity

In order to determine antioxidant enzyme activities, such as superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), and lipid peroxidation (LPO), 3 fish from each experimental group were scooped out randomly from net cages at 15th, 45th, and 75th days of the experiment. Fish were euthanized by an overdose of phenoxyethanol. Liver samples and white muscles of the fish were excised aseptically and frozen immediately in liquid nitrogen. All samples were then stored at − 80 °C until analysis. After thawing, liver samples were washed with sterile physiological saline, dried with filter paper, and approximately 1 g sample was homogenized in a Potter–Elvehjem homogenizer and put into homogenization medium (0.25 M sucrose, 0.5 mM EDTA and 10 mM Tris–HCl; pH 7.4) for centrifugation at 4 °C, 20000×*g* for 45 min (Hisar et al. 2009). After centrifugation, the supernatant was separated and used for enzyme activity assays. In the study, SOD was determined using SIGMA 19160-1KT-F SOD Assay Kit, catalase activity was determined using Cayman 707002 Catalase Assay Kit, glutathione peroxidase activity was determined using Cayman 703102 Glutathione Peroxidase Assay Kit, glutathione reductase was determined using Glutathione Reductase Assay Kit 703202, and lipid peroxidation was determined used Cayman 10009055 TBARS Assay Kit. All these methodologies were performed following the manufacturer's instructions.

Non-specific immune parameters

In this study, oxidative radical production was determined using the reduction of nitroblue tetrazolium ((NBT) Sigma–Aldrich, St. Louis, MO, USA) assay on the 15th, 45th, and 75th days, as per the previously

described method (Siwicki et al. 1994). LA was determined according to the method used by Ellis (1990). Total MPO activity present in the serum was measured according to the method described by Sahoo et al. (2005).

Statistical analyses

All statistical analyses were performed using statistical software package SPSS for Windows 22 (SPSS Inc., Chicago, IL, USA). Firstly, the variance of data was analyzed using one-way ANOVA, and Duncan's multiple range test was then performed to determine significant differences among groups. The accepted level of significance was $P < 0.05$.

Results

Growth performance

Results of the growth performance analysis are provided in Table 1. Results confirmed that weight gain and SGR were significantly higher ($P > 0.05$) in LB0.5 and LB1 groups compared with the control group (Table 1). There were no differences observed on FCR levels among different groups ($P > 0.05$).

Hematology

On the 15th day of the study, no differences were observed in groups WBC, RBC, HGB, and HCT values (Table 2). On the 45th day of the study, WBC increased significantly in LB0.1 and LB0.5, and decreased in LB1 group compared with the control ($P < 0.05$). Similar results were observed on hemoglobin and hematocrit values ($P < 0.05$). At the end of the study, WBC was the highest in fish of control group. In all other experimental groups, WBC decreased significantly. Similar results were observed on HCT and HGB.

Digestive enzymes

On the 15th, 45th, and 75th days of the study, amylase, lipase, pepsin, and trypsin activities in rainbow trout treated with lemon balm extract were determined (Fig. 1). Amylase increased on 15th day in LB0.5 group, and on 45th day in LB0.1 and LB0.5 groups compared with control. However, at the end of the study, amylase activity significantly decreased in all experimental groups ($P < 0.05$). Lipase was elevated at the end of the study in all experimental groups compared with control ($P < 0.05$). Pepsin activity in rainbow trout fed LB0.5 supplemented diet was significantly higher than in rainbow trout fed the control diet or LB0.1 and LB1 supplemented diets during all the sampling times ($P < 0.05$). Trypsin activity was the highest in LB1 supplemented diet–fed fish compared with the control fish at the end of the study ($P < 0.05$).

Antioxidant activities

In this study, to determine the effects of the experimental treatments, antioxidant activities (SOD, CAT, GR, GPx, lipid peroxidation) were evaluated (Fig. 2). SOD activity was decreased in LB0.1 and LB0.5 groups compared with the control, and no differences were observed between control and LB1 treated groups on 15th day

Table 1 Growth performance of rainbow trout fed with different doses of lemon balm (*Melissa officinalis*) methanolic extract

Dietary treatments				
	Control	LB0.1	LB0.5	LB1
IW (g)	22.75 ± 0.05	23.11 ± 0.07	23.11 ± 0.08	23.14 ± 0.06
FW (g)	87.72 ± 0.61^{b}	91.75 ± 0.77^{b}	103.48 ± 0.72^{a}	102.15 ± 0.73^{a}
WG (%)	278.89 ± 0.73	298.58 ± 0.68	350.19 ± 0.63^{a}	342.11 ± 0.48^{a}
SGR	0.87 ± 0.01	0.92 ± 0.01	1.07 ± 0.01^{a}	1.05 ± 0.01^{a}
FCR	1.004 ± 0.01	1.00 ± 0.01	1.00 ± 0.01	0.99 ± 0.01

Data are presented as mean ± SE of three replicates ($n = 3$). Means with different superscript letters in a row are significantly different ($P < 0.05$). LB0.1, LB0.5, and LB1 are extracts of lemon balm (*Melissa officinalis*) at 0.1 g kg^{-1}, 0.5 g kg^{-1}, and 1 g kg^{-1} diet, respectively

IW initial weight, *FW* final weight, *WG* weight gain, *SGR* specific growth rate, *FCR* feed conversion ratio

of the study. Differently, on 45th day of the study, SOD was higher in all treatment groups than in control. At the end of the study, SOD was the highest in LB1 group among the experimental groups ($P < 0.05$). CAT activity significantly reduced in LB1 group compared with other groups at the 15th day of the study. However, no differences were observed among groups at any sampling time ($P > 0.05$). GR activity was determined higher in experimental treatment groups than the control group during all experimental sampling times ($P < 0.05$). GPx activity also increased in LB0.5 and LB1 compared with other experimental groups on any sampling time ($P < 0.05$). No differences were determined between control and LB0.1 ($P > 0.05$). Lipid peroxidation levels in treatment groups showed no differences at the end of the study compared with control group ($P < 0.05$).

Immune responses

Non-specific immune responses of the rainbow trout treated with different doses of lemon balm are summarized in Table 3. ORP was higher in LB1 group than that of other groups at all sampling intervals ($P < 0.05$). Lysozyme activity increased in all experimental groups compared with control. However, MPO activity decreased in all experimental group compared with control during all sampling times ($P < 0.05$).

Discussion

Results of this study indicate that the administration of dietary methanolic extract of lemon balm at the doses of 0.5 and 1 g kg^{-1} can promote growth in rainbow trout. It was also observed that lemon balm extract promotes digestive enzyme activity, antioxidant activity, and non-specific immune responses. The increased growth promotion along with stimulated antioxidant and non-specific immune response could provide a better yield and an early protective immunity in rainbow trout.

In the study, growth performance parameters, such as final weight and SGR levels displayed improvement. Especially, LB0.5 and LB1 caused dose-dependent growth promotion. Similar to the present study, significantly increased final weight and SGR were reported in rainbow trout fed with capper and nettle extract, respectively (Bilen et al. 2016a, b). On the contrary, use of laurel caused no effects on growth of rainbow trout (Bilen and Bulut 2010).

In our study, lemon balm influenced no effects on WBC, RBC, HGB, and HCT values at the first sampling time. At the second sampling time, WBC significantly increased in fish of LB0.1 and LB0.5. At the end of the study, WBC was the highest in control group. In all other experimental groups, WBC decreased significantly. Similar results were observed on HCT and HGB. WBC count is a very important variable that determine non-specific responses in fish (Bilen et al. 2011). However, in this study, long-term use of the plant extract resulted in decreased WBC level. Haghighi et al. (2017) found no effects on WBC in rainbow trout fed diet supplemented with *Aloe vera* extract. Similarly, no influences were observed in carp fed with different doses of celery extract (Mohamed et al. 2018) and *Tilia tomentosa* (Almabrok et al. 2018). Mohamadi Saei et al. (2016) observed the highest RBC, WBC, and HCT in fish fed diets containing 300 g/kg of both savory and myrtle extracts. Awad and Austin (2010) noticed the increased values of RBC, HCT, and WBC in rainbow trout fed diets supplemented with *Lupinus perennis*, *Mangifera indica*, and *Urtica dioica*. It seems that, in terms of hematology, long-term use of lemon balm is ineffective.

Digestive enzymes play a significant role in hydrolysis of proteins, lipids, and carbohydrates, thereby assisting in assimilation of nutrients (Furne et al. 2005). In our study, FCR did not vary, whereas SGR and final weight increased significantly. These results demonstrate that feeding rainbow trout with diet containing lemon balm at the rate of 0.5 and 1 g kg^{-1} stimulated digestive enzymes except amylase at the end of the study (Fig. 1). Similarly, increased digestive enzyme activities were observed in rainbow trout fed diet containing lupin (*Lupinus perennis*), mango (*Mangifera indica*), and stinging nettle (*Urtica dioica*) (Awad and Austin 2010). Almabrok et al. (2018) determined no differences in digestive enzyme activities in carps fed diet supplemented with *Tilia tomentosa*. Iqbal et al. (2016) also demonstrated no effects on digestive enzyme activities in *Labeo rohita* fed with plant extracts.

Antioxidant activities of the study are summarized in the Fig. 2. SOD activity showed differences among groups at different sampling times. However, an increased activity was observed in LB1 group. It indicated that positive effects were observed on SOD activity of LB0.5 and LB1. Normally, an increased ORP must be resulted with increased SOD activity. Sonmez et al.

Table 2 Hematological profiles of the rainbow trout fed with different doses of lemon balm (*Melissa officinalis*) methanolic extract

	15th day				45th day				75th day			
	Control	LB 0.1	LB 0.5	LB 1	Control	LB 0.1	LB 0.5	LB 1	Control	LB 0.1	LB 0.5	LB 1
WBC (10^4/mm^3)	7.31 ± 1.0	7.02 ± 0.9	7.41 ± 1.2	7.91 ± 1.1	7.12 ± 2.1	7.43 ± 1.1	7.51 ± 0.9	7.32 ± 1.0	7.89 ± 0.1	7.65 ± 1.4	7.30 ± 1.7	7.69 ± 2.01
RBC (10^6/mm^3)	0.63 ± 0.00	0.66 ± 0.01	0.40 ± 0.00	0.86 ± 0.18	0.75 ± 0.00^c	1.25 ± 0.00^a	1.10 ± 0.00^b	0.57 ± 0.01^d	1.16 ± 0.00^a	0.98 ± 0.00^c	1.03 ± 0.00^b	1.02 ± 0.00^b
HGB (g/dl)	8.99 ± 0.00	8.37 ± 0.01	7.92 ± 0.00	5.92 ± 1.30	7.75 ± 0.01^c	8.07 ± 0.01^b	9.66 ± 0.01^a	3.69 ± 0.00^d	9.09 ± 0.01^b	8.28 ± 0.00^d	9.20 ± 0.01^a	8.68 ± 0.00^c
HCT (%)	14.32 ± 0.01	9.56 ± 0.01	10.24 ± 0.01	20.22 ± 4.12	21.27 ± 0.01^c	22.10 ± 0.01^a	21.48 ± 0.00^b	13.11 ± 0.01^d	30.34 ± 0.01^a	26.38 ± 0.01^c	25.43 ± 0.01^d	28.48 ± 0.04^b
MCV (fl)	223.65 ± 0.00^c	146.47 ± 0.01^d	256.29 ± 0.01^a	235.58 ± 0.07^b	284.12 ± 0.00^a	178.68 ± 0.01^d	195.28 ± 0.00^c	233.82 ± 0.05^b	264.14 ± 0.03^c	271.13 ± 0.04^b	245.76 ± 0.03^d	277.58 ± 0.04^a
MCH (pg/cell)	138.56 ± 0.01^b	128.50 ± 0.03^c	197.65 ± 0.05^a	67.36 ± 2.21^d	103.32 ± 0.03^a	65.26 ± 0.00^c	87.39 ± 0.03^b	63.56 ± 0.00^d	77.53 ± 0.03^c	85.71 ± 0.01^b	90.30 ± 0.04^a	85.87 ± 0.03^b
MCHC (%)	62.77 ± 0.05^c	87.34 ± 0.05^b	77.34 ± 0.08^b	290.25 ± 5.89^a	364.64 ± 0.03^b	364.67 ± 0.04^b	449.58 ± 0.03^a	28.11 ± 0.00^c	299.50 ± 0.03^d	314.74 ± 0.00^b	361.29 ± 0.01^a	303.78 ± 0.02^c

Data are presented as mean ± SE of three replicates ($n = 3$). Means at a particular sampling time with different superscript letters in a row are significantly different ($P < 0.05$). LB0.1, LB0.5, and LB1 are extracts of lemon balm (*Melissa officinalis*) at 0.1 g kg^{-1}, 0.5 g kg^{-1}, and 1 g kg^{-1} diet, respectively

WBC white blood cells, *RBC* red blood cells, *HGB* hemoglobin, *HCT* hematocrit value, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration

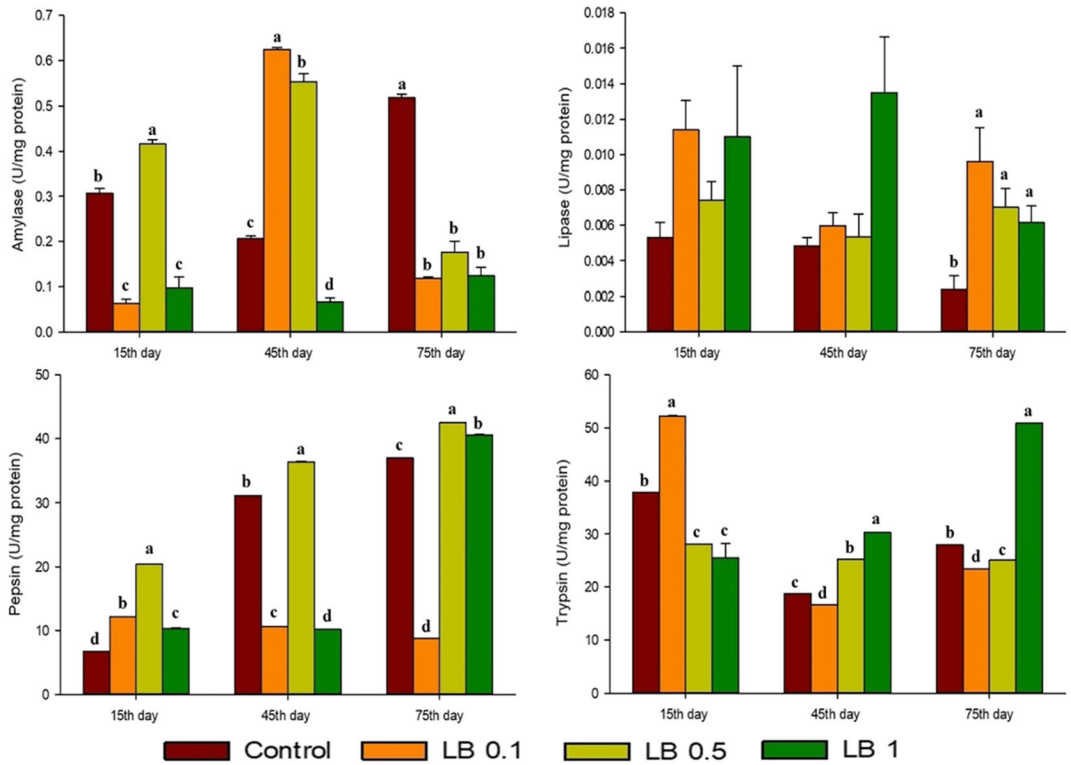

Fig. 1 Amylase, lipase, pepsin, and trypsin activities in rainbow trout juveniles fed diets containing different doses of lemon balm (*Melissa officinalis*) extract; 0 (control), 0.1 (LB0.1), 0.5 (LB0.5), and 1 (LB1) g kg^{-1}. Values are expressed as mean ± SE. Different letters indicate significant differences between groups ($P < 0.05$)

(2015a), Taheri Mirghaed et al. (2018), and Sahin et al. (2014) also observed similar results in rainbow trout. In our study, both parameters increased. It is clear that lemon balm has antioxidant and immunostimulant properties.

CAT displayed no differences on the 15th day of the study. Giannenas et al. (2012) determined a significant increase of CAT in rainbow trout fed diet containing carvacrol and thymol. GR increased significantly in treated fish at all sampling times compared with control. Increased GR activity was reported in several studies (Sahin et al. 2014; Sonmez et al. 2015a). Similar result on GPx was also recorded. GPx catalyzes peroxidation of H_2O_2 (Iskesen et al. 2006). Our study demonstrated an antioxidative response in the fish groups fed with lemon balm. Similarly, Sonmez et al. (2015a) also observed an increased GPx activity in rainbow trout fed with sage, mint, and thyme oils. Lipid peroxidation is an important index of oxidative stress (Fang et al. 2002). In our study, no differences were observed on lipid peroxidation levels. Taheri Mirghaed et al. (2018) determined a decrease in lipid peroxidation in rainbow trout fed diet containing 1,8-cineole. Ciftci et al. (2011) noticed a decrease lipid peroxidation in rats fed with curcumin. Citronellal is one of the main ingredients of the lemon balm (Cosge et al. 2009). Previous studies demonstrated a decreasing level of lipid peroxidation in rats treated with citronellal. In general, our study indicated no difference in lipid peroxidation in treated fish compared with control group.

Immune responses were also improved in rainbow trout fed diet containing lemon balm. ORP is an important immune response indicator. Similarly, Amhamed et al. (2018) determined an elevated ORP in carp treated with *Chenopodium album* extract. Neutrophils are the main source of the oxidative radical production. From the results of the study, it indicated that lemon balm usage could stimulate immune cells, and it can be resulted in an increased ORP. Previous studies also demonstrated that medicinal extract can stimulate immune cells (Amhamed et al. 2018; Bilen et al. 2018; Mohamed et al. 2018). Lysozyme is an important enzyme that disrupts the bacterial cell wall. Increased lysozyme activity was also observed in our study. Another ingredient

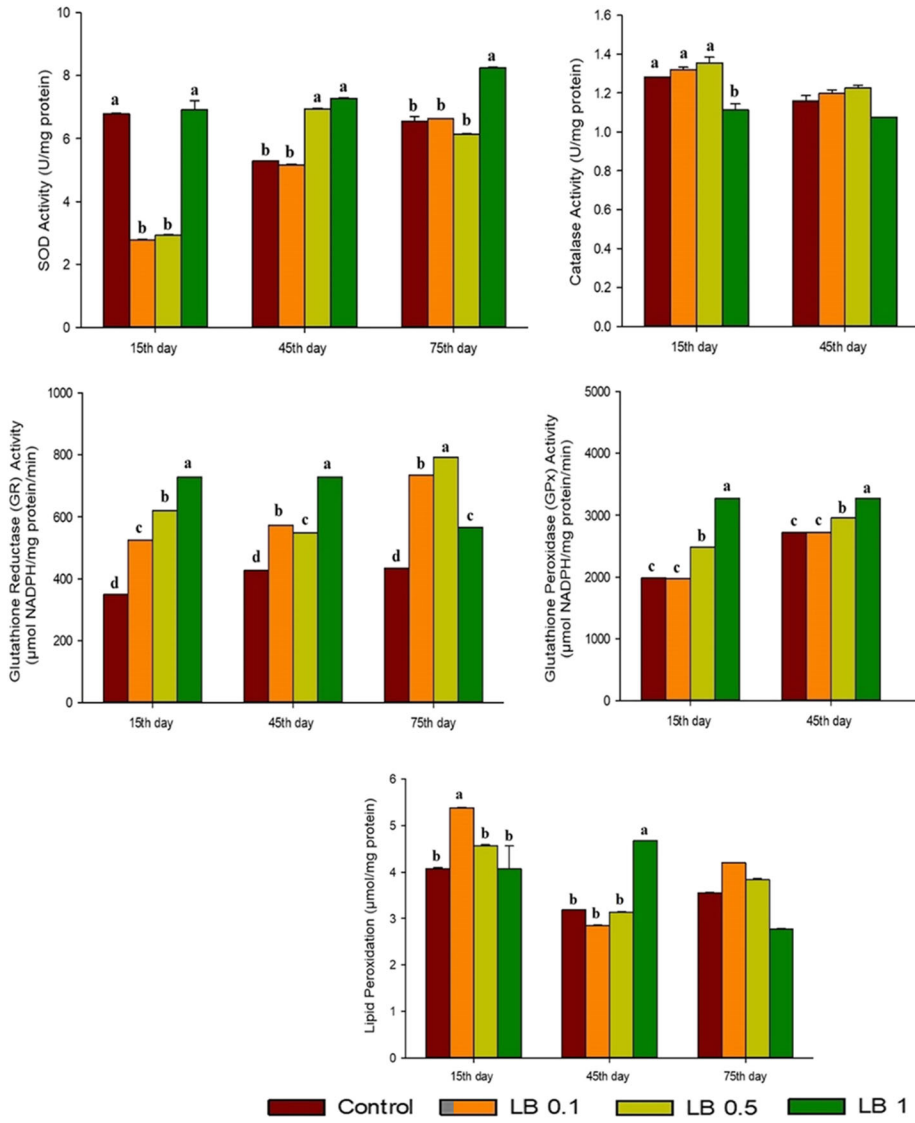

Fig. 2 Superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), and lipid peroxidation in rainbow trout juveniles fed diets containing different doses of lemon balm (*Melissa officinalis*) extract; 0 (control), 0.1 (LB0.1), 0.5 (LB0.5), and 1 (LB1) g kg^{-1}. Values are expressed as mean ± SE. Different letters indicate significant differences between groups ($P < 0.05$)

of the lemon balm, geraniol, has potent anti-inflammatory, anti-angiogenic, anti-cell proliferative, and apoptosis-inducing properties (Vinothkumar et al. 2012). Lysozyme increased in fish fed diets containing *Chenopodium album* (Amhamed et al. 2018), *Apium graveolens* (Mohamed et al. 2018), and *Lepidium sativum* (Bilen et al. 2018) in carp and *Cotinus coggygria* (Bilen et al. 2011), *Capparis spinose* (Bilen et al. 2016a), *Pleurotus ostreatus*, and *Urtica dioica* (Bilen et al. 2016b) in rainbow trout. Myeloperoxidase is also an important immune enzyme that helps in production of H_2O_2 in neutrophils and macrophages during inflammation (Lau et al. 2005). In this study, a decreased MPO was noticed during all the sampling times. Antioxidative properties of the lemon balm may be responsible for these results. Almabrok et al. (2018) reported no differences observed in carp fed diets containing *T. tomentosa* methanolic extract. On the contrary, various medicinal plants influenced an elevated MPO activity in fish (Tang et al. 2014).

Table 3 Non-specific immune response of rainbow trout fed with different doses of lemon balm (*Melissa officinalis*) methanolic extract

Groups	Oxidative Radical Production (mg/ml)			Lysozyme (U/ml)			MPO (OD_{540})		
	15th day	45th day	75th day	15th day	45th day	75th day	15th day	45th day	**75th day**
Control	4.32 ± 0.00^{b}	4.07 ± 0.01^{b}	2.56 ± 0.01^{d}	0.32 ± 0.01^{c}	0.04 ± 0.00^{d}	0.05 ± 0.00^{d}	448.87 ± 0.03^{a}	146.10 ± 0.13^{a}	178.84 ± 0.13^{a}
LB 0.1	$3.05 \pm .00^{e}$	3.05 ± 0.00^{c}	2.70 ± 0.00^{c}	0.39 ± 0.01^{b}	1.16 ± 0.01^{a}	1.86 ± 0.01^{a}	248.77 ± 0.20^{b}	29.61 ± 0.19^{b}	38.97 ± 0.03^{b}
LB 0.5	$3.05 \pm .01^{c}$	2.63 ± 0.00^{d}	3.16 ± 0.00^{b}	0.24 ± 0.00^{d}	1.10 ± 0.00^{b}	0.74 ± 0.00^{c}	154.05 ± 0.09^{c}	24.24 ± 0.08^{c}	13.73 ± 0.11^{c}
LB 1	$4.96 \pm .83^{a}$	5.97 ± 0.00^{a}	4.57 ± 0.01^{a}	1.08 ± 0.00^{a}	0.73 ± 0.00^{c}	1.22 ± 0.00^{b}	147.14 ± 0.10^{d}	21.67 ± 0.10^{d}	13.99 ± 0.11^{c}

Data are presented as mean ± SE of three replicates ($n = 3$). Means of a particular parameter with different superscript letters in a row are significantly different ($P < 0.05$). LB0.1, LB0.5, and LB1 are extracts of lemon balm (*Melissa officinalis*) at 0.1 g kg^{-1}, 0.5 g kg^{-1}, and 1 g kg^{-1} diet, respectively

Conclusion

In the present study, new information on lemon balm is provided by examining its effects on growth performance, antioxidant activity, and immune responses. Therefore, this is a new growth promoter, immunostimulant, and antioxidant for rainbow trout, and further studies needs to be produced, a cost-effective application of the plant in aquaculture industry.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures were approved in advance by the local ethics committee for animal research studies at the Kastamonu University (KUHADYEK-09.05.2016–2016.17).

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