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Growth performance and antioxidant enzyme activities in rainbow trout (*Oncorhynchus mykiss*) juveniles fed diets supplemented with sage, mint and thyme oils

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Abstract This study evaluated effects of dietary supplementation of sage (*Salvia officinalis*), mint (*Mentha spicata*) and thyme (*Thymus vulgaris*) oils on growth performance, lipid peroxidation level (melondialdehyde, MDA) and liver antioxidant enzyme activities (superoxide dismutase, SOD; catalase, CAT; glucose-6-phosphate dehydrogenase, G6PD; glutathione reductase, GR; glutathione-S-transferase, GST and glutathione peroxidase, GPx) in rainbow trout (*Oncorhynchus mykiss*) juveniles. For this purpose, triplicate groups of rainbow trout were fed daily ad libitum with diets containing sage, mint and thyme oils at 500, 1,000 and 1,500 mg kg^{-1} for 60 days. While weight gain percentage of fish fed the diets containing sage and thyme oils was significantly higher than the control group, that of fish fed mint oil was the lowest. Similarly, specific growth rate was found to be the highest in all groups of the sage and thyme oil feeding and the lowest in the mint groups. Moreover, feed conversion ratio was significantly higher in the mint oil administered groups. Survival rate was also significantly reduced in the fish fed the diet containing mint oil. It was observed that SOD, G6PD and GPx activities were significantly increased in liver tissues of all the treated fish groups compared to that of control diet-fed group. However, CAT, GST and GR activities were significantly decreased in experimental diet-fed fish groups at the end of the experiment. On the other hand, a significant reduction was found in MDA levels in the fish fed the diets with sage and thyme oils compared to control and mint diets on the 30th and 60th days of experiment. Overall, dietary inclusion of sage and thyme oils is effective in enhancing rainbow trout growth, reduction in MDA and least changing antioxidant enzyme activities at a low level of 500 mg kg^{-1} diet, and they can be used as important feed supplements for rainbow trout production.

Keywords Fish · Oxidative stress biomarkers · Plant oils · Feed supplement · Growth

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Introduction

Since ancient times, the medicinal plant extracts have been in use for different purposes, such as food supplements, cosmetics and drugs. They have been screened for their potential uses as alternative remedies for treatment of many infectious diseases and preservation of foods from toxic effects of oxidants (Kelen and Tepe 2008). Mainly, the antimicrobial properties of plant extracts and oils have constituted the basis of many applications, including pharmaceuticals, alternative medicines and natural therapies (Reynolds 1996). Turkey has a very rich floral diversity and a great knowledge of folk medicines and consequently represents potential resources for such studies (Hudson et al. 2000). To date, approximately 10,500 plant species have been identified within her borders and 30 % of them are endemic (Davis et al. 1988; Güner et al. 2000). In Turkey, the rate of endemism in plant species is relatively high when compared to other European countries (Ugulu et al. 2009). Folk medical researches about diseases in which herbal drugs are used colloquially in Turkey, and their effects and names have been gaining an increasing attention since Republican period (1923) (Baytop 1999).

Sage, the largest genus of Lamiaceae, includes about 900 species, widespread all over the world, and this genus is stood for in Turkish flora by 88 species and 93 taxa, 45 of which are endemic (Güner et al. 2000). *Salvia officinalis* has been described for many medicinal uses such as astringent, spasmolytic and antiseptic (Newall et al. 1996). Sage species were also reported to be used for memory-enhancing purposes in European folk medicines. Consumption of capsules containing 50 μL essential oil of *S. lavanduaefolia* plus 50 μL of sunflower oil (as a carrier) by human patients for 6 weeks resulted in reduction in neuropsychiatric syndromes and an improvement in attention (Perry et al. 2003). Mint, also known as *Mentha*, is a genus of plants in the family Lamiaceae (Harley et al. 2004) and was originally used as a medicinal herb to treat stomach ache and chest pains and also as sleeping aid, diuretic and an antipruritic. For instance, improvement in patients with acute and chronic symptoms of dyspepsia after administration of peppermint leaves (100 mg) in combination of other herbs (caraway, fennel, gentian) was noticed (Uehleke et al. 2002). The leaves of thyme can be used fresh or dried as a spice. Essential oils extracted from fresh leaves and flowers can be used as aroma additives in foods, pharmaceuticals and cosmetics (Javanmardi et al. 2002). Thyme also possesses various beneficial effects, for example, antiseptic, carminative, antimicrobial and antioxidative properties (Baranauskiene et al. 2003). Thyme oil or thymol administration with diets at 42.5 mg kg^{-1} body weight per day for 3 months to male Wistar rats caused significantly higher antioxidant enzyme activities and total antioxidant status (Youdim and Deans 2000). The antibacterial activities of spices and essential oils have been known for a long time, and a number of research projects on the antimicrobial effect of oregano, thyme and savoury plants, essential oils and their derivatives have been reported (Iscan et al. 2002; Lin et al. 1999; Moreno et al. 2002).

Rainbow trout (*Oncorhynchus mykiss*) is the most widely cultured fish species in the Turkish aquaculture industry (TUIK 2013). Organic fish farming has become popular over the last decade and therefore, natural antioxidative plants have been receiving more attention as important inputs. As far as our literature survey could ascertain, antimicrobial activities of *S. officinalis* have been previously reported (Haznedaroglu et al. 2001), but no information is available on the antioxidative activity of this plant in fish. However, porcine and bovine ground meat samples treated with sage (sage essential oil 3 % w/w) and stored as 4 °C had significantly reduced oxidation (Fasseas et al. 2007). To evaluate the effect of different antioxidant and antimicrobial sources, semi-fried mullet fish fillets were dipped into edible coating solution containing thyme and marjoram at 2.5 and 5.0 % and stored at 4 °C. Samples coated with 5 % thyme showed the lowest rate of peroxide formation (Yasin and Abou-Taleb 2007). Moreover, coated samples with thyme at 2.5 or 5 % showed the lowest incremental pattern in psychrophilic bacterial counts at any point of time during the cold storage. Combined effect of thyme oil (0.2 % v/w) and modified atmosphere packaging (MAP) showed a synergistic effect for shelf-life extension of fresh seabass fillets during refrigerated storage (4 ± 0.5 °C) for a period of 21 days (Kostaki et al. 2009). Therefore, these results indicate potential usefulness of these plant oils.

The antioxidant defence system of organism, comprising of enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx), is set to maintain the lowest potential levels of

reactive oxygen species (ROS) in cells, and recognised as an essential component of an organism's self-maintenance (Castex et al. 2010; Holmblad and Söderhäll 1999). Present in almost all cells are numerous antioxidant systems employed to scavenge free radicals, thus minimising cellular damages. SOD catalyses the dismutation reaction between superoxide radicals (SOR), yielding hydrogen peroxide (H_2O_2) and oxygen. The detoxification of H_2O_2 is then catalysed by GPx or CAT. In addition, other enzymes such as non-selenium-dependent GPx and glutathione reductase (GR) have also a role in protection against free radical damages. Numerous non-enzymatic defences are also employed to provide protection, these include vitamin E, vitamin C and glutathione (GSH) (Harman 1972). It has been reported that the concentrations of a number of these antioxidant enzymes are adequate to cope with the normal production of free radicals, but an imbalance between free radical production and their removal results with ageing allowing progressive damage to occur (Barja de Quiroga et al. 1991; Bunker 1992; Socci et al. 1995; Cao et al. 1992). The main aim of the present study was to investigate the antioxidant potentials of the essential oils of sage, mint and thyme. For this purpose, effects of dietary incorporation of these oils on antioxidant activities in rainbow trout was investigated by analysing different enzymes, viz. SOD, CAT, glucose-6-phosphate dehydrogenase (G6PD), GR, glutathione-S-transferase (GST) and GPx and melondialdehyde (MDA) that is a by-product of lipid oxidation. Besides this, influence of these products on growth, which is an essential trait for fish production in aquaculture, was also investigated.

Materials and methods

Experimental design

Rainbow trout juveniles, mean body weight of 5.06 ± 0.11 g, were provided from Research Center of Faculty of Fisheries at Atatürk University, Turkey, and the fish were maintained in 1,000-L tanks supplied with flow-through system for 15 days prior to the experimental use. The experiment was designed as three replicates of each treatment in 45-L tanks with 20 fish in each. Dechlorinated, aerated and recirculated tap water with a flow rate of 1.5 L min^{-1} was used to maintain a supply of freshwater; the flow rate was equivalent to one full exchange of water every 8.7 h. Other water parameters determined weekly were in the following ranges: temperature 9–11 °C; dissolved oxygen 6–8 mg L^{-1}; pH 7.8–8.2 and total hardness 100–105 mg as $CaCO_3$ L^{-1}.

Essential oils of sage (*Salvia officinalis*), mint (*Mentha spicata*) and thyme (*Thymus vulgaris*) were purchased from NURİ AGA LTD. (Izmir, Turkey) and added to a commercial trout extruded feed (Pınar™, Turkey) at a dose of 500, 1,000 and 1,500 mg kg^{-1} by a mixer. Briefly, after heating (40 °C) for 3 h, the diet was top dressed with fish oil containing the essential oil of herbs by slowly mixing in a food mixer. No herbal oil supplementation was made to control feed. Diets with different doses of sage, mint and thyme oils were called as S500, S1000, S1500, M500, M1000, M1500, T500, T1000 and T1500, respectively. Diets were offered to fish three times a day ad libitum for 60 days. The enzyme levels were determined at 30th and 60th day. Growth parameters, such as weight gain (%) = [100 × (final weight−initial weight)/initial weight], specific growth rate (SGR) (% d^{-1}) = 100 × [(ln final fish weight) − (ln initial fish weight)]/days fed, feed conversion ratio (FCR) = feed intake (g)/weight gain (g) and survival were calculated at the end of experiment.

This experimental design was approved by the Ethics Committee for Animal Experimentation of Atatürk University (2014-101).

Determination of enzyme activities

In order to determine antioxidant enzyme activities, such as SOD, CAT, G6PD, GR, GST and GPx, ten fish from each experimental group were scooped out randomly from holding tank at 30th and 60th day of the experiment and kept in a bucket containing aerated freshwater. Fish were euthanised by an overdose of phenoxyethanol. Liver samples of the fish were excised aseptically and frozen immediately in liquid nitrogen. All samples were then stored at −80 °C until analyses. 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 homogeniser and put into homogenisation medium (0.25 M sucrose, 0.5 mM EDTA and 10 mM Tris–HCl; pH 7.4) for centrifugation at 4 °C, 10000×*g* for 45 min (Hisar et al. 2009).

After centrifugation, the supernatant was separated and used for activity assays.

SOD

SOD (EC 1.15.1.1) activity was determined according to the method described by Kono (1978) with some modifications. The reaction mixture consisted of 0.725 mL of tris-buffer (50 mM; pH 8.3, Sigma, France), 0.1 mL nitro-blue tetrazolium (NBT, 500 μM; Sigma), 0.1 mL of nicotinamide adenine dinucleotide reduced, NADH (780 μM; Sigma) and phenazonium methosulfate (Sigma) with different volumes, 0.025, 0.050, 0.075, 0.1 and 0.050 mL of liver sample. The change in absorbance was recorded spectrophotometrically at 560 nm. Activity was reported by its ability to inhibit 50 % reduction in NBT, and the value is expressed as U/min/mg/protein.

CAT

The CAT (EC 1.11.1.6) activity was analysed following the method of Claiborne (1985) as described by Giri et al. (1996). The test mixture contained 1 mL H_2O_2 (0.019 M), 1.95 mL phosphate-buffered saline, PBS (0.05 M, pH 7.0) and 0.05 mL of liver sample in a final volume of 3 mL. Change in absorbance was recorded at 240 nm during incubation for 1 min at 20 °C. Catalase activity was calculated in terms of nmol H_2O_2 consumed per min per mg protein.

G6PD

The G6PD (EC 1.1.1.49) activity was assayed by the method of Beutler (1984). This activity assay was carried out by monitoring the increase in absorption at 340 nm because of the reduction in 2 mM $NADP^+$. The assay system contained 1 M Tris–HCl, 5 mM EDTA buffer (pH 8.0), 0.1 M $MgCl_2$, 2 mM $NADP^+$, 6 mM G6P, distilled water and liver tissue homogenate (50 μL).

GR

The GR (EC 1.6.4.2) activity was determined using reduced nicotinamide adenine dinucleotide phosphate (NADPH) (Sigma) and oxidized glutathione (GSSG) (Sigma) as substrates (4 min at 20 °C) by a technique described by Knörzer et al. (1996). One unit of GR was defined as the quantity of NADPH consumed per min, which catalysed the reduction in 1 mM of GSSG.

GST

The glutathione-S-transferase (EC 2.5.1.18) activity was evaluated using 1-chloro-2, 4 dinitrobenzene (CDNB) (Sigma) as substrate, as previously described by Habig et al. (1974). The measurements were performed at 25 °C during 5-min incubation. One unit of GST was defined as the quantity of enzyme that catalysed the formation of 1 μmol of product per min at 25 °C and pH of 6.5.

GPx

The GPx (EC 1.11.1.9) activity was assayed according to the method described by Beutler (1984). The assay mixture consisted of 100 μL Tris–EDTA, 100 μL GR (10 U/mL), 20 μL GSH (0.1 M), 100 μL NADPH (2 mM), 660 μL distillated water and homogenate (1:16) and was incubated for 10 min at 37 °C. Then, 10 μL 7 mM t-butilhydroperoxide was added to this mixture. Absorbance values were recorded in a spectrophotometer at 340 nm, and final absorbance was calculated after deducting the absorbance of t-butilhydroperoxide as blank.

MDA

Melanaldehyde (MDA) levels of the fish livers were estimated according to the method of Hisar et al. (2009). To 0.5 mL homogenate, 0.5 mL Tris/HCl buffer (50 mM, pH 7.4) was added followed by further mixing and incubation at room temperature for 10 min; 1.0 mL of 0.75 % thiobarbituric acid in 2 M Na2SO4 was added, and then the mixture was heated at 96 °C for 45 min. After cooling, 1.0 mL of 70 % TCA was added, the mixture was then vortexed and centrifuged at $1000 \times g$ for 10 min. The absorbance of supernatant was determined at 530 nm. Total thiobarbituric acid-reactive materials were expressed as MDA, using a molar extinction coefficient for MDA of $1.56 \times 105\ cm^{-1}\ M^{-1}$. MDA concentrations were expressed as μmoL per mg protein. All processes were carried out in the ice or at 4 °C. Protein levels were determined spectrophotometrically (595 nm) according to the Bradford method (1976) using bovine serum albumin (BSA) as the standard.

Statistical analysis

The data were checked for normality before analysis, and they followed normal distribution. One-way analysis of variance and LSD multiple range tests were run to ascertain any difference in enzyme activity values among different treatment groups at any one sampling time using SPSS for Windows v. 17.0 program (SPSS Inc., Chicago, IL, USA). Statistical analysis was performed in the same way for all parameters tested. The accepted level of significance was $P < 0.05$.

Results

Effects on antioxidant enzyme activities

The specific activity values of the SOD, CAT, G6PD, GR, GST and GPx from the liver samples are presented in Tables 1–6, respectively.

Except in M1500 and T1500 groups at 30th day, and S500–1500, M1500, T1000 and T1500 groups at 60th day of the study, the activities of SOD in other inclusion levels over the time course were significantly increased compared to the control group ($P < 0.05$) (Table 1). On the whole, CAT activity was decreased over the time course tested in all the treated fish compared to the control group with the lowest values in T1500 group (Table 2). G6PD enzyme activity was found higher in all experimental groups except T1500 than in control group ($P < 0.05$) at 30th day. It was also observed that at 60th day all values enhanced significantly compared with that of control and treated groups' results at 30th day ($P < 0.05$) (Table 3).

In all the treated fish groups, GR enzyme activity of the liver tissue was decreased compared to the control group over the experimental time course (Table 4). Similar results were observed at 60th day. GST activity had no differences among S1000, T1500 and control groups. S500 group exhibited a decrease in GST value at the 30th and 60th day of study. In comparison with control diet-fed fish, GST activity was significantly increased in rest of the treated groups (Table 5). GPx enzyme activity in all the experimental diet-fed groups was increased significantly compared to that of control group both at 30th and 60th day of experiment (Table 6). However, the values at 60th day decreased compared to 30th day's results.

In addition, a significant reduction was found in MDA levels in the fish fed the diets with sage and thyme oils compared with that of basal diet-fed fish on the 30th and 60th day of experiment (Table 7).

Table 1 Superoxide dismutase (SOD) activities in rainbow trout juveniles fed diets containing different doses of sage, mint and thyme oils

Diet	Time (days)		
	0	30	60
Control	40.33 ± 2.28^{a}	48.40 ± 5.57^{Aa}	40.58 ± 3.99^{Ba}
S500		55.02 ± 3.03^{Bb}	39.46 ± 2.17^{Bc}
S1000		56.23 ± 6.06^{Bb}	40.33 ± 4.35^{Bc}
S1500		56.23 ± 6.96^{Bb}	40.33 ± 4.99^{Bc}
M500		74.30 ± 7.75^{Cb}	53.29 ± 5.56^{Cc}
M1000		66.27 ± 3.61^{Cb}	47.53 ± 2.59^{Cc}
M1500		47.39 ± 5.57^{Ab}	33.99 ± 3.99^{Bc}
T500		73.10 ± 6.64^{Cb}	52.43 ± 4.76^{Cc}
T1000		57.03 ± 6.06^{Bb}	40.90 ± 4.35^{Bc}
T1500		32.93 ± 3.03^{Ab}	23.62 ± 2.17^{Ac}

Diets with three inclusion levels (500, 1,000 and 1,500 mg kg^{-1}) of sage, mint and thyme oils are denoted as S500, S1000, S1500, M500, M1000, M1500, T500, T1000 and T1500, respectively. Lowercase superscripts (a, b) indicate significant differences ($P < 0.05$) among different doses within each experimental diet-fed group, whereas superscripts in uppercase show significant differences ($P < 0.05$) among diet groups. Each value is the mean ± S.D. of ten individual observations. Specific activity is expressed as units per mg protein

Effects on growth, FCR and survival

Effects of different doses of sage, mint and thyme oils on growth, FCR and survival in rainbow trout fingerlings are presented in Table 8. Mint oil fed fish groups had significantly lower SGR and percentage weight gain percentage compared to the control group at the end of experiment. SGR and weight gain were similar in sage and thyme oil fed groups and found to be the highest in the former groups. FCR was affected negatively in all mint groups and found to be higher than in control and other groups ($P < 0.05$). Significantly lower FCR values were observed in the sage and thyme treated groups compared to that in control ($P < 0.05$) with no difference between each other's FCRs. Sage and thyme oil fed groups had similar survivals with that of control group ($P > 0.05$). However, mint oil supplemented diets affected survival rate negatively ($P < 0.05$).

Table 2 Catalase (CAT) activities in rainbow trout juveniles fed diets containing different doses of sage, mint and thyme oils

Diet	Time (days)		
	0	30	60
Control	165.95 ± 8.10^{a}	170.93 ± 8.35^{Aa}	164.38 ± 7.05^{Aa}
S500		99.79 ± 8.24^{Ba}	84.29 ± 6.96^{Da}
S1000		112.56 ± 10.33^{Ba}	95.07 ± 8.72^{Da}
S1500		96.82 ± 13.62^{Ba}	81.78 ± 11.51^{Db}
M500		152.82 ± 8.55^{Aa}	129.08 ± 7.23^{Cb}
M1000		88.29 ± 2.33^{Ba}	74.57 ± 1.97^{Db}
M1500		78.75 ± 13.00^{Ba}	66.52 ± 10.98^{Da}
T500		164.62 ± 2.72^{Aa}	139.05 ± 2.30^{Cb}
T1000		170.17 ± 1.01^{Aa}	143.74 ± 0.85^{Bb}
T1500		55.34 ± 3.48^{Ca}	46.75 ± 2.94^{Eb}

Diets with three inclusion levels (500, 1,000 and 1,500 mg kg^{-1}) of sage, mint and thyme oils are denoted as S500, S1000, S1500, M500, M1000, M1500, T500, T1000 and T1500, respectively. Lowercase superscripts (a, b) indicate significant differences ($P < 0.05$) among different doses within each experimental diet-fed group, whereas superscripts in uppercase show significant differences ($P < 0.05$) among diet groups. Each value is the mean ± S.D. of ten individual observations. Specific activity is expressed as μmol H_2O_2 metabolised per mg protein per min

Table 3 Glucose-6-phosphatase dehydrogenase (G6PD) activities in rainbow trout juveniles fed diets containing different doses of sage, mint and thyme oils

Diet	Time (days)		
	0	30	60
Control	0.10 ± 0.01^{a}	0.137 ± 0.04^{Aa}	0.155 ± 0.10^{Aa}
S500		0.521 ± 0.02^{Da}	1.270 ± 0.05^{Bb}
S1000		0.682 ± 0.01^{Ea}	1.660 ± 0.01^{CDb}
S1500		0.566 ± 0.09^{Da}	1.380 ± 0.22^{Cb}
M500		0.674 ± 0.01^{Ea}	1.640 ± 0.01^{Db}
M1000		0.357 ± 0.02^{Ba}	0.870 ± 0.05^{Ab}
M1500		0.427 ± 0.01^{Ca}	1.040 ± 0.01^{Eb}
T500		0.506 ± 0.01^{Da}	1.230 ± 0.01^{Bb}
T1000		0.454 ± 0.01^{Ca}	1.100 ± 0.02^{Bb}
T1500		0.199 ± 0.06^{Aa}	0.480 ± 0.14^{Ab}

Diets with three inclusion levels (500, 1,000 and 1,500 mg kg^{-1}) of sage, mint and thyme oils are denoted as S500, S1000, S1500, M500, M1000, M1500, T500, T1000 and T1500, respectively. Lowercase superscripts (a, b) indicate significant differences ($P < 0.05$) among different doses within each experimental diet-fed group, whereas superscripts in uppercase show significant differences ($P < 0.05$) among diet groups. Each value is the mean ± S.D. of ten individual observations. Specific activity is expressed as μmol NADP + per mg protein per min

Table 4 Glutathione reductase (GR) activities in rainbow trout juveniles fed diets containing different doses of sage, mint and thyme oils

Diet	Time (days)		
	0	30	60
Control	4.10 ± 0.41^{a}	4.25 ± 1.26^{Da}	4.05 ± 2.05^{Ea}
S500		7.28 ± 0.51^{Ea}	1.46 ± 0.10^{Db}
S1000		3.77 ± 0.44^{Da}	0.75 ± 0.09^{Cb}
S1500		3.93 ± 0.17^{Da}	0.79 ± 0.03^{Cb}
M500		1.34 ± 0.10^{ABa}	0.27 ± 0.02^{Ab}
M1000		2.70 ± 0.03^{Ca}	0.54 ± 0.06^{Bb}
M1500		4.46 ± 0.41^{Da}	0.89 ± 0.08^{Cb}
T500		0.62 ± 0.51^{Aa}	0.12 ± 0.10^{Ab}
T1000		3.92 ± 0.68^{Aa}	0.78 ± 0.14^{Cb}
T1500		1.70 ± 0.39^{Ba}	0.34 ± 0.08^{Ab}

Diets with three inclusion levels (500, 1,000 and 1,500 mg kg^{-1}) of sage, mint and thyme oils are denoted as S500, S1000, S1500, M500, M1000, M1500, T500, T1000 and T1500, respectively. Lowercase superscripts (a, b) indicate significant differences ($P < 0.05$) among different doses within each experimental diet-fed group, whereas superscripts in uppercase show significant differences ($P < 0.05$) among diet groups. Each value is the mean ± S.D. of ten individual observations. Specific activity is expressed as μmol NADPH per mg protein per min

Discussion

In this study, impacts of three different plant oils were examined on antioxidant enzyme activities and growth promoting effect in rainbow trout. Feeding sage, mint and thyme oil supplemented diets influenced antioxidant enzyme activities, growth, FCR and survival in varied manners in rainbow trout.

Table 5 Glutathione-S-transferase (GST) activities in rainbow trout juveniles fed diets containing different doses of sage, mint and thyme oils

Diet	Time (days)		
	0	30	60
Control	14.92 ± 1.10^{a}	14.83 ± 2.19^{Ba}	13.33 ± 1.57^{Ba}
S500		9.12 ± 0.22^{Aa}	6.52 ± 0.16^{Ab}
S1000		16.45 ± 0.04^{Ba}	11.76 ± 0.03^{Bb}
S1500		12.99 ± 3.20^{Ba}	9.29 ± 2.29^{ABb}
M500		25.72 ± 9.36^{BCDa}	18.39 ± 6.69^{Cb}
M1000		20.58 ± 0.78^{Ca}	14.71 ± 0.56^{Bb}
M1500		25.78 ± 0.52^{Da}	18.43 ± 0.37^{Cb}
T500		23.99 ± 2.74^{CDa}	17.15 ± 1.96^{Cb}
T1000		21.51 ± 0.74^{Ca}	15.38 ± 0.53^{BCb}
T1500		12.85 ± 0.75^{Ba}	9.19 ± 0.53^{ABb}

Diets with three inclusion levels (500, 1,000 and 1,500 mg kg^{-1}) of sage, mint and thyme oils are denoted as S500, S1000, S1500, M500, M1000, M1500, T500, T1000 and T1500, respectively. Lowercase superscripts (a, b) indicate significant differences ($P < 0.05$) among different doses within each experimental diet-fed group, whereas superscripts in uppercase show significant differences ($P < 0.05$) among diet groups. Each value is the mean ± S.D. of ten individual observations. Specific activity is expressed as μmol CDNB per mg protein per min

Table 6 Glutathione peroxidase (GPx) activities in rainbow trout juveniles fed diets containing different doses of sage, mint and thyme oils

Diet	Time (days)		
	0	30	60
Control	2.91 ± 1.02^{a}	2.33 ± 0.57^{Aa}	2.30 ± 0.27^{Aa}
S500		6.77 ± 0.31^{Ba}	3.85 ± 0.17^{Ab}
S1000		39.83 ± 1.07^{Ca}	22.64 ± 0.61^{Bb}
S1500		41.54 ± 0.47^{Ca}	23.61 ± 0.27^{Bb}
M500		36.39 ± 6.63^{Ca}	20.68 ± 3.77^{Bb}
M1000		37.08 ± 6.63^{Ca}	21.07 ± 3.77^{Bb}
M1500		51.63 ± 19.24^{Ca}	29.35 ± 10.94^{Ba}
T500		35.57 ± 2.92^{Ca}	20.22 ± 1.66^{Bb}
T1000		35.01 ± 0.79^{Ca}	19.90 ± 0.45^{Bb}
T1500		37.42 ± 3.82^{Ca}	21.27 ± 2.17^{Bb}

Diets with three inclusion levels (500, 1,000 and 1,500 mg kg^{-1}) of sage, mint and thyme oils are denoted as S500, S1000, S1500, M500, M1000, M1500, T500, T1000 and T1500, respectively. Lowercase superscripts (a, b) indicate significant differences ($P < 0.05$) among different doses within each experimental diet-fed group, whereas superscripts in uppercase show significant differences ($P < 0.05$) among diet groups. Each value is the mean ± S.D. of ten individual observations. Specific activity is expressed as units per mg protein

Table 7 The effects of sage, mint and thyme oils at the different concentrations on malondialdehyde (MDA) levels in rainbow trout (*Oncorhynchus mykiss*) juvenile livers

Diet	Time (days)		
	0	30	60
Control	121 ± 16	126 ± 13^{A}	115 ± 17^{A}
S500		30 ± 9^{C}	28 ± 7^{C}
S1000		45 ± 10^{C}	39 ± 10^{C}
S1500		52 ± 10^{BC}	46 ± 11^{BC}
M500		140 ± 21^{A}	138 ± 20^{A}
M1000		159 ± 23^{A}	144 ± 19^{A}
M1500		159 ± 20^{A}	144 ± 22^{A}
T500		77 ± 12^{B}	66 ± 12^{B}
T1000		82 ± 14^{B}	65 ± 11^{B}
T1500		85 ± 13^{B}	70 ± 12^{B}

Diets with three inclusion levels (500, 1,000 and 1,500 mg kg^{-1}) of sage, mint and thyme oils are denoted as S500, S1000, S1500, M500, M1000, M1500, T500, T1000 and T1500, respectively. Capital superscripts (A, B) show significant differences ($P < 0.05$) among diet groups. Each value is the mean ± S.D. of ten individual observations. MDA is expressed as μmol per mg protein

SOD and CAT involve in cellular defences against uncontrolled oxidative processes and catalyse the dismutation of superoxide radical and H_2O_2 (Otto and Moon 1996). These two antioxidant enzymes have related functions and are considered as the first line of defence against oxygen toxicity due to their inhibitory effects on oxygen radical formation (Pandey et al.

Table 8 Effects of different diets containing three levels of sage, mint and thyme oils on growth, feed conversion and survival in rainbow trout juveniles at 60th day of the study

Diet	SGR (% d^{-1})	Weight gain (%)	FCR	Survival (%)
Control	2.39 ± 0.08^{a}	320.21 ± 1.87^{a}	0.94 ± 0.02^{a}	100^{a}
S500	2.79 ± 0.06^{b}	422.38 ± 2.58^{b}	0.71 ± 0.1^{b}	100^{a}
S1000	2.74 ± 0.05^{b}	421.28 ± 4.74^{b}	0.75 ± 0.2^{b}	100^{a}
S1500	2.70 ± 0.09^{b}	411.50 ± 4.21^{b}	$0.74 \pm .05^{b}$	100^{a}
M500	2.05 ± 0.06^{c}	241.58 ± 6.34^{c}	1.25 ± 0.02^{c}	85 ± 2.5^{b}
M1000	2.00 ± 0.05^{c}	254.12 ± 2.85^{c}	1.20 ± 0.13^{c}	60 ± 3^{c}
M1500	1.87 ± 0.10^{d}	223.54 ± 1.24^{c}	1.35 ± 0.14^{c}	60 ± 3^{c}
T500	2.65 ± 0.06^{b}	375.84 ± 2.84^{d}	0.80 ± 0.12^{b}	100^{a}
T1000	2.61 ± 0.04^{b}	374.21 ± 3.21^{d}	0.80 ± 0.12^{b}	100^{a}
T1500	2.67 ± 0.01^{b}	385.21 ± 2.35^{d}	0.78 ± 0.11^{b}	100^{a}

Diets with three inclusion levels (500, 1,000 and 1,500 mg kg^{-1}) of sage, mint and thyme oils are denoted as S500, S1000, S1500, M500, M1000, M1500, T500, T1000 and T1500, respectively. All data represent the mean ± S.D. ($n = 10$). Within a column, values with different superscripts are significantly different from each other ($P < 0.05$)

SGR specific growth rate, *FCR* feed conversion ratio

2003; Li et al. 2009). In the present study, activity of SOD was increased significantly in M500, M1000 and T500 treated groups all through the time course. An increase in the activity of this enzyme probably neutralises the impact of increased ROS generation (John et al. 2001). H_2O_2 is a damaging by-product of many normal metabolic processes and to prevent damages to cells and tissues, it must be quickly converted into other less dangerous compounds. For this purpose, CAT is frequently used by cells to rapidly catalyse the decomposition of H_2O_2 into less reactive gaseous oxygen and water molecules (Gaetani et al. 1996). It is a fact that all known animals use CAT in every organ, with particularly high concentrations occurring in the liver. In our study, CAT activity was affected negatively and decreased at all sampling days and in different dietary levels of groups except M500, T500 and T1000. According to Gião et al. (2010), aqueous extracts of a few medicinal plants lead to a decrease in CAT activity Peroxisomes possess CAT, and designate that intracellular localisation of the enzyme could manage different responses to oxidative stress.

It was found that diets incorporated with sage, mint and thyme oils significantly affected the enzyme G6PD by increasing its activities ($P < 0.05$). Although larger trout requires less amount of NADPH production necessary to satisfy the growth demand (Peragón et al. 1998), and there has also been a clear evidence that during trout development liver makes less total NADPH available to cells (Barroso et al. 1999), our results may suggest that small size fish require more amount of NADPH production resulted from an increasing level of G6PD activity.

Although in all groups, GR enzyme activities are generally low in the polar fish compared with temperate species, aerobic enzyme cytochrome c oxidase consistently show greater activities in polar species than in temperate zone fish (Speers-Roesch and Ballantyne 2005), thus demonstrating that having a greater aerobic demand does not confer direct compensation for higher antioxidant enzyme activities in Antarctic fish. This may explain why GR activity was detected lower in all groups than in control.

The major role of the GST is cellular defence against chemically induced toxicity (Blanchette et al. 2007). According to Hisar et al. (2012), higher GST activities in liver of fish fed the diets with diludine tended to be higher. Similarly, in our study, GST activities in liver of fish fed the diets containing mint (all doses) and thyme (T500 and T1000) were increased. These findings suggest that mint and thyme supplementations provided enhanced antioxidant protection in rainbow trout.

GPx catalyse the reduction in H_2O_2 and lipid peroxides (Üner et al. 2006). An increase in GPx activity in almost all groups was observed at 30th and 60th day compared to control. However, values of 30th day were also higher than 60th day results in the treated fish groups. This could indicate that the

antioxidant capacity of fish liver was elevated at an early phase. According to the present and previous studies (Monteiro et al. 2006; Pandey et al. 2008; Zhang et al. 2008), GPx depletion may reduce the cellular ability to scavenge free radicals, raising the general oxidative potential in the cells. Lowering the intracellular glutathione level and decreasing glutathione-related antioxidant enzymes activity simultaneously lead to oxidative imbalance and induce oxidative processes, resulting in increased cell death (Pandey et al. 2008). Considering all results of the study, a change in activity of some enzymes probably reflected an adaptation to rearing conditions and diet compositions (Bastrop et al. 1991).

It can be assumed that the increased SOD enzyme activity during the experimental period may be attributed to the enhanced production of highly reactive superoxide anions associated with growth. It is well known that the increased production of H_2O_2 can be detoxified by CAT and GPx. While CAT activities were significantly decreased, GPx activity was increased in liver tissues of all the experimental diets fed fish. Therefore, it can be suggested that GPx may be mainly responsible for detoxifying H_2O_2. GPx catalyses the reduction in hydroperoxides using GSH. Glutathione disulphide (GSSG) is reduced to GSH by GR. This reaction enzymatically requires NADPH produced by glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase in the pentose phosphate pathway (Urso and Clarkson 2003). Hence, the increased G6PD activity is not a surprising result because of the increased NADPH demands at the biochemical reaction chain.

As an outcome of the study, the present work demonstrated changes in oxidative stress indices and antioxidant defence systems in liver of rainbow trout after long-term exposure to different plant oils. Sage and thyme oils have been determined as convenient and useful antioxidant enzyme stimulators and growth promoters. Although, mint oil generally caused an increase in almost all antioxidant enzyme levels, all growth parameters, FCR and survival were negatively affected by mint diets. Therefore, it is suggested that mint has some undesirable effects on rainbow trout physiology and is not a suitable feed additive. Overall, dietary inclusion of sage and thyme oils is effective in enhancing rainbow trout growth, reduction in MDA and least changing antioxidant enzyme activities at a low level of 500 mg kg^{-1} diet, and they can be used as important feed supplements for rainbow trout production.

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