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RESEARCH ARTICLE

Growth promoter, immunostimulant and antioxidant for rainbow trout (*Oncorhynchus mykiss*): Terebinth (*Pistacia terebinthus*) extract

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ABSTRACT

In this study, the effects of *Pistacia terebinthus* (PT) fruit extract supplemented diet on growth performance, haematology, digestive and antioxidant enzyme activities, and non-specific immune responses were evaluated in juvenile rainbow trout (*Onchoryhnchus mykiss*). The fish were fed diets containing three doses of *Pistacia terebinthus* extract (0.1, 0.5 and 1% of diet) and a control diet without extract for 63 days. Final weight, weight gain and specific growth rate were significantly improved in all the treated groups. In addition, feed conversion ratio was significantly reduced in all PT diet fed groups. Pepsin and lipase activities were significantly increased in all the treated groups. Trypsin was significantly improved in PT 0.1% and PT 1% groups. Amylase was significantly increased in PT 0.5 and 1% groups. In haematological assays, red blood cell, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration values were not changed among all experimental groups. Superoxide dismutase, catalase, glutathione peroxidase and glucose-6-phosphate dehydrogenase activities were significantly improved in all the treatment groups. However, catalase activity decreased in PT 0.5% group at the end of 63 days. In addition, hepatic and white muscle lipid peroxidation activities were significant decreased in all the treated groups compared to the control. Non-specific immune parameters, such as nitroblue tetrazolium reduction, myeloperoxidase and lysozyme activities were increased in all the treated fish groups. These results indicated that extract of *P. terebinthus* can be used to improve fish health in aquaculture.

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Introduction

Fish is one of the important protein sources in human diet. Over the last two decades, aquaculture sector has developed rapidly and expanded to many countries (FAO, 2018). However, this development causes various economic issues such as disease risk, use of expensive feed, wrong feeding strategy or formulation and others (Farahi et al., 2010; Syahidah et al., 2015; Erguig et al., 2015). Nutritional management is an important key factor for prevention of diseases and successful production in aquaculture (Farahi et al., 2010; Bilen & Bilen, 2013).

Inadequate nutritional factors are known to cause various diseases and poor health condition in fish (Waagbø, 1994). Adequate nutrients in diet directly affect to improve fish health and prevent disease. Therefore, strategies for nutritional management have critical role in the success of semi-intensive and intensive aquaculture (Zoral et al., 2018). Also, the increase of technological developments gave an opportunity to increase stocking densities in aquaculture facilities (Bilen et al., 2013), which in turns could also negatively affect fish health.

Fish farmers usually use antibiotics or some chemotherapeutics for the prevention and control of diseases (Boran et al., 2013; Cabello et al., 2016; Uney et al., 2021). However, antibiotics and chemotherapeutics may pose residual and toxic effects on organs in fish and human (Erguig et al., 2015; Syahidah et al., 2015). In addition, the use of antibiotics and chemotherapeutic agents for controlling diseases can pollute the aquatic environment (Biswas et al., 2010; Syahidah et al., 2015).

Since the past decade, application of medicinal plants and their bioactive compounds are being widely used to prevent diseases and maintain fish health in aquaculture (Hoseinifar et al., 2019; Bilen et al., 2020; Hoseinifar et al., 2020a; Hoseinifar et al., 2020b; Terzi et al., 2021; Yılmaz et al., 2022). Bioactive compounds of these herbs have various beneficial effects to animal (Güroy et al., 2022; Sönmez et al., 2019; Sönmez et al., 2021). For example, several reports demonstrated that medicinal plants have antimicrobial, anti-antioxidant, appetite stimulating, digestibility ameliorative, growth promoting, sperm quality improving and immunostimulant properties (Ranjbar et al., 2010; Banaee et al., 2011; Takaoka et al., 2011; Vaseeharan & Thaya, 2014; Sönmez et al., 2018; Sönmez et al, 2019; Karga et al., 2020).

Pistacia terebinthus L. (terebinth), belonging to the family Anacardiaceae, is a plant that widely grows in the Mediterranean region countries, such as Morocco, Portugal, Greece, Turkey, Syria and Lebanon (Topçu et al., 2007). It was reported that terebinth can be used to treat bronchitis, wounds, burns and stomach disorders (Topçu et al., 2007; Cakilcioglu & Turkoglu, 2010; Gogus et al., 2011). In addition, pharmacological studies have revealed that terebinth has antimicrobial, antifungal, antioxidant, anti-inflammatory, diuretic and antitussive properties (Topçu et al., 2007; Özcan et al., 2009). There are no reports on the application of this herb for improvement of growth and health of rainbow trout (*Onchoryhnchus mykiss*).

In this context, an experiment was conducted to investigate the efficacy of terebinth supplemented diet on rainbow trout health. We examined various parameters (growth performance, digestive and antioxidant enzyme activities, haematological profile, and immune responses) which are directly related to aquatic animal health.

Material and Methods

Source of fish and acclimatization

Rainbow trout juveniles (15.77±0.13 g) were purchased from a commercial fish farm in Kastamonu, Turkey. All experiments were performed at the indoor laboratory of Kastamonu University, Fisheries Faculty, Turkey. The fish were acclimatized for 2 weeks before commencement of the experiment. Water parameters (7.77-9.29 mg/L of dissolved oxygen, 6.5-7.5 pH, 12°C water temperature) were checked daily. Photoperiod was maintained at 12 h light: 12 h dark. During acclimation, the fish were fed *ad libitum* with a commercial diet twice a day.

Preparation of terebinth extract

Ripe fruits of terebinth were collected according to the procedure described by Pakravan et al. (2012) with a slight modification. The ripe fruits were ground in a mechanical grinder to a fine powder. Fifty gram of fruits and 1 L of 40% methanol (Sigma-Aldrich, St. Louis, MO, USA) were blended in a laboratory blender. The mixture was kept at room temperature for 3 days with daily shaking. After 3 days, fruit extract was filtered through a filter paper (Whatman filter No 1). Then, the filtrate was collected and evaporated in a rotary evaporator at 55-65°C to remove alcohol from the fruit extract. Final product (crude) was dissolved in distilled water and kept in a flask at 4°C until use for experiment. The final product was sprayed on fish diet (Commercial fish feed (Özpekler MoyFeed, 2 mm, Protein 48%, Fat 17%) at three selected doses, 0.1, 0.5 and 1% terebinth extract diet (Taee et al., 2017). All the

experimental diets were kept in plastic zipped packs and stored at -20°C until use. Diet composition is shown in Table 1.

Table 1. Composition of the experimental diets

Constituents	Concentration (%)			
	Control 0	PT 0.1%	PT 0.5%	PT 1%
Crude protein	46	46	46	46
Total lipid	14	14	14	14
Moisture	11	11	11	11
Ash	10	10	10	10
Phosphorus	1.2	1.2	1.2	1.2
Fiber	3	3	3	3
PT fruit extract	0	0.5	0.1	1

Note: PT: *Pistacia terebinthus*, PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract

Oral treatment trials and sampling

The fish were randomly divided into four main groups that were fed with 3 experimental diets, 0.1, 0.5 and 1% *Pistacia terebinthus* and a control diet without extract. Forty fish were distributed in each tank (500 L, 1 m diameter) in triplicate. All the groups were fed *ad-libitum* for 63 days twice in a day.

During oral administration experiment, fish (3 fishes from each experimental tanks) were randomly selected and were anesthetised (0.01 mg/L of fenoxyethanol) for sampling at 21, 42 and 63 days. Blood was collected from caudal vein puncture with heparinized syringes. The blood was transferred to EDTA tubes for analysis of haematological and non-specific immune parameters. The blood was centrifuged at 10000 rpm for 15 min at 4°C. And then, plasma was separated and stored at -80°C.

Stomach and anterior part of intestine were collected, and stored in plastic tubes at -80°C at the end of experiment. Liver and white muscle samples were collected at 21, 42 and 63 days and stored in liquid nitrogen at -80°C until antioxidant enzyme and lipid peroxidation analyses were performed.

All animal experiments were conducted according to the relevant international guidelines. Study protocols were approved in advance by the local ethics committee for animal research studies at the Kastamonu University (KUHADYEK-07.03.2016-2016.10).

Growth performance

Weight of fish was measured at the beginning and at the end of study. Each fish was individually weighed. Growth performance was calculated as follows:

$$\text{Weight gain (WG,\%)} = \left[100 \times \frac{\text{Final fish weight-Initial fish weight}}{\text{Initial fish weight}}\right] \quad (1)$$

$$\text{Specific growth rate (SGR,\%/day)} = \left[100 \times \frac{\ln(\text{final fish weight}) - \ln(\text{initial fish weight})}{\text{Experimental days}}\right] \quad (2)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Weight gain (g)}} \quad (3)$$

$$\text{Survival rate (SR,\%)} = \left[\frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100\right] \quad (4)$$

Digestive enzyme activities

The stomach and anterior part of intestine tissues (0.1 g) were homogenized in 1 mL cold double distilled water using ISOLAB homogenizer (Germany). And then, samples were centrifuged at 9000 rpm for 20 min at 4°C. The supernatant was separated and stored at –80°C until digestive enzyme activity assay. Protein contents of stomach and intestine supernatants were determined according to Bradford (1976).

Pepsin activity in stomach supernatant was determined as described by Anson (1938). Trypsin activity in intestinal supernatant was determined using benzoyl-dl-arginine-p-nitroanilide following Erlanger et al. (1961). Lipase activity in the intestinal supernatant was determined by hydrolysis of 4-nitrophenyl myristate (Gawlicka et al., 2000). Amylase activity in the intestinal supernatant was determined by starch hydrolysis (Worthington, 1991).

Heamatological analysis

Red blood cell (RBC, $\times 10^6/mm^3$) was measured as described by Blaxhall & Daisley (1973). Hemoglobin (Hb, g dL^{-1}) was determined according to Drabkin & Austin (1932). Hematocrit (Hct, %) was determined as described by Billett (1990). Red blood indices such as mean cell volume (MCV, fL), mean cell hemoglobin (MCH, pg) and mean cell hemoglobin concentration (MCHC, %) were calculated according to White et al. (2006).

$$\text{Mean cell volume (MCV, } \mu m^3) = \left[\frac{\text{Hct (\%)}}{\text{RBC}(\times 10^6 \mu L)} \times 10\right] \quad (5)$$

$$\text{Mean cell hemoglobin (MCH, pg)} = \left[\frac{\text{Hb (g } dL^{-1})}{\text{RBC}(\times 10^6 \mu L)} \times 10\right] \quad (6)$$

$$\text{Mean cell hemoglobin concentration (MCHC, \%)} = \left[\frac{\text{Hb (g } dL^{-1})}{\text{Hct(\%)}}\right] \quad (7)$$

Antioxidant activity

Liver (0.1 g) and white muscle (0.1 g) samples were washed with physiological saline. Samples were dried on a filter paper and then homogenized separately on ice with 1 mL of phosphate buffer. After that, the samples were centrifuged at 10000 rpm for 15 min at 4°C. The supernatant was then separated and stored at -80°C until use for oxidative stress

activity assay. The protein contents of liver supernatants were determined similarly as described above (Bradford, 1976).

Superoxide dismutase (SOD) in liver tissue was determined with using Sigma-Aldrich kit (Cat. No. 19160). Catalase (CAT), glutathione peroxidase (GPx), glucose-6-phosphate dehydrogenase (G6PDH) and lipid peroxidation (LPO) were determined using Cayman chemical kits, item No. 707002, 703102, 700300 and 10009055, respectively.

Non-specific immune parameters

Nitroblue tetrezolium (NBT) reduction activity was determined using fresh blood (Siwicki et al., 1994). Myeloperoxidase (MPO) and lysozyme activity (LA) assays were determined using plasma according to (Ellis, 1990; Sahoo et al., 2005) with some modifications.

Statistical analysis

The data were expressed as mean ± SEM (standard error of means) for all parameters tested. Differences between treatments for various parameters were determined by one-way analysis of variance (ANOVA) followed by Tukey tests at 5% level of significance ($p<0.05$) using SPSS (16.0) program.

Results

Growth performance

All diet administered groups demonstrated that final weight and weight gain were significantly higher ($p<0.05$) than in control (Table 2). In addition, rate of WG and SGR were significantly increased compared to the control (Table 2). FCR in all the treatment groups significantly decreased (Table 2). During the feeding experimental period, no mortality was observed in all the groups.

Digestive enzyme activities

Pepsin and amylase activity in 0.1, 0.5 and 1% PT diet fed groups significantly increased ($p<0.05$) in a dose-dependent manner (Table 3). Trypsin activity significantly increased in 0.1 and 1% PT diet fed groups (Table 3). In addition, lipase activity significantly increased in all the treatments (Table 3).

Haematological analysis

RBC, Hct, MCV, MCH and MCHC values did not vary among treatment groups and control (Table 4). Only 0.5% PT diet fed fish had significantly increased Hb level compared to that of other diet fed groups and control (Table 4).

Antioxidant activity

After 21 days of feeding, SOD activity increased significantly ($p<0.05$) in 0.1 and 0.5% PT diet administered groups (Fig. 1). Additionally, after 42 days, all treatment groups showed a significantly higher SOD activity compared to that of control. SOD activity was higher in 0.1 and 1% PT diet fed groups at the end of experiment (Figure 1). Throughout the feeding period, CAT activity in 0.1% PT diet fed group was significantly higher than that in control (Figure 2). In addition, other treatment groups displayed higher CAT activity compared to control at different sampling days (Figure 2). GPx activity in all PT diet fed groups increased in a dose-dependent manner after 21 and 63 days (Figure 3). We observed that G6PDH activity in all the treatment groups increased at 21 and 42 days (Figure 4). Only 0.5% PT diet fed fish did not display any significant result compared to control at the end of study (Figure 4). On the other hand, LPO activity significantly decreased in all treatment groups compared to that of control (Figure 5).

Table 2. Growth performance parameters and survival of rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days

Treatment Groups	Growth Parameters					
	Initial weight (g)	*Final weight (g)*	*Weight gain (%)*	*FCR*	*SGR (%)*	*Survival (%)*
Control	15.58±0.06	42.12± 0.35^{b}	170.37± 2.70^{b}	1.18± 0.00^{a}	1.58±0.02^{b}	100
PT0.1%	15.38± 0.14	51.01± 0.36^{a}	232.5.1± 2.67^{a}	1.02± 0.00^{b}	1.91± 0.01^{a}	100
PT0.5%	15.41± 0.12	50.79± 0.26^{a}	229.82± 3.87^{a}	1.03± 0.00^{b}	1.89± 0.02^{a}	100
PT1%	15.27± 0.18	51.54± 0.40^{a}	236.66± 4.95^{a}	1.04± 0.00^{b}	1.92± 0.02^{a}	100

Note: Each value is mean ± SE. Different superscript letters in the same column indicate significant differences ($p<0.05$) between treatment groups. FCR, feed conversion ratio; SGR, specific growth rate. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

Table 3. Digestive enzyme activities of rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days

Treatment Groups	Digestive Enzyme Activities			
	Pepsin U/mg protein	***Trypsin U/mg protein***	***Lipase U/mg protein***	***Amylase U/mg protein***
Control	47.95 ± 0.69^{d}	8.29 ± 0.16^{b}	0.15 ± 0.11^{b}	0.51 ± 0.01^{c}
PT0.1%	67.40 ± 0.65^{b}	12.62 ± 0.57^{a}	0.23 ± 0.11^{a}	0.56 ± 0.03^{c}
PT0.5%	75.45 ± 0.88^{b}	8.31 ± 0.48^{b}	0.19 ± 0.02^{a}	0.68 ± 0.03^{a}
PT1%	81.68 ± 0.81^{a}	11.45 ± 0.55^{a}	0.20 ± 0.01^{a}	0.59 ± 0.02^{b}

Note: Each value is mean ± SE of 3 fish. Different superscript letters in the same column indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

Table 4. Haematological parameters of rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days

Treatment Groups	Haematological Parameters					
	RBC ($x10^6/mm^3$)	***Hb (g/dl)***	***Hct (%)***	***MCV (μm^3)***	***MCH (pg)***	***MCHC (%)***
Control	2.14 ± 0.03	8.47±0.44^{b}	23.00 ± 0.57	107.43 ± 3.73	43.79 ±0.34	441.66 ± 1.20
PT0.1%	1.94± 0.10	9.63± 0.43^{b}	22.43 ± 0.80	112.37± 1.52	46.49 ±0.58	440.33 ± 0.66
PT0.5%	2.31 ± 0.06	9.90± 0.36^{a}	23.37 ± 0.46	101.53 ± 2.49	43.45 ±1.48	440.66 ± 1.20
PT1%	2.07 ± 0.05	9.37± 0.35^{b}	22.07 ± 0.76	104.83 ± 3.27	45.13 ±0.78	442.00 ± 1.52

Note: Each value is mean ± SE of 3 fish. Different superscript letters in the same column indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract. RBC, Red blood cell; Hb, haemoglobin; Hct, haematocrit; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration.

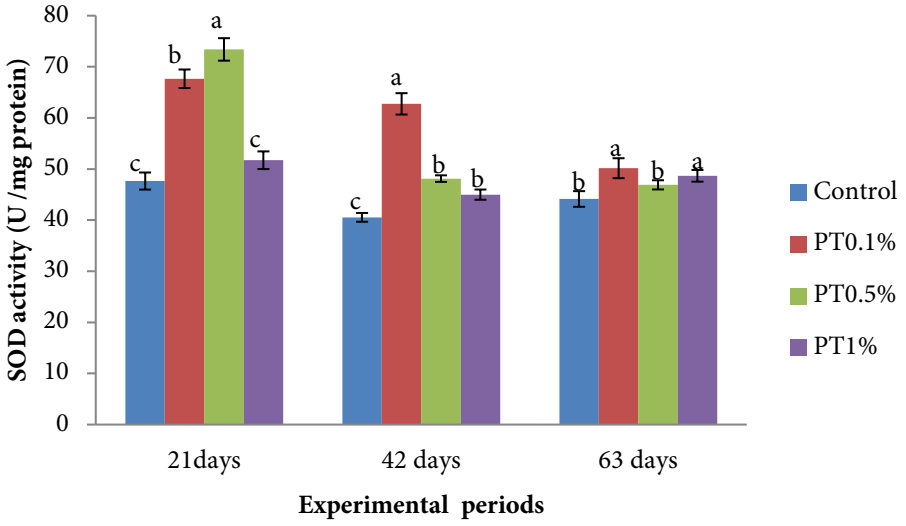

Figure 1. Superoxide dismutase (SOD) activity in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days. Different letters on the bars indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

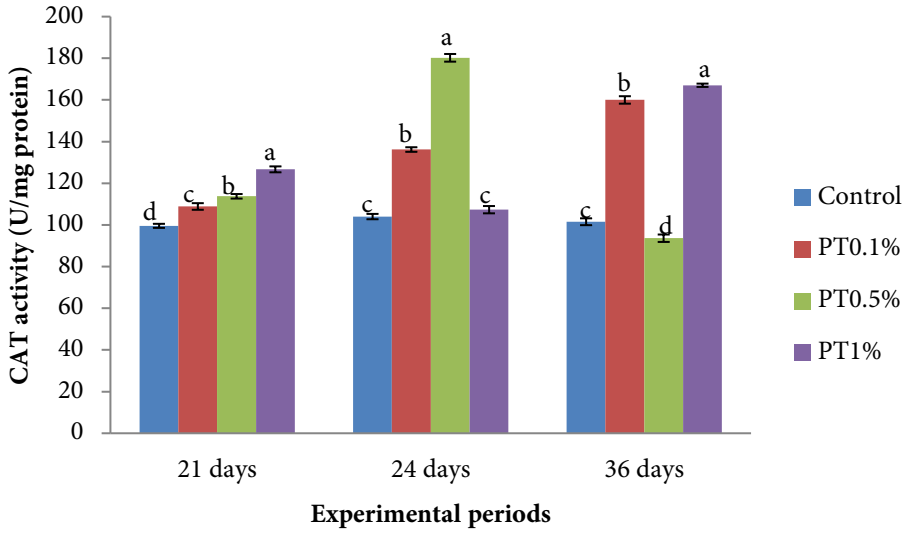

Figure 2. Catalase (CAT) activity in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days. Different letters on the bars indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

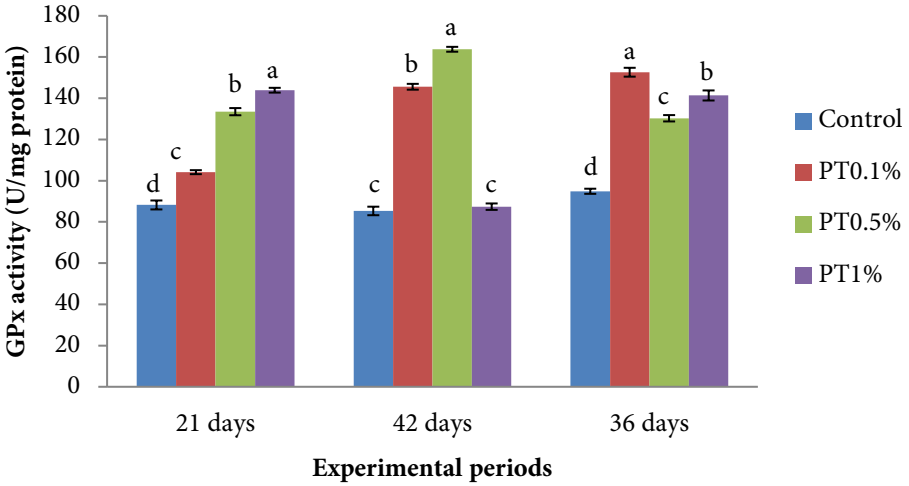

Figure 3. Glutathione peroxidase (GPx) activity in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days. Different letters on the bars indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

Non-specific immune parameters

During the experiment, MPO activity significantly increased in fish of all treatment groups (Table 5). In addition, we observed that NBT level in all PT diet fed treatments increased at the end of 21, 42 and 63 days (Table 6). Increasing LA activity was observed on different sampling day in treatment groups (Table 7).

Discussion

In the present study, dietary administration of PT extract supplemented feed on the growth parameters (FW, WG and SGR) indicated beneficial effects in rainbow trout. Moreover, FCR decreased significantly in all PT extract treated fish groups. Major bio-active compounds of terebinth, such as vitamins, minerals, polysaccharide, alkaloids, fatty acids and flavonoids could affect the growth rate. Previous studies reported that these compounds stimulated appetite which caused enhanced feed consumption, digestion and absorption of nutrients (Zahran et al., 2014; Bilen et al., 2020; Elbesthi et al., 2020). In contrast, Bilen & Bilen (2012) found no differences on growth performance in rainbow trout fed with tetra and laurel powder. The performance of digestive system in fish and other animals directly depends on enzyme (pepsin, trypsin, amylase and

lipase) activities (Furne et al., 2012). Pepsin is one of the most important acid proteases secreted in stomach. This enzyme is responsible for the digestion of dietary proteins to polypeptides such as phenylalanine, tyrosine and other aromatic amino acids (Darias et al., 2007). In this study, pepsin activity significantly increased in all PT diet fed groups. This increase may be due to protein contents in terebinth extract, which could stimulate pepsin activity in the stomach. Previous studies reported that pepsin activity was significantly increased in rainbow trout fed garlic (*Allium sativum*), lupin (*Lupinus perennis*), stinging nettle (*Urtica dioica*) and mango (*Mangifera indica*) incorporated diets (Nya & Austin, 2011; Awad et al., 2012). Trypsin enzyme, one of the alkaline proteases, is secreted in intestine lumen. It completes protein digestion process and hydrolysis of amino acids when chyme arrives at the intestine (Darias et al., 2007; Napora-Rutkowski et al., 2009). The current study revealed that PT extract at 0.1 and 1% of diet caused a significant improvement in the intestinal trypsin activity compared to that of the control and 0.5% PT extract treated group. Probably, terebinth compounds accelerated trypsin activity in the intestine. Ester bonds among the fatty acids and glycerol are dissolved by lipase enzyme which is secreted from pancreas to be discharged into intestine lumen (Ojha et al., 2014). An enhanced trypsin activity was also observed in carp fed with *Chenopodium album* (Amhamed et al., 2018) and *Apium graveolens* extracts (Mohamed et al., 2018). Similar to trypsin activity, lipase activity increased in all the treated fish groups compared to the control. This is probably due to terebinth compounds that stimulated lipase activity. The amylase enzyme is mainly responsible for degradation of carbohydrates and secreted in pancreas and finally discharged into intestine lumen. It is responsible for the hydrolysis of glycosidic bonds among sugar residues in large carbohydrates. It digests carbohydrates into glucose (Ojha et al., 2014). Amylase activity increased in all the treated groups. The increase in amylase activity might have happened due to carbohydrate content in fruit extract of PT or may be due to augmentation for proliferation and maintenance of intestinal microflora which can produce enzymes that may improve digestibility and absorption of feed ingredients (Aly & Mohamed 2010; Citarasu, 2010; Bulfon et al., 2015). These findings agree with the previous studies which indicated that medicinal herbal extracts stimulated digestion with enhanced bile acid concentration and induced the pancreas for increased secretion of digestive enzymes in fish (Bhosale et al., 2010).

Table 5. Myeloperoxidase activity (OD 450 nm) in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days

Treatment Groups	Experimental Period		
	21 days	***42 days***	***63 days***
Control	108.67 ± 1.34^{d}	116.80 ± 2.02^{d}	110.89 ± 1.75^{c}
PT0.1%	128.81 ± 2.92^{c}	156.39 ± 2.26^{a}	111.12 ± 3.42^{c}
PT0.5%	151.22 ± 2.67^{a}	139.65 ± 3.00^{b}	114.64 ± 2.57^{b}
PT1%	137.11 ± 1.45^{b}	126.59 ± 1.76^{c}	122.87 ± 3.61^{a}

Note: Each value is mean ± SE of 3 fish. Different superscript letters in the same column indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

Table 6. Nitroblue tetrezolium activity (mg ml^{-1}) in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days.

Treatment Groups	Experimental Period		
	21 days	***42 days***	***63 days***
Control	1.38 ± 0.02^{c}	2.45 ± 0.04^{c}	2.33 ± 0.05^{c}
PT0.1%	1.63 ± 0.05^{b}	2.81 ± 0.05^{a}	2.76 ± 0.06^{a}
PT0.5%	1.67 ± 0.04^{b}	2.58 ± 0.04^{b}	2.51 ± 0.06^{b}
PT1%	2.59 ± 0.02^{a}	2.58 ± 0.01^{b}	2.74 ± 0.02^{a}

Note: Each value is mean ± SE of 3 fish. Different superscript letters in the same column indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

Table 7. Lysozyme activity (U ml-1) in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days

Treatment Groups	Experimental Period		
	21 days	***42 days***	***63 days***
Control	0.45 ± 0.00^{b}	0.02 ± 0.00^{b}	0.02 ± 0.00^{c}
PT0.1%	0.47 ± 0.03^{b}	0.05 ± 0.01^{b}	0.02 ± 0.01^{c}
PT0.5%	0.61 ± 0.02^{a}	0.03 ± 0.00^{b}	0.03 ± 0.01^{b}
PT1%	0.37 ± 0.03^{c}	0.27 ± 0.02^{a}	0.04 ± 0.01^{a}

Note: Each value is mean ± SE of 3 fish. Different superscript letters in the same column indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

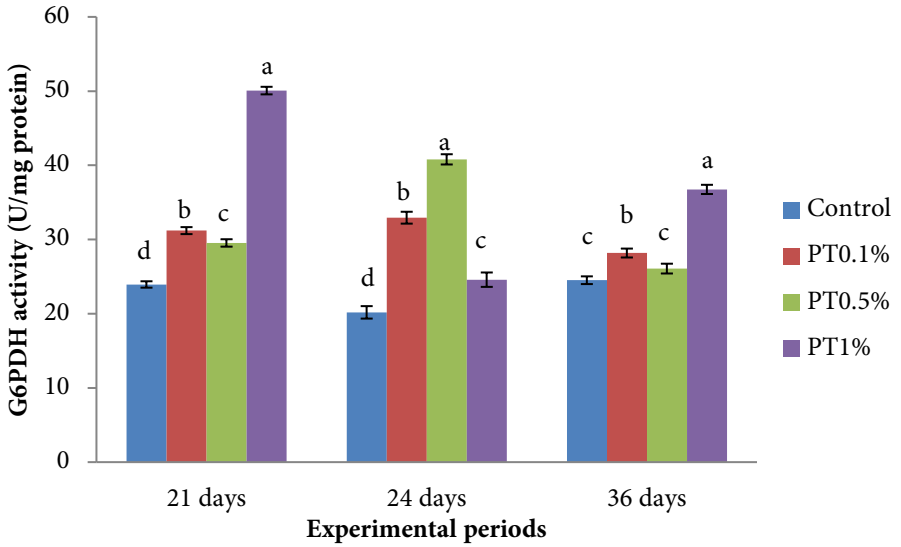

Figure 4. Glucose-6-phosphate dehydrogenase (G6PDH) activity in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days. Different letters on the bars indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

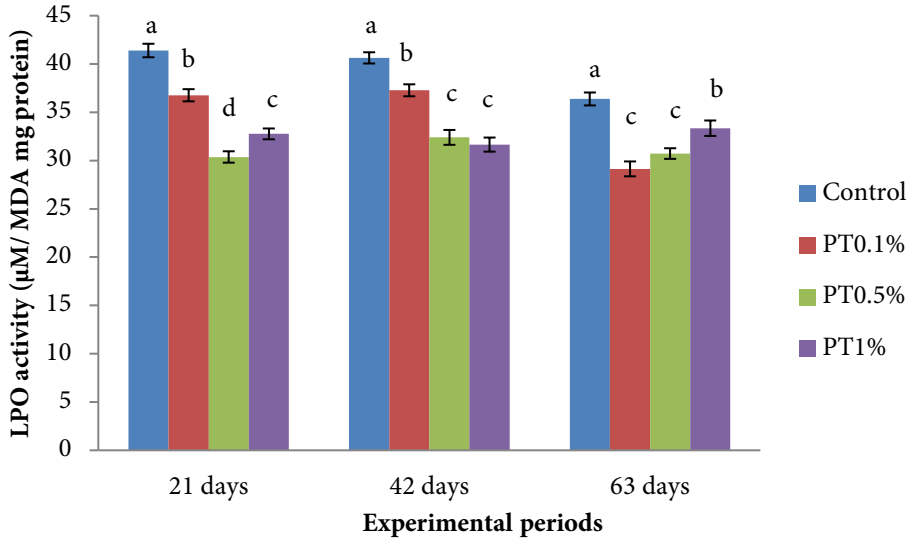

Figure 5. Lipid peroxidation (LPO) activity in rainbow trout juveniles fed diets supplemented with *Pistacia terebinthus* (PT) fruit extract for 63 days. Different letters on the bars indicate significant differences ($p<0.05$) between treatment groups. PT0.1, PT0.5 and PT1% are diets containing 0.1, 0.5 and 1% PT fruit extract.

The present study showed that RBC, Hb, Hct, MCV, MCH and MCHC values did not vary among treatments and control. This result established that supplementation of terebinth extract at 0.1, 0.5 and 1% of feed does not exert any adverse effect on haematological parameters in rainbow trout.

SOD and CAT are responsible for removal of superoxide anions (Vutukuru et al., 2006). In the present study, SOD and CAT activities increased in all the treatment groups at all sampling times, except in 0.5% PT extract administered group after 63 days. This increase in both hepatic SOD and CAT activities was probably due to the antioxidant property of fruit extract which could further stimulate antioxidant defence mechanism through enhancing the activity of endogenous antioxidant enzymes or stimulating the synthesis of hepatic SOD and CAT to prevent generation of excessive free radicals (O_2^- and H_2O_2) during normal cell metabolism and respiratory burst activity (Topçu et al., 2007; Yilmaz et al., 2010). GPx is one of the important enzymes involved in cellular antioxidant enzymatic system. In addition, the GPx activity is an indicator of cell damage (Uner et al., 2006; Liang et al., 2017). In the present study, the hepatic GPx activity was significantly increased in all the experimental groups throughout the experimental period. The antioxidant property of terebinth extract might have stimulated GPx activity. Ellagic acid is one of the main phenolic compounds of *Pistasia spesices* (Mehenni et al., 2016). In line with the present finding, Mişe Yonar et al. (2014) observed that GPx activity in rainbow trout increased significantly during feeding with ellagic acid. Similar result of increased GPx activity was also reported in rainbow trout fed with thyme (*Thymus vulgaris*), sage (*Salvia officinalis*) and mint (*Mentha spicata*) extracts (Sönmez et al., 2015). G6PDH is considered as a secondary antioxidant enzyme (Rayeni, 2016). It cannot neutralise free radicals directly, however, it has a supporting role to other endogenous antioxidants (Carocho & Ferreira, 2013). The current study demonstrated that G6PDH activity increased in all the treatment groups after 21 and 42 days. Only in fish fed with 0.5% PT diet, no significant enhancement was observed after 63 days. Sönmez et al. (2015) reported similar results of elevated G6PDH activity in rainbow trout fed with thyme, sage and mint extracts. Furthermore, clove (*Syzygium aromaticum*) and cardamom (*Elettaria cardamomum*) containing diets increased the activity of G6PDH in rohu fish (*Labeo rohita*) (Asimi & Sahu, 2016). LPO was significantly reduced in all the experimental fish groups. This result may be attributed to free radical scavenging property of terebinth extract which is associated to the bioactive compounds such as polyphenols, flavonoids and vitamins. Mişe Yonar et al. (2014) suggested ellagic acid as a polyphenolic compound (Mehenni et al., 2016) that caused a significant decrease in LPO in liver, kidney and spleen tissues in rainbow trout. In addition, LPO activity decreased in rainbow trout and silver catfish (*Rhamdia quelen*) fed with the medicinal herb, garlic which contains flavonoid compounds (Mohebbi et al., 2012; Pês et al., 2016).

Superoxide anions are important antimicrobial effectors. Thus, the respiratory burst activity of phagocytes has been used frequently as an indicator of non-specific immunity in fish (Sahoo et al., 2005). NBT reduction activity improved in fish of the all experimental groups. This effect may be due to a strong immunostimulant property of this fruit extract which contains bioactive compounds, such as vitamins, oils, polyphenolic and flavonoid compounds (Topçu et al., 2007; Karga et al., 2020). In line with our results, *Aloe vera* extracts caused enhanced respiratory burst activity in rainbow trout fry (Haghighi et al., 2014). MPO is an enzyme secreted by macrophages and neutrophils of fishes. It utilizes halide and hydrogen peroxidase to kill bacteria (Hampton et al., 1996). The MPO can act as an indicator of phagocyte and neutrophil activities, which are used as an indicator of non-specific immune response (Johnston, 1978). MPO activity increased in all treatment fish groups. Similar result was observed in Mozambique tilapia (*Oreochromis mossambicus*) fed a diet supplemented with a medicinal herb, guduchi (*Tinospora cordifolia*) leaves (Alexander et al., 2010) and rainbow trout fed with cherry stem extract (Amoush et al., 2021). LA is an important element in non-specific defence mechanism (Evelyn, 2002). Lysozyme is secreted by lysosomes present in neutrophils (Uribe et al., 2011). In our study, LA increased on different sampling days in treatment groups. Some previous studies indicated that medicinal herbs and their bioactive compounds have positive effects on LA activity in rainbow trout (Salem et al., 2021; Lakwani et al., 2022). Haghighi et al. (2014) reported that LA activity increased significantly in rainbow trout fry fed with 1% of *Aloe vera* extract.

Conclusion

In conclusion, our results indicated that terebinth extract supplemented diet has positive effect on growth, digestive enzyme activities, antioxidant activities and non-specific immune response in rainbow trout. It is also considered that this type of medicinal plants can be used in aquaculture sector as a therapeutic and immunostimulant supplement material.

Compliance With Ethical Standards

Authors' Contributions

GAM: During experimental period fish car, laboratory studies and writing.

SB: Experimental design preparing, laboratory studies, statistical analyses and writing.

KG: The plant collection, preparing of the extraction and GS-MS analyses.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

All animal experiments were conducted according to the relevant international guidelines. Study protocols were approved in advance by the local ethics committee for animal research studies at the Kastamonu University (KUHADYEK-07.03.2016-2016.10).

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