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DANDELION ROOT EXTRACT IN TROUT FEED AND ITS EFFECTS ON THE PHYSIOLOGICAL PERFORMANCE OF *ONCORHYNCHUS MYKISS* AND RESISTANCE TO *LACTOCOCCUS GARVIEAE* INFECTION

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Abstract

In this study, we investigated the dietary effects of dandelion root extract (DRE) on growth performance, body composition, serum biochemical parameters, intestinal and liver histology, and fish resistance against *Lactococcus garvieae* infection in the rainbow trout. In total, 540 rainbow trout fry (22.05±1.740 g and 13.03±0.470 cm) were used. Six experimental groups (Control, 2.5 mL/kg (DRE-1), 5 mL/kg (DRE-2), 10 mL/kg (DRE-3), 20 mL/kg (DRE-4), and 40 mL/kg (DRE-5) dandelion root extract) with three replicates were formed. In each tank, 30 fish were placed and fed for 90 days, and the volume of water was adjusted to 80 L with a flow-through system. The results showed that the final fish weight, weight gain, specific growth rate, protein efficiency ratio, and protein deposition rate were significantly higher in the DRE-4 group, while the feed conversion ratio and fat deposition ratio were significantly lower compared to that in the control group (P<0.05). The viscerosomatic index and the hepatosomatic index decreased in the experimental groups depending on the concentration of the extract used, while the condition factor was higher than that in the control (P<0.05). The DRE significantly decreased glucose, cholesterol, triglyceride, low-density lipoprotein, alanine aminotransferase, and aspartate aminotransferase levels in blood serum in the fish from the experimental groups, but it significantly increased high-density lipoprotein, alkaline phosphatase, and total protein levels (P<0.05). Similarly, the DRE positively affected intestinal histology by increasing the stratum compactum, submucosa, lamina propria, villi length, villi width, absorption area, tunica muscularis thickness, mucosal folds, and goblet cell count. In the liver, depending on the high-fat diet (crude lipid = 20%), vacuolization areas, hepatocellular degeneration areas, apoptotic hepatocyte nucleus, and necrotic areas recorded in the control group decreased or even disappeared completely in the extract groups. The results of the challenge test showed that adding 2.5 mL/kg of the DRE to the feed positively affected the disease resistance against *Lactococcus garvieae* infection. The results of the quadratic polynomial regression showed that an average level of 23.91±1.048 mL/kg of DRE would be suitable for optimum fish growth.

Key words: *Taraxacum officinale*, medicinal plant, serum biochemistry, histology, *Lactococcus garvieae*

The demand of consumers for protein is increasing with an increase in the global population. The aquaculture sector has shown an average growth rate of 7.5% since 1970 (FAO, 2020) and supplies 17% of the total animal protein and 7% of all proteins consumed globally (FAO, 2017). According to the FAO, the demand for animal protein is estimated to rise from 20 kg/day to 40 kg/day in 2030 (FAO, 2018). The increase in protein demand has increased the demand for fish meal and fish oil, which are limited resources, and thus, feed costs have increased. To meet the increasing demand for protein and to reduce production costs, the aquaculture sector has increased the stock density and the fat content of feeds. An increase in stock density has enhanced environmental stress, leading to slower growth and economic losses (Paknejad et al., 2020; Srichaiyo et al., 2020). Although increasing the fat content in the diet can slightly accelerate the development of fish and save protein (Lu et al., 2013), high-fat diets can cause fat accumulation in the tissues of cultured fish, resulting in metabolic disorders, suppression of the immune system, reduction of meat quality, and fatty liver (Cheng et al., 2009; Li et al., 2012; Zheng et al., 2014). To resolve such problems, various plant extracts, enzymes, vitamins, minerals, hormones, antibiotics, and carotenoids have been tested as additives in feeds (Kaya et al., 1997). The European Union (EU) banned the use of antibiotics as feed additives on 1 January 2006 (Hermann et al., 2003; Goda, 2008). Several researchers found that supplementing species of medicinal plants in the feed might be a useful alternative strategy to promote disease resistance, protect the gastrointestinal biota, improve growth performance, and increase the survival rates of fish in fish farms (Goda, 2008; Keser and Bilal, 2008; Bilen et al., 2011; Newaj-Fyzul and Austin, 2015).

As the medicinal plants from which the extracts are obtained are available locally, cheap, and environmentally friendly, their use in aquaculture is advantageous. Medicinal plant extracts contain some active compounds, such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids, and essential oils, which have various biological functions, including growth promotion, appetite stimulation, tonicity, immune system stimulation, maturation of cultivated species, aphrodisiac, and anti-stress effects (Galina et al., 2009; Chakraborty and Hancz, 2011; Reverter et al., 2014). Dandelion (*Taraxacum officinale*) is a medicinal plant that occurs commonly throughout the northern hemisphere (Domitrović et al., 2010; Wang, 2014). It is a member of the Asteraceae (Compositae) family (You et al., 2010; Berezi et al., 2013) and contains several compounds and phytochemicals, including oligosaccharides, polysaccharides, alkaloids, phenolic acids, flavonoids, terpenes, amino acids, organic acids, fatty acids, vitamins, and minerals (Schmidt, 1979; Jackson, 1982; Gail, 1994; Kisiel and Barszcz, 2000; Jung et al., 2011; Qian et al., 2014; Grauso et al., 2019 a, b). Some studies have reported that dandelion has several health benefits, including growth promotion (Al-Kassie et al., 2008; Qureshi et al., 2016; Tan et al., 2017; Tan and Sun, 2020), hepatoprotective effects (You et al., 2010; Gulfraz et al., 2014; Qureshi et al., 2016; Mahboubi and Mahboubi, 2020), immune enhancement (Schütz et al., 2006; Tan et al., 2017, 2018 b; Tan and Sun, 2020), hypoglycemic effects (Önal et al., 2005; Ricky and Silitonga, 2019), hypolipidemic effects (Choi et al., 2010; Kim et al., 2014), antibacterial and antiparasitic effects (Lans and Turner, 2011; Yan et al., 2012; Qian et al., 2014; Qureshi et al., 2015), antioxidant effects (Hu and Kitts, 2003; Ghaima et al., 2013; Gulfraz et al., 2014), and anti-inflammatory effects (Hu and Kitts, 2005; Jeon et al., 2008).

Studies on the effects of dandelion on growth performance, disease resistance, and intestinal and liver histology in fish are limited. Thus, based on the reports on the beneficial effects of dandelion in mammals and some other vertebrates, we investigated its potential biological activities in rainbow trout. However, no published studies are available on *Lactococcus garvieae* infection, although it can cause over 85% loss of rainbow trout at water temperatures above 18°C (Royo, 1999). As the antibiotics used for treating bacterial infections are expensive and may cause problems such as environmental contamination and bacterial resistance, we selected dandelion to conduct our study as it is environmentally friendly, cheap, and easily available. In this study, we evaluated the effects of different concentrations of dandelion root extract (DRE) added to trout feed on growth performance, body composition, serum biochemical parameters, intestinal and liver histology, and resistance to *Lactococcus garvieae* infection of the rainbow trout. We also determined the optimal concentration of DRE for optimum fish growth performance.

Material and methods

Preparation of experimental fish and experimental groups

This study was conducted between 1 March and 15 June 2022 at Recep Tayyip Erdogan University, Faculty of Fisheries, İyidere R&D Unit. In total, 540 rainbow trout with minimum and maximum lengths of 11.80 cm and 14.90 cm (mean 13.03±0.470 cm), respectively, and minimum and maximum weights of 17.60 g and 26.07 g (mean 22.05±1.740 g), respectively, were used. The fish were obtained from Recep Tayyip Erdogan University, Faculty of Fisheries, İyidere R&D Unit. The experiment was conducted in fiberglass fry-rearing tanks (100 L) with a flow-through system. Each tank received water (flow rate of 0.20 L/s) and boosted aeration. The tanks were adjusted to 80 L of water, and 30 fish were placed in each tank. Six experimental groups (Control, 2.5 mL/kg (DRE-1), 5 mL/kg (DRE-2), 10 mL/kg (DRE-3), 20 mL/kg (DRE-4), and 40 mL/kg (DRE-5) dandelion root extract) were formed with three replications. All fish were fed for 90 days and their growth metrics, blood parameters, and histological parameters were recorded, followed by rearing for another 14 days for the challenge test. All fish were housed following a 12-h/12-h light/dark cycle and were fed thrice a day (9:00 a.m, 1:00 p.m, and 5:00 p.m) at 2% of their body weight. Throughout the study, the parameters of the water were as follows: minimum and maximum temperature: 6.7°C and 16.2°C (mean: 12.25±2,28°C), minimum and maximum dissolved oxygen: 7.0 mg/L and 7.6 mg/L (mean: 7.3±0.21 mg/L), minimum and maximum pH: 7.7 and 8.2 (mean: 8.01±0.09), minimum and maximum conductivity: 120 µs/cm and 152 µs/cm (mean: 137.16±8.80 µs/cm), and minimum and maximum dissolved organic matter (TDS): 60 ppm and 76 ppm (mean: 68.54±4.38 ppm).

Dandelion extract and experimental feeds

Dandelion was extracted following the method described in our previous study (Köse and Ariman Karabulut, 2022). Briefly, fresh dandelion roots (164.56 g) were dried in a fan-drying oven at 40°C for 3−4 days until constant weight (weight after drying root: 37.84 g), and then, they were ground and sieved through a 500 µm sieve. Next, 10 g of the ground root was extracted with a 30:70 v/v mixture of methanol and distilled water. The antioxidant activity of the extracts was determined using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) antioxidant activity method (Brand-Williams et al., 1995) ($y = 0.0977x + 0.2316$; $R^2 = 0.9833$; 85.04±0.495 mg Trolox eq/100 g d.m). Total phenolic compound (TPC) was analyzed using the Folin-Ciocalteu phenol reagent and following the method described by Singleton and Rossi (1965) ($y = 31.914x + 428.7$; $R^2 = 0.9719$; 159.21±7.833 mg GAE eq/100 g d.m).

We used 4 mm commercial fry trout feed (crude protein 45%, crude fat 20%, ash 9.5%, crude cellulose 1.7%,

calcium 2.3%, phosphorus 1.5%, and sodium 0.3%, GÜMÜŞ DOĞA SU ÜRÜNLERİ A.Ş, Muğla, Türkiye), which was ground into a powder and sieved through a 500-µm sieve. Then, stock solutions were created for the trial groups. Briefly, 2.5, 5, 10, 20, and 40 mL of DRE were made up to 400 mL with distilled water. The powdered commercial feed was kneaded with these solutions (so that the DRE was homogeneously distributed to all the feed material in the experimental groups) and passed through a pellet machine (EFE KULUÇKA, Model: EFE-1312, Antalya, Türkiye). Then, the pellets were dried in a fan feed drying oven (POL-EKO-APARATURA SP. J. SLW 400 STD) at 45°C until the moisture content was below 10% and stored at –18°C for later use.

Sample collection

At the end of the study, the fish were starved for 24 h. The fish in all groups were sedated with clove oil at a dose of 2−5 mg/L (Küçük et al., 2016), and six fish from each group were randomly sampled. These fish were used for determining the proximate composition and blood parameters and performing histological examinations. The growth parameters were calculated by measuring the length and weight of all individuals in each group.

Proximate composition of fish

At the end of the study, three of the six fish sampled were used for muscle analysis. Additionally, three of these fish were used for liver proximate analysis and the other three were used for conducting histological examinations. The procedures recommended by the Association of Official Analytical Chemists (AOAC) were used to determine the crude protein, crude fat, crude ash, and dry matter of the fish samples. Briefly, for determining the dry matter, the samples were brought to a constant weight at 105°C. The level of crude protein was determined by the Kjeldahl method (N×6.25) (Method No. 978.04) (AOAC, 2005). The crude lipid content was determined by the solvent extraction method (Soxhlet) using petroleum benzine (40−60°C, 2 h) with Velp SER 148/6 (Velp Scientifica. Milano, Italy) (Method No. 930.09) (AOAC, 2005). For determining the crude ash content, 3 g of the sample placed in a porcelain crucible was incinerated in a muffle furnace (ŞİMŞEK Laborteknik KF-908) at 550°C for 6 h (Method No. 930.05) (AOAC, 2005).

Blood biochemical analysis

Three of the six sampled fish were used for conducting the blood biochemical analysis. All blood samples were collected from the caudal vein (Val et al., 1998) with a 5-mL sterile syringe and transferred to blood collection tubes with a clot activator (gel and coagulant). The blood samples were left undisturbed for 30−45 min to coagulate, and then, the samples were centrifuged at 1180×g and 4°C for 10 min to separate the serum. Blood sera were collected in 1-mL cryotubes and stored at –20°C for one day and then analyzed. Biochemical serum examinations were performed at the Biochemistry Laboratory of Recep Tayyip Erdoğan University Faculty of Medicine Training and Research Hospital, Rize, Turkey. The levels of glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), amylase (AML), cholesterol (Chol), triglyceride (TG), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total protein (TP) were analyzed using the Beckman Coulter AU5800 device (Beckman Coulter Inc. Brea, California, USA) by the spectrophotometric method. The device was fully automated and operated in a closed system using commercial assay kits. The analysis kits used in the study were as follows: OSR6221 for GLC (absorbance measured at 340/660 nm), OSR6107 for ALT (absorbance measured at 340/660 nm), OSR6109 for AST (absorbance measured at 340/660 nm), OSR6604 for ALP (absorbance measured at 410/480 nm), OSR6106 for amylase (absorbance measured at 410/480 nm), OSR6116 for cholesterol (absorbance measured at 540/600 nm), OSR6118 for triglycerides (absorbance measured at 660/800 nm), OSR6196 for LDL (absorbance measured at 540/660 nm), OSR6195 for HDL (absorbance measured at 600/700 nm), and OSR6132 for TP (absorbance measured at 540/660 nm) (Beckman Coulter Inc. Brea, California, USA).

Intestinal and liver histology

The gut morphology of all six fish sampled was examined and the liver morphology of three fish was examined. The intestinal regions were determined based on the method described by Løkka et al. (2013). The samples taken from the second segment of the midgut in each fish were used for histological examinations. The intestinal samples were incubated with Davidson's fixative solution and liver samples were incubated with 10% Neutral Buffer for 36 h. Then, they were transferred to 70% ethyl alcohol and kept in the dark until histological sections were prepared. Histological staining of the specimens was performed following the method described by Luna (1968). Briefly, sufficiently sized pieces (approximately 5 mm) were cut from the samples in 70% alcohol, placed in cassettes, and passed through a series of alcohol and xylene. The samples were treated with paraffin at 65°C and then blocked. Then, 5−6 µm sections were cut using a microtome. They were stained with hematoxylin and eosin and covered with a coverslip using Entellan. All histological sections were observed under a light microscope (Leica DM 500) with an integrated video camera (Leica ICC50). Measurements were made from the sections using the software LAS EZ Version 3.4.0 (Leica Microsystems Limited, Switzerland).

Liver sections were evaluated based on vacuolization in hepatocytes and the position and diameter of cell nuclei (Caballero et al., 2004; Martínez-Llorens et al., 2012; Aydın and Gümüş, 2020). Intestinal morphology was eval-

uated following the methods described by Bullerwell et al. (2016), Ye et al. (2016), and Aydın and Gümüş (2020). To avoid wide ranges of standard deviations due to large variations in the length of villi in the intestinal sections and perform more meaningful statistical analyses, villi below 600 µm were arbitrarily categorized as short villi, and those above 600 µm were categorized as long villi (Verdile et al., 2020). Goblet cell number was expressed as the number of goblet cells per villus, i.e., the average number of goblet cells in the villus (Baeza-Ariño et al., 2016; Ye et al., 2016; Aydın and Gümüş, 2020). The absorption area of the villi was calculated by multiplying the length and width of the villi recorded in the intestine. The width of the villi was determined by averaging the values measured at five different points along each villus (Figure 1).

Calculation of growth parameters

The length and weight of the fish were measured every 15 days. While taking measurements, the fish were sedated with 2−5 mg/L clove oil (Küçük et al., 2016). The growth parameters of the fish were calculated using the formulae presented below.

Weight gain (WG) = [(final body weight – initial body weight)];

Specific growth rate (SGR, % day^{-1}) = 100 × [ln(final body weight, g) – ln(initial body weight, g)/day];

Feed conversion ratio (FCR) = dry feed intake (g)/wet weight gain (g);

Protein efficiency ratio (PER) = wet weight gain/protein intake;

Protein deposition rate (PDR) = 100 × [(final body weight (g) × final average whole body protein (% dry basis) – initial body weight (g) × initial average whole body protein (% dry basis))]/total protein intake (g);

Lipid deposition rate (LDR) = 100 × [(final body weight (g) × final average whole body lipid (% dry basis) – first body weight (g) × first average whole body lipid (% dry basis))]/total lipid intake (g); (adapted from Tan et al., 2017).

Condition factor (CF, g/cm^3) = 100 × [(body weight, g)/(body length, cm)^3];

Viscerosomatic index (VSI, %) = 100 × (viscera weight, g)/(whole body weight, g);

Hepatosomatic index (HSI, %) = 100 × (liver weight, g)/(whole body weight, g).

***Lactococcus garvieae* challenge assay**

The challenge trial was initiated seven days after the completion of the study and continued for 14 days. Six experimental groups (Control, DRE-1, DRE-2, DRE-3, DRE-4, and DRE-5) were established with two replications. Initially, 15 fish were placed in each tank containing 80 L of water, which had a flow-through system. The water temperature and fish mortality were recorded daily. *Lactococcus garvieae* were obtained from the Disease Laboratory Department of Recep Tayyip Erdogan University, Faculty of Fisheries (Catalog ID: RTEÜ.127-*Lg*). The laboratory had isolated and preserved *Lactococcus garvieae* from the kidney tissue of the rainbow trout that showed signs of disease at Recep Tayyip Erdogan University, Faculty of Fisheries, Iyidere R&D unit. The challenge test procedure was as follows. First, the bacteria were incubated in TSB (tryptic soy broth) for 24 h. Then, they were centrifuged at 7392×g and 4°C for 5 min to the supernatant was discarded. The precipitated bacteria were mixed with FTS (physiological saline) to McFarland 0.5 turbidity and prepared for injection (Er et al., 2021; Köse et al., 2021). Based on our previous experience, 0.1 mL (2.8×10^8 CFU/mL) of bacteria were injected intraperitoneally with an insulin syringe (Er et al., 2021; Köse et al., 2021). During the experiment, the fish were fed thrice a day (9:00 a.m, 1:00 p.m, and 5:00 p.m) at 2% of their body weight with the experimental feed of their respective groups. At the end of the trial, the cumulative survival rate was calculated by the Kaplan–Maier test using the following formula:

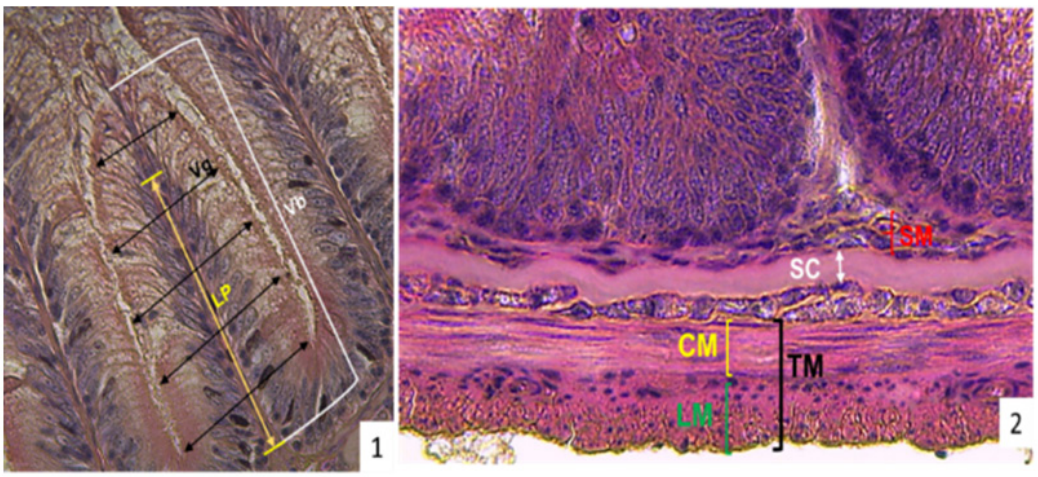


Figure 1. Measurement points in the intestinal regions of rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of DRE. 1: Vg indicates villi width, Vb indicates villi height and LP indicates laminapropria. 2: SM indicates submucosa, SC indicates stratum compactum, CM indicates circular muscularis, LM indicates longitudinal muscularis, and TM indicates tunica muscularis (H&E, 40X)

Cumulative survival rate (%) = 100 – [100 × (Number of fish alive at the start – number of fish that died)/number of fish alive at the start)].

The cause of mortality was confirmed by re-isolating bacteria from dead fish. Briefly, the samples were taken from the spleen, liver and kidneys of dead fish and streaked on trypticase-soy agar plates (TSA) and incubated at 22°C for 48 h. Morphological, physiological, biochemical, and enzymatic characterizations of the isolates obtained were carried out. Cell morphology was ascertained using the gram staining technique. To determine phenotypic characteristics, motility, oxidase and catalase activities, nitrate reduction test, supplemented with 1% glucose Oxidation/Fermentation (O/F) test, H_2S production on triple-sugar iron (TSI) medium were performed. Some other tests were performed using API 20 E and API 50 CH systems and bacteria were identified.

Statistical analysis

All data were presented as the mean ±standard deviation (±SD) using Microsoft Office Excel 2016 pro. The Sigma Plot 14.0 package program (Systat Software Inc., San Jose, CA, USA) was used to perform statistical analyses. To determine whether the data were normally distributed, the Shapiro-Wilk test was conducted. After indicating all data were normally distributed with equal variance, a one-way analysis of variance (ANOVA) was performed and the differences among groups were determined by Tukey's post-hoc for growth parameters and Holm-Sidak post-hoc for biochemical blood parameters and histological data. For estimating the optimum dandelion extract level, Spearman's rank correlation was first applied to the data, and it was confirmed that the data passed the test. Then, the optimum extract level (X value) calculated by quadratic polynomial regression according to Yossa and Verdegem (2015) was considered to be the appropriate extract level. Cumulative survival rates were determined by conducting the Kaplan-Maier test on the challenge trial data. All tests were considered to be statistically significant when the associated P-value was 0.05.

Results

Growth performance, nutrient use, and body indices

We found significant improvements in growth performance, nutrient utilization, and body indices in all DRE groups compared to that in the control group ($P<0.05$). The highest FBW (142.64±0.795), WG (120.59±0.769), FI (106.66±0.956), SGR (2.07±0.021), PDR (41.10±0.101), and CF (1.45±0.028) values and the lowest FCR (0.89±0.008) value were recorded in the DRE-4 group (Table 1). When the extract level increased in the DRE groups, the LDR level decreased significantly compared to the control group and the PDR value increased significantly in the DRE groups compared to the control group, except for DRE-1 ($P<0.05$). The FI values were higher in the DRE groups compared to that in the control group, but no statistical difference was recorded ($P>0.05$). Adding the DRE significantly improved the PER values in the DRE groups depending on the level of extract added ($P<0.05$). The HSI values were lower in the DRE groups compared to that in the control group, and within the DRE groups, the HSI decreased as the extract level increased. The difference was significant between the DRE groups (except for the DRE-1 group) and the control group ($P<0.05$). The differences in the VSI values between groups were similar to those of the HSI values. The VSI values decreased as the extract level increased in all DRE groups compared to the VSI values in the control group. The VSI of the DRE-4 and DRE-5 groups were significantly different from that of the Control group ($P<0.05$). The effects of the DRE added to trout feed on growth performance, nutrient utilization, and body indices are presented in Table 1.

Table 1. Changes in growth parameters, nutrient utilization, and body index values of rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of dandelion root extract (DRE) at the end of the study

Parameters	Control	DRE-1	DRE-2	DRE-3	DRE-4	DRE-5
IBW (g)	22.05±0.028	22.05±0.040	22.07±0.023	22.04±0.027	22.05±0.034	22.05±0.029
FBW (g)	133.10±0.483 b	136.00±0.413 c	136.39±2.032 c	139.09±0.514 d	142.64±0.795 a	138.69±0.567 d
WG (g)	111.05±0.496 b	113.94±0.382 c	114.32±2.036 c	117.05±0.511 d	120.59±0.769 a	116.64±0.585 d
FI(g)	104.19±0.973	104.96±0.891	105.03±1.579	105.29±0.71	106.66±0.956	105.39±0.542
FCR	0.94±0.009 a	0.92±0.008 ac	0.92±0.014 ac	0.90±0.006 bc	0.89±0.008 b	0.91±0.005 bc
SGR (%)	2.01±0.012 b	2.03±0.009 ba	2.03±0.021 ba	2.06±0.017 a	2.07±0.021 a	2.04±0.006 ba
PER	2.48±0.007 b	2.50±0.006 d	2.51±0.002 d	2.53±0.003 c	2.55±0.012 a	2.55±0.009 a
PDR (%)	38.84±0.367 b	39.28±0.327 bc	39.90±0.344 cd	40.71±0.526 ad	41.10±0.101 a	39.81±0.151 c
LDR (%)	7.72±0.073 a	7.28±0.061 d	7.04±0.061 c	7.05±0.091 c	7.00±0.017 c	6.68±0.025 b
CF (g/cm^3)	1.37±0.025 b	1.45±0.026 a	1.44±0.027 ba	1.44±0.029 ba	1.45±0.028 a	1.43±0.023 ba
VSI (%)	14.27±1.189 a	14.08±0.977 a	13.80±0.905 ac	13.35±1.251 ab	12.72±0.802 bc	12.45±1.725 b
HSI (%)	1.13±0.111 a	1.12±0.160 a	0.98±0.145 b	0.93±0.128 b	0.93±0.102 b	0.99±0.093 b

Values are the mean±SD of three replicates (n=3). Means in the same row with different letters indicate significantly different ($P<0.05$). IBW: initial body weight, FBW: final body weight, WG: weight gain, FI: feed intake, FCR: feed conversion ratio, SGR: specific growth rate, PER: protein efficiency ratio, PDR: protein deposition rate, LDR: lipid deposition rate, CH: condition factor, VSI: viscerosomatic index, and HSI: hepatosomatic index. Control, DRE-1, DRE-2, DRE-3, DRE-4, and DRE-5 are experimental groups supplemented with 0, 2.5, 5, 10, 20, and 40 ml DRE/kg feed, respectively.

Muscle and liver composition of the rainbow trout

We found that the crude protein content increased and the crude fat content decreased with an increase in the extract levels compared to their respective contents in the Control group. The DER-4 group had the highest muscle and liver crude protein contents (72.36±0.928 and 67.21±0.102, respectively), which were significantly different than those in the control group ($P<0.05$). In contrast, the lowest crude lipid contents in muscle and liver (14.84±0.426 and 5.08±0.245, respectively) were significantly lower in the DRE-5 groups than those in the control group ($P<0.05$). The ash and dry matter contents were not significantly different between the groups ($P>0.05$). The muscle and liver proximate results of the DRE added to trout feed are presented in Table 2.

Serum biochemical parameters

We found that the serum biochemical blood parameters were significantly affected by DRE. Compared to the control group, all DRE groups showed a significant reduction in the levels of glucose, ALT, AST, amylase, cholesterol, triglycerides, and LDL ($P<0.05$). In contrast, the ALP, HDL, and TP levels were significantly greater in all DRE groups than those in the control group ($P<0.05$) (Figure 2).

Determination of the optimum extract level

The DRE-4 group had the highest FI, WG, FCR, and SGR parameters at the end of the experiment. Using the data from this group, we performed quadratic polynomial regression analysis according to Yossa and Verdegem (2015) to determine the optimal level of DRE (X value) for these parameters and created graphs (Figure 3). The results showed that when the extract level was increased to 22.5 mL/kg, the SGR value reached 2.072 ($y = –0.00012x^2+0.0054x+2.012$; $r^2 = 0.954$). This value was significantly different from the control group ($P<0.05$). Similarly, we found that when the extract level was increased to 23.90 mL/kg, the FI value reached 106.72 g ($y = 0.004665x^2+0.2193x+104.19$, $r^2 = 0.8667$), and the difference with the other groups was not significant ($P>0.05$). When the extract level was increased to 24.26 mL/kg, we found that 120.91 g of WG could be achieved ($y = –0.0165x^2+0.8007x+11.2$, $r^2 = 0.9760$), which was significantly different from all groups ($P<0.05$). When the extract level was increased to 25.00 mL/kg, the FCR value decreased to 0.8863 ($y =-0.00008x^2+0.0004x+0.9353$, $r^2 = 0.9789$), and this value was significantly different between the groups ($P<0.05$). Our results showed that an average extract level of 23.91±1.048 mL/kg would be suitable for optimum growth performance.

Liver morphology

The diameter of the hepatocyte nucleus was the lowest in the DRE-1 group and the highest in the DRE-5 group (4.55±0.332 and 4.68±0.465, respectively) (Table 3). However, the difference between the groups was not significant ($P>0.05$). We found that the control group had some vacuolization areas, some hepatocellular degeneration areas, apoptotic hepatocyte nuclei, and very few detectable necrotic areas, which decreased and improved as the extract levels increased in the DRE groups (Figure 4).

Intestinal morphology

Morphometric measurements of tunica muscularis (TM), circular muscularis (CM), longitudinal muscularis (LM), stratum compactum (SC), submucosa (SM), and goblet cell (Gb) numbers in the second segment of the middle intestine of the fish were higher in all DRE groups and increased with an increase in the extract level. The highest CM (56.03±2.114), LM (60.23±4.772), TM (116.26±5.673), SC (26.03±2.783), SM (18.23±1.338), and Gb (12.10±0.508) values were recorded in the DRE-4 group, and these values were significantly different from those in the control group (Table 4). No abnormalities in CM, LM, TM, SC, and SM were observed (Figure 5).

Table 2. Changes in muscle and liver proximate values of rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of dandelion root extract (DRE) at the end of the study

Parameters	Control	DRE-1	DRE-2	DRE-3	DRE-4	DRE-5
Muscle (%)						
crude protein	68.81±0.744 b	69.77±1.187 bc	70.87±0.555 cd	71.86±1.535 ad	72.36±0.928 a	70.82±0.966 cd
crude lipid	21.58±0.243 a	19.00±0.384 b	18.06±0.597 c	17.49±0.507 d	16.18±0.525 e	14.84±0.426 f
ash	5.82±0.455	5.27±0.224	5.46±0.357	5.68±0.506	5.61±0.453	5.71±0.232
dry matter	25.60±0.330	24.67±0.815	24.43±0.803	25.13±0.707	24.51±0.488	24.42±0.521
Liver (%)						
crude protein	60.01±0.370 f	62.73±0.272 d	65.56±0.120 c	66.27±0.108 b	67.21±0.102 a	60.69±0.259 e
crude lipid	6.07±0.319 a	5.28±0.179 b	5.12±0.264 b	5.02±0.430 b	4.90±0.139 b	5.08±0.245 b
ash	10.70±0.498	10.21±0.487	10.91±0.646	10.62±0.436	10.82±0.583	10.75±0.530
dry matter	18.93±0.595	18.95±0.675	18.66±0.475	18.41±0.642	18.86±0.688	19.06±0.610

The table shows the raw data determined according to dry matter. Values are the mean ± SD of three replicates (n=3). Means in the same row with different letters indicate significantly different ($P<0.05$). Control, DRE-1, DRE-2, DRE-3, DRE-4, and DRE-5 are experimental groups supplemented with 0, 2.5, 5, 10, 20, and 40 ml DRE/kg feed, respectively.

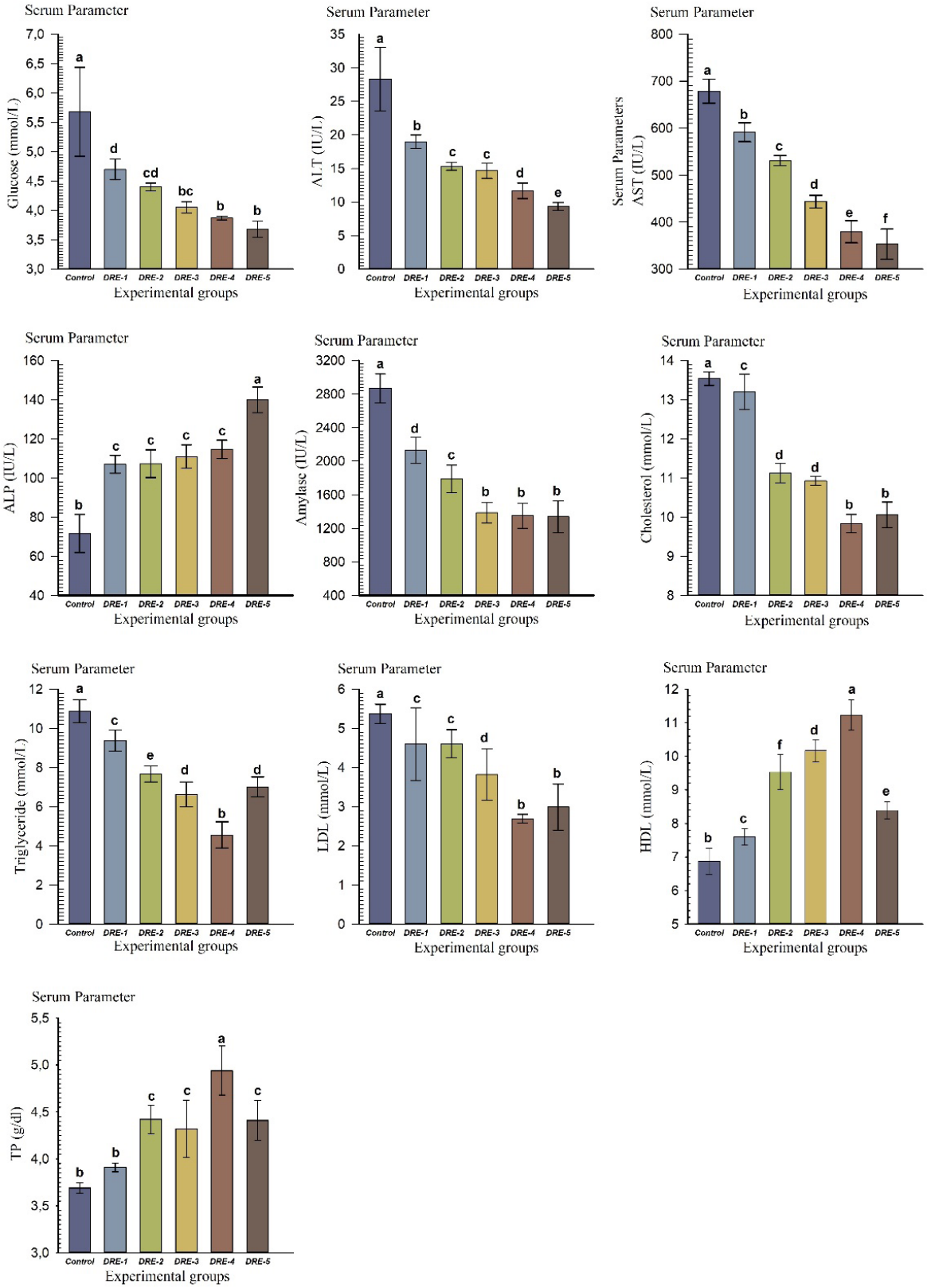


Figure 2. Changes in serum blood parameters in rainbow trout (*Oncorhynchus mykiss*) after dietary administration of dandelion root extract (DRE) (2.5, 5, 10, 20, and 40 ml DRE/kg feed; DRE-1, DRE-2, DRE-3, and DRE-4 diets, respectively) for 90 days. The control group fish fed a basal diet without herbal supplements. Bar values are the mean±SD of three replicates (n=3). Different letters above the bars indicate that the difference among groups is significant ($P<0.05$)

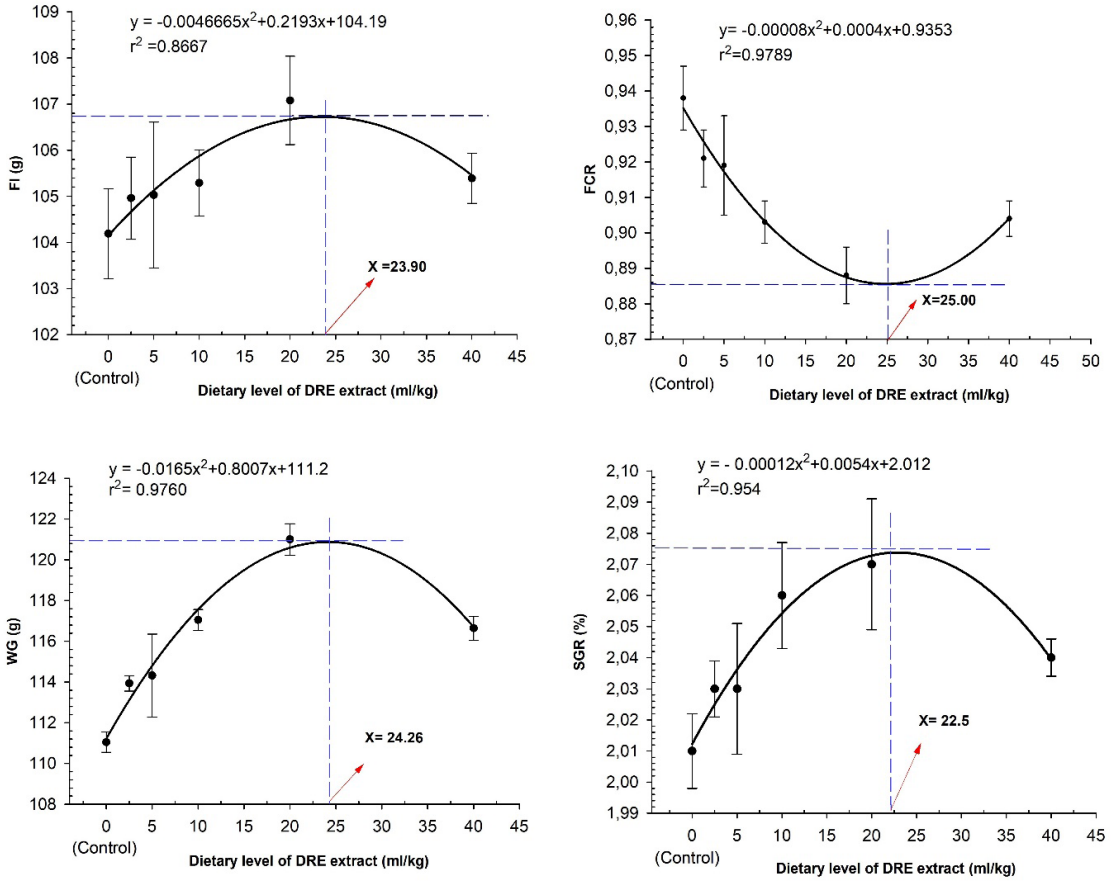


Figure 3. Significant quadratic relationships and polynomial regressions analysis (P< 0.05) among feed intake (FI), feed conversion ratio (FCR), weight gain (WG), and specific growth rate (SGR) in rainbow trout (*Oncorhynchus mykiss*) fed with different levels of dandelion root extract (DRE) for 90 days. In the graph, the point and error bars (mean±SD, n=3) are the values determined in the study on the y-axis (growth parameters) corresponding to the respective x-axis (DRE level) value. The trend line shows the optimum growth curve of the parameters of interest. The trend-line equation ($y = -ax^2+bx+c$) indicated in each chart represents the "x" value corresponding to the apex of the equation. The X value refers to the calculated DRE level estimated for optimal growth. R^2 is the measure of the success of the trend equation and can be max 1

Table 3. Hepatic histologic changes in rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of dandelion root extract (DRE) at the end of the study

Parameters	Control	DRE-1	DRE-2	DRE-3	DRE-4	DRE-5
Hepatocyte nucleus (µm)	4.63±0.457	4.55±0.332	4.59±0.313	4.60±0.418	4.65±0.411	4.68±0.465
Vacuolization	+++	+	+	–	–	–
Necrotic area	+	–	–	–	–	–
Hepatocellular degeneration	+++	+	+	+	–	–
Apoptotic hepatocyte nucleus	+++	+	–	–	+	–

Note: The symbols defined are none (-), very little (+), little (++), moderate (++++), much (++++), and very much (++++++). Control, DRE-1, DRE-2, DRE-3, DRE-4, and DRE-5 are experimental groups supplemented with 0, 2.5, 5, 10, 20, and 40 ml DRE/kg feed, respectively.

Table 4. Changes in the intestinal wall and other structures in the second segment of the midgut determined at the end of the study in rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of dandelion root extract (DRE)

Parameters	Control	DRE-1	DRE-2	DRE-3	DRE-4	DRE5
CM (µm)	52.07±2.609 b	53.19±2.330 c	54.07±2.451 cd	54.74±2.710 d	56.03±2.114 a	53.78±2.108 c
LM (µm)	57.89±2.926 b	58.47±3.120 b	58.48±3.102 b	59.30±4.385 ba	60.23±4.772 a	57.88±2.575 b
TM (µm)	109.96±5.433 b	111.66±5.269 bd	112.52±2.915 dc	114.04±3.712 c	116.26±5.673 a	111.66±3.290 bd
SC (µm)	22.25±1.250 b	23.96±0.735 d	23.69±1.393 cd	24.20±0.629 d	26.03±2.783 a	23.27±1.697 c
SM (µm)	16.32±0.537 b	18.10±1.390 ac	18.04±2.308 ac	18.42±1.357 a	18.23±1.338 a	17.54±1.471 c
Gb per/villi	8.42±0.638 b	9.22±0.490 cd	9.37±0.495 d	10.28±0.634 e	12.10±0.508 a	9.05±0.760 c

Values are the mean ± SD of three replicates (n= 90; 6 samples x 3 replicates x 5 observations). Means in the same row with different letters indicate significantly different (P<0.05). CM: circular muscularis, LM: longitudinal muscularis, TM: tunica muscularis, SC: stratum compactum, SM: submucosa, and Gb: goblet cell. Control, DRE-1, DRE-2, DRE-3, DRE-4, and DRE-5 are experimental groups supplemented with 0, 2.5, 5, 10, 20, and 40 ml DRE/kg feed, respectively.

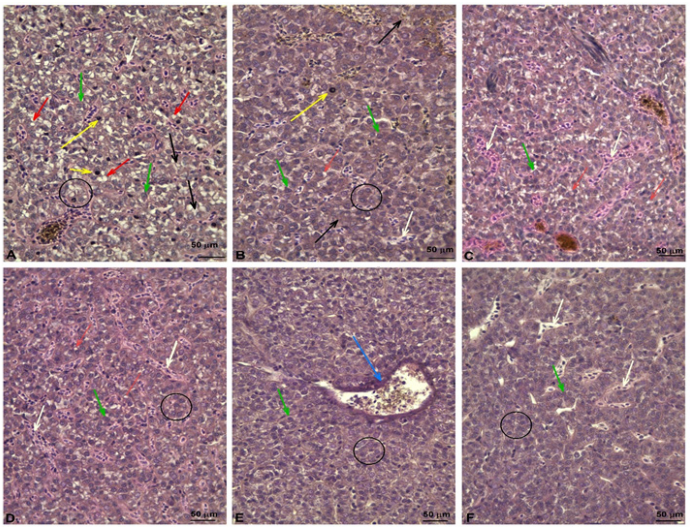


Figure 4. Changes in liver morphology determined by histological studies in rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of dandelion root extract (DRE) at the end of the study. A: Control, B: DRE-1, C: DRE-2, D: DRE-3, E: DRE-4, and F: DRE-5 groups in which they were supplemented with 0, 2.5, 5, 10, 20, and 40 ml DRE/kg feed, respectively, for 90 days (H&E, 40X). The red arrow indicates vacuolization (lipid drop), the black arrow indicates foci of hepatocellular degeneration, the green arrow indicates hepatocyte nuclei, the yellow arrow indicates apoptotic hepatocyte nuclei, the black circle indicates polygonal and hexagonal hepatocyte areas, the white arrow indicates blood vessel sinusoids and surrounding epithelial cells, and the blue arrow indicates portal vein and surrounding connective tissue

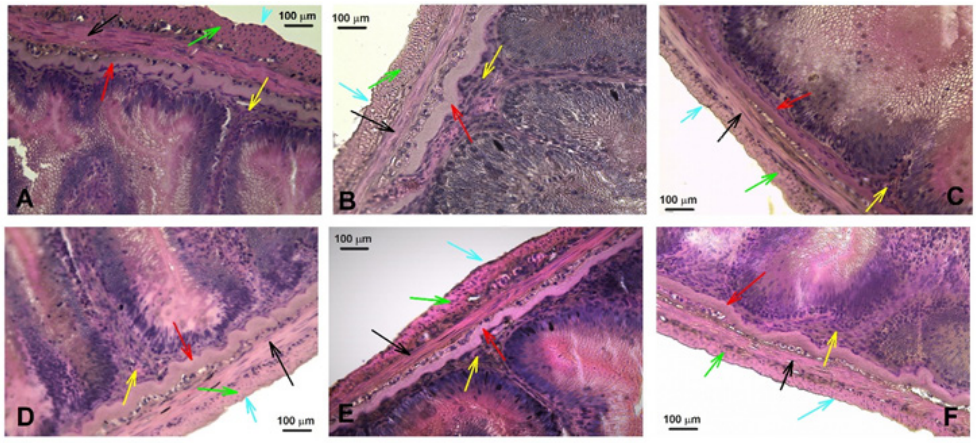


Figure 5. Morphological changes on the intestinal wall of rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of dandelion root extract (DRE) at the end of the study as determined by histological studies. A: Control, B: DRE-1, C: DRE-2, D: DRE-3, E: DRE-4, and F: DRE-5 groups in which they were supplemented with 0, 2.5, 5, 10, 20, and 40 ml DRE/kg feed, respectively, for 90 days (H&E, 40X). The red arrow indicates Stratum compactum, the black arrow indicates circular muscular, the green arrow indicates longitudinal muscularis, the yellow arrow indicates submucosa and the blue arrow indicates serosa layer

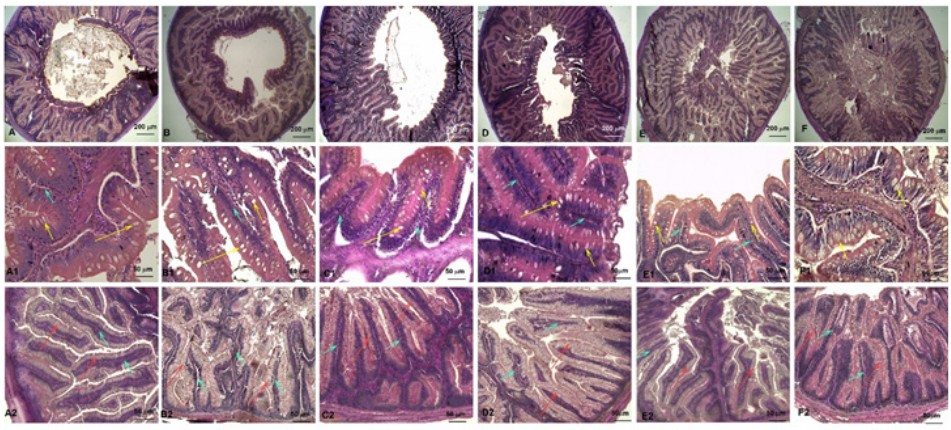


Figure 6. Morphological changes on intestinal villi structures in rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of dandelion root extract (DRE) at the end of the study as determined by histological studies. A, A1, and A2: Control; B, B1, and B2: DRE-1; C, C1, and C2: DRE-2; D, D1, and D2: DRE-3; E, E1, and E2: DRE-4; F, F1, and F2: DRE-5 groups in which they were supplemented with 0, 2.5, 5, 10, 20, and 40 ml DRE/kg feed, respectively, for 90 days (H&E, A, B, C, D, E, and F 10X, and the others 40X). The yellow arrow indicates goblet cells in the apical mucosal fold, the red arrow indicates enterocytes with pinocytic vacuolization, and the blue arrow indicates lamina propria

Table 5. Changes in villi and other structures in the second segment of the midgut determined at the end of the study in rainbow trout (*Oncorhynchus mykiss*) fed diets supplemented with different levels of DRE

Groups	Short				Long			
	LP (mm)	VL (mm)	VW (mm)	AA (mm^2)	LP (mm)	VL (mm)	VW (mm)	AA (mm^2)
Control	0.34±0.031 b	0.51±0.047 b	0.17±0.004 b	0.09±0.007 b	0.95±0.025 b	1.16±0.006 b	0.18±0.022 b	0.21±0.055 b
DRE-1	0.37±0.068 a	0.52±0.074 bc	0.18±0.006 c	0.10±0.017 d	0.97±0.026 bc	1.18±0.015 c	0.20±0.039 c	0.24±0.037 c
DRE-2	0.37±0.046 a	0.55±0.086 a	0.19±0.008 d	0.10±0.016 d	0.97±0.020 b	1.20±0.008 d	0.22±0.044 de	0.27±0.046 d
DRE-3	0.39±0.049 a	0.56±0.071 a	0.19±0.005 d	0.11±0.016 c	0.99±0.092 c	1.27±0.051 e	0.23±0.059 d	0.26±0.058 d
DRE-4	0.39±0.040 a	0.57±0.055 a	0.20±0.007 a	0.12±0.014 a	1.08±0.038 a	1.29±0.053 a	0.25±0.033 a	0.32±0.062 a
DRE-5	0.38±0.066 a	0.55±0.092 ac	0.18±0.010 c	0.10±0.011 d	0.99±0.070 c	1.19±0.070 cd	0.21±0.032 ce	0.24±0.056 c

Values are the mean ± SD of three replicates (n= 90; 6 samples x 3 replicates x 5 observations). Means in the same row with different letters indicate significantly different (P<0.05). LP: lamina propria, VL: villi length, VW: villi width, and AA: absorption area. Control, DRE-1, DRE-2, DRE-3, DRE-4, and DRE-5 are experimental groups supplemented with 0, 2.5, 5, 10, 20, and 40 ml DRE/kg feed, respectively.

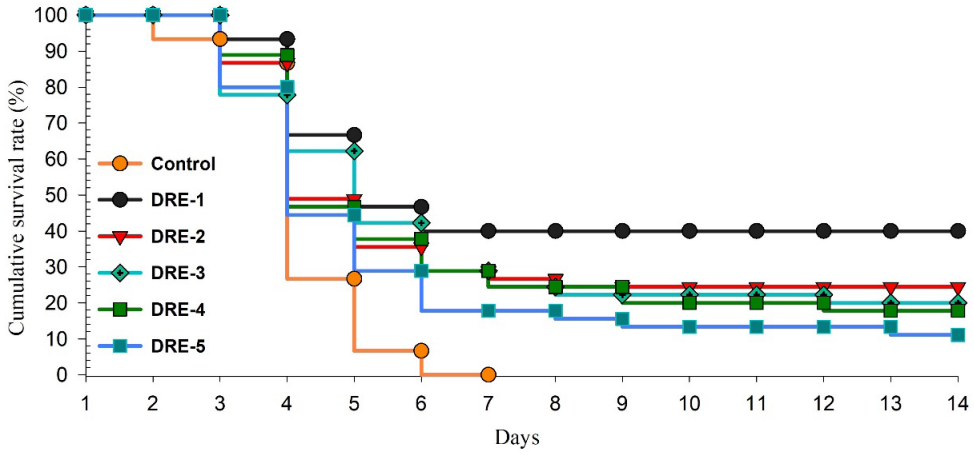


Figure 7. Cumulative survival (Kaplan–Meier survivorship curves) of rainbow trout (*Oncorhynchus mykiss*) challenged with *Lactococcus garvieae* after dietary administration of dandelion root extract (DRE) (2.5, 5, 10, 20, and 40 ml DRE/kg of feed; DRE-1, DRE-2, DRE-3, and DRE-4 diets, respectively) and the control (the fish fed with the basal diet with no herbal supplements) for 90 days (n=15)

The villi were categorized as short (<600 µm) and long (≥600 µm) to obtain more meaningful statistical results. The values of the lamina propria (LP), villi length (VL), villi width (VW), and absorption area (AA) were higher in all DRE groups than in the control group, and the values increased with an increase in the amount of extract, for both short and long villi. These findings indicated that the values of LP, VL, VW, and AA were significantly higher in all DRE groups, primarily the DRE-4 group (P<0.05). In both short and long villi, we found that the DRE increased the surface area of absorption (P<0.05) (Table 5).

Morphological analysis showed considerably more dense and complex mucosal folds in the experimental groups than those in the control group (Figures 6 A, B, C, D, E, and F). Swollen goblet cells were prevalent in the apical regions of the mucosal folds that faced the intestinal lumen, and these cells were relatively more abundant in the DRE groups (Figures 6 A1, B1, C1, D1, E1, and F1). In the basal parts, the goblet cells were not prominent. Enterocytes did not exhibit pinocytic vacuolization in the apical regions. However, in the basal parts, enterocytes were the predominant cell type in all groups (Figures 6 A2, B2, C2, D2, E2, and F2).

Challenge test with *Lactococcus garvieae*

The average water temperature during the challenge experiment was 18.2±1.31°C. The first fish death occurred in the control group on the second day of the experiment. Mortality was the highest in all groups between days 3 and 5. By day 7, all fish in the control group were dead. No more deaths were recorded on the 7th day in the DRE-1 group and on the 8th day in the DRE-2 group. Since no more deaths occurred after the 14th day, the 14th day was set as the end of the study. At the end of the study, the DRE-1 group had the highest survival rate at 40%, which was significantly different from all other groups (P<0.05). Thus, we determined that DRE supplementation provided resistance against *Lactococcus garvieae* infection, but this effect decreased as the extract level increased, as determined by the cumulative survival rates (Figure 7).

Discussion

Researchers have recently started investigating the application of dandelion in the field of aquaculture, although many researchers in other disciplines have studied it. Only a few studies have assessed the growth performance and histological, physiological, and immunological parameters in some fish (e.g., *Trachinotus ovatus* (Tan et al., 2017; Tan and Sun, 2020), hybrid grouper (Sun et al., 2019, 2022), *Cyprinus carpio* (Sirakov et al., 2019), and *Oncorhynchus mykiss* (Koshinski, 2020; Salem et al., 2021; Mostafavi et al., 2022; Köse and Ariman Karabulut, 2022). Additionally, no other bacterial disease resistance has been investigated except for *Vibrio harveyi* (Tan et al., 2017) and *Streptococcus iniae* infection (Shekarabi et al., 2021). In this study, DRE was added to trout feed and fed to rainbow trout for 90 days. The FBW, WG, FCR, SGR, PER, PDR, and CF values increased significantly in all DRE groups, while the FCR, LDR, VSI, and HSI values decreased significantly in the DRE groups. These results showed an increase in the values of FBW, WG, FI, SGR, and PDR from 0 to 20 mL/kg of DRE and a decrease in the values with a further increase in the concentration of DRE, which was still higher than the values in the control group. Similar trends were reported in other studies that used dandelion (Tan et al., 2017; Sirakov et al., 2019; Sun et al., 2019; Tan and Sun, 2020; Koshinski, 2020; Salem et al., 2021; Mostafavi et al., 2022; Sun et al., 2022). Additionally, some studies in which rainbow trout were treated with different plant extracts showed similar (Lee et al., 2005; Adineh et al., 2020) and different (Cagiltay et al., 2011; Farahi et al., 2012) trends. Although similar results were obtained in all studies conducted with dandelion on different species, the reason for different plant extracts showing different or similar results on the same species might be associated with the lack of any toxic effect of dandelion. Our argument is supported by the fact that no toxic effect of dandelion was reported in any study.

We calculated the LDR value using the PDR formula of Tan et al. (2017) for the first time. The values of PER and PDR, which are used as feed quality indicators in fish diets, can be used to evaluate protein utilization (Lee et al., 2014; Tan et al., 2017). Since the LDR value expresses the utilization value of oil in the feed, it can be used as a feed quality indicator, along with the PER and PDR values. In contrast to the PDR value, the steady decrease in the LDR values in the experimental groups probably occurred because the fish consumed the protein in the feed for growth while increasing the utilization of oil for survival, which occurred because of the effect of dandelions.

Condition factor (CF) is a good criterion for monitoring the nutrition and growth of fish in aquaculture (Yiğit and Aral, 1999). In this study, the CF value, which increased with dandelion extract supplementation, was similar to that reported by Mostafavi et al. (2022) but differed from that found in a study on *Trachinotus ovatus* (Tan et al., 2017). The VSI and HSI values were high in fish that were administered high-energy feeds (Cheng et al., 2006). In this study, the decrease in the VSI and HSI values with the application of dandelion matched the findings of a study on hybrid grouper (Sun et al., 2019); however, the trends were different from those reported in other studies (Su et al., 2017; Tan et al., 2017). These differences might be related to several factors, such as different plant extraction methods, different contents of the trial feeds, and different levels of extracts used in the trials.

The DRE added to the diet increased the crude protein content in the muscle and liver as the concentration of the extract increased from 0 to 20 mL/kg, while the crude lipid content decreased compared to that in the control group. Similar findings were reported in studies on the rainbow trout and *Trachinotus ovatus* fish with dandelion extract applied to the diet (Tan et al., 2017; Koshinski, 2020; Köse and Ariman Karabulut, 2022; Mostafavi et al., 2022). As mentioned in our previous study, the feed administered to the fish is utilized more effectively in the presence of dandelion, which positively affects protein and lipid accumulation. A low crude lipid content in the liver indicated that dandelion had hepatoprotective properties. This argument was also supported by the results of liver histology and the serum liver enzyme content (ALT, AST, and ALP) in this study. Our results confirmed the hepatoprotective effects of dandelion reported by other researchers (Domitrović et al., 2010; Hfaiedh et al., 2014; García-Carrasco et al., 2015).

Biochemical blood parameters can reflect the physiological and metabolic status of fish depending on their nutritional status (Tan et al., 2018 a). As these parameters vary in response to changes in the nutritional status, they can help in assessing the health status of fish (Gholamhosseini et al., 2021). Glucose is the main indicator of stress in fish. It is released into the blood to meet the increasing energy requirements of cells and is obtained via glycogenolysis (Mohammadi et al., 2020). In our study, the glucose levels of the fish in all DRE groups were significantly lower than those of the fish in the control group. This indicated that dandelion extract had a hypoglycemic effect on the feed. Other studies on dandelion found similar results (Sirakov et al., 2019; Sun et al., 2019; Mostafavi et al., 2022; Sun et al., 2022). Choi et al. (2018) showed that the α-glucosidase inhibitor derived from dandelion can decrease glucose absorption by suppressing carbohydrate digestive enzyme activities in the intestine. Some polysaccharides in the dandelion extract might reduce blood glucose levels as they can be directly consumed by host cells (Al-Kassie et al., 2008; Mostafavi et al., 2022). The low glucose levels observed in this study can be explained by these factors.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are important liver enzymes that catalyze the transfer of amino groups from alpha-amino to alpha-keto acids. Damaged liver cells release large quantities of these enzymes into the blood. Therefore,

the increase in the levels of these two enzymes in blood serum indicates liver damage (Soltan et al., 2008). In this study, serum ALT and AST levels in the DRE groups decreased gradually as the amount of extract increased and were significantly lower than those in the control group. Similar results were reported in some studies that used dandelion (Tan et al., 2017; Sirakov et al., 2019; Koshinski, 2020; Mostafavi et al., 2022; Sun et al., 2022). The rich polysaccharide, antioxidant, and anti-radical properties of dandelion prevent the release of ALT and AST enzymes into the plasma by preventing lipid peroxidation in the cell membrane, thus protecting the liver (Choi et al., 2010). The low levels of ALT and AST recorded in this study might be related to the mechanism reported by Choi et al. (2010).

The total protein (TP) level in blood serum is very important. ALP is a liver enzyme that regulates some basic functions in organisms. It is an indicator of the health condition of fish (Zhou et al., 2015). In this study, DRE significantly increased the values of serum ALP and TP in all treatment groups. Similar results were reported in some studies (Tan et al., 2017; Mostafavi et al., 2022). However, other studies reported low ALP and high TP values (Koshinski, 2020; Gholamhosseini et al., 2021; Sun et al., 2022). A strong innate immune response in fish is associated with higher TP and ALP values (Zhou et al., 2015; Tan et al., 2017). The high TP and ALP levels determined in this study might be related to the increase in non-specific immune responses in fish mediated by the dandelion extract. The differences between the results of our study and other studies can be explained by several factors, such as different fish species, environmental conditions, extract levels, extraction methods, etc.

Cholesterol is synthesized in the liver and transported to other tissues via LDL. In contrast, HDL transports cholesterol from peripheral tissues to the liver followed by excretion in bile, which lowers the level of cholesterol in fish blood (Asgary et al., 2000). In this study, serum cholesterol, triglyceride, and LDL levels were significantly decreased, and HDL levels were increased in all DRE groups. Similar results were reported in previous studies (Sun et al., 2019; Koshinski, 2020; Gholamhosseini et al., 2021; Mostafavi et al., 2022). Quercetin is a plant flavone that belongs to the flavonoid group of polyphenols, and its presence in dandelion was first reported by Schütz et al. (2005). Quercetin inhibits cholesterol biosynthesis by preventing the activity of fatty acid synthesis (Yamamoto and Oue, 2006). The lower levels of cholesterol, triglyceride, and LDL in this study might be attributed to quercetin.

Amylase hydrolyzes dietary starch and glycogen, and amylase activity is a key indicator of the digestive capacity and nutritional and physiological status of fish (Rønnestad et al., 2013). The level of amylase in blood serum reflects the balance between the release and removal of amylase from the blood. In humans, almost all serum amylase is produced in the pancreas, salivary glands, and liver (Bhutta and Rahman, 1971; Pieper-Bigelow et al., 1990). Whether plasma amylase activity in fish originates from these tissues is unclear (Kawanago et al., 2014). In this study, the amylase levels were significantly lower in the DRE groups. The amylase levels reported in healthy individuals of *Oncorhynchus mykiss* (Aydın et al., 2001) matched the amylase levels determined in this study. The decrease in the amylase levels with an increase in the amount of extract might be attributed to the α-amylase and α-glycosidase inhibitors present in dandelion flavonoids, as reported by Huang et al. (2021). On the other hand, the low amylase levels recorded in the DRE groups might be due to the rapid digestion of dietary starch and glycogen facilitated by the action of dandelion on the digestive system. This might have accelerated its release into the blood and removal from the blood. Additionally, as the fish in all groups were healthy throughout the study, no deaths were recorded during the study, and better growth parameters were obtained in the DRE groups. Thus, we inferred that the addition of DRE to the diet did not adversely affect the liver and pancreas of the fish, and we also concluded that dietary starch and glycogen might have contributed to growth. High-fat feeds can increase the accumulation of lipids in the body and fatty acids in the liver, which can cause liver diseases (Li et al., 2014; Yan et al., 2015). Hepatocyte surface area, hepatocyte nuclei area, hepatocyte number, and lipid and glycogen content in the cytoplasm can be used as indicators of metabolic activity in the liver (Strussmann and Takashima, 1990). In this study, no difference occurred in the diameter of the hepatocyte nucleus between the DRE and control groups. The commercial feed to which we added DRE was a high-fat feed (CL = 20%). Therefore, moderate vacuolization, apoptotic structures in the hepatocyte nucleus, areas with hepatocellular degeneration, and necrotic areas were observed in the control group. We found that these abnormal structures that occurred in the control group gradually decreased or even completely disappeared in the DRE groups. These results indicated that dandelion had a hepatoprotective effect. The hepatoprotective effects of dandelion were also reported in other studies (Park et al., 2010 a, 2010 b; You et al., 2010; Sun et al., 2019, 2022) . The protective effect of dandelion might be mediated by biochemical compounds that are responsible for hepatoprotective effects, such as inulin, taraerol, laevulin, and taraxol (Mahboubi and Mahboubi, 2020).

In Salmonids, the distal intestine (segment II of the midgut in this study), which commonly experiences changes (Martínez-Llorens et al., 2012), is the site of fat and protein absorption (Ezeasor, 1978). In aquatic organisms, an increase in the gut length, villi height, villi width, and villi number indicates the development of absorption areas (Awad et al., 2009; Torrecillas et al., 2015). An increase in intestinal muscle thickness improves the ability to digest and absorb (Chen and Wang, 2013). Our results showed that administering DRE increased the thickness of the intestinal CM, LM, and TM in the fish of the DRE group. Similarly, higher values were detected in

the parameters LP, VL, and VW. Additionally, mucosal folds were more complex and dense in the experimental groups, and the apical parts had more goblet cells. Similar results were reported in previous studies in which dandelion and other plant species were administered to rainbow trouts and other fish species (Heidarieh et al., 2012; Tan et al., 2018 b; Diler and Görmez, 2019; Tan and Sun, 2020). The improvement in the intestine due to the addition of dandelion might be associated with inulin which is abundant in chicory species. Inulin is a carbohydrate that cannot be digested in the small intestine but is fermented in the large intestine (Apolinário et al., 2014) and is stored in plants. Inulin has a probiotic effect as it promotes the growth, metabolism, and health of bacteria, especially lactobacilli and bifidobacteria, which are limited in the intestine (Roberfroid, 2000; Karimi et al., 2015).

Bacterial challenge tests are widely used to determine the effect of alternative feeds or additives found in fish diets on fish pathogens (Austin and Zhang, 2006). Except for testing the effects on *Vibrio harveyi* (Tan et al., 2017) and *Streptococcus iniae* (Shekarabi et al., 2021), no study has performed bacterial resistance testing on fish using dandelion extract. Resistance to *Lactococcus garvieae* was recorded for the first time in this study. *Lactococcus garvieae* is a zoonotic pathogen that can cause serious infections in humans and animals. It is a gram-positive and non-motile bacterial species. It severely affects fish in their natural habitat and artificial habitat (Vendrell et al., 2006). In *Lactococcus garvieae* infections, mortality rates can be over 85% in rainbow trout at water temperatures above 18°C (Royo, 1999). Although all fish in the control group died in this study, a survival rate of 40% in the DRE-1 group was similar to previously reported values. On the other hand, the ALP and TP values were higher in the DRE-supplemented groups than in the control group. Some studies found that a strong innate immune response in fish was associated with higher TP and ALP values (Zhou et al., 2015; Tan et al., 2017). At the end of the challenge trial, some fish were still alive in the DRE groups, but the survival rate decreased with the increase in the extract level. These results suggested that although the effect decreased with an increase in the extract level, overall, DRE supplementation enhanced the innate immune response and increased the resistance of the fish to *Lactococcus garvieae* infection.

Conclusions

The DRE-4 group, which received 20 mL/kg of DRE, had the best growth, nutrient utilization, and body index parameters. However, the results of the quadratic polynomial regression analysis of the FI, WG, FCR, and SGR parameters against DRE levels showed that the optimum level should be 23.91±1.048 mL/kg. An increase in the length of the intestinal villi, intestinal muscle thickness, absorption areas, mucosal folds, and the number of goblet cells with DRE supplementation indicated that dandelion had growth-promoting effects. Additionally, the dandelion extract exhibited hypolipidemic and hypoglycemic effects and promoted the healing of fish muscles, the biochemical blood parameters, and liver morphology. The survival rates recorded in the DRE groups after *Lactococcus garvieae* infection indicated that dandelion might enhance fish immunity.

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

Özay Köse: writing − original draft, conceptualization, data curation, visualization, methodology. Huriye Arıman Karabulut: supervision, conceptualization, review and editing, validation. Akif Er: methodology, validation, writing − review and editing.

Ethics approval

This research was checked and approved of by Recep Tayyip Erdogan University Animal Experiments Local Ethics Committee (Decision No: 2020/11 Date: 31.03.2020). All procedures subject to the research were carried out within the scope of all guidelines for the scientific use of animals.

Data availability

The corresponding author will provide all study datasets upon reasonable request.

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