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RESEARCH PAPER

Determination of Some Hematological and Non-Specific Immune Defences, Oxidative Stress and Histopathological Status in Rainbow Trout (*Oncorhynchus mykiss*) Fed Rosehip (*Rosa canina*) to *Yersinia ruckeri*

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Abstract

The effect of the *Yersinia ruckeri* infection in the different tissues of 225 rainbow trout (*Oncorhynchus mykiss*) fed with rosehip (*Rosa canina*) was researched by evaluating a range of factors such as hematological and histopathological findings, non-specific immune parameters, liver antioxidant parameters, and then determined mortality and relative percentage survival (RPS) rates.

The study comprises control groups (C+, C−) and rosehip experimental groups (REGs): R_{10} (10%), R_{20} (20%) and R_{30} (30%). Following the 50-day feeding period, the fish were infected with *Y. ruckeri* and then blood, liver, spleen and kidney samples were taken. RBC, WBC, Hb, Hct and RBC indices, non-specific immune parameters, NBT, phagocytic activity and leukocyte formulas all significantly increased in R_{20} compared to other groups. Antioxidant parameters SOD, CAT and GSH values increased in the R_{20} and R_{30} groups, and the lowest RPS value was detected in R_{30} at 26.31%, while it increased in the R_{20} group to 42.09%. R_{30} showed no significant hematological and immunological effects, however, the immune cells were suppressed. In this research, R_{20} was determined to be the ideal dose, taking into account both health indicators and hematological parameters, as a defense mechanism against *Y. ruckeri*, showing the best antibacterial and antioxidant effects.

Keywords: Antioxidant parameters, hematology, histopathology, immunostimulant, *Oncorhynchus mykiss*.

Introduction

In aquaculture, the use of antibiotics employed for prophylactic or therapeutic purposes, hormones and of irrational drug use over time leads to accumulation in fish tissues and an increase in the resistant bacterial strains in the natural environment. Due to the negative effects of these pharmacological substances or hormones on humans and the environment, the majority of the studies conducted in the last 20 to 25 years have focused on the use of immunostimulants that will strengthen the immune system, rather than the treatment of fish (Ergönül *et al*., 2012). In aquaculture, vitamin C (ascorbic acid-AA) which is one of the most commonly studied micronutrients of immunostimulants is an important antioxidant in many teleost species and has various functions in hematopoiesis and in the immune system (Lim *et al*., 2000). In many species, including teleosts, due to the lack of the L-gulonolactone oxidase enzyme that catalyzes ascorbic acid synthesis, and the limited period (6 to 8 weeks) for the aquatic animals to store the water soluble vitamins, the vitamin C requirement was met by mixing it into the feed (Kubat *et al*., 2013). Rosehip (*Rosa canina* L.) is a traditional medicinal herb and a strong immunostimulant containing high levels of vitamin C. Therefore, it protects cells from stress-induced damage caused by free radicals in the organism – due to infections and various other reasons it prevents the formation of radicals and it has an important role in antioxidant defense. In studies on immunostimulants, it has been reported that immunostimulants increased the resistance of fish against various bacterial, viral and parasitic diseases, decreased the pathogen-based mortality in the larval stage, increased growth and overcame cases of stress-induced immunosuppression (Barman *et al*., 2013). *Yersinia ruckeri*, which was first isolated in 1991 in rainbow trout (*Oncorhynchus mykiss*, Walbaum, 1792) in Turkey, causes enteric redmouth disease (ERM). It is a Gram-negative rod-shaped Enterobacterium that causes significant economic losses (Altun and Diler, 1999). Important findings have been obtained from the studies investigating the use of medicinal plants on the protection of fish and reduced mortality from *Y. ruckeri*, which is a pathogen that can be controlled by immunostimulants (Madhuri *et al*., 2012). This study

revealed some hematological and non-specific immune system parameters and liver antioxidant enzyme activities to combat pathogenic *Y. ruckeri* in rainbow trout given feed fortified with rosehip which contains an important antioxidant vitamin and the histopathological effects of rosehip were investigated.

Materials and Methods

Fish Investigation Method

Two hundred and twenty-five rainbow trout with an average weight of 50 to 60 g were procured from a private fish farm and placed in 300 L cylindrical-conical tanks with 15 fish per tank in a flow-through freshwater system in the Fish Diseases Unit of the Faculty of Fisheries in Çukurova University. Three replication of rosehip experimental (REGs) and control groups (C+, C-) were designed as shown in Table 1. Water temperature, oxygen level and pH values in the tanks were measured using a YSI 6600 CTD multi parameter instrument. In addition, a Spectroquant NOVA 60 (Merck) was used for measuring ammonia (NH_3), nitrite (NO_2) and nitrate (NO_3) (mg/L) values in the tanks.

Preparation of Rosehip (RH) and Basal (Control) Diets

Basal trout fish feed (no:3) and rosehip-fortified feed were prepared in the feed preparation units of the Akuamax Aquaculture Marine Company (Table 2 and Table 3). Rosehip purchased from a local herbalist store was dried in an oven at 70˚C, ground in a Retsch Agate Mortar Grinder and mixed with basal trout fish feed in three different ratios (10%, 20% and 30%).

The measurement of the nutritional content of all experimental and control feeds (basal diet) and of the AA (vitamin C) in rosehip were conducted at the food engineering laboratories of the Instrumental Analyses and Agriculture Faculty and Fisheries Faculty at Çukurova University. For the AA analysis, rosehip extracts were injected into an Agilent 1260 model High Performance Liquid Chromatography (HPLC) device containing a Diode Array Detector (DAD) detector, and the AA values were determined using the calibration curves obtained by an external standard method (Lee and Coates, 2000). The ratios of rosehip added to the experimental feed were determined according to the effective doses of rosehip in rainbow trout that have been detected and reported in previous studies. Additionally, the fish were fed 2% of their body weight, two times a day for 50 days.

Experimental Infection with *Yersinia ruckeri*

In all experimental groups, at the end of the 50-day feeding period, the fish were anaesthetized with quinaldine sulfate (20 ml/L, 4 to 5 min) (Sigma Chemical Co., Germany) using a bathing method application. Then, 3×10^8 cfu / 0.1 mL per fish of highly virulent *Y. ruckeri* isolate isolated in Turkey was applied to all the experimental group fish intraperitoneally (i.p.) except for the C− group. In order to ensure the equality of stress between the groups caused by the application, the fish in the C− group were injected with the same amount of physiological saline as the injections to fish in the other experimental groups (REGs), and all the groups were monitored for mortality every 12 hours.

By day seven, the clinical symptoms of the pathogen intensified and deaths were observed in the C+ group. In the sampling carried out at day seven after the injection, *Y. ruckeri* was re-isolated from the visceral organs and blood of moribund fish using a tryptic soy agar (TSA) medium (Austin *et al*., 2003, Sousa *et al*., 2001). Hematological and non-specific immune parameters, oxidative stress indices (OSI) and histopathological examinations were analyzed in twenty-one fish from each experimental group (the total number of fish: 105). Ninety-six fish (except the C− group) were left in the tanks in order to observe the intergroup mortality and relative percent survival (RPS) rates at 10 days.

Disease Resistance

Mortality rates were recorded daily for 96 fish in the experimental groups (C+ and REGs) for 10 days following the infection. Relative percent survival (RPS) was calculated using the Ellis (1988) formula

$$\text{RPS} = 1 - \frac{(\text{Percent mortality in treated group})}{(\text{Percent mortality in control group})} \times 100$$

Hematological and Non-Specific Immune Analyses

Before the hematological and non-specific immune analyses, fish in all groups were anaesthetized with quinaldine sulfate (20 ml/L 4 to 5 min.). Blood samples were taken from the caudal vein using a syringe, transferred into tubes with Ethylenediamine tetraacetic acid (EDTA) and stored at 4°C. Leukocyte (WBC), erythrocyte (RBC), hematocrit (Hct) and hemoglobin (Hb) were measured in the hematology laboratory of the Veterinary Faculty of Selçuk University, with an MS4-e Veterinary Hematology Analyzer (Hemocell counter) of trout fish scales. Erythrocyte indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated according to Stolen *et al*. (1994). Additionally, peripheral blood smears were stained with May-Grünwald-Giemsa. Leukocyte formulas (lymphocyte, monocyte, and granulocyte) were determined using blood smears from each fish (Fujimaki and Isoda, 1990). Phagocytic activities of leukocyte cells and Nitro blue tetrazolium activity (NBT) were determined using a spectrophotometric method

Table 1. Rosehip experimental groups (REGs) and control groups

	Y.ruckeri	Rosehip (RH)
Control Groups		
Positive Control (C+)	+	-
Negative Control (C-)	-	-
REGs		
10% Rosehip (R_{10})	+	+
20% Rosehip (R_{20})	+	+
30% Rosehip (R_{30})	+	+

Table 2. Formulation of basal trout diet

Basic Content	Used amounts
Fish meal	0.3 g
Soybean meal	0.3 g
Corn gluten	0.2 g
Wheat gluten	0.02 g
Cholorella	50 g
Schizochytrium	100 g
Spirulina	30 g
Garlic	100 g
Lipids	
Fish oil	0.04 g
Vitamins and Minerals	
Vitamin premix	24 g
Mineral premix	10 g
DL Methionine	14 g
Choline Cloride	10 g
Carophyl Pink (8%)	1 g
E Vitamin	30 g
Additional Ingredients	
Anti mold	50 g
Food coloring (red)	5 g

Table 3. Proximate composition of rosehip (RH) (100%) and diet groups with RH at different ratios

	RH (%)	Basal Diet (Control)	REGs		
			R_{10} (10%)	R_{20} (20%)	R_{30} (30%)
Crude protein (%)	5.50±1.0	47.09±2.0	45.64±1.7	43.76±1.0	42.48±1.5
Moisture (%)	8.06±0.1	7.11±0.2	9.43±1.0	10.17±0.3	10.86±0.5
Ash (%)	1.75±0.0	8.43±1.0	8.5±0.5	7.78±0.1	7.66±1.0
Crude fat (%)	3.70±0.1	20.22±0.5	20.80±0.5	21.55±1.0	22.72±0.0

REGs: Rosehip experimental groups. Data are represented as mean ± SD.

(Seeley *et al*., 1990; Siwicki *et al*., 1985).

Measurement of Oxidative Stress Indices (OSI)

For the OSI analyses, liver tissue with strong hepatocellular damage was taken from all the anesthetized experimental fish. Chopped liver tissue prepared for Malondialdehyde (MDA), Reduced Glutathione (GSH), Superoxide Dismutase (SOD) and Catalase (CAT) analyses were homogenized using a Heidolph 50110 R2R0 homogenizer in 5 (w/v) volume containing a 1.15% KCl solution.

MDA analysis and SOD activity were determined according to Sloof *et al*. (1983) and Fridovich (1974), respectively. CAT and GSH were analyzed according to Beutler (1975).

Histopathological Examinations

Spleen, kidney and liver tissues were taken from the C+ groups and REGs for the histopathological examinations. Sample tissues were fixed in 5% neutral buffered formalin, dehydrated in a graded ethanol series and embedded in paraffin. Sections of 5 µm tissue samples stained with Hematoxylin-Eosin (H&E) were examined for histopathological changes under an Olympus BX51 light microscope (Bullock, 1978).

Statistical Analyses

Comparisons of hematological parameters and antioxidant enzyme results were conducted using one-way analysis of variance (ANOVA). A Duncan multiple comparison test of the one-way ANOVA was used to compare the mean differences. The differences were considered to be significant at $P \leq 0.05$.

Results

The nutritional contents of the basal trout diet feed and rosehip-fortified feed along with the results for the moisture, ash, crude fat and crude protein analyses of 100% rosehip are shown in Tables 2 and 3. In Table 3, there were no significant differences between the nutritional contents of the basal trout diet feed and the rosehip-fortified feed ($P>0.05$). Also, the AA (vitamin C) content for 1 g of grinded rosehip was determined to be 2.15 mg/g. Accordingly, the ratio of AA in the experimental groups containing rosehip were 215 mg/kg for R_{10}, 430 mg/kg for R_{20} and 645 mg/kg for R_{30}. During the trial, the average water temperature, dissolved oxygen amounts and pH in the tanks were measured to be $6 \pm 0.01^{\circ}C$, 7.5 ± 0.02 mg/L, and pH 7.8, respectively. Nitrite (NO_2), nitrate (NO_3) (mg/L) and ammonia (NH_3) values measured in the tanks were found to be 0.02 ± 0.01 mg/L, 23.34 ± 15.5 mg/L and 0.08 ± 0.04 mg/L, respectively.

Clinical and Necropsy Findings in Fish Infected with *Y. ruckeri*

In the C+ group, by day 5 following the *Y. ruckeri* injections, clinical observations revealed reluctance in the fish to receive feed, as well as fatigue, abdominal swelling and redness around the anus. In the R_{10} group, the clinical findings of the pathogen intensified towards day 7, and hemorrhages were detected in the pectoral and ventral fin bases and around the anus. According to the re-isolation results of *Y.ruckeri*, no negative conditions were observed in the experimental groups containing rosehip until day 7, except for the clinical symptoms.

Hematological and Non-Specific Immune Responses

RBC, Hb and Hct values decreased in the C+ and R_{10} groups, whereas they significantly increased in R_{20} ($P<0.05$) (Table 4). On the other hand, there were no significant differences between the MCV values of the groups ($P>0.05$) while the changes in MCH and MCHC values were parallel to those determined for RBC and Hct values ($P<0.05$) (Table 4). There were significant differences between the control groups and REGs in terms of WBC and non-specific immune parameters ($P<0.05$) (Table 4).

NBT and phagocytic activity values increased significantly in the C+ and R_{10} groups. This increase reached a maximum in R_{20} ($P<0.05$). On the other hand, in R_{30}, non-specific immune parameters and WBC values decreased significantly ($P<0.05$) (Table 4).

Oxidative Stress Indices

SOD and CAT values increased significantly in the R_{20} and R_{30} groups containing high levels of rosehip ($P<0.05$). However, no significant differences were observed between the experimental groups in terms of MDA values ($P>0.05$) (Table 5).

Disease Resistance

Mortality ratios, survival and relative percent survival (RPS) were calculated for the C+ and REGs groups (Table 6). The highest mortality was in the C+ group (79.17%) and the lowest mortality was seen in R_{20} (33.33%) after 10 days (Table 6). In addition, the highest RPS value was detected in R_{20} (42%).

Histopathological Findings

Histopathological findings obtained from liver, spleen and kidney tissues in the C+ group and the REGs are shown in Figures 1, 2 and 3. The examinations revealed similar pathologies in the C+ group and REGs including hemorrhages in parenchyma tissue, erythrocyte and leukocyte infiltration, vacuolar degeneration, liquefactive necrosis, necrotic cells and melanomacrophage centers. No significant histopathological differences were observed between the C+ group and the REGs.

Discussion

It was found that measured temperature, oxygen content and the pH values of the water in the tanks were in accordance with trout aquaculture criteria, also, nitrite (NO_2), nitrate (NO_3) and ammonia (NH_3) values were within the limits recommended by the Ministry of Food, Agriculture and Livestock for trout production in closed systems (http://www.tarim.gov.tr/, 2016).

In studies conducted on fish health, it has been reported that natural immunostimulants were preferred due to their easy absorption by the body, leaving no residuals as is the case of chemicals, quick disposal and their antibiotic, antioxidant properties and vitamin content (Dörücü *et al*., 2009). Antioxidant vitamin C found in the composition of rosehip is also an important immunostimulant forming bactericidal and viral resistance and activity against pathogens in fish, and stimulating the proliferation and phagocytosis of the immune cells (Ergönül *et al*., 2012). The study material, rosehip, is an important herbal immunostimulant used for the

Table 4. Hematological and some non-specific immune parameters of *O.mykiss* fed with rosehip (RH)

Experimental Groups	Control Groups		REGs		
	C+ *Y.ruckeri* (+) RH (-)	C- *Y.ruckeri* (-) RH (-)	R_{10} *Y.ruckeri* (+) 10% RH	R_{20} *Y.ruckeri* (+) 20% RH	R_{30} *Y.ruckeri* (+) 30% RH
Hematol. Param.					
RBC ($\times 10^6/mm^3$)	0.65±0.0[a]	1.25±0.0[c]	0.81 ± 0.0[b]	0.98±0.0[c]	0.77±0.0[a]
Hb (g/dL)	6.44±0.5[a]	10.47±0.2[b]	6.99 ± 0.6[a]	7.08±0.5[a]	6.74±0.3[a]
Hct (%)	21.15 ± 1.7[a]	32.21±0.7[b]	17.27±0.8[a]	27.81±1.2[b]	20.30±1.8[a]
Erythrocyte Indices					
MCV (μ^3)	247.14±3.4	258.10±2.1	257.12±3.0	263.30±2.4	255.37±2.9
MCH (pg)	67.25±4.9[a]	104.84±5.6[c]	85.29±6.7[a]	89.84±5.8[b]	84.34±3.7[a]
MCHC (%)	24.07±1.6[a]	32.62±1.2[a]	33.01±2.2[a]	39.80±1.9[b]	35.15±2.0[b]
Ns. Immun. Param.					
WBC ($\times 10^3/mm^3$)	13.98±0.7[b]	11.76±0.8[a]	14.42±0.4[b]	16.39±0.1[c]	13.86±0.5[b]
Leukocyt. Formuls.					
Lymphocyte	58.15±3.3[b]	45.8±0.9[a]	60.44±2.5[b]	65.90±2.7[b]	61.20±3.3[b]
Monocyte (%)	3.29±0.0[b]	2.09±0.0[a]	3.30±0.1[b]	4.32±0.1[c]	3.01±0.1[b]
Granülocyte	43.90±3.0[b]	32.87±2.2[a]	44.05±2.1[b]	53.82±2.1[c]	43.20±2.2[b]
NBT (mg/mL)	2.20±0.2[a]	1.59±0.3[a]	2.50±0.3[ab]	3.82±0.4[b]	2.89±0.1[b]
Phagocytic Activity (O.D.510nm)	0.30±0.2[b]	0.20±0.1[a]	0.33±0.3[b]	0.47±0.2[c]	0.34±0.1[b]

O.D.: Optical Density. C+: Positive control, C-: Negative control. REGs: Rosehip experimental groups. Hematol. Param.: Hematological parameters RBC: Red blood cell, Hb: Hemoglobine, Hct: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: Leukocyte cell, Ns. Immun. Param.: Non-specific immune parameters, Leukocyt. Formuls.: Leukocyte Formulas, NBT: Nitroblue tetrazolium activityData are represented as mean ± SD. The values in the same line with different letters are significantly different (P<0.05)

Table 5. Liver oxidative stress indices of infected *O.mykiss* fed with rosehip (RH) at different ratios

Experimental Groups	MDA	Oxidative Stress Indices		
		GSH	SOD	CAT
	(nmole/mg protein)	(µmole/mg protein)	(U/mg protein)	(U/mg protein)
Control Groups				
C+ *Y.ruckeri* (+) RH (-)	6.78±1.1	0.16±0.0[b]	22.53±0.5[b]	240.01±10.2[b]
C-*Y.ruckeri* (-) RH (-)	7.50±1.0	0.09±0.0[a]	12.41±1.2[a]	177.57±10.0[a]
REGs				
R_{10} *Y.ruckeri* (+) RH(10%)	7.56±1.1	0.14±0.0[b]	26.96±9.9[b]	242.30±13.2[b]
R_{20} *Y.ruckeri* (+) RH (20%)	7.77±1.1	0.20±0.0[b]	92.80±7.0[c]	348.27±21.2[d]
R_{30} *Y.ruckeri* (+) RH (30%)	7.60±0.4	0.39±0.0[c]	90.00±4.8[c]	358.16±18.7[d]

C+: Positive control, C-: Negative control. REGs: Rosehip experimental groups. MDA: Malondialdehyde, GSH: Reduced Glutathione, SOD: Süperoxide Dismutase, CAT: Catalase Values are represented as mean ± SD. The values in the same column with different letters are significantly different (P<0.05).

stimulation of carotene pigment erythropoiesis in foods, treatment of wounds and lesions, and also in skeletal deformities (Kılıçgün, 2008; Lim *et al*., 2000).

In a previous study conducted on rainbow trout infected with *Y. ruckeri*, it was observed that hematological parameters decreased because of necrosis in hematopoietic tissues and resulted in anemia; reductions in the RBC indices were also observed (De Kinkelin, *et al*., 1985). In our study, by day 7 of *Y. ruckeri* infection in line with the positive re-isolation results obtained from the tissues – the results, featuring a decrease in RBC, Hb, Hct values and RBC indices observed in the C+ group in our study, were similar to those obtained in the previous studies. However, in the R_{20} group, sudden increases were observed in these parameters. The increases reported by Lim *et al*. (2000) have been regarded as the best indicator of the stimulating effect of AA in rosehip on erythropoiesis. On the other hand, hematological values decreased in most of the fish in the R_{30} group. For the effective use of immunostimulants, timing, dosage, method of administration and the health status of the fish need to be taken into consideration (Barman *et al*., 2013). Cook *et al*. (2001) reported that lower doses of β-1.3/1.6 glucan are an important immunostimulant induced immune response, whereas overdoses (1.0%, 2.0%) caused immunosuppression. In studies on natural products with immunostimulant properties, it

Table 6. Mortality rate, survival and relative percentage survival (RPS) of infected *O.mykiss* fed with rosehip (RH) at different ratios

Experimental Diets	Number of sampled fish	Mortality (%)	Survival (%)	RPS (%)
Control Groups				
C+ *Y.ruckeri* (+) RH (-)	24	79.17^{c}	20.83^{a}	-
C- *Y.ruckeri* (-) RH (-)	-	-	-	-
REGs				
R_{10} *Y.ruckeri* (+) RH (10%)	24	54.17^{b}	45.83^{b}	31.58^{b}
R_{20} *Y.ruckeri* (+) RH (20%)	24	33.33^{a}	66.67^{c}	42.09^{c}
R_{30} *Y.ruckeri* (+) RH (30%)	24	58.34^{b}	41.66^{b}	26.31^{a}

C+: Positive control, C-: Negative control, REGs: Rosehip experimental groups. The values in the same column with different letters are significantly different (P<0.05).

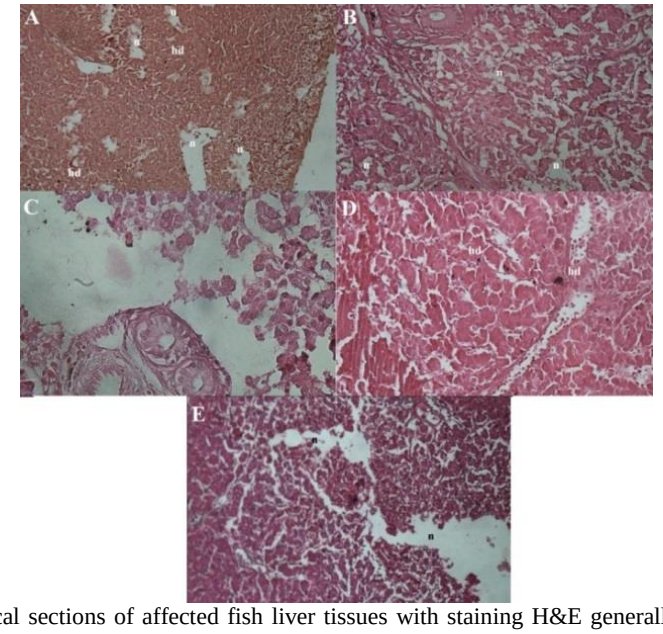

Figure 1. Histopathological sections of affected fish liver tissues with staining H&E generally showed diffuse vacuoler degeneration, hyperaemia, multifocal liquefactive necrosis (n) and hemosiderine deposits (hd) in all groups. A, B: C+ group. 20 × (H&E), C: R_{10} group. Degeneration and necroses in hepatic cells. 40 × (H&E), D: R_{20} group. 20 × (H&E) and E: R_{30} group. Haemorrhage in parenchyma cells and cellular infiltration. 20 × (H&E).

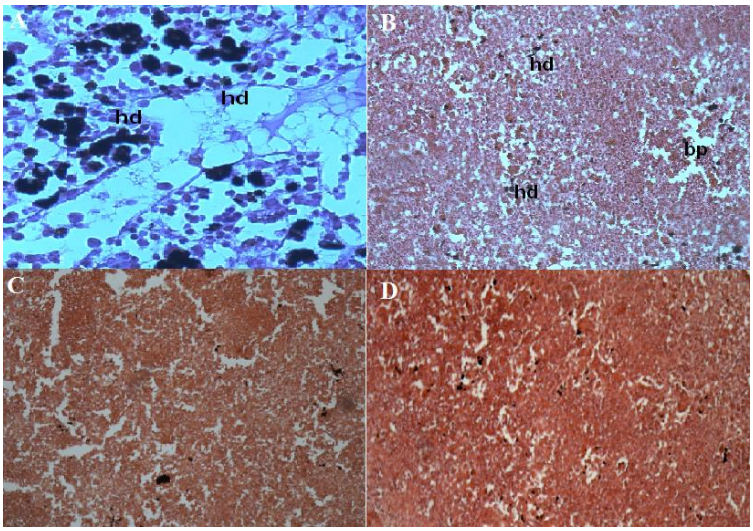

Figure 2. Histopathological sections of affected fish spleen tissues with staining H&E generally showed depletion of white pulp, multifocal liquefactive necrosis (n) and hemosiderine deposits (hd) in all groups. A. C+ group. 40 × (H&E), B. R_{10} group. 20 × (H&E), C. R_{20} Group . 20 × (H&E), D. R_{30} Group. 20 × (H&E).

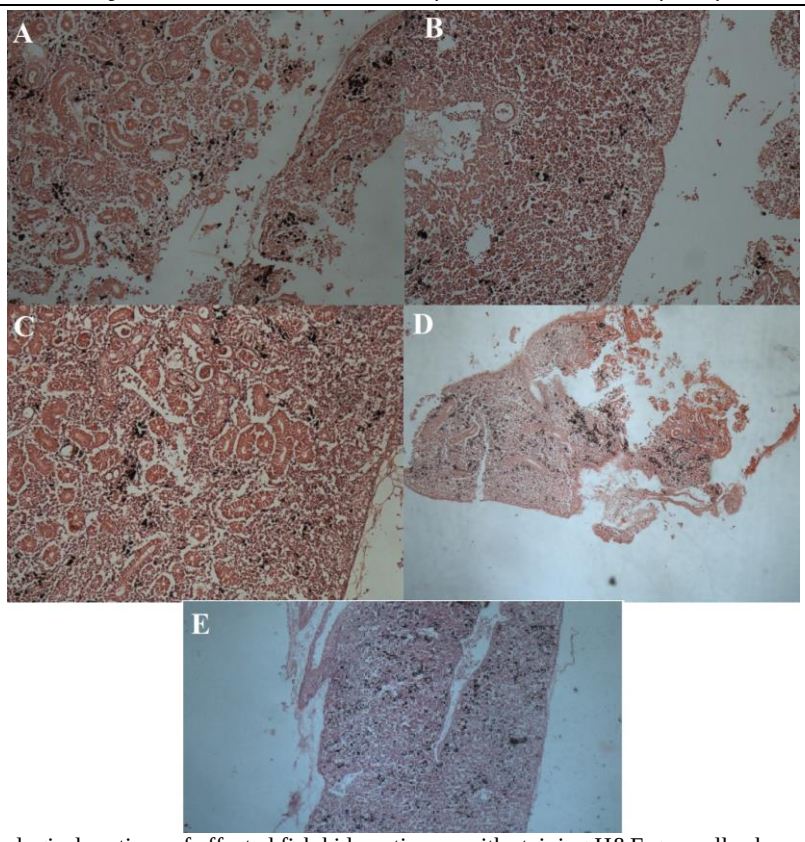

Figure 3. Histopathological sections of affected fish kidney tissues with staining H&E generally showed periglomerular edema, tubular necrosis and melanomachrophage centers in all groups. A, B. C+ group 10 × (H&E), C. R_{10} group 20 × (H&E), D. R_{20} group 4 × (H&E) and E. R_{30} group 4 × (H&E).

has been reported that the lowest doses of these products caused the highest NBT activity and this result was supported by lymphocyte and eosinophil cell counts (Jeney *et al*., 1997; Şahan *et al*., 2015). In fish, phagocytosis is the primary mechanism of the non-specific immune response to pathogenic microorganisms. The NBT activity test is one of the methods used to assess the phagocytic activity of neutrophils and monocytes (Şahan *et al*., 2015). In this study, the phagocytic activity (a non-specific immune system indicator), the leukocyte cell activity indicator, NBT values and the leukocyte counts significantly increased in R_{20} (430 mg/kg AA) in comparison to those obtained in R_{30} (645 mg/kg AA).

Similar to the previous studies, the especially low levels of immunomodulators we used activated NBT leukocyte cells and leukocyte cell formulas, and the results were in line with each other. Sadigh-Eteghad *et al*. (2011) found that 250 to 500 mg/kg of rosehip extract significantly increased the neutrophil and monocyte counts as well as the phagocyte activity in comparison with the control rat groups.

In a different study, it was reported that lycopene, which is an important source of carotenoid, and an antioxidant with an important role in the regulation of the immune response, was found to be present in quantities of between 12.9 to 35.2 mg/100g in rosehip and between 2.3 to 5.0 mg/100g in processed products (Koca *et al*., 2008). In the studies on lycopene in fish, it was reported that the lycopene had positive effects on the hematological and immunological parameters, stimulated the antioxidant parameters and inhibited the stress effects induced by pathogens and intensive stocking (Şahin *et al*., 2014). It has been stated that high doses of vitamin C (500 to 2000 mg/kg) increased the immune function and resistance, stimulated hematopoiesis, repaired tissue damage and had direct positive effects on the healing of wounds in rainbow trout against most viral and bacterial pathogens including *Y. ruckeri* (Tewary and Patra, 2008).

In this study, it was observed that R_{20} (430 mg/kg AA) stimulated all defense cells, whereas R_{30} (645 mg/kg AA) suppressed all the hematological parameters including both health indicators and defense parameters, and it had an immunosuppressive effect on the immune cells. It was found that, similarly to the results obtained in previous studies, R_{20}, which is a rich source of vitamin C and lycopene, had an effect on hematological and immunological parameters. On the other hand, in R_{30}, which included the highest dose of rosehip, the decrease observed in hematological and immunological parameters were in line with the immunosuppressive effects of overdoses of immunostimulants used in different studies on immune parameters (Cook *et al*., 2001). Shahkar *et al*.

(2015), studied the effects of different doses of vitamin C on the hematological parameters and the non-specific immune response in Japanese eels**.** The researchers reported that an 840 mg/kg AA level promoted the immune response; however, higher doses could damage the immune functions and have immunosuppressive effects. The immunosuppressive effect observed in the 645 mg/kg AA application in our study was also observed in the study by Shahkar *et al*. (2015) in their 840 mg/kg AA application. This result was associated with using vitamin C in a commercially pure AA form. However, in experimental studies on animals, including fish, it has been reported that AA products procured in commercial or natural forms revealed different results both in terms of both hematological and immunological parameters (Kubat *et al*., 2013). It has been stated that low doses of vitamin C obtained from natural vegetable and fruits had a stronger effect on immunological and hematological parameters compared to those in commercial products (Özaslan *et al*., 2004).

In the studies of the effects of immunostimulants on many bacteria, including *Y. ruckeri*, positive results have been obtained including the formation of resistance and the reduction of mortality rates (Barman *et al*., 2013). In this study, as a result of 10-day monitoring of *Y.ruckeri*, the lowest mortality rate (33.33%) and the highest RPS ratio (42.09%) was obtained in the R_{20} group. On the other hand, R_{20} not only induced the non-specific immune response through leucopoiesis and enhanced proliferation of lymphocytes, but also stimulated erythropoiesis and provided significant protection against bacterial pathogens.

As in all aerobic organisms, the fish increased their antioxidant SOD and CAT (enzymatic), and their GSH and MDA (non-enzymatic) levels were also increased. These results showed increased oxidative stress. SOD and CAT are the defense mechanisms in erythrocytes, and in the liver, against oxidative stress, while GSH reacts with free radicals and peroxides to protect the cells against oxidative damage. MDA which is a product of lipid peroxidation in fish forms as a result of the oxidation of unsaturated fatty acids and is an important indicator of the oxidative stress. Both GSH and MDA have important roles in the body's defense against reactive oxygen species (ROS) (Keleştemur and Özdemir, 2011). In this study, it was observed that SOD and CAT enzymes significantly increased in R_{20}, containing high doses of rosehip compared to those in the C+ group, and similar increases were also observed in GSH values. However, no significant differences were observed between the groups in terms of MDA levels. In the groups with high doses of AA (430 to 645 mg/kg), high levels of SOD, CAT and GSH activities prevented the oxidative stress caused by bacterial infections before it reached levels that can cause damage to the cells and thus supported the body's defense. There were no significant changes between the groups in MDA values, which is the most important indicator of oxidative stress, and this was regarded as the most important indicator of the above mentioned antioxidant effects.

It has been reported that lycopene, which gives the red color in fruits including tomato, water melon, pink grapefruit and rosehip deactivate hydrogen peroxide (H_2O_2) and nitric oxide (NO) radicals and increase the antioxidant parameters (Şahin *et al*., 2014). Yonar and Sakin (2010) reported that lycopene prevented oxidative stress in rainbow trout, reduced the MDA values and increased the CAT and GSH. In this study, the increase in antioxidant parameters was mostly observed in the experimental groups containing high levels of rosehip. This result was associated, as supported by the previous studies, with the antioxidant properties of AA and lycopene (Rayes, 2012; Barman *et al*., 2013). Wid´en *et al*. (2012), in their study on rosehip, reported that rosehip showed antioxidant activity against oxidative damage and provided maximum protection for erythrocyte cells. Kılıçgün and Altuner (2009) reported that *Rosa canina* prevented liver lipid peroxidation due to its antioxidant effect in rats with damaged livers due to carbon tetrachloride.

Histopathological studies provide information on the diet quality, metabolism, and nutrition status of fish (Caballero *et al*., 2004). In different studies, it has been reported that some vegetables and fruits rich in vitamin C had no significant healing effect on the tissues (Kubat *et al*., 2013; Özaslan *et al*., 2004). In our study, the destruction and damage detected in the C+ group, especially in the kidney, liver and spleen, was higher than that detected in the R_{20} and R_{30} groups. It was determined that even higher concentrations of rosehip could not prevent damage to the tissues and it was histopathologically ineffective. These results support the other results obtained in the study. In this study, it was determined that the antioxidant mechanism functioned in a mutually supporting manner in terms of both enzymatic (SOD, CAT) and non-enzymatic (MDA) activities in the REGs with high AA (430 to 645 mg/kg) content.

In addition, it was observed that the increase in antioxidant enzymes against oxidative stress, hematological and immunological parameters also increased the resistance of the fish and, in spite of observing some acute histopathological findings, there were no high mortality rates at the 430 mg/kg AA level. It was concluded that the R_{20} group (430 mg/kg AA) contained the most effective dose in this study in terms of antibacterial activity. In terms of fish health, it was concluded that obtaining vitamin C from the natural, immunostimulant and eco-friendly rosehip was of importance in terms of the prevention of irrational drug use, culturing fish resistant to disease and reducing costs in aquaculture. It would also make a basis for future studies and create a different perspective.

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