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Effect of Dietary Sumac (*Rhus coriaria* L.) Supplementation on Non-Specific Immune Response and Hematology of Rainbow Trout (*Oncorhynchus mykiss*), Resistance Against *Vibrio anguillarum*

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Research Article

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Abstract

This study aimed to investigate the effects of sumac (*Rhus coriaria* L.) fruit powder in different concentrations as feed additives on non-specific immune response, hematology, and the disease resistance in rainbow trout (*Oncorhynchus mykiss*). Fish (35.67±0.88 g) were fed with experimental diets (1.0, 3.0, 5.0 and 10.0 g kg^{-1}) at four different concentration. There were no significant differences between groups fed with sumac fruit powder and control group by the mean of red blood cell count (RBC), white blood cell count (WBC), differential leukocytes count (monocyte, lymphocyte, and neutrophile), hematocrit (Hct), hemoglobin (Hb), cell hemoglobin (MCH pg), cell hemoglobin concentration (MCHC %), cell hemoglobin volume (MCV μm^3) and plasma lysozyme, Immunoglobulin M (IgM), total protein level on the 8 weeks. After 8 weeks of feeding, fish were challenged with *Vibrio anguillarum* and cumulative mortality was recorded over 21 days. Dietary administration of 1.0, 3.0, and 5.0 g kg^{-1} sumac fruit powder significantly increased fish survival rate ($p<0.05$). The 10.0 g kg^{-1} diet received fish showed no mortality post challenged with *V. anguillarum*. These results showed that the sumac fruit powder improved disease resistance when added to the rainbow trout diet.

Keywords: Hematology, non-specific immune response, *Oncorhynchus mykiss*, *Rhus coriaria* L., *Vibrio anguillarum*.

Yeme İlave Edilen Sumak (*Rhus coriaria* L.)' ın Gökkuşağı Alabalıkları (*Oncorhynchus mykiss*)' nın Spesifik Olmayan Bağışıklık Tepkisi, Hematoloji ve *Vibrio anguillarum*' a Karşı Direnç Üzerine Etkisi

Özet

Bu çalışmanın amacı, yem katkı maddesi olarak farklı düzeylerde kullanılan sumak (*Rhus coriaria* L.) meyvesi tozunun gökkuşağı alabalığında (*Oncorhynchus mykiss*) spesifik olmayan bağışıklık yanıtı, hematoloji ve hastalık direnci üzerindeki etkilerini araştırmaktır. Balıklar (35,67±0,88 g) dört farklı konsantrasyonda (1,0, 3,0, 5,0 ve 10,0 g/kg) sumak ilaveli yemlerle beslenmişlerdir. Sumak meyve tozu ilaveli yemle beslenen gruplar ve kontrol grubu arasında kırmızı kan hücresi sayısı (RBC), beyaz kan hücresi sayısı (WBC), diferansiyel lökosit sayısı (monosit, lenfosit ve nötrofil), hematokrit (Hct), hemoglobin (Hb), ortalama hücre hemoglobin değeri (MCH pg), hücre hemoglobin konsantrasyonu (% MCHC), hücre hemoglobin hacmi (MCV µm3) ve plazma lizozim, immunoglobulin M (IgM) ve toplam protein düzeyinde 60. günde önemli bir farklılık görülmemiştir. 8 haftalık besleme sonrasında balıklara *Vibrio anguillarum* ile deneysel enfeksiyon uygulaması yapılmış ve 21 gün boyunca kümülatif mortalite kaydedilmiştir. Yeme 1,0, 3,0 ve 5,0 g/kg oranında ilave edilen sumak meyve tozu balık yaşam oranını önemli ölçüde artırmış olduğu tespit edilmiştir ($p<0.05$). 10,0 g/kg oranında *V. anguillarum* ile deneysel enfeksiyon sonrasında mortalite görülmemiştir. Bu sonuçlar gökkuşağı alabalığı yemine eklenen sumak meyve tozunun hastalık direncini artırdığını göstermektedir.

Anahtar kelimeler: Hematoloji, non-spesifik bağışıklık tepkisi, *Oncorhynchus mykiss*, *Rhus coriaria* L., *Vibrio anguillarum*.

INTRODUCTION

Infectious disease is the main problem for the development and sustainability of aquaculture. One of the most important bacterial diseases that causes mortality in rainbow trout is vibriosis and *Vibrio anguillarum* is the most common pathogen of disease. Recently, there has been increased interest in

the possibility of using medicinal herbs as disease resistance of cultured fish (Terzioğlu and Diler, 2016; Diler et al., 2017a; Diler et al., 2017b; Uluköy et al., 2018). Sumac (*Rhus coriaria* L.) is a plant species in the Anacardiaceae family that is used as a spice and a native medicine and grows wild in the region extending from Canary Island over the Mediterranean coastline. It has a long history of use by indigenous people for medicinal and other applications. *Rhus coriaria* has been reported to possess antibacterial (Iauk et al., 1998; Ali-Shtayeh et al., 2013; Kossah et al., 2013; Al-Boushi et al., 2014), antifungal (Onkar et al., 2011), antioxidant Aliakbarlu et al., 2014), anti-inflammatory (Panico et al., 2009). Sumac fruits contain phenolic acids, flavonols, hydrolysable tannins, anthocyanin, and organic acids (Mavlyanov et al., 1997). A large number of active metabolites have been reported in sumac including gallic acid, quercetin, vanillic acid (Abu-Reidah et al., 2014; Al-Boushi et al., 2014). *R. coriaria* extract had a strong *in vitro* antibacterial activity against tested bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Shigella* spp., *Staphylococcus aureus*, *Pseudomonas aerugenisis*, *Salmonella enteric* and *Bacillus cereus* (Gabr et al., 2014; Mahdavi et al., 2018). Currently, *in vivo* studies linking the antimicrobial effect of sumac (*R. coriaria* L.) in animal production are scarce. In a study, sumac fruit powder was determined to increase growth performance and not effected immune response of broiler chicks (Toghyani and Faghan, 2017). The determination of health status of fish, the blood parameters are useful tool (Campbell, 2004). Some authors reported which blood parameters such as hematocrit, hemoglobin and total erythrocyte count below the normal ranges are all signs of anaemia. Anaemia is caused that reduced tolerance to secondary stressors and fish are sensitive to certain secondary pathogens (Gatlin, 2007; Rios et al., 2005). This study aimed to determine the effects of sumac fruit powder (SFP) on hematology, non-specific immune response, and to disease resistance against *Vibrio anguillarum* in rainbow trout (*Oncorhynchus mykiss*, Walbaum).

MATERIALS and METHODS

Experimental design

This study was carried out in the commercial trout farming in Aksu-Isparta. The fish for the experiment was obtained from a commercial rainbow trout farm in Aksu (Isparta, Turkey). Fish were stocked into fiberglass tanks and an adaptation period of 20 days was applied prior to the trial. A total of 450 fish (35.67±0.88 g) were randomly allotted as 30 fish in each tank (5 groups with 3 triplicate). During the feeding experiment, the average water temperature was 10.2±0.5ºC, the dissolved oxygen was 8.9±0.2 mg L^{-1} and the pH was 7.2±0.3. Sumac fruit powder (SFP) was purchased from a local market in Isparta province. Different concentration (0, 1.0, 3.0, 5.0 and 10.0 g kg^{-1}) of the SFP were added into experimental diet. Five diets were formulated (40.26 g kg^{-1} crude protein and 16.72 g kg^{-1} crude lipid) based on the study of New (1987) (Table 1). Fish fed twice daily for a total of 8 weeks.

Table 1. Formulation of experimental diets and proximate analysis

Ingredients (g kg $^{-1}$)	Groups				
	Control (0 g kg^{-1})	**Sumac (1 g kg^{-1})**	**Sumac (3 g kg^{-1})**	**Sumac (5 g kg^{-1})**	**Sumac (10 g kg^{-1})**
Fish meal	31.00	31.00	31.00	31.00	31.00
Soybean meal	44.00	44.00	44.00	44.00	44.00
Corn Starch	1.00	0.90	0.70	0.50	0.00
Wheat flour	11.00	11.00	11.00	11.00	11.00
Soybean oil	11.70	11.70	11.70	11.70	11.70
Vit-Min*	1.00	1.00	1.00	1.00	1.00
Pellet binder	0.30	0.30	0.30	0.30	0.30
Sumac fruit powder	0.00	0.10	0.30	0.50	1.00
Chemical composition					
Dry matter	92.23	92.24	92.25	92.27	92.30
Crude protein	40.26	40.27	40.27	40.28	40.29
Total lipid	16.72	16.72	16.71	16.71	16.70
Crude fiber	2.81	2.81	2.80	2.80	2.80
Crude ash	10.64	10.64	10.65	10.65	10.66
Energy (kcal/kg)	4067	4064	4059	4053	4040

*Vitamin premix contained the following per kilogram; 4 000 000 IU vitamin A, vitamin D3 480 000 IU, 2400 mg vitamin E, 2400 mg vitamin K3, 4000 mg vitamin B1, 6 000 mg vitamin B2, 4 000 mg Niacin, 10 000 mg Cal.D. Pantothenate, 4 000 vitamin B6, 10 mg vitamin B_{12}, 100 mg D-Biotin, 1200 mg folic acit, 40 000 mg vitamin C, 60 000 mg inositol.

*Mineral premix contained the following per kilogram; 23 750 mg manganese, 75 000 mg zinco, copper 5 000 mg, cobalt 2 000 mg, iodine 2750 mg, selenium 100 mg, magnesium 200 000 mg.

Analysis of phenolic constituents

The procedure for the phenolic contents has been described by Capanio et al. (1999). High-performance liquid chromatography was used. Detection and quantification was carried out with a SLC-10Avp system controller (Shimadzu, Japan) SIL-10 AD vp Autosampler, LC-10 AD vp pump, DGU-14a degasser, CTO-10 A vp column heater and diode array detector set at 278 nm. AnAgilent Eclipse XDB C-18 column (250x4.6mm,5mikrometre) was used. The flow rate was 0.8 mL/min, injection volume was 10 microlitre and the column temperature was set at 30°C. Methanol and 3% acetic acid were used for mobile phases. The data were integrated and analyzed using the Shimadzu Class-VP (Chromatography Laboratory Automated Software System (Tokyo, Japan). Plant samples, standard solutions, and mobile phases were filtered using a 0.45 micrometer pore size membrane filter (Vivascience AG, Hannover, Germany). The amount of phenolic contents in the plant sample was calculated as g kg^{-1} herb using external calibration curves, constructed for each pure phenolic standart. All determinations were carried out in triplicate and the results were presented as mean ± standard error.

Blood collection

Blood samples of randomly selected five fish were collected. Fish were anesthetized with clove oil and then blood was taken from the caudal vein by using hypodermal heparinized syringe (1 mL). The 200 µL blood volume was transferred to ethylenediaminetatraacetic acid (EDTA) tubes for hematological analysis. The rest of the blood sample (600 µL) was put in plastic tubes for biochemistry and immunological analysis. These blood samples were coagulated, the tubes were centrifuged at 5000 x g for 10 min at 4°C for serum separation, which was stored below -20°C.

Hematological analysis

Red blood cells (RBC, 10^6 mm^3), hematocrit (Hct, %) and hemoglobin (Hb, g dL^{-1}) were determined by using the method by Blaxhall and Daisley (1973). RBC was counted with a Thoma hemocytometer using Dacie' s diluting fluid. Hct was determined using a capillary hematocrit tube. Hb concentration was determined by the cyanomethahaenoglobin method in the spectrophotometer (540 nm). The hematological findings of mean cell haemoglobin concentration (MCHC: g dL^{-1}), mean cell haemoglobin (MCH: pg), and mean cell volume (MCV: fl) were calculated using the total RBC count, Hb concentration, and Ht. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated using the following formula.

(1) MCV (μm^3) = [(Hct, %) x 10] / (RBC, x 10^6 per mm^3),
(2) MCH (pg) = [(Hb, g/dl) x 10] / (RBC, x10^6 per mm^3),
(3) MCHC (%) = [(Hb, g/dl)x100] / (Hct, %)

Immunological analysis

Lysozyme activity

The lysoplate assay was performed as described by Ellis (1996) with a final concentration of 0.12% *M. lysodeikticus* cells in 100 mL of 0.5% agarose in 66 mM sodium phosphate, pH 6.2. The zones of lysis were measured with a microcaliper after 20 h of incubation at 36°C. The results for standards were plotted on semilogarithmic graph paper and sample values extrapolated from this standard curve.

Immunoglobulin M

Immunoglobulin M (IgM) level in fish serum samples was assayed by enzyme-linked immunosorbent assay (ELISA) using a fish Immunoglobulin M (IgM) ELISA Kit (Cusabio Biotech Co. Ltd., CSB-E12045Fh, MD, USA). The manufacturer's instructions were followed. The absorbance of samples was read at a wavelength of 630 nm with a Bio-Tek FLX 800 plate reader.

Biochemical analysis

Biochemical findings in serum including total protein (TPROT), albumin (ALB), globulin (GLO) were determined using bioanalytic test kits (Bioanalytic Diagnostic Industry, Co) and measured in a spectrophotometer (PG Instruments, UK).

***V. anguillarum* challenge test**

Experimental groups were included dietary administration of the SFP (1.0, 3.0, 5.0, 10.0 g kg^{-1}) and control group in which diet had no sumac feed supplementation. After 8 weeks of feeding, a challenge test was performed on each group with *Vibrio anguillarum* which was isolated before in our preliminary study. The LD_{50} value of the *V. anguillarum* pathogen was calculated by determining the cumulative mortality rates post-injection at different doses. The challenge was performed in thriplicate. Total 30 fish in each group (10 fish/replicate) at the end of the trial were transferred to challenge tanks. Fish were inoculated with 0.1 mL suspension (containing 2.10^5 CFU/mL (LD_{50} dose) of *V. anguillarum* by intraperitoneal injection in each fish. Mortalities were recorded daily for up to 21 days. The collected all dead fish were examined bacteriologically to determine the presence of the pathogen. The relative percent survival (RPS) was calculated according to Amend (1981).

RPS= (1- % mortality in experiment group % mortality in control) x 100.

Statistic

In this study, the variation analyses were carried out with Duncan multiple comparison tests, and the differences between groups were carried out by SPSS 18 statistics program to evaluate the relationships between the data were obtained from the test groups. Statistical significance was established at $p<0.05$.

RESULTS

Phenolic constituents

The results of the chemical analysis of sumac were presented in Table 2. Major components of sumac were determined as gallic acid and quercetin.

Table 2. Phenolic content (g kg^{-1}) of sumac

Component	
Gallic acid	5206.2
Quercetin	1106.4
Protocatechic acid	391.5
Catechin	80.7
Syringic acid	24.3
Epicatechin	*nd
p-coumaric acid	*nd
Ferulic acid	*nd
Hesperidin	*nd
Cinnamic acid	*nd

Hematological, biochemical and immunological analysis

No significant differences were observed between treatment groups with SFP and control group in terms of haematological indices, differential leukocytes count, hematocrit (Hct), hemoglobin (Hb), and all the values of red blood cell findings at the end of the study (Table 3).

Table 3. The values of hematological parameters in rainbow trout fed with different levels of SFP at 8th weeks

		Groups				
		Control	**1.0 g kg^{-1}**	**3.0 g kg^{-1}**	**5.0 g kg^{-1}**	**10.0 g kg^{-1}**
Haematological indices	**WBC (10^3/µ)**	401.10 ±10.46	365.80 ±102.95	436.40 ±7.49	346.90 ±65.76	406.60 ±29.98
	RBC (10^6/µ)	0.86 ±0.11	0.88 ±0.09	1.02 ±0.02	0.87 ±0.03	0.95 ±0.04
Differential leukocytes count	**LY (%)**	82.30 ±2.54	80.65 ±9.97	76.25 ±0.49	75.70 ±13.15	78.55 ±0.35
	MO (%)	14.50 ±4.24	14.65 ±6.29	10.80 ±3.25	15.30 ±3.11	18.05 ±1.20
	NE (%)	2.75 ±1.76	4.40 ±3.25	11.70 ±1.27	8.30 ±9.61	2.80 ±0.98
	LY (10^3/µ)	330.45 ±18,87^{a}	289.75 ±46.45^{ab}	332.45 ±3.32^{a}	258.45 ±4.45^{b}	319.40 ±21.92^{ab}
	MO (10^3/µ)	57.80 ±15.41	56.80 ±38.18	47.25 ±15.06	54.05 ±20.71	73.60 ±10.32
	NE (10^3/µ)	11.00 ±7.35	17.75 ±16.33	51.15 ±4.59	31.80 ±38.74	11.25 ±3.04
	HCT (%)	51.66 ±2.88	53.66 ±7.76	50.00 ±5.56	50.66 ±1.52	48.66 ±4.16
	Hb (g/dL)	10.15 ±1.20	7.65 ±4.03	11.50 ±0.14	8.40 ±1.55	11.35 ±0.35
The values of red blood cell findings	**MCV (µm^3)**	613.06 ±39.54	590.17 ±58.54	496.34 ±90.02	577.12 ±0.92	501.75 ±33.22
	MCH (pg)	118.12 ±1.56	84.53 ±36.76	112.76 ±1.74	96.43 ±21.67	118.91 ±2.46
	MCHC (%)	19.30 ±0.98^{ab}	14.08 ±4.83^{b}	23.06 ±3.83^{ab}	16.71 ±3.78^{ab}	23.76 ±2.06^{a}

*Data are presented as the means ±SEM (n=3) values within the same row having different superscipts are significantly different (p<0.05).
*Hb, haemoglobin, Hct, hematocrit, RBC, red blood cells, MCV, meancell hemoglobin volume MCH meancell haemoglobin, MCHC, meancell haemoglobin concentration. Data represent as mean ±SE. Within a row, means with differing letters are significantly different (p<0.05).

At the end of the trial, biochemical and immunologic values in serum samples showed no significant result in experimental groups and control group (Table 4).

Table 4. Biochemical and immunological analysis of rainbow trout fed with different levels sumac powder for 8 weeks.

		Groups				
		Control	**1.0 g kg^{-1}**	**3.0 g kg^{-1}**	**5.0 g kg^{-1}**	**10.0 g kg^{-1}**
Serum biochemical values	**T.PRO. g/dL**	3.65±0.43	3.19±0.13	3.89±0.16	3.72±0.37	3.84±0.07
	GLOB g/dL	2.21±0.24	1.99±0.12	2.35±0.05	2.26±0.26	2.30±0.12
	ALB g/Dl	1.44±0.19^{ab}	1.20±0.01^{b}	1.54±0.10^{a}	1.46±0.10^{ab}	1.53±0.04^{a}
Serum immunologic values	**IgM (µg/ml)**	62.33±15.14	55.00±15.00	65.00±17.43	67.00±9.84	67.66±11.01
	Lysozyme (mg/ml)	0.005±0.00	0.006±0.00	0.006±0.00	0.006±0.00	0.007±0.00

*Values are mean ±SEM (n=6) Within a row, means with differing letters are significantly different (p<0.05).

Challenge results indicated that the fish fed with SFP supplemented diets had determined better survival rates against *V. anguillarum*. All four treated groups (1.0, 3.0, 5.0 and 10.0 g kg^{-1}) showed reduced mortality compare to the control. The fish received 10.0 g kg^{-1} SFP supplemented diet

performed best result that did not seen mortality. Sumac supplemented diets caused higher relative percentage survival (RPS) than control group (Table 5).

Table 5. The mortality rate of fish fed with diets containing different concentrations of SFP for 8 week and challenged with *V. anguillarum* pathogen.

Groups	Mortality (%)	RPS
Control	56.66±0.00^{a}	-
1.0 g kg^{-1}	33.42±0.13^{b}	41.08±0.12^{d}
3.0 g kg^{-1}	7.31±0.60^{c}	87.41±0.58^{c}
5.0 g kg^{-1}	5.65±0.14^{d}	90.10±0.14^{b}
10.0 g kg^{-1}	0.00±0.00^{e}	100.00±0.00^{a}

*Values are mean ±SEM (n=3) Within a column, means with differing letters are significantly different ($p<0.05$).

DISCUSSION

The use of plant products as health promoters is a very topical concept in aquaculture (Citarasu, 2010; Yılmaz et al., 2013; Ahmadifar et al., 2014; Gormez and Diler, 2014; Metin et al., 2015; Diler et al., 2017b). In some studies, the *in vitro* antibacterial assays carried out on sumac (*R. coriaria* L.) used either ethanol or water extracts (Nimri et al., 1999; Nasar-Abbas and Halkman, 2004; Candan and Sökmen, 2004; Gulmez et al., 2006; Akrayi et al., 2016). The water and hydro-methanol extracts obtained from the fruits of sumac were found to have a great inhibitory activity against bacterial species (Nasar-Abbas and Halkman, 2004).

In this study, fish fed with the sumac-supplemented diet challenged with *V. anguillarum* had better survival rates against *V. anguillarum*. All four experimental groups (1.0, 3.0, 5.0, and 10.0 g kg^{-1}) reduced mortality compared to the control. The 10.0 g kg^{-1} diet showed no mortality. The fish fed with a sumac supplemented diet showed high relative percentage survival (RPS). There was an inverse relationship between the mortality rate and the amount of plant extract in the diet. As the amount of plant extract increased in the diet, the rate of mortality decreased. Similar results have been reported by Gharaei et al. (2020) that sumac increased resistance to the *Yersinia ruckeri* in rainbow trout. In another study performed by Diler et al. (2015) that *Artemisia vulgaris* provided resistance to challenge with pathogenic bacteria, *Vibrio anguillarum* in rainbow trout. Nya and Austin (2011) dietary garlic application reduced the mortality rate of *A. hydrophila* infection compared to control in rainbow trout. Further, Feng et al. (2020) reported that dietary administration of *Rehmannia glutinosa* polysaccharide significantly reduced fish mortality.

The knowledge of the mechanism for the antimicrobial activity of spices and herbs is very limited. In the present study, gallic acid was determined as a major component of phenolics of sumac (Al-Boushi et al., 2014). Phenolics have antibacterial effects against bacteria due to their toxicity and effects on bacterial enzymes (Cowan, 1999). Also, Gabr et al. (2014) analyzed sumac extracts by GC-MS methods. They found that phenols (41.8%), glycosides (19.4%), alkaloids (17.5%) and terpenoids (11.3%) were major components. The abundance of polyphenols may explain the mechanisms for the antimicrobial activity of sumac (Abu-Reidah et al., 2014). Therefore, in the present study, the percentage mortality was significantly decreased in fish fed sumac powder supplemented diet challenged with *Vibrio anguillarum*.

The blood parameters have been used as a diagnostic parameter for the investigation of disease and physiological disorders (Fazio, 1999). In this study, haematological parameters (RBC, WBC), differential leukocytes count (LY, MO, NE), Hct, Hb, the mean values of cell hemoglobin (MCH pg), cell hemoglobin concentration (MCHC %), and cell hemoglobin volume (MCV μm^3) were no adversely affected in fish feeding with sumac powder when compared control group. These observations are in agreement with the obtained results of other researchers, who reported that rainbow trout treated with *Origanum vulgare* extract and carvacrol powder were no significant differences in RBC, Hb, MCV, MCH, MCHC parameters (Ahmadifar et al., 2011; Haghighi and Rohani, 2015; Yılmaz et al., 2015). In contrast, Gharaei et al. (2020) observed that WBC and RBC, lymphocyte, monocyte, and neutrophil value was significantly increased in fish fed a sumac supplemented diet.

Serum proteins are various humoral elements of the non-specific immune system, measurable total protein albumin and globulin levels suggest that high concentrations are likely to be a result of the enhancement of the non-specific immune response of fish. In this study, total protein (TP), globulin

(GL), and albümin (ALB) values had no significant differences on the 8th week. Similar results was reported in *Dicentrarchus labrax* fed carvacrol supplemented diet after 4 and 8 weeks (Volpatti et al., 2014) and in nile tilapia fed the chinese herbs supplemented diet (Ardo et al., 2008). In contrast, the use *Laurus nobils*, *Cotinus coggyria* and *Origanum vulgare* enhanced the non-specific immune parameters in rainbow trout (Bilen ve Bulut, 2010; Bilen et al., 2011; Haghighi and Rohani, 2015).

Immunoglobulin M (IgM) is the major component of humoral immune system. Also, lysozyme is a humoral component of the non-specific defense mechanism which can prevent the growth of bacteria by splitting β-1,4 glycosidic bonds in the peptidoglycan of bacterial cell walls, resulting in bacteriolysis (Ellis, 1999). However, this study was found no effect on plasma lysozyme and Immunoglobulin M (IgM) level at the 8th week. This result is opposed to Gharaei et al. (2020) that reported significant stimulation in lysozyme activity of rainbow trout with sumac diet. Similarly, Feng et al. (2020) reported that the lysozyme activity increased *Rehmannia glutinosa* polysaccharide in common carp.

CONCLUSION

In conclusion, administration of *Rhus coriaria* (sumac) fruit powder as a dietary supplement reduced the mortality rate against *Vibrio anguillarum* infection. This is the first study to provide data that the sumac powder evaluated against *V. anguillarum* possess *in vivo* potential antibacterial activity. *Rhus coriaria* can be utilized as a health promoter in rainbow culture.

REFERENCES

Abu-Reidah, I.M., Jamous, R.M., & Ali-Shtayeh, M.S. (2014). Phytochemistry, pharmacological properties and industrial applications of *Rhus coriaria* L. (Sumac). *Jordan Journal of Biological Sciences*, *7*(4), 233-244.

Ahmadifar, E., Falahatkar, B., & Akrami, R. (2011). Effects of dietary thymol-carvacrol on growth performance, hematological parameters and tissue composition of juvenile rainbow trout, *Oncorhynchus mykiss*. *Journal of Applied Ichthyology*, *27*(4), 1057-1060.

Ahmadifar, E., Mansour, M.R., Amirkolaie, A.K., & Rayeni, M.F. (2014). Growth efficiency, survival and haematological changes in great sturgeon (*Huso huso* Linnaeus, 1758) juveniles fed diets supplemented with different levels of thymol–carvacrol. *Animal Feed Science and Technology*, *198*, 304-308.

Akrayi, H.F., Salih, R.M., & Hamad, P.A. (2016). *In vitro* screening of antibacterial properties of *Rhus coriaria* and *Origanum vulgare* against some pathogenic bacteria. *Aro-The Scientific Journal of Koya University*, *3*(2), 35-41.

Al-Boushi, M.A., Hamdo, H.H., & Herbali, J. (2014). Extraction and study of the phenolic compounds in the leaves and sticks of the Syrian sumac plant (*Rhus coriaria* L.). *International Journal of Food Science & Technology*, *6*(4), 2414-2420.

Aliakbarlu, J., Mohammadi, S., & Khalili, S. (2014). A study on antioxidant potency and antibacterial activity of water extracts of some spices widely consumed in Iranian diet. *Journal of Food Biochemistry*, *38*(2), 159-166.

Ali-Shtayeh, M.S., Al-Assali, A.A., & Jamous, R.M. (2013). Antimicrobial activity of Palestinian medicinal plants against acne-inducing bacteria. *African Journal of Microbiology Research*, *7*(21), 2560-2573.

Amend, D.F. (1981). Potency testing of fish vaccines. International symposium on fish biologics: serodiagnostics and vaccines. *Developments Biological Standardization*, *49*, 207-264.

Ardo, L., Yin, G., Xu, P., Váradi, L., Szigeti, G., Jeney, Z., & Jeney, G. (2008). Chinese herbs (*Astragalus membranaceus* and *Lonicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. *Aquaculture*, *275*(1-4), 26-33.

Bilen, S., & Bulut, M. (2010). Effects of laurel (*Laurus nobilis*) on the non-specific immune responses of rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Animal and Veterinary Advances*, *9*(8), 1275-1279.

Bilen, S., Bulut, M., & Bilen, A.M. (2011). Immunostimulant effects of *Cotinus coggyria* on rainbow trout (*Oncorhynchus mykiss*). *Fish & Shellfish Immunology*, *30*(2), 451-455.

Blaxhall, P.C., & Daisley, K.W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology*, *5*(6), 771- 781.

Campbell, T.W. (2004). *Hematology of lower vertebrates.* In 55 th Annual Meeting of the American College of Veterinary Pathologists (ACVP) & 39 th Annual Meeting of the American Society of Clinical Pathology (ASVCP), International Veterinary Information Service. Ithaca NY USA (pp. 1104-1108).

Candan, F., & Sökmen, A. (2004). Effects of *Rhus coriaria* L. (Anacardiaceae) on lipid peroxidation and free radical scavenging activity. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *18*(1), 84-86.

Capanio, F., Alloggio, V., & Gomes, T. (1999). Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chemistry*, *64*(2), 203-209.

Citarasu, T. (2010). Herbal biomedicines: a new opportunity for aquaculture industry. *Aquaculture International*, *18*(3), 403-414.

Cowan, M.M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews*, *12*(4), 564-582.

Diler, O., Gormez, O., Diler, I., & Metin, S. (2017a). Effect of oregano (*Origanum onites* L.) essential oil on growth, lysozyme and antioxidant activity and resistance against *Lactococcus garvieae* in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquaculture Nutrition*, *23*(4), 844-851.

Diler, Ö., Görmez, Ö., Metin, S., İlhan, İ., & Diler, İ. (2017b). Effect of *Origanum vulgare* L. essential oil on growth, lysozyme and antioxidant activity and resistance against *Vibrio anguillarum* in rainbow trout. *Süleyman Demirel University Journal of Eğirdir Fisheries Faculty*, *13*(1), 42-57.

Diler, Ö., Görmez, Ö., Terzioğlu, S., & Atabay, A. (2015). Effect of wormwood (*Artemisia vulgaris* L) on resistance against diseases and nonspecific immune system in rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Journal of Aquaculture Engineering and Fisheries Research*, *4*(1), 1-11.

Ellis, A.E. (1996). *Lysozyme Assay*. In Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S., Mulswink, W.B. (Eds), Techniques in Fish Immunology, 101-103. SOS Publication, 258p, Australia.

Ellis, A.E. (1999). Immunity to Bacteria in Fish. *Fish and Shellfish Immunology*, *9*(4), 291-308.

Fazio, F. (2019). Fish hematology analysis as an important tool of aquaculture: A review. *Aquaculture*, *500*, 237-242.

Feng, J.C., Cai, Z.L., Zhang, X.P., Chen, Y.Y., Chang, X.L., Wang, X.F., Qin, C.B., Yan, X., Ma, X., Zhang, J.X., & Nie, G.X. (2020). The Effects of oral *Rehmannia glutinosa* polysaccharide administration on immune responses, antioxidant activity and resistance against *Aeromonas hydrophila* in the common carp, *Cyprinus carpio* L. *Frontiers in Immunology*, *11*, 904.

Gabr, S.A., El-Metwally, M.M., & Al-Ghadir, A.H. (2014). Antioxidant and antibacterial active constituents of *Rhus coriaria*. *Biotechnology*, *13*(2), 37-45.

Gatlin, D.M. (2007). *Dietary supplements for the health and quality of cultured fish*: Dietary supplements for the health and quality of cultured fish. Cabi Press, USA.

Gharaei, A., Shafie, M., Mirdar Harijani, J., Hasanein, P., & Arshadi, A. (2020). Immune responses and haematological parameters change of rainbow trout (*Oncorhynchus mykiss*) under effects of dietary administration of sumac (*Rhus coriaria* L.). *Journal of Agricultural Science and Technology*, *22*(1), 173-186.

Gormez, O., & Diler, O. (2014). *In vitro* antifungal activity of essential oils from *Tymbra, Origanum, Satureja* species and some pure compounds on the fish pathogenic fungus, *Saprolegnia parasitica*. *Aquaculture Research*, *45*(7), 1196-1201.

Gulmez, M., Oral, N., & Vatansever, L. (2006). The effect of water extract of sumac (*Rhus coriaria* L.) and lactic acid on decontamination and shelf life of raw broiler wings. *Poultry Science*, *85*(8), 1466-1471.

Haghighi, M., & Rohani, M.S. (2015). Non-specific immune responses and heamatological parameters of rainbow trout (*Oncorhynchus mykiss*) fed with *Origanum vulgare* extract diets. *American Advanced Journal Biological Science*, *1*(1), 1-9.

Iauk, L., Caccamo, F., Speciale, A.M., Tempera, G., Ragusa, S., & Pante, G. (1998). Antimicrobial activity of *Rhus coriaria* L. leaf extract. *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, *12*(S1), S152-S153.

Kossah, R., Nsabimana, C., Zhang, H., & Chen, W. (2013). Evaluation of antimicrobial and antioxidant activities of Syrian sumac fruit extract. *Journal of Natural Products*, *6*, 96-102.

Mahdavi, S., Hesami, B., & Sharafi, Y. (2018). Antimicrobial and antioxidant activities of Iranian sumac (*Rhus coriaria* L.) fruit ethanolic extract. *Journal of Applied Microbiology and Biochemistry*, *2*(2:5), 1-5.

Mavlyanov, S.M., Islambekov, S.Y., Karimdzhanov, A.K., & Ismaikov, A.I. (1997). Anthocyans and organic acids of the fruits of some species of sumac. *Chemistry of Natural Compounds*, *33*(2), 209-209.

Metin, S., Diler, O., Didinen, B.I., Terzioglu, S., & Gormez, O. (2015). *In vitro* and *in vivo* antifungal activity of *Satureja cuneifolia* ten essential oil on *Saprolegnia parasitica* strains isolated from rainbow trout (*Oncorhynchus mykiss*, Walbaum) Eggs. *Aquaculture Research*, *46*(6), 1396-1402.

Nasar-Abbas, S.M., & Halkman, A.K. (2004). Antimicrobial effect of water extract of sumac (*Rhus coriaria* L.) on the growth of some food borne bacteria including pathogens. *International Journal of Food Microbiology*, *97*(1), 63-69.

New, M.B. (1987). *Feed and feeding of fish and shrimp: a manual on the preparation and presentation of compound feeds for shrimp and fish in aquaculture*. Rome: Food and Agriculture Organization of the

United Nations and United Nations Environment Programme, www.fao.org/docrep/s4314e/s4314e00.htm#Contents.

Nimri, L.F., Meqdam, M.M., & Alkofahi, A. (1999). Antibacterial activity of Jordanian medicinal plants. *Pharmaceutical Biology*, *37*(3), 196-201.

Nya, E.J., & Austin, B. (2011). Development of immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic. *Fish Shellfish Immunology*, *30*(3), 845-850.

Onkar, S., Mohammed, A., & Nida, A. (2011). New antifungal aromatic compounds from the seeds of *Rhus coriaria* L. *International Research Journal of Pharmacy*, *2*(1), 188-194.

Panico, A., Cardile, V., Santagati, N.A., & Messina, R. (2009). Antioxidant and protective effects of sumac leaves on chondrocytes. *Journal of Medicinal Plants Research*, *3*(11), 855-861.

Rios, F.S., Oba, E.T., Fernandes, M.N., Kalinin, A.L., & Rantin, F.T. (2005). Erythrocyte senescence and haematological changes induced by starvation in the neotropical fish traíra, Hoplias malabaricus (Characiformes, Erythrinidae). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, *140*(3), 281-287.

Terzioğlu, S., & Diler, Ö. (2016). Effect of dietary sage (*Salvia officinalis* L.), licorice root (*Glycyrrhize glabra* L.), blueberry (*Vaccinium myrtillus* L.) and echinaceae (*Echinacea angustifolia* Hell) on nonspecific immunity and resistance to *Vibrio anguillarum* infection in rainbow trout, (*Oncorhynchus mykiss*). *Süleyman Demirel University Journal of Eğirdir Fisheries Faculty*, *12*(2), 110-118.

Toghyani, M., & Faghan, N. (2017). Effect of sumac (*Rhus coriaria* L.) fruit powder as an antibiotic growth promoter substitution on growth performance, immune responses and serum lipid profile of broiler chicks. *Indian Journal of Pharmaceutical Education and Research*, *51*(3), S295-S298.

Uluköy, G., Kubilay, A., Didinen, B.I., Metin, S., Altun, S., Diler, Ö., Mammadov, R., & Dulluç, A. (2018). Immunostimulant effects of geophyte plant extract on non-specific defence mechanisms of rainbow trout (*Oncorhynchus mykiss*). *Journal of Limnology and Freshwater Fisheries Research*, *4*(1), 36-41.

Volpatti, D., Bulfon, C., Tulli, F., & Galeotti, M. (2014). Growth parameters, innate ımmune response and resistance to *Listonella (Vibrio) anguillarum* of *Dicentrarchus labrax* fed carvacrol supplemented diets. *Aquaculture Research*, *45*, 31-44.

Yılmaz, S., Ergün, S., & Çelik, E.Ş. (2013). Effect of dietary herbal supplements on some physiological conditions of sea bass *Dicentrarchus labrax*. *Journal of Aquatic Animal Health*, *25*(2), 98-103.

Yılmaz, E., Ergün, S., & Yılmaz, S. (2015). Influence of carvacrol on the growth performance, hematological, non-specific immune and serum biochemistry parameters in rainbow trout (*Oncorhynchus mykiss*). *Food and Nutrition Sciences*, *6*(6), 523-531.