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Immune Responses and Haematological Parameters Changes of Rainbow Trout (*Oncorhynchus mykiss*) under Effects of Dietary Administration of Sumac (*Rhus coriaria* L.)

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ABSTRACT

The purpose of this study was to evaluate the effect of sumac (*Rhus coriaria* L.) administration on the growth performance, immune response, and haematological parameters of rainbow trout (*Oncorhynchus mykiss*). Rainbow trout juveniles with an initial weight of 38±2.65 g were allocated into 12 tanks at a density of 15 fish per tank. Fish were fed four experimental diets containing 0% (as control diet), 0.5%, 2%, and 5% sumac-supplementation for 56 days. After the feeding trial, fish were challenged with *Yersinia ruckeri*, and survival rate was calculated for 15 days. Sumac diet significantly increased resistance to the pathogen and led to the control of infection in rainbow trout without changing weight. The leukocyte (WBC) and erythrocyte (RBC), lymphocyte, monocyte, and neutrophil value was significantly higher in fish that were fed a sumac-supplemented diet when compared with the control. Serum lysozyme, and alternative complement pathway haemolytic activity (ACH50) as well as the hepatic expression of TNF-α and IL-1b were recorded to be the highest in fish fed 2% and 5% sumac-supplement. Meanwhile, mRNA expression levels of IL-10 significantly decreased in fish fed 5% sumac supplementation for 56 days. These results suggest that sumac administration, especially 2% and 5%, may effectively enhance the immune system, resistance to the pathogen, and hematopoiesis in rainbow trout.

Keywords: Gene expression, Cytokine, Immune system, Lyzozyme.

INTRODUCTION

Today, aquaculture has gained a special place among other human food-sourcing activities. One of the basic practices to supply the world's need for protein is rearing fish **(**Ringø *et al*., 2018; Iswarya *et al*., 2018; Faggio *et al*., 2015; Hoseinifar *et al*., 2018; Guardiola *et al*., 2016). Rainbow trout is one of the most valuable and important economic fish species in the global cold-water aquaculture industry, and efforts to improve the growth indices and immunity of the fish against multiple bacterial diseases are increasing (Alishahi *et al.*, 2010).

Among the several ways to improve health conditions in the rearing of aquatic organisms, biologic and natural compounds have been shown to be economical for fish farmers and to enhance non-specific immune system of the cultivated fish (Thanikachalam *et al.*, 2010). Herb or spices have been reported to promote various functions like growth (Yilmaz *et al*., 2014; Van Hai, 2015), immune functions (Ardo *et al*., 2008; Awad *et al*., 2013; Nootash *et al.*, 2013), skin coloration (Yilmaz and Ergün, 2014), egg-hatching rates (Yilmaz and Ergün 2012a), haematological and biochemical status (Yilmaz and Ergün 2012b; Bilen *et al*., 2013; Gholampoor *et al*., 2011),

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and also increased disease resistance (Yilmaz *et al*., 2013a; Yilmaz *et al*., 2013b; Yılmaz and Ergün, 2014) in fish culture due to different active components.

Rhus coriaria (sumac), belonging to Anacardiaceae, is a deciduous shrub that can grow to 2-5 m in height in the Mediterranean region, North Africa, Southern Europe, Iran, and Afghanistan. Sumac is commonly used as a spice by grinding the dried fruits and as a medicinal agent to relieve stomach disease, bowl complains, fever, and dermatitis. Moreover, it is used as an appetizer, diuretic and antiseptic (Onkar *et al.,* 2011). Although *Rhus coriaria* extract is most notable for its antimicrobial, antifungal, and anti-inflammatory activities (Haghparast *et al*., 2011; Yılmaz and Ergün, 2012; Gharaei *et al.*, 2013; Khalilpour *et al.,* 2018), there is no study on the effect of dietary sumac administration on the immune response of rainbow trout. On the other hand, the assessment of hematological parameters are commonly used methods in aquatic toxicology and biomonitoring programs of aquatic animals **(**Faggio *et al*., 2014; Fazio *et al*., 2012; Aliko *et al*., 2018; Fazio *et al*., 2013). Therefore, the current study aimed to evaluate the effects of sumac dietary supplementation on the humoral immunity and expression of some important genes like TNF-α, IL-1b and IL-10 in the kidney of rainbow trout. We also aimed to investigate the growth performance and resistance to pathogen *Yersinia ruckeri*.

MATERIALS AND METHODS

Experimental Animals and Procedure

Rainbow trout fingerlings with mean weight of 38±2.65 g from Khash Reproduction and Rearing Centre (Blouchestan, Iran) were collected. The fish were fed with the basal diet for two weeks before the beginning of the feeding trial in order to adapt with the experimental conditions. Then, 180 fish were randomly allocated to 12 tanks (100 L, 15 fish per tank) supplied with flow through spring water (0.8 m^3/s) and divided into four groups with three tanks (as replicate) in each group. Fish were fed with various concentrations of sumac powder (Golha Corporation, Iran) that was mixed with the basal diet at levels of 0% (control), 0.5%, 2%, and 5% (Table 1) for 56 days. During the experimental period, the fish were fed 3% of biomass and three times per day (08:00, 12:00 and 16:00 h). Water temperature, dissolved oxygen and pH were measured daily and maintained at 16±1.5 °C, 6.2±0.6 mgL^{-1}, and 7.9±0.3, respectively. Continuous aeration was provided in each tank. Sumac powder was added to fishmeal mixed with other ingredients using a blender in the first step. Then, the other ingredients of experimental diets were mixed with water and the ingredients were passed through a meat grinder equipped with a 2-mm-diameter strand to obtain uniform pellets (Cerezuela *et al.*, 2008). The pellets were air-dried, ground, sieved to produce a suitable crumble, and stored at 4 °C until use.

Assessment of Growth Performance

After an eight-week feeding period, weight gain (WG%), specific growth rate (SGR %/day), feed conversion ratio (FCR), and survival rate (%) were calculated according to following equations (Mahghani *et al.*, 2014):

$$WG(\%) = (Wt - W_0) \times 100$$

$$SGR = (Ln\ Wt - Ln\ W_0) \times 100/t$$

$$FCR = \text{dry feed fed / wet weight gain}$$

$$\text{Survival rate} = (Nt/N_0) \times 100$$

Where, Wt and W_0 are final and initial body weights (g), respectively, while t is the duration of experimental days. N_0 is the initial number of fish and Nt is the final number of fish.

Assessment of Haematological Parameters

Prior to sampling, the fish were anesthetized with 200 mgL^{-1} MS_{222} (Faggio *et al.*, 2013). To measure hematologic parameters at the end of the feeding period, a 2 mL blood

Table 1. Composition of the basics diet (a) and the experimental diets (b).

(a)		(b)			
Ingredient	Percentage (%)	Control	0.5 %	2 %	5 %
Fish meal	21.5	21.5	21.5	21.5	21.5
Wheat meal	13.5	13.5	13.5	13.5	13.5
Corn meal	16.5	16.5	16.5	16.5	16.5
Soybean meal	25	25	25	25	25
Vitamin premix [a]	2	2	2	2	2
Mineral premix [a]	2	2	2	2	2
Soybean oil	14.5	14.5	14.5	14.5	14.5
Filler	5	5	4.5	3	0
Sumac powder		0	0.5	2	5
Chemical composition (%)					
Crud protein %	39				
Crud fat %	9.2				
Fiber %	0.6				
Moisture %	11.5				
Digestible energy (Kcal/kg)	3900				

[a] Supplements provided as the following: Trace mineral mix (zinc, iron, manganese, copper, iodine, cobalt, and selenium), Vitamin mix (vitamin A, D3, K, E, riboflavin, pyridoxine, panthothenic acid, nicotinic acid, folic acid, biotin, vitamin B12, vitamin C, choline chloride, L-ascorbyl acid-2-polyphosphate, celufil.

sample from the caudal vein (18 G×1 ½ syringe) of three fish from each tank was drawn. Each blood sample was collected into heparinized micro tubes (50 IU mL), and the red blood cell (RBC), white blood cell (WBC), neutrophil, lymphocyte and monocyte values were measured (Faggio *et al.*, 2014a; Faggio *et al.*, 2014b).

Assessment of Immunological Parameters

Blood sera obtained by centrifuging the samples at 3,000 rpm for 10 min (Heraeus Labofuge 400) were collected in a disposable transfer pipette (Akrami *et al.*, 2015). Serum lysozyme activity was determined by turbidometric assay. Briefly, 250 µL of each sample were mixed with 1.75 ml of *Micrococcus lysodeikticus* (Sigma) suspention (0.375 mg/mL, 0.05 M sodium phosphate buffer, pH 6.2) and the optical density was read at 670 nm at 15 and 180 seconds (Soltani and Pourgholam, 2007).

Alternative Complement Pathway Haemolytic *A*ctivity (ACH50)

Serum complement activity was measured based on method describe by Gharaei *et al*. (2016). Hemolytic activity driven by the alternative complement pathway was measured using rabbit RBC in EGTA, magnesium, gelatin buffer (GVB) as described by Chen *et al*. (2003). Golden shiner serum (25 µL) was diluted in 175 µL of GVB and serial doubling dilutions made down a 96-well plate. The optical density of the diluted serum solution was measured at 414 nm by a Kinetic microplate reader. After reading, 25 µL of rabbit RBC washed in GVB was added to each well. The plate was incubated at 20 °C for 90 min with manual shaking. After incubation, each sample was transferred to a 48-well plate with 1 mL of cold 20 mmol/L EDTA-GVB buffer to stop the hemolytic reaction. The 48-well plates were centrifuged at 600 × g for 5 min. The upper supernatant (200 µL)

from each well was transferred to a new 96-well plate. The extent of hemolysis was determined by measuring the optical density of the supernatant at 414 nm. Complete (100%) and no (0%) hemolysis were determined by adding 25 µL of the washed rabbit RBC suspension to 100 µL of distilled water, and 25 µL of the washed rabbit RBC suspension to 100 µL of GVB buffer, respectively. The alternative complement pathway hemolytic activity (ACH) was reported as the reciprocal of the serum dilution causing 50% lysis of rabbit RBC (ACH50).

Immune Related Genes Expression

In order to compare mRNA expression levels, individual liver tissues from each treatment group (N= 5) were randomly collected at the end of trial, were frozen and kept at -80 °C until use. Total RNA extraction was carried out in the liver samples by using the Takapou Zist Kit (Tehran, Iran) following the manufacturer's instructions. RNA integrity was verified by ethidium bromide staining of the 28S and 18S ribosomal RNA bands (as marker) on 1.2% agarose gel. To remove DNA contaminants, the extracted RNA was treated with RNA-Free DNase (Takara, Japan), and reverse transcribed to cDNA by a Superscript cDNA synthesis kit (AccuPawer® CycleScript RT PreMix, Germany), following the manufacturer's instructions. The mRNA expression levels of TNF-α, IL-1β, and IL-10 genes in the livers of the rainbow trout were evaluated by fluorescent real-time quantitative PCR. The specific primers for TNF-α, IL-1β, IL-10, and β-actin (housekeeping gene) were designed according to the cDNA sequences of rainbow trout in GenBank (Nootash *et al*., 2013) and thermocycling conditions, as indicated in Table 2. TakapouZist Co., Ltd. synthesized all primers and amplified fragments length of 70–295 bp. Real-time quantitative PCR was conducted in a quantitative thermal cycler (Mastercycler® eprealplex; Eppendorf, Germany). Three replicates for each sample were performed.

Table 2. Real-time PCR primer sequences and thermocycling condition.

Target genes	Primer	Primer sequence (5′— 3′)	Thermocycling condition	Accession no.	Product length (bp)
IL-1β	F	ACATTGCCAACCTCATCATCG	95 °C 30 s, 35 cycles of 95 °C 5 s, 62 °C 30 s and 72 °C 30 s	AJ223954	91
	R	TTGAGCAGGTCCTTGTCCTTG		AJ298294	
IL-10	F	CGACTTTAAATCTCCCATCGAC	95 °C 30 s, 35 cycles of 95 °C 5 s, 60 °C 30 s and 72 °C 30 s	AB118099	70
	R	GCATTGGACGATCTCTTTCTTC			
TNF-α	F	TGGAGGGGTATGCGATGACACCTG	95 °C 30 s, 35 cycles of 95 °C 5 s, 60 °C 30 s and 72 °C 30 s	AJ249755.1	116
	R	TGAGGCCTTTCTCTCAGCGACAGC			
β-Actin	F	TCACCCACACTGTGCCCATCTACGA	95 °C 30 s, 35 cycles of 95 °C 5 s, 60 °C 30 s and 72 °C 30 s	AC006483.3	295
	R	CAGCGGAACCGCTCATTGCCAATGG			

The threshold cycle (CT) was determined manually for each run. PCR efficiency for each set of primers was determined using serial 10-fold dilutions of cDNA, and the resulting plots of CT vs. the logarithmic cDNA dilution, using the efficiency equation (E):

$E = 10^{(-1/slope)}$

Gene expression data were analyzed using the $2^{-\Delta\Delta CT}$ method after verification that the primers were amplified with an efficiency of 97- 99% (Gharaei *et al.*, 2011), and that data for all treatment groups were compared to the control group.

After the 56-day trial, the remaining fish from each group were challenged by *Y. ruckeri* BCCM/LMG3279) strain. The bacterial density used was 0.5×10^7 CFU mL^{-1}. Therefore, bacterial culturing, preparation, and challenge experiment ware carried out as described in detail by Hoseinifar *et al*. (2015). At the end of the 15-day resistance test period, the mortality percentage of fish in each group was determined and the relative percentage of survival (RPS) was calculated according to the following formula:

RPS=100-[(test mortality/control mortality) ×100]

Statistical Analysis

All statistical analyses were performed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA) with significance determined at $p<0.05$. The data were analysed by one-way analysis of variance (ANOVA). Where differences occurred, Tukey's post hoc multiple comparisons was applied. Data are presented as mean ± S.E.M.

RESULTS

Effects of Sumac Administration on Growth Performance

The effects of sumac powder in rainbow trout on growth performance and survival rate are represented in Table 3. There were no significant differences in final weight, weight gain, SGR, and FCR in fish fed with sumac diets when compared with the control diet at the end of experiment.

Effects of Sumac Administration on Haematological Parameters

After 8 weeks of treatments, there were significant differences in the RBC, WBC, neutrophil, lymphocyte, and monocyte values between the control groups and other treatment groups (Table 4)(all $p> 0.05$). All the haematological parameters values showed a significant increase in fish fed 2% and 5% sumac-supplemented diets if compared with the control group(all $p<0.05$).

Effects of Sumac Administration on Immunological Parameters

As shown in Table 5, lysozyme activity levels and ACH50 increased in fish fed 2% and 5% sumac-supplemented diets when compared with the control ($p<0.05$).

The mRNA expression levels of rainbow trout liver genes, relative to β-Actin, are shown in Table 6. The expression of TNF-α, IL-1b, and IL-10 genes in fish liver was affected by sumac supplementation. Fluctuation of the TNF-α gene expression level increased alongside the increase of sumac supplementation from 0.5% to 5%, when compared with the control. IL-1b gene expression levels showed significant increases ($p<0.05$) in fish that received 2% and 5% sumac-supplemented diets when compared with the control. In contrast, the gene expression level of IL-10 significantly decresed ($p<0.05$) only in fish treated with 5% sumac-supplemented diet.

Effects of Sumac Administration on the Pathogen Challenge Test

In the challenge test, the effects of

Table 3. Growth parameters of rainbow trout fed sumac-supplemented diets for 56 days feeding trial. [a]

Performance indices	Diet group			
	Control	0.5%	2%	5%
Initial weight (g)	38.99± 0.69	38.30 ± 0.53	38.73± 0.64	38.38± 0.64
Final weight (g)	100.67± 2.9	103.17± 3.1	102.47± 3	101.23± 2.5
WG(%)	15.81±1.43	16.94±1.08	16.82±1.63	16.62± 1.01
SGR (%/day)	1.93± 0.06	2.02± 0.05	2.00± 0.03	1.98± 0.04
FCR	1.47± 0.08	1.44± 0.1	1.45± 0.2	1.48± 0.12

[a] Data expressed as mean ± SEM ($p<0.05$).

Table 4. Haematological parameters of rainbow trout fed sumac-supplemented diets for 56 days feeding trial. [a]

Parameters	Diet group			
	control	0.5%	2%	5%
WBC (10^{-3} cell / mm^3)	62.45±5.1[a]	65.1±6.3[a]	78.2±5.7[b]	85.3±5.5[b]
RBC (10^{-3} cell / mm^3)	92.8±7.3[a]	104.1±10.2[a]	108.4±11.8[b]	107.6±9.8[b]
Lymphocytes (%)	85.50±1.5[a]	86.50±1.81[a]	91.0±2.5[b]	93.33±2.4[b]
Monocytes (%)	3.6±1.1[a]	3.8±1.03[a]	4.5±0.9[b]	5.1±1.07[c]
Neutrophils (%)	5.1±0.5[a]	5.3±0.9[a]	7.2±1.4[b]	8.5±1.61[c]

[a] Data expressed as mean ± SEM ($p<0.05$).

Table 5. Effects of sumac-supplemented diets for 56 days on ACH50 and lysozyme activity of rainbow trout in different groups. [a]

Parameters	Diet group			
	control	0.5%	2%	5%
ACH50	122.50±0.95[a]	129.00±2.54[a]	140.00±1.00[b]	146.00±1.47[b]
Lysozyme	47.50±1.04[a]	51.12±1.49[a]	63.50±3.50[b]	77.25±2.9[c]

[a] Data expressed as mean ± SEM ($p<0.05$).

Table 6. Relative mRNA levels of rainbow trout liver fed sumac-supplemented diets for 56 days feeding trial. [a]

Genes	Diet group			
	control	0.5%	2%	5%
TNF-α	0.38±0.01[a]	1.43±0.08[b]	1.45±0.1[b]	1.50±0.50[b]
IL-1β	0.96±0.11[a]	1.06±0.13[a]	1.81±0.05[b]	1.85±0.78[b]
IL-10	1.07±0.19[a]	1.12±0.43[a]	0.96±0.54[a]	0.64±0.3[b]

[a] Data expressed as mean±SEM ($p<0.05$).

different sumac diets on rainbow trout resistance tested against *Y. ruckeri,* are shown in Figure 1. The results indicated that sumac supplementation significantly increased ($p<0.05$) the rainbow trout's resistance against *Y. ruckeri,* when compared with the control diet. The highest resistance against *Y. ruckeri* was recorded in fish received a 5% sumac-supplemented diet. In addition, in all treatment groups, The results of dead fish recorded at day five after the bacterial injection showed the highest relative percectentage of survival was in fish fed the 5% sumac-supplemented diet (71.25%), followed by the 2% sumac-supplemented diet (62.74%), and the 0.5% sumac-supplemented diet (28.31%).

DISCUSSION

Recently, there is a great focus on the use of natural immuno-stimulants in aquaculture to prevent diseases and avoid the use of hazardous antibiotics (Hai, 2015). Therefore, it is expected that herbs and spices can successfully replace antibiotics in fish culture **(**Carbone and Faggio, 2016; Aragona *et al*., 2017; Nath *et al*., 2019; Rashidian *et al*., 2018). The results of the current study showed that sumac administration, especially at 2% and 5%, in rainbow trout diet augmented the immunity, haematopoesis and survival rate without affecting the growth performance.

Sumac administration (0.5%, 2%, and 5%) for 56 days did not affect WG, SGR and FCR of rainbow trout. These results are opposed to those studies that reported significant improvement in weight performance of rainbow trout with a ginger diet (Nya and Austin, 2009; Shaluei *et al.*, 2016) but are in accordance with the unchanged weight gain of rainbow trout after green tea administration at 100 mg/kg (Nootash *et al.*, 2013) .

Hematological parameters are considered indicators of toxicity in fish (Sancho *et al.*, 2000). Blood cell, including erythrocyte, leukocyte, lymphocyte, monocyte, and neutrophil represent valuable information in the assessment of fish health (Fazio *et al*., 2015; Burgos-Aceves *et al*., 2018; Faggio *et*

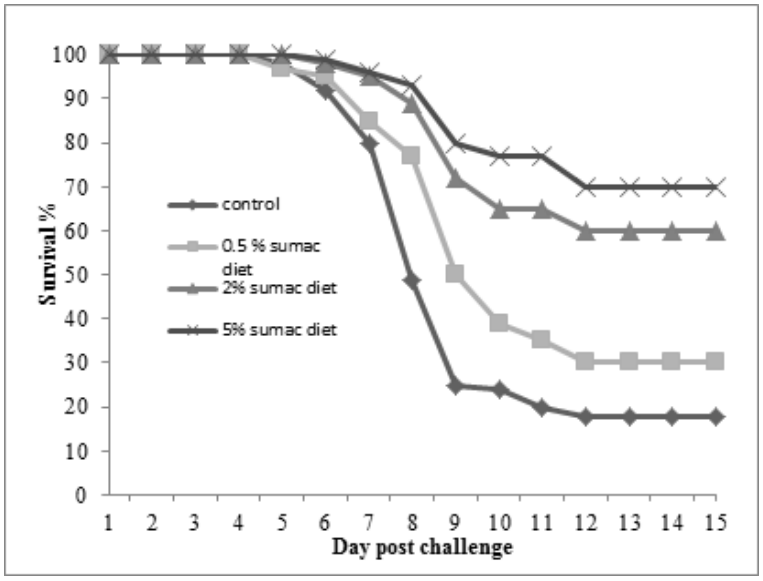

Figure 1. Survival rate of rainbow trout, *Oncorhynchus mykiss* fed different doses of dietary sumac (0, 0.5%, 2% and 5%) during 15 days post-challenge with *Y. ruckeri*.

al., 2016). The significant increase in WBC values in fish fed sumac-supplemented diet may indicate that sumac is capable of improving non-specific immunity in rainbow trout. Sumac polysaccharides with large molecular size and having antigenic properties may be involved in stimulating immunity responses (Steinmüller *et al.,* 1993). Our findings are also compatible with the previous studies by Choudhury *et al.* (2005) and Thanikachalam *et al.* (2010), who found that WBC count increased with administration of dietary natural immuno-stimulants such as garlic, ascorbic acid, and b-glucan.

The increased RBC count induced by sumac in this study may be related to its stimulatory effect on the kidneys, encouraging them to release the hormone erythropoietin, which is a stimulant in producing more red blood cells. Red blood cell membrane is rich in polyunsaturated fatty acids, which are very susceptible to free radical mediated peroxidation. Eventually, hemolysis is induced by membrane lipid peroxidation (Niki *et al*., 1988). In this way, compounds with antioxidant activity have an important role in scavenging free oxygen radicals and stabilizing red blood cell membrane. Previous studies have shown that sumac has a potent antioxidant activity, which may be related to polyphenolic constituents, especially gallic acid and its derivatives (Najjar *et al*., 2017). Therefore, the increased RBC count induced by sumac in this study may be related to its antioxidant effect in protecting red blood cells membranes against hemolysis.

To evaluate the effects of treatments with sumac on inflammatory and immune responses, we measured several markers. We assayed lysozyme activity and ACH50, which are the major actors of humoral immunity (Saurabh and Sahoo, 2008). Our findings showed that lysozyme activity and ACH50 of fishes fed with 2% and 5% sumac treatment during 56 days increased compared to the control group, presenting an optimum concentration of sumac to act as an immunostimulant in this study. The same results were recorded in fish, after supplementing their diets with the various spices (Talpur and Ikhwanuddin, 2013; Cho and Lee, 2012; Yılmaz *et al*., 2016) or herbs (Yin *et al.,* 2006; Yılmaz *et al*., 2015; Yılmaz, 2019). In continue, we evaluated gene expression levels of three pro-inflammatory cytokines, TNF-α, IL-10 and IL-1β in liver. Gene expression levels of TNF-α, which acts as an initiator in inflammatory processes, were significantly higher in fish fed with sumac powder supplementation compared to the control in all treatments, implying sumac involvement through this immune response mediator, and this effect was dose-dependent. It has been reported that TNF-α expression levels in gill and spleen tissue of rainbow trout increase after green tea (*Camellia sinensis*) administration (Nootash *et al.,* 2013). Furthermore, turbot treated with nucleotide-supplemented diets increased expression of TNF- α gene levels (Low *et al.,* 2003). Polyphenols, especially anthocyanins and proanthocyanidins extracted from staghorn sumac (*Rhushirta* L.), may mediate signalling events of TNF-α and subsequently induce anti-inflammatory effects (Peng *et al.,* 2016).

IL-1b has a pivotal role in the host response against tissue injury and is produced by many cells especially monocytes and macrophages (Corripio-Miyar *et al.,* 2007; Lauriano *et al*., 2016). Gene expression levels of IL-1β in fish fed 2% and 5% sumac-supplemented diets increased significantly. Same results on IL-1β expression were obtained by administration of green tea (Nootash *et al.,* 2013), spirulina algae (Watanuki *et al.,* 2006) and ergosan (Hodge *et al.,* 2005) in treated fish.

IL-10 is a regulatory cytokine, which shows immunosuppressive action. This cytokine inhibits chemokine receptors and the effect of pro-inflammatory cytokines and, therefore, minimizes damages to the host cells (Raida and Buchmann, 2008). In the current study, gene expression levels of

IL-10 only in fish fed 5% sumac-supplemented diets decreased, compatible with the results of this cytokine expression in liver and spleen of rainbow trout following oral administration of green tea (100 mg. kg^{-1}) (Nootash *et al.*, 2013).

The present study revealed that the dietary supplementation with sumac significantly increased resistance to *Y. ruckeri* and the highest survival rates were recorded in the fish fed with the 5% sumac-supplemented diet. Similarly, in *O. mykiss* fed diets with various dietary additives increased survival rate against *Yersinia ruckeri* (Yılmaz *et al*., 2018a; Yılmaz *et al*., 2018b; Yılmaz and Ergun 2018).

The significant enhancement in survival rate in this study may be related to the bioactive compounds of sumac, including polyphenols, flavonoids, tannins and saponins that play a role of anti-infection in fish, due to induction of non-specific immune defences.

Antioxidant activity of sumac may improve cell viability and reinforce the defences against free radical species inducing diseases. Furthermore, we observed that sumac supplementation increased mRNA expression levels of anti-inflammatory cytokines-related factors, interleukin IL-1β, and TNF-α. Meanwhile, it decreased expression of pro-inflammatory cytokines-related factor like IL-10. Therefore, sumac supplementation may increase fish immunity and improve survival against the bacterium challenge by altering these factors as indicated in previous studies (Yilmaz, 2013a; Xu *et al*., 2016).

In conclusion, the current study highlighted and served as a more useful and comprehensive study by showing the interaction among the cellular agents, humoral agents, and the expression of three immune-related genes. Results of the present study revealed that dietary administration of sumac at 5 % levels could stimulate the immune responses, increase disease resistance, and improve heamatological indices in rainbow trout. In addition, this is even more interesting because of the lack of information on the beneficial functional mechanisms of sumac in rainbow trout. Therefore, further studies are needed to have more insights into the protective mechanisms of sumac extracts.

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پاسخ ایمنی و تغییرات فاکتورهای خونی ماهی قزل آلا (*Oncorhynchus mykiss*) تحت تاثیر سماق خوراکی (*Rhus coriaria* L.)

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چکیده

این تحقیق به منظور بررسی تأثیر میوه سماق خوراکی بر شاخص‌های سیستم ایمنی در ماهی قزل‌آلای‌رنگین کمان طراحی شد. در این آزمایش بچه ماهیان قزل‌آلا با وزن میانگین ۲/۶۵ ± ۳۸ گرم در ۱۲ مخزن با تراکم ۱۵ ماهی در هر مخزن ذخیره شدند. این ماهیان با چهار غلظت صفر درصد(شاهد)، ۰/۵ درصد، ۲درصد و ۵ درصد از پودر میوه سماق به مدت ۵۶ روز تغذیه شدند. پس ازپایان دوره تغذیه، ماهیان تیمار شده درمعرض باکتری یرسنیا راکری قرار داده شدند و نرخ بقاء طی ۱۵ روز بررسی شد. سماق خوراکی بطور معنی داری مقاومت به عامل بیماریزا رو افزایش داد و منجر به کنترل عفونت در ماهیان قزل آلا بدون تغییر در وزن آنها شد. تعداد گلبول‌های سفید و قرمز، لیمفوسیت ها، منوسیت ها و نوتروفیل بطور معنی داری در ماهیان تغذیه شده با سماق نسبت به گروه کنترل بیشتر بود. همچنین میزان آنزیم لیزوزیم، میزان فعالیت همولیتیک کمپلمان (ACH50) در سرم خون و بیان ژن های TNF-α و IL-1β در کبد افزایش معناداری در ماهیان تغذیه شده با ۲ و ۵ درصد سماق خوراکی نشان داد در حالیکه میزان بیان ژن IL-10 بطور معنی داری در ماهیان تغذیه شده با ۵ درصد سماق خوراکی طی ۵۶ روز کاهش نشان داد. نتایج این تحقیق نشان می دهد که سماق خوراکی در سطح ۲ و ۵ درصد بطور موثری سیستم ایمنی، میزان مقاومت به عوامل بیماریزا و خونسازی در قزل الا را بهبود می بخشد.