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Research Article

Study on the effects of red pepper (*Capsicum annuum*) extract on immune responses and resistance of rainbow trout (*Oncorhynchus mykiss*) juveniles against *Yersinia ruckeri*

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Abstract

The aim of this study was to investigate the effect of red pepper (*Capsicum annuum*) extract on growth, blood, biochemical and non-specific immune parameters of 360 rainbow trout (*Oncorhynchus mykiss*) juveniles with average weight of 9.38±0.01 g. The fish were fed with a commercial diet containing 0, 0.25, 0.5 and 1% of *C. annuum* extract in 12 tanks (300L) with three replicates (30 fish per tank) for 30 days. Then, *Yersinia ruckeri* was injected by intraperitoneal to evaluate fish resistance. At the end of fish feeding period, growth and survival, as well as blood and immune parameters were assessed. The highest level of weight gain (%) and SGR and also the least level of FCR were observed in fishes fed by 0.5% red pepper extract (p<0.05). Hematological parameters (RBC, WBC and hemoglobin) significantly increased in fish fed with 0.25 and 0.5% of *C. annuum* extract for 30 days and after challenging with *Y. ruckeri* as compared to the control group. Lysozyme and complement contents indicated significant increases in all treatments fed with red pepper extract after bacterial infection (p<0.05). Generally, it can be concluded that rainbow trout fed with 0.5 % red pepper extract for a month could gain more weight and enhance immune responses against infectious diseases.

Keywords: Red pepper extract, Immunity, Rainbow trout, *Yersinia ruckeri*, Blood, Growth

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Introduction

The critical role of immune system in keeping aquatics′ health which ensures their survival and proper growth rate during rearing period forced researchers to apply a wide variety of chemical and natural compounds stimulating fish immunity. Like other lower vertebrates, fishes commonly rely on their non-specific immune system when facing pathogens. Therefore, immune stimulants, as suitable alternatives to antibiotics, are used to enhance activities associated with non-specific defense mechanism which raises resistance against diseases in rearing fish (Ravelo *et al.*, 2006). Plant-derived compounds are mainly categorized into tannins, alkaloids, pigments, essential oils, steroids, terpenoids, flavonoids, and phenolics (Soltani *et al.*, 2019; Elumalai *et al.*, 2021). Phytochemicals or plant-derived active compounds exert immunostimulant, biocidal, anti-stress, and growth promoting effects (Elumalai *et al.*, 2021). Immune stimulants are biological extracts and chemical compounds which stimulate immune reactions by increasing cell phagocytosis, bacterial inactivation, producing non-specific intracellular toxins and antibiotics. Among immune stimulants, herbal extracts have been considered due to their accessibility, low price, non-resistance against pathogens and the least damage to fish and surrounding environment (Rao *et al.*, 2006). In recent years, the used of herbal medicine as an alternative method has been introduced to prevent diseases in aquaculture and also oral administration of plant extracts is considered as the best way for stimulation of target fish immune functions (Haghighi *et al.*, 2014; Azizi *et al.*, 2016; Soltani *et al.*, 2019; Van Doan *et al.*, 2020).

The use of plant extracts as anti-fungal, antibacterial and immune system stimulant has been widely confirmed in several commercial fish species (Soltani *et al.*, 2019; Van Doan *et al.*, 2020; Mehrabi and Firouzbakhsh, 2020) including rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*). The functions of herbs vary depending on species of the herb, which may be due to variation in active substances. Adel *et al.* (2016) investigated potential effects of peppermint on blood parameters and immune responses of rainbow trout against *Yersinia ruckeri* infection. Bilen *et al.* (2016) evaluated growth parameters and immune system of rainbow trout fed by different levels of nettle, *Urtica dioica*, at 0.1 and 0.5 g/kg of diet for 30 days. They also assessed the fish resistance against *Aeromonas hydrophila.* Moreover, fish feeding by the extract of *Ferulago angulata* for 8 weeks could increase the number of some blood cells as well as increase the complement and lysozyme contents (Bohlouli and Sadeghi, 2016).

Red pepper (*Capsicum annuum*), from the family Solanaceae contains vitamin C, β-carotene, lycopene and astaxanthin (Topuz and Ozdemir, 2007). Capsanthin in red pepper is known as an immune stimulant and resistance booster against infections in fish (Talebi *et al.*, 2013). Components of red pepper could increase secretion of intestine, pancreas and bilirubin which eventually increase nutrient absorbance, weight gain and growth performance of fishes.

Yersinia ruckeri is the agent of enteric red mouth disease (ERM) in salmonids (Ross *et al.*, 1966; Rucker, 1966). Yersiniosis, as an important bacterial fish disease, damages rainbow trout culture in many parts of the world as well as Iran (Tobback *et al.*, 2007; Soltani *et al.*, 2014).

Considering positive impacts of immune stimulants in controlling several microbial and fungal diseases and activating immune systems in aquatics, in this work, the effect of red pepper extract on immune responses and resistance of rainbow trout was investigated against *Yersinia ruckeri*.

Materials and methods

Fish collection and experimental design

Fish samples were bought from a culture and breeding center of cold water fishes located in Babol, Mazandaran Province. They were transported to the rearing room of fisheries department, Sari Agricultural Sciences and Natural Resources University and then fed by a commercial diet for 10 days to adapt with indoor captive condition. After adaptation, 300 specimens of rainbow trout weighing 9.38±0.01g in average were randomly selected and stocked in 12 equal sized fiberglass tanks (300 L) containing well aerated water. Water quality parameters were monitored on a daily basis. Mean water physicochemical parameters were 11.0±0.5°C for temperature, 7.5 for pH and 600 mg/l for hardness.

Diet preparation and feeding

To prepare experimental diets, red pepper extract was produced by Kashan Barij Co., Iran, and was added to the commercial diet of Beyza feed mill 21, Shiraz-Iran (Crude protein 45%, Raw fat 14%, Crude fiber 2%, moisture 10%) at 0, 0.25, 0.5 and 1% to obtain four triplicated treatment combinations. Then for fixing the extract on food plates, it was dissolved in 6 mL of ethanol and sprayed on plates per each kg of the food. The diet of control group was only sprayed with the solvent. Thereafter, plates were dried under a sterilized hood for 24h, oil-covered (2.5 ml/kg) food was packed and stored in a refrigerator at 4°C. Fish were fed daily by 3% of body weight, three times a day (9.00 am, 13.00 pm and 16.30 pm) and for 30 days. During the experimental period, the feces and uneaten food were syphoned from the tank and two third of the whole tank water was replaced daily.

Calculation of growth indices

During the experiment, biometric indices were measured fortnightly. Parameters of weight gain percent (WG), specific growth rate (SGR), and food conversion ratio (FCR) were calculated according to the following formula (Mahghani *et al.*, 2014):

$$SGR = \frac{Ln_{W2} - Ln_{W1}}{t} \times 100$$

$$WG = \frac{w_2 - w_1}{w_1} \times 100$$

$$FCR = \frac{F}{w_2 - w_1} \times 100$$

Where t is the rearing period, Ln_{w2} and Ln_{w1} is natural log of final and initial fish weights (g), respectively, and F is the amount of consumed food (g).

Calculation of biochemical and hematological parameters

After 30 days of fish feeding, three specimens were randomly selected from each replicate and anesthetized in a solution containing 150 ppm of clove oil. Blood sampling was performed by cutting fish caudal peduncle. Red and white blood cell counts (RBC and WBC) were measured according to the method described by Houston (1990). Hematocrit percent (Htc) was measured by micro centrifugation method and hemoglobin measurement was done using the method of Drabkin (1945). Total protein and albumin contents were calculated as demonstrated by Lowry *et al.* (1951) and King and Wotton (1956), respectively. Serum globulin was obtained by subtracting albumin from total protein. Immune factors of lysozyme and complement contents were analyzed based on Ellis's method (1990) using ELISA device.

Challenging with bacteria

To assess fish resistance, *Yersinia ruckeri* was intraperitoneally injected (1.2×10^8 CFU/ml) at the end of experiment (day 30). Total number of dead fish in each replicate was calculated after 10 days of bacterial injection. Moreover, blood sampling, as described previously, was taken for measurement of serum biological and hematological factors after challenging period.

Statistical analyses

This experiment was conducted in a completely-randomized design. Firstly, data normality was checked using Shapiro-wilk test. Blood parameters data (the percent of eosinophil, neutrophil, monocyte, lymphocyte, hematocrit), and total mortality were square root transformed to approximate a normal distribution. One-way ANOVA was applied to find any significant difference among various treatments. Mean comparisons were performed by Duncan's multiple range test and paired-samples T-test. Statistical analyses were done using the statistical software package of SPSS Ver. 20 and diagrams were prepared by Microsoft Excel 2012.

Results

Changes in growth parameters

Results of growth parameters comparison in rainbow trout fish fed with red pepper extract are shown in Figures 1-4. As presented in Figure 1, differences in the values of fish weight exposed to different feeding treatments at first day of the experiment were statistically non-significant ($p>0.05$) while final weight of the fish fed with 0.5 % red pepper extract was significantly higher than that of the other groups ($p<0.05$). Weight gain (%) and SGR of 0.5%-treated fish was the most whereas the control and 1%-treated fish showed the least amount of these parameters (Figs. 2 and 4). Furthermore, the rainbow trout fish fed with 0.5 % and 1 % of red pepper extract had the least

(1.22±0.034) and the highest (1.44±0.038) level of FCR, respectively (Fig. 3).

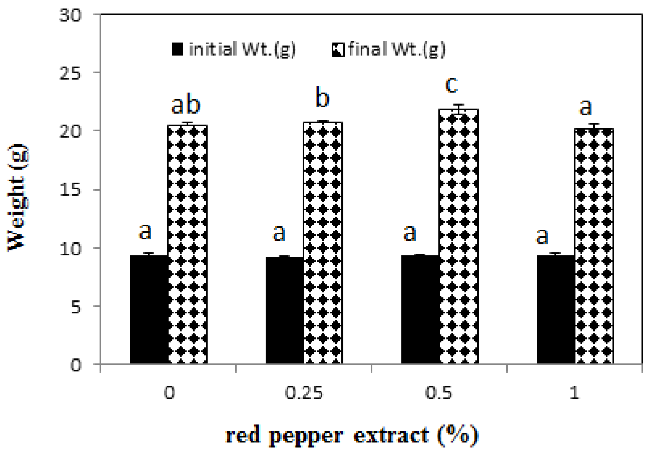

Figure 1: Comparison of initial and final mean weight (g) in rainbow trout fed with different levels of red pepper extract for 30 days. Error bards show standard deviation.

Figure 2: Comparison of mean weight gain (%) in rainbow trout fish fed with different red pepper extract for 30 days. Error bars show standard deviation.

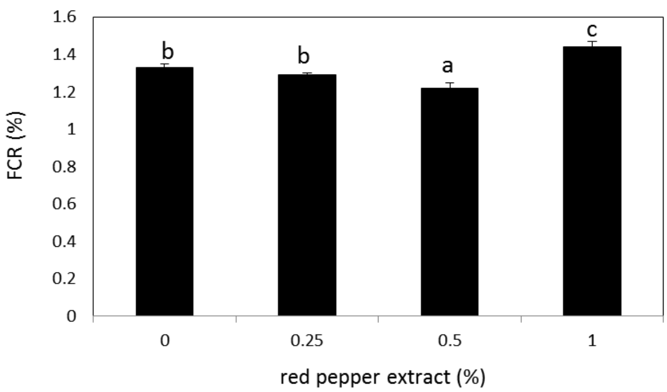

Figure 3: Comparison of mean food conversion ratio (FCR) in rainbow trout fish fed with different red pepper extract for 30 days. Error bars show standard deviation.

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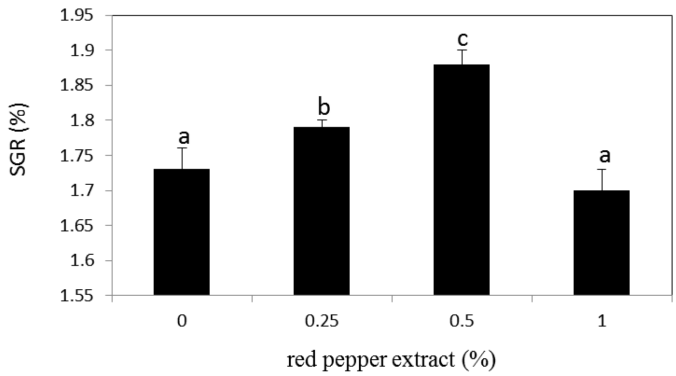

Figure 4: Comparison of mean specific growth rate (SGR) in rainbow trout fish fed with red pepper extract for 30 days. Error bars show standard deviation.

Changes in proximate composition

Results of proximate composition comparison in rainbow trout carcass among different treatments at the end of rearing period are presented in Table 1. Based on the results, no significant difference was discovered among various levels of pepper extract in humidity, dry matter, protein, and ash of the fish carcass ($p>0.05$). But fat content in the control fish was significantly less than the other treatments ($p<0.05$).

Table 1: Proximate composition of rainbow trout carcass fed with red pepper extract during the 30-day rearing period (mean ± standard deviation).

	0 %	0.25%	0.5 %	1 %
Humidity	77.55±0.02 ^{A}	77.73±0.19 ^{A}	77.73±0.19 ^{A}	77.79±0.25 ^{A}
Dry matter	22.44±0.02 ^{A}	22.28±0.16 ^{A}	22.17±0.15 ^{A}	22.27±0.18 ^{A}
Protein	73.67±2.27 ^{A}	71.57±0.52 ^{A}	74.72±6.12 ^{A}	71.22±0.87 ^{A}
Fat	19.51±0.49 ^{A}	20.05±0.21 ^{B}	20.11±0.08 ^{B}	20.43±0.06 ^{B}
Ash	10.74±0.16 ^{A}	10.52±0.14 ^{A}	10.17±0.02 ^{A}	9.90±1.65 ^{A}

Different letters (A-D) indicate significant difference among different concentration in each row ($p<0.05$).

Changes in hematological and biochemical parameters

According to the results of blood parameter measurements in Table 2, there was no significant difference between pre and 10 days post-challenging in the control treatment ($p>0.05$). However, treating with different concentrations of red pepper extract had a significant effect on hemoglobin and WBC as both parameters showed a significant decline after challenging with bacteria ($p<0.05$). Among the evaluated parameters, significant differences were detected between pre and 10 days post-challenging of bacteria for neutrophil and lymphocyte in 0.25 % treated fish, monocyte in 0.5 % treated fish, and hematocrit in 1% treated fish ($p<0.05$). The fish fed with 0.5 % and 1 % of red pepper extract revealed a significant difference in RBC as a remarkable decline was observed in the number of red blood cells at 10 days post-challenge ($p<0.05$).

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Table 2: Changes in hematological and biochemical parameters of rainbow trout fed with different red pepper extract on pre and 10 days post-challenge (mean ± standard deviation).

		Red pepper extract			
		0 %	**0.25 %**	**0.5 %**	**1 %**
RBC ($\times10^6$/mm^3)	Pre-challenge	$0.92\pm0.04^{A}_{a}$	$1.04\pm0.04^{A}_{b}$	$1.02\pm0.03^{A}_{b}$	$0.95\pm0.02^{A}_{a}$
	Post-challenge	$0.80\pm0.01^{B}_{a}$	$0.89\pm0.03^{B}_{c}$	$0.88\pm0.03^{B}_{bc}$	$0.85\pm0.02^{B}_{b}$
Hematocrit (%)	Pre-challenge	$32.67\pm1.53^{A}_{a}$	$37.67\pm1.53^{A}_{a}$	$36.00\pm2.00^{A}_{a}$	$31.00\pm1.00^{A}_{a}$
	Post-challenge	$28.00\pm1.00^{A}_{a}$	$30.00\pm1.00^{A}_{a}$	$31.00\pm1.00^{A}_{a}$	$28.33\pm1.53^{B}_{a}$
Hemoglobin (g/dl)	Pre-challenge	$7.89\pm0.49^{A}_{a}$	$8.87\pm0.32^{A}_{b}$	$8.83\pm0.16^{A}_{b}$	$7.49\pm0.15^{A}_{a}$
	Post-challenge	$6.17\pm0.25^{A}_{a}$	$6.45\pm0.08^{B}_{b}$	$6.84\pm0.09^{B}_{c}$	$5.99\pm0.12^{B}_{a}$
WBC ($\times10^3$/mm^3)	Pre-challenge	$13.93\pm4.50^{A}_{a}$	$19.23\pm4.72^{A}_{c}$	$19.07\pm6.42^{A}_{c}$	$16.03\pm3.05^{A}_{b}$
	Post-challenge	$13.13\pm3.78^{A}_{a}$	$16.10\pm4.35^{B}_{b}$	$16.30\pm8.00^{B}_{b}$	$14.03\pm4.04^{B}_{a}$
Neutrophil (%)	Pre-challenge	$34.33\pm5.13^{A}_{b}$	$19.33\pm1.53^{A}_{a}$	$21.67\pm3.51^{A}_{a}$	$30.00\pm3.61^{A}_{b}$
	Post-challenge	$23.00\pm3.61^{A}_{a}$	$28.33\pm3.21^{B}_{a}$	$18.67\pm4.62^{A}_{a}$	$22.00\pm8.72^{A}_{a}$
Monocyte (%)	Pre-challenge	$1.00\pm1.00^{A}_{a}$	$3.00\pm1.00^{A}_{ab}$	$3.67\pm1.53^{A}_{b}$	$2.33\pm1.53^{A}_{ab}$
	Post-challenge	$2.33\pm0.58^{A}_{a}$	$3.67\pm1.53^{A}_{a}$	$2.00\pm1.00^{B}_{a}$	$2.67\pm1.53^{A}_{a}$
Lymphocyte (%)	Pre-challenge	$63.33\pm3.06^{A}_{a}$	$75.33\pm1.53^{A}_{c}$	$72.67\pm4.73^{A}_{bc}$	$67.00\pm2.00^{A}_{ab}$
	Post-challenge	$73.67\pm2.08^{A}_{ab}$	$65.67\pm2.08^{B}_{a}$	$78.00\pm2.65^{A}_{b}$	$74.33\pm9.29^{A}_{ab}$
Eosinophil (%)	Pre-challenge	$1.33\pm1.53^{A}_{a}$	$2.33\pm1.53^{A}_{a}$	$2.00\pm1.00^{A}_{a}$	$0.67\pm0.58^{A}_{a}$
	Post-challenge	$1.00\pm1.00^{A}_{a}$	$2.33\pm0.58^{A}_{a}$	$1.33\pm1.15^{A}_{a}$	$1.00\pm0.00^{A}_{a}$
Total protein (g/dl)	Pre-challenge	$3.23\pm0.12^{A}_{a}$	$3.87\pm0.17^{A}_{b}$	$3.75\pm0.11^{A}_{b}$	$3.68\pm0.14^{A}_{b}$
	Post-challenge	$3.25\pm0.11^{A}_{a}$	$3.96\pm0.17^{A}_{b}$	$3.86\pm0.22^{A}_{b}$	$3.82\pm0.05^{A}_{b}$
Albumin (g/dl)	Pre-challenge	$1.25\pm0.16^{A}_{a}$	$1.72\pm0.13^{A}_{b}$	$1.62\pm0.08^{A}_{b}$	$1.56\pm0.09^{A}_{b}$
	Post-challenge	$1.21\pm0.07^{A}_{a}$	$1.79\pm0.14^{A}_{b}$	$1.73\pm0.08^{A}_{b}$	$1.69\pm0.08^{A}_{b}$
Globulin (g/dl)	Pre-challenge	$1.97\pm0.25^{A}_{a}$	$2.15\pm0.16^{A}_{a}$	$2.14\pm0.05^{A}_{a}$	$2.12\pm0.22^{A}_{a}$
	Post-challenge	$2.04\pm0.04^{A}_{a}$	$2.17\pm0.05^{A}_{a}$	$2.13\pm0.17^{A}_{a}$	$2.13\pm0.12^{A}_{a}$

Different letters (a-d) indicate significant difference among different concentration in each row ($p<0.05$). Different letters (A-D) indicate significant difference between pre and post-challenge for each parameter ($p<0.05$).

Of all the studied parameters, increasing red pepper extract in feeding diet of rainbow trout fish caused no significant difference in globulin, hematocrit and eosinophil at pre and 10 days post-challenge. Also, the number of white blood cells, neutrophils and monocytes indicated no difference among the fish treated by various levels of red pepper extract at 10 days post-challenge ($p<0.05$). At pre-challenge, the fish treated by 0.5 % red pepper extract had the highest number of monocytes while the control fish showed the lowest number of this cell. By contrast, neutrophil count was the least in 0.25 % and 0.5 % treated fish but the most in 1 % treated rainbow trout fish. At pre and 10 days post-challenge, there was no significant difference in total protein and albumin contents of those fish fed by various concentrations of red pepper extract ($p>0.05$), while they had significant difference with the control ($p<0.05$). The 0.25 % and 5% treated fish showed the most number of lymphocytes at pre and 10 days post-challenge, respectively. In 0.25% and 0.5% treated fish, the most number of WBC, RBC and hemoglobin content were perceived at pre-challenging of bacteria. Hemoglobin content of fish fed by 0.5 % red pepper extract was significantly higher than that of other fish at 10 days post-challenge ($p<0.05$).

Changes in immune responses

Results of mean comparison of immune responses in rainbow trout fish fed with different concentrations of red pepper extract at pre- and 10 days post-challenge are depicted in Table 3. Regardless of red pepper extract concentrations, all the reared rainbow trout fish indicated a significant difference between pre- and 10 days post-challenge of bacteria in immune responses ($p<0.05$). In addition, there were significant differences among various treatments of red pepper extract at each sampling occasion for each indicator of immune responses ($p<0.05$). Notably, an increasing trend was observed in lysozyme and complement contents of the fish with increased level of red pepper extract in their feeding diet.

Table 3: Changes in immune responses of rainbow trout fed with different concentrations of red pepper extract on pre and 10 days post-challenge (mean ± standard deviation).

		Red pepper extract			
		0 %	**0.25 %**	**0.5 %**	**1 %**
Lysozyme (mg/ml)	Pre-challenge	$316.92\pm0.40\ ^{A}{}_{a}$	$379.80\pm0.21\ ^{A}{}_{b}$	$395.35\pm1.64\ ^{A}{}_{c}$	$406.39\pm2.33\ ^{A}{}_{d}$
	Post-challenge	$382.90\pm1.78\ ^{B}{}_{a}$	$434.38\pm0.54\ ^{B}{}_{b}$	$442.83\pm1.12\ ^{B}{}_{c}$	$455.69\pm2.91\ ^{B}{}_{d}$
Complement (mg/ml)	Pre-challenge	$16.03\pm0.29\ ^{A}{}_{a}$	$22.06\pm0.28\ ^{A}{}_{b}$	$24.56\pm0.91\ ^{A}{}_{c}$	$28.21\pm0.25\ ^{A}{}_{d}$
	Post-challenge	$43.21\pm1.09\ ^{B}{}_{a}$	$76.23\pm1.09\ ^{B}{}_{b}$	$84.82\pm0.57\ ^{B}{}_{c}$	$88.31\pm1.08\ ^{B}{}_{d}$

Different letters (a-d) indicate significant difference among different concentrations in each raw ($p<0.05$).

Changes in fish mortality

Results of cumulative fish mortality (%) after 10 days of bacterial challenge are depicted in Figure 5. Comparison among various treatments significantly exhibited less mortality rate (%) in the fish treated by higher concentrations of red pepper extract (p<0.05).

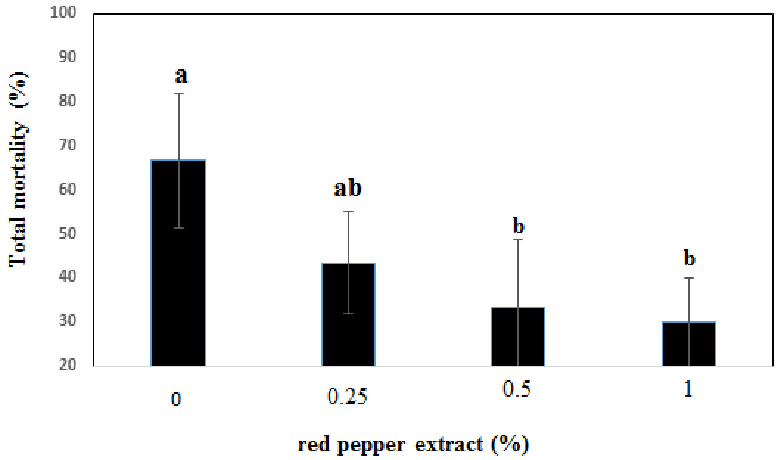

Figure 5: Cumulative mortality (%) in rainbow trout fish fed by red pepper extract at 10 days post-challenge with *Y. ruckeri*.

Discussion

Farmed fish species are exposed to immunosuppression due to increased rearing density and adverse environmental conditions. Therefore, feed additives as immunostimulants are widely used to enhance the growth performance, immune responses, and resistance to diseases in aquatic animals.

The present study indicates that dietary red pepper extract for a month could be a potential feed additive to enhance fish growth performance, hematological and biochemical factors and also immune response in rainbow trout. In this study, the highest increase in body weight and specific growth rate was observed in 0.5% *C. annuum* extract. These results are parallel with results of Talebi *et al.* (2013) who obtained higher weight gain and significant growth after feeding rainbow trout (*Oncorhynchus mykiss*) with red bell pepper diet. Similar results are indicated by Parrino *et al.* (2020) who observed remarkable changes in weight gain and SGR of the rainbow trout treated by hot pepper (*Capsicum* sp.) oil. Also, Shamsaie Mehrjan *et al.* (2020) demonstrated that supplementing diet with 5% *C. annuum* powder improved growth performance and immunity of rainbow trout fry in 45 days of feeding. These results may be because potentially inhibitory pathogens would enhance the population of beneficial microorganisms and may improve feed digestibility and nutrient absorption. Thus, digestibility increases and in turn the energetic benefits enhance the growth rate. In this investigation, higher concentration of red pepper extract (1%) indicated no positive influence on growth performance which might be due to

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changes in flavor and fish appetite. Moreover, this might be likely because of anti-nutritional materials in higher concentrations of red pepper extract added to the diet which would impressively disrupt fish growth.

Necessarily, body chemical composition is always affected by diet components and daily feeding percentage. Feeding by red pepper extract in the present work affected only fat content and the lowest fat level was detected in the control treatment. As reported by Mahdavi *et al.* (2014), feeding by fennel oil (*Foeniculum vulgare*) for 60 days showed no significant effect on protein and ash contents of Caspian white fish (*Rutilus frisii*), but fat amount in 100 and 400 mg/kg treatments were statistically significant. Also Setiawati *et al.* (2016) reported that the addition of 1% cinnamon (*Cinnamomum burmannii*) leaf to Asian catfish (*Pangasianodon hypophthalmus*) dietary improved flash fat content.

Hematology tests and analysis of fish blood serum are suitable tools for detection of metabolic disorders, fish health evaluation in intensive culture systems, non-specific resistance of fish and brood stocks, assessment of feeding condition and additive ingredients effects. The obtained results of this study significantly indicated increases the RBC, WBC and hemoglobin content in the fish treated by red pepper extract. These increases in WBC and RBC are in line with Nya and Austin (2009) who obtained increased WBC and RBC after feeding rainbow trout with ginger diet. The increase in hematological parameters following red pepper supplemented diet feeding indicates the immunostimulants properties of *C. annuum*. It could be explained that the bioactive compounds in red pepper extract prevented rainbow trout from diseases by immune system stimulation.

Serum protein level is an important index of humoral defense system and health condition in fish species. Actually, immune stimulants activate immunity system cells by stimulating protein synthesis (Rao *et al.*, 2006). In the present research, total protein contents of the treated groups were significantly different than that of control after 30 days of rearing. Also, total protein and albumin contents increased in the fish fed by pepper extract than those in the control after bacterial challenging. However, some researchers reported no impact of herbal extracts on protein content, which is incompatible with the present work (Ispir and Dörücü, 2005; Misra *et al.*, 2006). Furthermore, Nya and Austin (2011) demonstrated non-significant effect of garlic powder added to rainbow trout diet on plasma protein after 14 days when compared to the control. Serum protein and albumin play significant roles in immune system of fishes. However, albumin is vital to sustaining the osmotic pressure needed for proper distribution of body fluids and acts as plasma carrier (Talpur and Ikhwanuddin, 2013).

The use of immunostimulants is one of the methods used to enhance the immune system, particularly non-specific defense mechanisms, for prevention and control of diseases in aquaculture. Lysozyme is considered as an important component of non-specific immunity in fishes which damages bacterial membrane, activates complement and spreads phagocytosis. Also complement has a substantial bactericidal activity in fish serum and mucus (Ellis, 2001; Holland and Lambris, 2002) interfering in phagocytosis as opsonin by binding to particular parts of an invasive agent in the host body. In the present study, serum lysozyme and complement of the fish fed by red pepper (*C. annuum*) extract was significantly more than that of the control fish. Reports of increased complement and lysozyme contents after feeding by herb extracts have been presented by many researchers, including the application of 1 and 2% wolf bean (*Lupinus perennis*), mango (*Mangifera indica*), nettle (*Urtica dioica*) after 14 days (Awad and Austin, 2010), garlic at 0.5 and 1 g per 100 kg of diet (Nya and Austin, 2011), nettle (0.1 and 0.5 g/kg) for 30 days (Bilen *et al.*, 2016) and 5% *C. annuum* powder for 45 days (Shamsaie Mehrjan *et al.*, 2020). Lysozyme acts an important role in the immune system of fish following its stimulation, which in addition to blood, greatly accumulates and secretes in the kidney (Soltani *et al.*, 2010). The increase of lysozyme content in fish fed with red pepper extract in our study could be due to flavonoids which stimulate phagocytosis.

The present work revealed no significant difference in survival rate between the fish groups fed by pepper extract and control group after a month. In a previous research by Sotoudeh *et al.* (2018) on different levels of dietary purple coneflower (*Echinacea purpurea*) and marjoram (*Origanum majorana*) extracts, the survival rate of rainbow trout was 100% at all treatments. Health promoting effect of red pepper and the presence of phenol and flavonoids components in its extract during one month feeding could prevent fish mortality in this investigation.

More of the fish fed by red pepper extract in this survey were survived than the control fish after *Yersinia ruckeri* injection. Bilen *et al.* (2016) found higher rate of survival in the rainbow trout fed by 0.1 and 0.5 g/kg of nettle extract in comparison with the control after challenging by *Aeromonas hydrophila*. Supplemented diet of peppermint (*Mentha piperita*) indicated higher survivability and significant difference by contrast to the control treatment against *Y. ruckeri* (Adel *et al.*, 2016). In an investigation by Amirkhani and Firouzbakhsh (2015) on common carp, most fish fed by diets containing 400 mg/kg of basil leaf extract (*Ocimum basilicum*) were survived against *A. hydrophila*. The application of red pepper extract at certain levels in the diet could improve non-specific immunity mechanisms and reduce mortality rates after challenge.

Results presented here suggest positive effects of red pepper extract as a growth promoter and survival enhancer in feed of rainbow trout. Addition of red pepper extract, particularly at 0.5 %, to the diet of rainbow trout can increase their

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weight gain (%) and stimulate immune system against infectious disease of yersiniosis, because of its different flavonoids and anti-oxidative components.

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References

Adel, M., Pourgholam, R., Zorriehzahra, J. and Ghiasi, M., 2016. Hemato-Immunological and biochemical parameters, skin antibacterial activity, and survival in rainbow trout (*Oncorhynchus mykiss*) following the diet supplemented with *Mentha piperita* against *Yersinia ruckeri*. *Fish and Shellfish Immunology*, 55: 267-273. DOI:10.1016/j.fsi.2016.05.040.

Amirkhani, N. and Firouzbakhsh, F., 2015. Protective effects of basil (*Ocimum basilicum*) ethanolic extract supplementation diets against experimental *Aeromonas hydrophila* infection in common carp (*Cyprinus carpio*). *Aquaculture Research*, 46(**3**), 716-724. DOI:10.1111/are.12217.

Awad, E. and Austin, B., 2010. Use of lupin, *Lupinus perennis*, mango, *Mangifera indica*, and stinging nettle, *Urtica dioica*, as feed additives to prevent *Aeromonas hydrophila* infection in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, 33(**5**), 413-420. DOI:10.1111/j.1365-2761.2009.01133.x.

Azizi, E., Yeganeh, S., Firouzbakhsh, F., and Janikhalili, K., 2016. Effects of dietary supplemental thyme essence (*Thymus vulgaris* L.) on growth, hematological and serum biochemical parameters of Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). *Journal of Applied Ichthyology Research*, 4(**2**), 45-61. in Persian.

Bilen, S., Ünal, S. and Güvensoy, H., 2016. Effects of oyster mushroom (*Pleurotus ostreatus*) and nettle (*Urtica dioica*) methanolic extracts on immune responses and resistance to *Aeromonas hydrophila* in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 454, 90-94. DOI:10.1016/j.aquaculture.2015.12.010.

Bohlouli, S. and Sadeghi, E., 2016. Growth performance haematological and immunological indices of rainbow trout (*Oncorhynchus mykiss*) fingerlings supplemented with dietary *Ferulago angulata* (Schlecht) boiss. *Acta Veterinaria Brno*, 85, 231-238. DOI:10.2754/avb201685030231.

Drabkin, D., 1945. Hemoglobin, glucose, oxygen and water in the erythrocyte: a concept of biological magnitudes, based upon molecular dimensions. *Science*, 101(**2627**), 445-

451. DOI:10.1126/science.101.2627.445.

Ellis, A.E., 1990. *Lysozyme assays*. In: Techniques in fish immunology. Stolen, J.S., Fletcher, T.C., Anderson, D.P., Roberson, B.S. and Van Muiswinkel W.R. editors. SOS Publications, Fair Haven, NJ, USA: 101-103.

Ellis, A.E., 2001. Innate host defense mechanisms of fish against viruses and bacteria. *Developmental Comparative Immunology*, 25(**8-9**), 827-839. DOI:10.1016/s0145-305x(01)00038-6.

Elumalai, P., Kurian, A., Lakshmi, S., Faggio, C., Esteban, M.A. and Ringø, E., 2021. Herbal Immunomodulators in Aquaculture. *Reviews in Fisheries Science and Aquaculture*, 29(**1**), 33-57. DOI:10.1080/23308249.2020.1779651.

Haghighi, M., Sharif Rohani, M., Samadi, M., Tavoli, M., Eslami, M. and Yusefi, R., 2014. Study of effects *Aloe vera* extract supplemented feed on hematological and immunological indices of rainbow trout (*Oncorhynchus mykiss*). *International Journal of Advanced Biological and Biomedical Research*, 2(**6**), 2143-2154. DOI:10.33945/SAMI/IJABBR

Holland, M.C.H. and Lambris, J.D., 2002. The complement system in teleosts. *Fish and Shellfish Immunology*, 12(**5**), 399-420. DOI:10.1016/fsim.2001.0408.

Houston, A.H., 1990. *Blood and circulation.* In: Methods in fish biology. Shreck, C.B. and Moyle, P.B. editors. American Fisheries Society, Bethesda, Maryland, USA. pp. 273-335.

Ispir, Ü. and Dörücü, M., 2005. A study on the effects of levamisole on the immune system of rainbow trout (*Oncorhynchus mykiss*, Walbaum). *Turkish Journal Veterinary and Animal Sciences*, 29(**5**), 1169-1176.

King F.J. and Wotton, I.D.P., 1956. *Microanalysis in medical biochemistry*, second edition. Churchill Livingston, London, UK, 228 P.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, 193(**1**), 265-275. DOI:10.1016/s0021-9258(19)52451-6.

Mahdavi, S., Yeganeh, S., Firouzbakhsh, F. and Jani Khalili, Kh., 2014. Effects of supplementary fennel (*Foeniculum vulgare*) essential oil of diet on growth, survival, body composition, and hematological parameters of *Rutilus frisii kutum* fry. *Fisheries Science and Technology*, 3(**3**), 79-90, in Persian.

Mahghani, F., Gharaei, A., Ghaffari, M. and Akrami, R., 2014. Dietary synbiotic improves the growth performance, survival and innate immune response of Gibel carp (*Carassius auratus* gibelio) juveniles. *International Journal of Aquatic Biology*, 2(**2**), 99-104. DOI:10.22034/ijab.v2i2.37.

Mehrabi, Z. and Firouzbakhsh, F., 2020. Short-term effects of feeding powdered Aloe vera (*Aloe barbadensis*) and nettle (*Urtica dioica*) on growth performance and stimulation of innate immune responses in rainbow trout (*Oncorhynchus mykiss*). *Comparative Clinical Pathology*, 29(**3**), 441-449. DOI:10.1007/s00580-019-03068-w.

Misra, C.K., Das, B.K., Mukherjee, S.C. and Meher, P.K., 2006. The immunomodulatory effects of tuftsin the non-specific immune system of Indian major carp, *Labeo rohita*. *Fish and Shellfish Immunology*, 20(**5**), 728-738. DOI:10.1016/j.fsi.2005.09.004.

Nya, E.J. and Austin, B., 2009. Use of dietary ginger, *Zingiber officinale* Roscoe, as an immunostimulant to control *Aeromonas hydrophila* infections in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, 32(**11**), 971-977. DOI:10.1111/j.1365-2761.2009.01101.x.

Nya, E.J. and Austin, B., 2011. Development of immunity in rainbow trout (*Oncorhynchus mykiss*, Walbaum) to *Aeromonas hydrophila* after the dietary application of garlic. *Fish and Shellfish Immunology*, 30(**3**), 845-850. DOI:10.1016/j.fsi.2011.01.008.

Parrino, V., Kesbiç O.S., Acar, Ü. and Fazio, F., 2020. Hot pepper (*Capsicum* sp.) oil and its effects on growth performance and blood parameters in rainbow trout (*Oncorhynchus mykiss*). *Natural Product Research*, 34(**22**), 3226-3230. DOI:0.1080/14786419.2018.1550769.

Rao, Y.V., Das, B.K., Jyotyrmayee, P. and Chakrabarti, R., 2006. Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. *Fish and Shellfish Immunology*, 20(**3**), 263-273. DOI:10.1016/j.fsi.2005.04.006.

Ravelo, C., Magariños, B., Herrero, M.C., Costa, L., Toranzo, A.E. and Romalde, J.L., 2006. Use of adjuvanted vaccines to lengthen the protection agaist Lactococcusis in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture*, 251(**2-4**), 153-158. DOI:10.1016/j.aquaculture.2005.05.027.

Ross, A.J., Rucker, R.R. and Ewing, W.H., 1966. Description of a bacterium associated with redmouth disease of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Microbiology*, 12(**4**), 763-770. DOI:10.1139/m66-103.

Rucker, R.R., 1966. Redmouth disease in rainbow trout (*Salmo gairdneri*). Bulletin of office *International of Epizooties*, 65(**5**), 825-830.

Setiawati, M., Jusadi, D., Laheng, S., Suprayudi, M.A. and Vinasyiam, A., 2016. The enhancement of growth performance and feed efficiency of Asian catfish, *Pangasianodon hypophthalmus* fed on *Cinnamomum burmannii* leaf powder and extract as nutritional supplementation. *AACL Bioflux*, 9(**6**), 1301-1309.

Shamsaie Mehrjan, M., Hosseini Shekarabi, S.P., Mahjob Zardest, M. and Mohammadi, N., 2020. Effect of dietary supplementation of red chili pepper (*Capsicum annuum*) powder on growth parameters, survival rate, hematological indices and some immunity responses of rainbow trout (*Oncorhynchus mykiss*) fry. *Iranian Scientific Fisheries Journal*, 29(**1**), 131-140, in Persian. DOI:10.22092/isfj.2019.120988.

Soltani, M., Sheikhzadeh, N., Ebrahimzadeh-Mousavi H.A. and Zargar, A., 2010. Effects of *Zataria multiflora* essential oil on innate immune responses of common carp (*Cyprinus carpio*). *Journal of Fisheries and Aquatic Sciences*, 5(**3**), 191-199. https://doi.org/10.3923/jfas.2010.191.199.

Soltani, M., Mousavi, Sh., Ebrahimzadeh Mousavi, H.A., Mirzargar, S.S., Taheri Mirghaed, A., Shafiei, Sh., Shohreh, P. and Mohamadian, S., 2014. Molecular study of *Yersinia ruckeri* distribution, the causative agent of yersiniosis in some farmed rainbow trout of Iran. *Iranian Veterinary Journal*, 10(**1**), 59-67, in Persian. DOI:10.22059/jvr.2019.262756.2828

Soltani, M., Lymbery, A., Song, S.K. and Hosseini Shekarabi, P., 2019. Adjuvant effects of medicinal herbs and probiotics for fish vaccines. *Reviews in Aquaculture*, 11(**4**), 1325-1341. DOI:10.1111/raq.12295.

Sotoudeh, E., Mardani, F., Jafari, M., Habibi, H. and Moradyan, S.H., 2018. Growth indices, feeding efficiency and survival of rainbow trout (*Oncorhynchus mykiss*) fed diets containing different levels of *Echinacea angustifolia* and *Origanum majorana* extracts. *Journal of Aquatic Animals Nutrition*, 4(**1**), 1-12, in Persian.

Talebi, M., Khara, H., Zoriehzahra, J., Ghobadi, S., Khodabandelo, A. and Mirrasooli, E., 2013. Study on effect of red bell pepper on growth, pigmentation and blood factors of rainbow trout (*Oncorhynchus mykiss*). *World Journal of Zoology*, 8(**1**), 17-23. DOI:10.5829/idosi.wjz.2013.8.1.7136.

Talpur, A.D. and Ikhwanuddin, M., 2013. *Azadirachta indica* (neem) leaf dietary effects on the immunity response and disease resistance of Asian seabass, *Lates calcarifer* challenged with *Vibrio harveyi*. *Fish and Shellfish Immunology*, 34(**1**), 254-264. DOI:10.1016/j.fsi.2012.11.003.

Tobback, E., Decostere, A., Hermans, K., Haesebrouck, F. and Chiers, K., 2007. *Yersinia ruckeri* infections in salmonids fish. *Journal of Fish Diseases*, 30(**5**), 257-268. DOI:10.1111/j.1365-2761.2007.00816.x.

Topuz, A and Ozdemir, F., 2007. Assessment of carotenoids, capsaicinoids and ascorbic acid composition of some selected pepper cultivars (*Capsicum annuum* L.)

[DOR: 20.1001.1.15622916.2021.20.6.7.6]

grown in Turkey. *Journal of Food Composition and Analysis*, 20(**7**), 596-602. DOI:10.1016/j.jfca.2007.03.007.

Van Doan, H., Soltani, E., Ingelbrecht, J. and Soltani, M., 2020. Medicinal herbs and plants: potential treatment of Monogenean infections in fish. *Reviews in Fisheries Science and Aquaculture*, 28(**2**), 260-282. DOI:10.1080/23308249.2020.1712325.