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Research Article

Effects of dietary Persian hogweed (*Heracleum persicum*) powder on growth performance, hematological and immune indices, and resistance against *Yersinia ruckeri* in rainbow trout (*Oncorhynchus mykiss*)

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Abstract

The current study was performed to evaluate the effect of dietary Persian hogweed (*Heracleum persicum*) powder (PHP) on growth, hematological and immunological parameters in rainbow trout. The fish with an average weight of 23.38±0.58 (g) were randomly assigned into five groups in triplicates. Diets were prepared by supplementing five levels of 0 (control), 0.5, 1, 1.5, and 2% PHP to a basal diet and fed for 60 days. The addition of 2% PHP significantly improved weight gain, specific growth rate, and feed conversion ratio compared to 0.5% PHP during 30 days ($p<0.05$). PHP also diminished whole-body fat, after 30 days ($p<0.05$). Hematological factors showed the enhancements of white and red blood cell counts in the fish fed 1 and 1.5% PHP ($p>0.05$). Moreover, the addition of 2% PHP improved Hb ($p<0.05$) after 30 days. On the day 60th, WBC count was significantly higher in the fish that received 1.5% PHP than 0.5% PHP ($p<0.05$). According to the biochemical analysis the highest concentration of alkaline phosphatase was observed in the fish fed with the control diet and 0.5% PHP ($p<0.05$). In contrast, cholesterol and triglyceride values reduced by PHP levels on day 60 ($p<0.05$). Immunological parameters indicated that total protein and globulin were significantly improved by the addition of 1.5 and 2% PHP during 60 days ($p<0.05$). Lysozyme activity was enhanced by the diets containing 1 and 1.5% PHP after 30 and 60 days ($p<0.05$). Besides, higher resistance against *Yersinia ruckeri* was obtained by the administration of 1 and 1.5% PHP. Altogether, supplementation of 1.5% PHP is suggested in the diet of rainbow trout because of partially increased immune indices without showing damages to the liver during 30 days.

Keywords: *Heracleum persicum*, Persian hogweed, Immunological parameters, *Yersinia ruckeri*, Rainbow trout

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Introduction

Aquaculture is a fast-growing industry supplying a great part of protein requirements for society. Hence, there is a need for the development of fish culture due to a constant fishing ratio in the world. Nowadays, increasing fish density, mostly in extensive rearing systems, has caused many challenges for fish farms, including the spread of various diseases, one of which is the enteric red mouth disease caused by a Gram-negative bacterium, known as *Yersinia ruckeri*. Fish can easily be infected by *Y. ruckeri* through direct contact, especially in stressful conditions. Therefore, the fish immune response is an important issue in such situations (Dorucu *et al*., 2009). Fish responds to pathogens in two specific or non-specific ways, depending mostly on the non-specific immune system. Thus, stimulating the non-specific immune system enhances fish health and resistance against pathogens (Harikrishnan *et al*., 2009). Immunostimulators are natural or synthetic compounds able to activate the immune system. Most of these compounds affect the non-specific immune system, such as macrophages, granulocytes, and monocytes, as well as humoral elements, including lysozyme, as the main components of the innate immune system (Kuebutornye and Abarike, 2020). Herbal-originated immunostimulants are not as efficient as vaccination or antibiotics, but they can be used as suitable alternatives because of having more accessibility, low cost, fewer side effects, and eco-friendliness (Sakai, 1999).

Heracleum is a genus with the common name of hogweed. *Heracleum persicum* (Persian hogweed) is a flowering herb in the Apiaceae family, which grows in humid mountainous regions of Iran. There are 300 genera and more than 3,000 species of hogweed currently known (Gharachorloo *et al*., 2017). The pharmaceutical application of hogweeds, besides their use as a spice, has been common since ancient times. Hogweed bioactive compounds, such as alkaloids, volatile substances, terpenoids, triterpenes, and furanocoumarins, are extracted from different parts of the fruit (Hajhashemi *et al*., 2009). Natural chemicals, including volatile (aliphatic esters, carbonyls, phenyl propenes, and terpenes) and nonvolatile (flavonoids, furanocoumarins, tannins, and alkaloids) ingredients, have been recognized in phytochemical analysis of *H. persicum* (Majidi and Sadati Lamardi, 2018). Moreover, antioxidant, antispasmodic, anti-inflammatory, and immune modifying activities (Asgarpanah *et al*., 2012), as well as antimicrobial (Kousha and Bayat, 2012) and lipid regulatory (Panahi *et al*., 2011) properties, have been discovered in Persian hogweed.

According to a study by Hoseinifar *et al*. (2016), the inclusion of Persian hogweed improved immunoglobulin, lysozyme, and protease in common carp (*Cyprinus carpio*). Similar results were reported in the carp injected with 60 and 600 mg of hogweed aqueous extract, indicating a higher immune response and resistance against *Aeromonas hydrophila* (Soltanian *et al*., 2017). Sharififar *et al*. (2009) also found that Persian hogweed aqueous extract stimulated cellular and humoral immune performance in mice. In the current study, the effect of Persian hogweed powder inclusion in the diet of rainbow trout was investigated on its growth performance, hematological, biochemical indices, and immune response to *Y. ruckeri* infection.

Materials and methods

Fish and the experimental system

Juvenile rainbow trout (n = 225) with an average weight of 23.38 ± 0.58 g were obtained from a trout farm (Sari city, northern Iran) and transferred to experimental rearing tanks of Sari Agricultural and Natural Resources University, Iran. Fifteen circular tanks were filled with 250 L of well water and aerated to supply adequate dissolved oxygen. Fish were randomly distributed into the experimental tanks after two weeks of acclimation and then fed with the diets containing Persian hogweed powder (PHP) until the last day of the trial. Water dissolved oxygen, nitrite, temperature, and pH were maintained at 7.8±0.14 mg L^{-1}, 0.05±0.02 mg L^{-1}, 12.7±0.5˚C, and 7.5±0.12, respectively, during the experiment.

Experimental diet

A desirable amount of *H. persicum* was collected from Mazandaran province, Iran, oven-dried (40˚C) overnight, and then pounded in a mortar to prepare a fine powder (Kousha and Ringo, 2015). A commercial diet with the chemical composition of 40% protein, 14% fat, 2% fiber, 9% ash, and 6% moisture was ground and mixed completely with different levels of 0.5, 1, 1.5, and 2% of PHP. Then, water was added before pelleting in a meat grinder with a 2 mm mesh size die. The pellets were well-dried at room temperature and stored at −18˚C until use (Hoseinifar *et al*., 2016). A PHP-free commercial diet was used as the control diet. Fish were fed with five PHP-containing experimental diets, namely 0% (control), 0.5%, 1%, 1.5%, and 2%, thrice a day (9.00, 13.00, and 17.00 hrs.) to apparent satiation for 60 days.

Sampling procedure

On the 30th and 60th days, the fish were anesthetized with 150 mg L^{-1} of clove powder to perform biometry for each tank. Then, five fish per tank were randomly taken to collect 6 mL blood samples from the caudal vein and transferred immediately to 2 mL heparin-containing tubes. 4 mL of blood samples were transferred to non-heparinized tubes, centrifuged after clotting for 10 min to obtain serum, and then kept at −18˚C. On the same sampling days, the same five fish were used to determine the whole-body proximate composition. Nine of the remaining fish were left in each tank for two more weeks to perform the bacterial challenge test after the final sampling.

Fish performance and feed efficiency

Growth performance parameters were calculated by the following formula:

Body weight (g)=FW-IW

Weight gain (%)=100 (FW-IW)/IW

Specific growth rate (SGR, %)=100 (ln FW- ln IW)/t

Feed conversion ratio (FCR)=FI/(FW-IW)

Where, FW is final weights (g); IW is initial weights (g), t is time (day); and FI denotes feed intake (g) estimated daily after the last feeding.

Proximate composition

The fat, crude protein, moisture, and ash contents in the fish whole-body were analyzed following the standard procedures (AOAC, 2005). Body moisture was evaluated by oven-drying at 105˚C for 24 h to reach a constant weight. Fat content was determined using a Soxhlet apparatus, and fat was extracted by petroleum ether until complete defatting after 3-6 h. Crude protein was measured after acid digestion at 200˚C using a Kjeldahl system and calculating the acid used for titration. Ash content was determined by weighing samples for 6 h after burning them in a muffle furnace at 550˚C (Yazdanpanah *et al*., 2021).

Hematological indices

WBC and RBC were counted by the method described by Hoston (1990). Hemoglobin (Hb) and hematocrit (Hct) measurements followed the procedure of Drabkin (1945). Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were assessed according to the formulas below:

M.C.V=Hct (%)×10/ RBC (million)

M.C.H=HB×10/ RBC (million)

M.C.H.C=HB×100/ Hct (%)

The WBC differential count was calculated according to the procedure of Brown *et al*. (1993) and expressed as a relative number of each type of WBC divided by the total 100 WBCs counted on microscopic slides.

Biochemical indices

Serum samples were used to determine total protein, glucose, albumin, triglyceride, and cholesterol using commercial kits (Pars Azmun, Karaj, Iran), and the absorbance was read on a spectrophotometer at 576 nm as described previously (Olesen and Jorgensen, 1986). Alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH) were measured spectrophotometrically by placing serum and kits (Pars Azmoon) directly into an AutoAnalyzer (BS-120 model). Superoxide dismutase (SOD) specific enzyme assay was obtained spectrophotometrically using a commercial kit (Nasodox-RBC) at 420 nm based on the instruction of the manufacturer.

Catalase activity equals an enzyme value (i.e. unit) for converting H_2O_2 into water and oxygen in 1 minute at 240 nm using H_2O_2 as the substrate prepared in a sodium, potassium, and phosphate buffer. Phosphate buffer is prepared by dissolving 1.1 g of Na_2HPO_4 and 0.27 g of KH_2PO_4 in 100 mL of distilled water. To this end, 1000 µL of H_2O_2 was mixed with 100 µL of serum and incubated at 37°C for 3 min. Then, 4000 µL of ammonium molybdate was added and read in an Elisa reader (Hadwan and Abed, 2016).

Immunological parameters

The activity of the alternative complement pathway was assessed using the hemolytic assay (Kumari, 2006). ACH50 units were defined as the concentration of plasma giving 50% hemolysis of rabbit red blood cells (RBCs). Rabbit RBCs were washed with 10 mM of EGTA, 10 mM of MgCl2, and 0.1% gelatin three times. Then, 50 µL of the solution was added to serum samples and incubated at 20˚C for 120 min. The reaction was stopped by adding 1.575 mL of 10 mM EDTA and 0.1% gelatin, and the solution was then centrifuged at 1600 g for 5 min. Distilled water (1.7 mL) and 50 µL of the RBC solution were used as the blank, and the optical density (OD) was measured at 414 nm wavelength.

The turbidimetric assay was performed for determining lysozyme activity as described by Kumari (2006). *Micrococcus lysodeikticus* (2 mg/mL) was added to 0.02 M sodium acetate buffer at pH 5.5 to prepare *M. lysodeikticus* suspension. Then, 15 µL of the sample was injected into microplates, followed by adding 150 µL of the *M. lysodeikticus* solution. Initial OD was measured at 450 nm immediately after adding the substrate. Then, samples were incubated at 24˚C for 1 h, and the final OD was measured afterward.

Bacterial challenge

The effect of dietary PHP on fish immune response was studied by challenging fish resistance against *Y. ruckeri*. A standard method was used for bacterial culture in TBS medium prior to transferring to Mueller Hinton Broth in sterile conditions. Then, the bacterial suspension was diluted in a ratio of 1:100 and adjusted to a final concentration of 1.5×10^6 cells mL^{-1}. Sampled fish (n=27) per group were anesthetized with 150 mg l^{-1} of clove powder, and 0.1 mL of *Y. ruckeri* was injected intraperitoneally into rainbow trout (9 fish per tank). Mortality was recorded during 2 weeks of the challenge (Heydari *et al*., 2020).

Statistical analysis

A completely randomized design was carried out for the present research. One-way ANOVA was run for data using SPSS 24 statistical software, and all results are represented as means of each treatment±standard deviation. Different groups were compared using Duncan's post hoc test at 0.05% probability after the Shapiro-Wilk test to verify the normality.

Results

Growth performance

There were significant differences in growth performance on the 30th and 60th days of sampling (p<0.05; Table 1) by the dietary addition of PHP. On the 30th day, the inclusion of 2% PHP led to increases in weight gain, and specific growth rate (SGR) values; however, the lowest values belonged to 0.5% PHP (*p*<0.05). The diet containing 0.5% PHP increased the feed conversion ratio (FCR) in comparison to the other diets (*p*<0.05). On the 60th day, the weight gain and SGR of the fish fed diets containing 1, 1.5, and 2% PHP were similar to those of the control group (*P*>0.05). However, the addition of 0.5% PHP led to significant reductions in weight gain and SGR compared to the control (*p*<0.05). The survival rate was not affected by dietary PHP after the 30th and 60th days (*p*>0.05).

Table 1: Growth performances and feed efficiency of rainbow trout fed different levels of Persian hogweed powder (PHP) after 30th and 60th days.

	Diets				
	Control	**PHP0.5%**	**PHP1%**	**PHP1.5%**	**PHP2%**
			the 30th day		
Initial weight (g)	23.35±0.36	23.86±0.34	23.66±0.11	23.22±0.89	22.81±0.34
Final weight (g)	50.15±0.96	48.20±1.42	49.15±2.27	49.17±1.10	50.62±0.62
Body weight (g)	26.79±0.96^{ab}	24.34±1.08^{b}	25.48±2.37^{ab}	25.95±1.17^{ab}	27.80±1.02^{a}
Weight gain (%)	114.76±4.73^{ab}	101.99±3.19^{b}	107.71±10.48^{ab}	111.89±8.15^{ab}	121.99±7.50^{a}
Specific growth rate (SGR) (%day^{-1})	2.54±0.01^{ab}	2.34±0.05^{b}	2.43±0.17^{ab}	2.49±0.12^{ab}	2.65±0.11^{a}
Feed conversion ratio (FCR)	0.62±0.02^{b}	0.83±0.16^{a}	0.66±0.06^{b}	0.66±0.07^{b}	0.58±0.02^{b}
Survival rate (%)	97.77±3.85	100.00±0.00	100.00±0.00	95.55±3.85	95.55±3.85
			the 60th day		
Initial weight (g)	23.35±0.36	23.86±0.34	23.66±0.11	23.22±0.89	22.81±0.34
Final weight (g)	76.00±0.96^{a}	72.49±0.46^{b}	75.43±2.45^{a}	72.01±1.15^{b}	72.48±1.13^{b}
Body weight (g)	52.64±0.54^{a}	48.63±0.69^{c}	51.77±2.55^{ab}	48.78±1.58^{bc}	49.66±1.75^{abc}
Weight gain (%)	225.44±1.57^{a}	203.87±5.53^{b}	218.78±5.53^{ab}	210.36±13.93^{ab}	217.92±13.77^{ab}
Specific growth rate (SGR) (%day^{-1})	1.96±0.01^{a}	1.85±0.03^{b}	1.93±0.06^{ab}	1.88±0.07^{ab}	1.92±0.07^{ab}
Feed conversion ratio (FCR)	0.78±0.03^{b}	1.00±0.11^{a}	0.92±0.05^{ab}	0.89±0.06^{ab}	0.93±0.09^{ab}
Survival rate (%)	97.77±3.85	100.00±0.00	100.00±0.00	95.55±3.85	95.55±3.85

All values are means of three replicates (tanks)/treatment ± standard deviation. Different superscript letters show significant differences (*p*<0.05).

Hematological indices

According to hematological indices (Table 2), the administration of 1 and 1.5% PHP enhanced the number of RBC and WBC, while these numbers decreased in the control and 0.5% PHP after the 30th day (*p*>0.05). Moreover, the addition of 2% PHP improved Hb and MCH values (*p*<0.05). The lowest values of Hb, Hct, MCV, and MCH were achieved by the dietary addition of 0.5% PHP (*p*>0.05). Furthermore, lymphocytes and monocytes were elevated significantly in the fish fed 1.5% and 1% PHP (86.33 and 6%), respectively (*p*<0.05). On the 60th day, RBC, MCV, and MCH were not influenced by different levels of PHP (*p*>0.05). The number of WBCs was significantly higher in the fish received 1.5% PHP than that in 0.5% PHP (*p*<0.05). The inclusion of 1, 1.5, and 2% PHP caused an increase in hemoglobin (*p*<0.05). MCHC and monocyte numbers significantly improved by the supplementation of 1% PHP compared to the control diet ($p<0.05$). Eosinophil and neutrophil did not change PHP levels during 60 days ($p>0.05$).

Table 2: Hematological indices of rainbow trout fed different levels of Persian hogweed powder (PHP) after the 30th and 60th days.

	Diets				
	Control	**PHP0.5%**	**PHP1%**	**PHP1.5%**	**PHP2%**
			the 30th day		
RBC ($\times10^6$/mm^3)	0.82±0.01^{bc}	0.79±0.02^{c}	0.86±0.01^{ab}	0.88±0.04^{a}	0.84±0.02^{abc}
Hb (g/l)	5.82±0.68^{b}	5.01±0.10^{c}	6.07±0.33^{b}	6.31±0.22^{ab}	6.78±0.18^{a}
Hct (%)	35.33±1.52^{a}	28.33±5.68^{b}	35.33±2.51^{a}	38.66±2.08^{a}	38.66±0.57^{a}
MCV (fl)	407.66±19.85^{ab}	355.00±67.22^{b}	426.33±21.00^{a}	436.00±1.00^{a}	459.66±14.22^{a}
MCH (pg)	67.30±8.68^{bc}	63.00±2.18^{c}	73.29±2.77^{ab}	71.30±2.54^{ab}	80.67±2.92^{a}
MCHC (%)	16.46±1.45	18.19±3.64	17.21±1.04	16.33±0.57	17.56±0.73
WBC ($\times10^3$/mm^3)	12.90±0.17^{c}	13.13±0.30^{abc}	13.40±0.30^{ab}	13.60±0.10^{a}	12.98±0.28^{bc}
Lymphocytes (%)	78.33±1.52^{b}	84.00±1.00^{ab}	83.33±2.51^{ab}	86.33±3.51^{a}	83.33±4.72^{ab}
Monocytes (%)	3.00±1.00^{b}	3.00±1.00^{b}	6.00±1.00^{a}	4.00±1.00^{ab}	4.00±1.00^{ab}
Neutrophil (%)	12.00±1.00	12.00±1.00	12.00±2.00	11.00±3.00	12.33±2.51
Eosinophil (%)	0.67±0.57	1.00±1.00	0.67±0.57	0.67±0.57	0.33±0.57
			the 60th day		
RBC ($\times10^6$/mm^3)	0.98±0.14	0.96±0.15	1.09±0.15	1.05±0.14	1.11±0.08
Hb (g/l)	4.15±0.10^{c}	4.52±0.23^{bc}	5.35±0.11^{a}	5.02±0.19^{ab}	5.00±0.64^{ab}
Hct (%)	40.00±1.00^{b}	40.33±1.52^{b}	41.00±1.00^{ab}	43.00±1.00^{a}	42.66±0.57^{ab}
MCV (fl)	410.95±55.76	426.61±78.76	379.54±49.26	412.96±51.86	380.06±34.32
MCH (pg)	42.80±6.81	47.94±9.54	49.51±5.79	48.12±4.77	45.23±6.76
MCHC (%)	10.14±0.44^{c}	11.21±0.16^{bc}	13.39±0.59^{a}	11.67±0.37^{b}	11.89±1.30^{b}
WBC ($\times10^3$/mm^3)	14.10±0.51^{ab}	13.23±0.35^{b}	13.66±0.20^{ab}	14.30±0.62^{a}	13.96±0.56^{ab}
Lymphocytes (%)	83.00±2.00	80.00±1.00	80.00±2.00	83.33±2.30	81.00±2.00
Monocytes (%)	4.00±0.00^{c}	5.33±0.57^{b}	6.33±0.57^{a}	5.33±0.57^{b}	4.67±0.57^{bc}
Neutrophil (%)	12.33±2.00	14.00±1.00	13.00±2.64	11.67±1.52	13.00±2.00
Eosinophil (%)	0.67±0.57	0.67±0.57	0.67±0.57	1.00±1.00	1.33±0.57

All values are means of three replicates (tanks)/treatment ± standard deviation. Different superscript letters show significant differences ($p<0.05$).

Biochemical indices

According to Table 3, there were no significant differences between cholesterol, triglyceride, glucose, ALT, and CAT concentrations among different PHP levels on the 30th day ($p>0.05$). AST levels rose in the fish fed the control diet ($p<0.05$). ALP concentrations displayed no reductions in the fish fed the control diet and 0.5% PHP compared to the other groups ($p<0.05$). SOD was enhanced by the administration of 1 and 1.5% PHP among different diets ($p<0.05$). Furthermore, feeding fish with diets containing 1, 1.5, and 2% PHP led to increased LDH activity ($p<0.05$).

In contrast, the addition of PHP to the diet reduced cholesterol and triglyceride values on the 60th day ($p<0.05$). Moreover, glucose, CAT, and SOD amounts were not changed by different levels of PHP inclusion ($p>0.05$). The control and 2% PHP diets caused no reductions in ALT and AST levels between different diets ($p<0.05$). Diets containing PHP lowered ALP levels in comparison to the control. Moreover, the LDH value rose in the fish that received 1 and 1.5% PHP ($p<0.05$).

Table 3: Biochemical indices of rainbow trout fed different levels of Persian hogweed powder (PHP) after the 30th and 60th days

	Diets				
	Control	PHP0.5%	PHP1%	PHP1.5%	PHP2%
			the 30th day		
Cholesterol (mg/dl)	394.62±34.52	402.15±24.79	380.53±55.39	377.67±23.27	383.62±25.84
Triglyceride (mg/dl)	262.44±44.12	175.54±55.61	217.65±37.17	220.81±58.95	223.82±70.95
Glucose (g/dl)	80.48±0.45	96.05±15.99	95.52±21.80	85.07±4.80	95.52±11.53
AST (u/L)	52.00±6.00^{a}	48.33±2.08^{ab}	42.00±3.00^{ab}	43.00±2.00^{ab}	41.66±8.96^{b}
ALT(u/L)	20.66±1.52	20.00±1.00	16.00±1.00	17.33±1.52	15.33±4.93
ALP(u/L)	765.33±2.51^{a}	772.33±33.50^{a}	731.00±9.00^{b}	688.00±15.00^{c}	684.00±11.53^{c}
CAT (u/mL)	99.33±1.52	106.00±18.00	103.00±9.53	106.33±0.57	106.33±11.45
SOD (u/mL)	45.00±2.00^{c}	48.33±2.08^{bc}	53.50±4.50^{a}	50.33±2.51^{ab}	47.00±1.00^{bc}
LDH (u/L)	429.00±6.00^{b}	428.00±23.00^{b}	487.66±2.51^{a}	486.33±0.57^{a}	456.66±30.33^{ab}
			the 60th day		
Cholesterol (mg/dl)	688.11±77.18^{a}	614.77±43.02^{ab}	549.28±58.94^{bc}	519.53±56.47^{bc}	481.83±21.09^{c}
Triglyceride (mg/dl)	370.80±78.38^{a}	253.12±61.01^{b}	264.73±39.85^{ab}	203.10±46.51^{b}	278.57±50.49^{ab}
Glucose (g/dl)	89.52±15.63	90.94±11.74	81.95±8.11	82.39±10.97	85.20±6.08
AST (u/L)	85.33±22.50^{a}	63.33±21.03^{b}	65.66±7.50^{b}	61.00±20.42^{b}	82.00±4.00^{a}
ALT(u/L)	29.50±1.50^{a}	22.00±1.00^{b}	17.00±3.00^{c}	17.00±3.46^{c}	29.33±1.52^{a}
ALP(u/L)	904.33±92.01^{a}	721.33±65.01^{b}	774.33±19.03^{b}	722.00±37.51^{b}	791.66±1.52^{b}
CAT (u/ml)	92.23±13.01	88.00±6.00	97.66±4.50	94.66±14.68	87.66±3.05
SOD (u/ml)	44.33±2.08	48.33±1.52	46.50±1.50	46.00±1.73	45.33±3.51
LDH (u/L)	581.66±13.03^{c}	585.66±90.91^{c}	784.33±4.04^{a}	812.33±23.50^{a}	654.33±56.00^{bc}

All values are means of three replicates (tanks)/treatment ± standard deviation. Different superscript letters show significant differences ($p<0.05$).

Immunological parameters

On the 30th day, immunological parameters indicated no changes in ACH50 and albumin concentrations between groups ($p>0.05$; Table 4). Diets containing 1 and 1.5% PHP led to an increase in lysozyme activity. Total protein and globulin enhanced in the fish fed 1, 1.5, and 2% PHP after the 30th day of feeding ($p<0.05$). On day 60, the ACH50 level was not influenced by dietary PHP ($p>0.05$). On the contrary, lysozyme activity increased by the addition of 1, 1.5, and 2% PHP to diets ($p<0.05$). Albumin values were higher in the fish fed PHP than in the control group. Total protein and globulin significantly improved in fish fed 1.5 and 2% PHP ($p<0.05$).

Table 4: Immunological responses of rainbow trout fed different levels of Persian hogweed powder (PHP) after the 30th and 60th days

	Diets				
	Control	**PHP0.5%**	**PHP1%**	**PHP1.5%**	**PHP2%**
			the 30th day		
ACH50 (U%)	137.66±1.52	137.00±1.00	135.33±0.57	136.33±3.51	134.00±3.00
Lysozyme (u/ml/min)	26.00±2.00^{b}	22.83±13.25^{b}	38.66±1.52^{a}	30.00±1.00^{ab}	26.33±5.13^{b}
Albumin (g/dl)	1.40±0.05	1.57±0.23	1.68±0.37	1.39±0.24	1.37±0.08
Total protein (g/dl)	2.69±0.50^{b}	2.78±0.83^{b}	3.32±0.19^{ab}	3.15±0.25^{ab}	3.95±2.18^{a}
Globulin (g/dl)	1.29±0.45^{b}	1.08±0.77^{b}	1.74±0.42^{ab}	1.75±0.37^{ab}	2.58±0.13^{a}
			the 60th day		
ACH50 (U%)	137.00±3.00	134.66±3.05	135.33±1.52	135.33±3.05	139.00±3.00
Lysozyme (u/ml/min)	32.33±3.51^{b}	31.00±31.60^{b}	40.33±2.08^{a}	40.33±1.52^{a}	36.33±5.03^{ab}
Albumin (g/dl)	1.79±0.18^{b}	2.04±0.12^{ab}	2.04±0.32^{ab}	2.44±0.20^{a}	1.95±0.36^{ab}
Total protein (g/dl)	4.47±0.62^{b}	4.04±0.29^{b}	4.52±0.60^{b}	6.67±0.46^{a}	5.93±0.51^{a}
Globulin (g/dl)	2.68±0.68^{b}	1.99±0.16^{b}	2.62±0.36^{b}	4.23±0.37^{a}	3.97±0.43^{a}

All values are means of three replicates (tanks)/treatment ± standard deviation. Different superscript letters show significant differences ($p<0.05$).

Body proximate composition

Whole-body fat content decreased significantly with the inclusion of PHP after the 30th day ($p<0.05$; Table 5). The fish fed diets containing different levels of PHP had higher body ash and moisture contents than the control ($p<0.05$). Contrarily, dry matter diminished in the fish fed PHP, and the lowest value was observed in fish that received 2% PHP ($p<0.05$). On day 60, body protein, fat, moisture, and dry matter contents were not affected by PHP inclusion ($p>0.05$). However, the addition of 1% PHP led to the highest value of body ash content ($p<0.05$).

Table 5: Proximate whole-body composition of rainbow trout fed different levels of Persian hogweed powder (PHP) after the 30th and 60th days

	Diets				
	Control	**PHP0.5%**	**PHP1%**	**PHP1.5%**	**PHP2%**
			the 30th day		
Protein (%)	58.68±2.05	57.98±0.88	55.53±0.88	58.91±2.33	58.80±4.90
Fat (%)	37.93±4.17^{a}	31.22±3.93^{b}	30.74±1.53^{b}	33.02±4.29^{ab}	30.39±1.23^{b}
Ash (%)	5.45±0.78^{b}	6.81±0.25^{a}	7.04±0.41^{a}	6.31±0.17^{ab}	6.42±0.55^{a}
Moisture (%)	72.38±5.06^{b}	75.24±0.80^{ab}	76.61±0.50^{ab}	76.19±0.22^{ab}	77.37±0.72^{a}
Dry matter (%)	27.61±5.06^{a}	24.75±0.80^{ab}	23.38±0.50^{ab}	23.80±0.22^{ab}	22.62±0.72^{b}
			the 60th day		
Protein (%)	57.98±0.68	55.36±1.79	55.71±4.42	56.63±3.80	56.65±2.19
Fat (%)	29.69±3.78	29.28±0.62	30.70±5.07	28.72±0.57	29.17±2.99
Ash (%)	8.46±0.27^{a}	7.89±0.60^{ab}	7.47±0.55^{b}	7.87±0.71^{ab}	8.71±0.12^{a}
Moisture (%)	74.12±0.93	74.44±0.08	72.80±2.20	74.06±1.01	74.16±2.22
Dry matter (%)	25.87±0.93	25.55±0.08	27.19±2.20	26.01±0.01	25.84±2.22

All values are means of three replicates (tanks)/treatment ± standard deviation. Different superscript letters show significant differences ($p<0.05$).

Bacterial challenge

According to the results of Figure 1, the survival rate of fish after the 2-w challenge with *Y. ruckeri* was significantly affected by different levels of PHP ($p<0.05$), and fish that received 1 and 1.5% PHP showed the highest rates of 59.25% and 55.55%, respectively. Moreover, the lowest survival rate was observed in groups that received the control (25.92%), 0.5% PHP (25.92%), and 2% PHP (37.03%) diets.

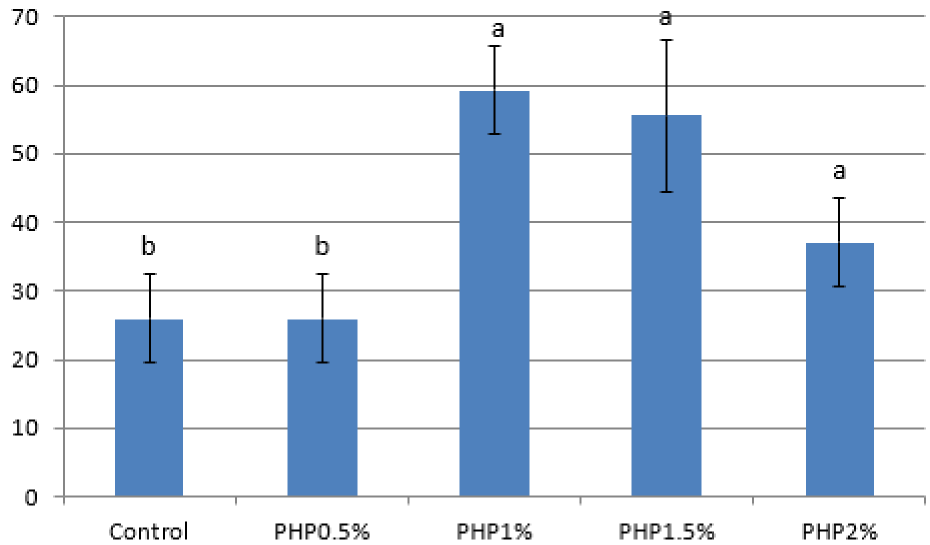

Figure 1: Survival rates (%) of rainbow trout (27 fish per tank) fed different levels of Persian hogweed powder (PHP) after 2 weeks of *Yersinia ruckeri* challenge.

Discussion

The hogweed plant is composed of 8.31% protein and 42.9% fiber and contains other micronutrients, such as Mg, Fe, Cu, Zn, Co, Cr, Na, Mn, K, Ca, P, and S (Tunçtürk and Özgökçe, 2015). Growth performance is expected to improve by hogweed administration considering the role of this plant in the stimulation of nutrient digestion and absorption (Eftekhari *et al*., 2018). In the present study, growth performance improved by the inclusion of 2% PHP during 30 days ($p<0.05$). On the 60th day, a slightly decreasing trend was observed in growth performance by the addition of PHP different levels ($p<0.05$). The reason for the decreased growth performance after the 60th day may be the loss of appetite in the fish fed PHP diets because of the pungent taste or herbal anti-nutritional elements in long term. Furthermore, herbal optimum doses should be considered before administration because of having anti-nutritional factors. The inclusion of 5 g kg^{-1} diet PHP resulted in the BWI, SGR, and FCR improvements in common carp (*C. carpio*) (Hoseinifar *et al*., 2016). Jamshidparvar *et al*. (2017) represented weight gain increase in broilers fed with 1, 1.5, 2, and 2.5 mL/l of hogweeds dissolved in water, but FCR was not affected by the treatments. In another study, Javandel *et al*. (2019) observed BWI and FCR enhancements in broilers that received a diet containing 0.75% hogweed. An increase in the villus height induced by hogweed led to better nutrient absorption (Amat *et al*., 1996). On the other hand, digestive enzymes (e.g. pepsin) are stimulated by hogweed and help improve nutrient digestion (Shahrani, 2006). There is also proof that bile salt secretion increased by herbs and caused viscosity reduction in the intestine (Lee *et al*., 2003; Manzanilla *et al*., 2004). On the contrary, Eftekhari *et al*. (2018) reported that growth performance and FCR did not change in broilers fed a diet supplemented with hogweed hydroalcoholic extract. Different results and optimum levels of hogweed could be obtained depending on the species.

Fish body composition represents health status and metabolism. The antihyperlipidemic properties of hogweed because of having flavonoids (Hajhashemi *et al*., 2009) probably caused the reduction of body fat on the 30th day. Moreover, the stimulation of bile could help fat digestion and lower fat residual in tissues. Furthermore, body ash elevation in diets containing different levels of PHP is probably related to the existence of minerals and fiber in this plant. After the 60th day of feeding with different levels of PHP, similar body composition was observed between groups, except for body ash.

Hematological parameters are indicators of fish health conditions. There are numerous studies suggesting increased WBCs and stimulation of the immune system by plants (Alishahi *et al*., 2011; Lin *et al*., 2011). Similarly, the addition of PHP led to improved hematological factors, such as RBC, WBC, and Hb on the 30th and 60th days in all levels, except for 0.5% PHP, showing that this level is probably insufficient for immune stimulation. The increased blood cells could be attributed to bioactive compounds in PHP, affecting hematopoietic tissues. When β-glucagon in herbal immunostimulants attaches to its specific receptor in WBCs, it activates the defense system by surrounding, killing, and digesting pathogens (Andrews *et al*., 2009). Thus, an increase in WBC count might be connected to fish protection (Sandnes *et al*., 1988). In the present research, lymphocyte and monocyte numbers rose in treatments containing PHP diets. It seems that PHP stimulates WBC production because of having furanocoumarins and flavonoids (Sharififar *et al*., 2009). These two bioactive compounds cause a humoral response by inducing macrophages and lymphocytes. In tissues, monocytes turn into macrophages to do phagocytosis, by which fish responds initially to infection (Sivagurunathan *et al*., 2011). On day 60, the highest number of monocytes was achieved in the fish fed 1% PHP (p<0.05). Similar results were reported by the injection of 6, 60, and 600 mg kg^{-1} BW of hogweed aqueous extract (HAE) into common carp (Soltanian *et al*., 2017). Moreover, the administration of 20 mg mL^{-1} of HAE led to increased macrophage activity in mice (Naeini *et al*., 2013), which is in accordance with our work.

Furthermore, lysozyme activity was enhanced in the fish fed 1 and 1.5% PHP during 30 and 60 days. High lysozyme activity improves fish immunity considering the role of lysozyme in destroying the bacterial peptidoglycan cell wall. Similar results were obtained by the addition of 5 g kg^{-1} diet of common carp, which increased lysozyme and ACH50 levels (Hoseinifar *et al*., 2016). Soltanian *et al*. (2017) indicated higher lysozyme activity in carp injected with 6, 60, and 600 mg kg^{-1} BW of HAE. The elevated serum protein in the present study is confirmed by the idea of Rao *et al*. (2006), who found immunostimulants stimulated lysozyme and complement synthesis by increasing serum proteins. Increased levels of albumin, total protein, and globulin in fish are related to the stimulation of the non-specific immune system, as observed in this study after 60 days. On the 30^{th} day, the dietary inclusion of PHP improved total protein and globulin, except for 0.5% PHP. After 60 days, the albumin value was also elevated in groups fed PHP.

On the contrary, serum cholesterol and triglyceride decreased by PHP addition. In agreement with our results, the hypolipidemic activity of hogweeds was observed in different studies (Panahi *et al*., 2011; Kheiri *et al*., 2014; Eftekhari *et al*., 2018). According to Javandel *et al*. (2019), this reduction probably happens because of inhibiting intestinal absorption of cholesterol.

Damage to the membrane of liver cells causes an increase in the release of hepatic enzymes in the blood. Therefore, measuring the level of hepatic enzymes in serum can be evaluated as an indicator of liver health (Banaee *et al*., 2011). According to our results, AST and ALP declined by the dietary supplementation of PHP after 30 days, suggesting liver health protection of PHP. On day 60, higher AST and ALT values were observed in 2% PHP and the control than the other doses of PHP. The administration of herbs in fish diets should be considered in optimum doses, especially in long term, otherwise, it can harm tissues (e.g., the liver) due to the presence of anti-nutritional factors (Rezaie and Jaymand, 2002); though, there were no signs of damage to the liver during 60 days in this study. The inclusion of 50, 100, and 200 mg kg^{-1} of HAE in mice did not affect AST and ALT levels after 5 days (Sharififar *et al*., 2009).

SOD enzyme is part of the important antioxidant defense that catalyzes superoxide radicals. Therefore, increased SOD values in the fish fed 1 and 1.5% PHP might show that it could protect fish by improving the antioxidant system capacity. The antioxidant activity of phenol compounds existing in hogweeds was previously found by Windisch *et al*. (2008). Moreover, furanocoumarins in hogweeds inhibit linoleic acid peroxidation and moderate antioxidant activity (Souri *et al*., 2004).

A 2-week challenge against *Y. ruckeri* suggested higher fish survival rates in 1% (59.25%) and 1.5% PHP (55.55%) treatments, respectively. Apparently, the antibacterial effect of PHP is related to its bioactive compounds, such as flavonoids and coumarins, improving the fish immune system by increasing the number of WBCs, lysozyme activity, and antioxidant capacity. The levels of administration may differ depending on the species and the form of using hogweeds. For instance, the antimicrobial activity of *H. persicum* was also shown in broilers in the forms of methanolic extracts, volatile oil, and hydroalcoholic extract at concentrations of 15 mg, 26.67 µg/mL, and 200-400 mg/kg, respectively (Kousha and Bayat 2012; Zandi and Aboee-Mehrizi, 2012; Eftekhari *et al*., 2018). *In vitro* studies also reported the antibacterial effect of hogweed essential oil as well as its alcoholic and aqueous extracts on *Acinetobacter baumanni*, *Streptococcus mutans*, *Enterococcus faecalis*, *Bacillus polymixa*, *Bacillus subtilis*, and *Staphylococcus aureus* (Javadian *et al*., 2016; Abdoli *et al*., 2017). Moreover, Nazemi *et al*. (2004) exhibited the similarity of hogweed extract to ampicillin.

Altogether, PHP caused immunity against bacterial infection probably by stimulating specific (*i.e.* lymphocytes) and non-specific (*i.e.* macrophages, lysozyme, and serum proteins) immune indices of *O. mykiss* in the present study. The growth performance was also improved by dietary 2% PHP, during 30 days of the feeding trial. However, it appears that long-term use of PHP could adversely affect growth. An optimum level of hogweed is short-term (30 days) inclusion of 1.5% PHP in the rainbow trout diet as an immunostimulant with no damage to the liver.

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