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ORIGINAL ARTICLE

Effects of *Hypericum perforatum* powder on immune responses, hepatic enzymes levels, and resistance against *Yersinia ruckeri* in rainbow trout

Najmeh TABASINEZHAD[1], Farid FIROUZBAKHSH[*1], Khosro JANI-KHALILI[1]

Department of Fisheries, Faculty of Animal Science and Fisheries, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.

Correspondence
f.firouzbakhsh@sanru.ac.ir

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Abstract

Immunostimulants play a crucial role in activating nonspecific defense mechanisms that protect fish against pathogens and are valuable for controlling fish diseases. The aim of this study was to investigate the effects of *Hypericum perforatum* powder on immunological responses, biochemical parameters, hepatic enzymes levels, and resistance against *Yeserina ruckeri* in rainbow trout (*Oncorhynchus mykiss*). Fish with an average weight of 5±1g were fed a diet supplemented with a powder of *H. perforatum* at doses of 0 (control), 0.5% (H0.5%), 1% (H1%), 2% (H2%), and 4% (H4%) for 8 weeks. At the end of 8 weeks, fish were challenged with *Y. ruckeri,* and mortality was recorded for up to 10 days after the challenge. Immunological (lysozyme and alternative complement activity) and biochemical (glucose, total protein, albumin and globulin) parameters, and hepatic enzymes levels (LDH, AST, ALT and ALP) were measured before and after challenge. Result showed that supplementation with *H. perforatum* significantly increased serum lysozyme, complement activity, and albumin levels ($P<0.05$) and reduced glucose levels in the treatment groups (per-challenge and post-challenge) compared with the control ($P<0.05$). However, AST and LDH levels were significantly increased in the H2% and H4% groups compared with the control ($P<0.05$). After challenge, the highest survival rates were recorded in groups that received 0.5%, 1%, and 2% *H. perforatum* compared with the control ($P<0.05$). The Results of the present study demonstrated that feeding *Oncorhynchus mykiss* a diet containing *H. perforatum* powder at lower dose (0.5% and 1%) improved the immune status, and increased the survival rate and resistance to yersiniosis .Therefore, this plant can be used in aquaculture to improve fish health, reduce mortality, and increase production efficiency.

Keywords: Medicinal plants, Lysozyme, Complement, Yersiniosis, *Hypericum perforatum*, Aquaculture

INTRODUCTION

High densities under cultivation conditions and environmental stressors can negatively affect the immune system and fish health (Bilen et al. 2020; Avani et al. 2022; Saha et al. 2023). As a result, resistance to pathogens is reduced, which the basis of outbreaks of diseases is found in cultivation environments (Subasinghe 2009; Pothriaj et al. 2023). These problems can increase mortality especially in larval stage, and reduce aquaculture production efficiency (Mousavi-Sabet et al. 2019). Yersiniosis or enteric red mouth disease is a systemic infection that causes significant economic losses in salmonids aquaculture worldwide. *Yersinia ruckeri* is a gram-negative bacterium that causes disease in salmonids, especially rainbow trout (Furones et al. 1993). In aquaculture, antibiotics and disinfectants are used to control infectious diseases (Cilinger et al. 2017; Saha et al. 2023). However, the use of antibiotics and chemotherapy has other problems, including high costs, the presence of drug-resistant bacteria, immunosuppression, environmental pollution (Bricknell et al. 2005; Bilen et al. 2016). On the other hand, immunostimulants are biological extracts and synthetic chemicals that stimulate specific and non-specific defense systems in fish and enhance resistance to pathogens during stressful periods (Saha et al. 2023; Pothriaj et al. 2023). Recently, natural products, such as medicinal plants, have attracted the attention of many researchers worldwide due to their low cost, reliability, safety, non-toxicity, and biocompatibility (Nazeemashahul et al. 2023). Many

studies have shown that plants can act as immunostimulants and increase the hematological parameters and immunological responses of rainbow trout (Nya and Austin 2011; Mehrabi & Firouzbakhsh 2020; Bilen et al. 2020; Mohammadi et al. 2020). In this regard, scores of plants have been tested and used in aquaculture with acceptable results as immunestimulant, anti-bacterial agents, and growth-promoting (Citarasu et al. 2003; Kumar et at. 2012; Cilinger et al. 2017).

Hypericum perforatum (St. John's Wort) is a flowering plant of the genus *Hypericum* (Egilmez et al. 2015) is widely distributed in Europe, Asia (as Iran), northern Africa, and the United States (Gambarana et al. 1999). *H. perforatum* contains active principal compounds such as naphthodianthron, hypericin, pseudohypericin, flavonoid, flavon, and essential (Nahrstedt & Butterweek 1997). The medical effects of *H. perforatum* include antibiotics (Mennini & Gobbi 2004), anti-depression (Chatterjee et al. 1998; Zou et al. 2004), antiviral (Meruelo et al. 1988), antioxidant (Cuzzocrea et al. 2001; Benedi et al. 2004), anti-stress (Kumar et al 2001a; Franklin et al. 2004), anti-inflammatory (Kumar et al. 2001b), and anticancer (Hostanska et al. 2003). For the first time, Wilasrusmee et al. (2002) verified the immunomodulatory effect of *H. perforatum* in mice. In other studies, this plant exerted positive effects on the immune system in rat (Aghili et al. 2014), hen (Jiang et al. 2012), chicken (Shang et al. 2012), and fish (Cilinger et al. 2017; Ghiasi et al. 2018; Farzollahi et al. 2019; Mohammadi et al. 2020).

Rainbow trout, *Oncorhynchus mykiss* (Walbaum 1792) is a freshwater fish (Coad 2021; Abdoli et al. 2022), and Iran is among the pioneers in the cultivation of this species. Rainbow trout is currently one of the main species in Iranian aquaculture production and has a significant influence on the economy and food security in the country (Avani et al. 2022). Therefore the present study aimed to evaluate the effects of dietary *H. perforatum* supplementation on immune responses, hepatic enzymes levels, biochemical parameters, and resistance against *Y. ruckeri* in rainbow trout.

MATERIALS AND METHODS

Preparation of *H. perforatum* and feeding: The aerial parts (leaves and flowers) of *H. perforatum* were collected from a cultivation area (Mazandaran, Iran). The plant was dried (under shade), ground, and the powder was then kept in a dry, clean, and airtight container. In order to prepare the diets, the commercial diet (Bayza) for rainbow trout was ground and mixed with *H. perforatum* powder to achieve 0 % (control), 0.5 % (H0.5%), 1% (H1%), 2% (H2%), and 4% (H4%) of feed. Then, water was added to make a paste. The paste was generated using a meat grinder, and strings were pelletized. Finally, the diets were dried, sealed in plastic bags, and stored at 4°C (Navarrete et al. 2010) until feeding.

Fish, rearing conditions, and experimental setup: Fish (average weight 5±1g) were procured from a commercial fish farm in Mazandaran province, northern Iran. The fish were transported to a laboratory. Before the initiation of the feeding experiment, the fish were acclimated to the experimental conditions for 2 weeks and fed a standard commercial diet. After the acclimation period, the fish (300) were randomly distributed in 15 fiberglass tanks (300L). During the experiment, aeration was provided in each tank, and 70% of the water was exchanged daily. The tanks were cleaned daily by siphoning out fish feces and uneaten food debris. The fish were fed three times a day at 9.00 a.m, 1.00 p.m, and 4.00 p.m at 3% of their body weight until the end of the pre-challenge experiment. After the challenge fish were fed a *H. perforatum* diet for (10 days) at same ratio described above, the control fish were fed a basal diet without *H. perforatum*. During the experimental period, the water quality parameters were monitored daily and maintained at optimal levels by regular water exchange (temperature 15 °C, dissolved oxygen 8.26 mg/L^{-1}, hardness 600ppt, pH 7.5 4 unit).

Blood sampling: At the end of 8 weeks, blood samples were collected from the specimens. After anesthetization with 150 ppm gillyflower powder,

Table 1. Lysozyme and complement activity of rainbow trout after feeding with different levels of *H. perforatum* for 8 weeks (per-challenge) and post-challenge with *Y. ruckeri.*

Groups	Lysozyme Mg/ml (per-challeng)	Lysozyme Mg/ml (post-challenge)	ACH50 (per-challeng)	ACH50 (post-challenge)
Control	435.58±1.9^{a}	433.54±4.1^{a}	87.70±1.8^{a}	74.68±3.1^{a}
H0.5%	436.38±2.0^{b}	435.29±3.2^{c}	210.11±1.5^{c}	131.06±1.6^{c}
H1%	436.38±1.9^{b}	435.29±2.0^{c}	146.51±1.1^{b}	120.41±1.9^{bc}
H2%	436.38±3.1^{b}	433.70±3.2^{a}	144.86±2.1^{b}	114.69±2.5^{bc}
H4%	435.58±1.9^{a}	433.69±2.6^{a}	138.63±1.6^{b}	92.76±2.1^{ab}

Data are presented as mean±S.D. Data in the same columns with different superscript letters are significantly different (P<0.05).

blood samples were collected from each group of five randomly selected fish (n=5, pre-challenge and post-challenge) thorough caudal-vein puncture using sterile syringes. To prevent clotting, samples were transferred into sterile vachette tubes containing heparin as an anticoagulant. Blood samples were also collected without heparin, allowed to clot for 2 h at room temperature, centrifuged at 6000 rpm for 15 min, and stored at -80^{0}C.

Serological parameters: Serum glucose, total protein, albumin, globulin, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels were determined using commercial kits (Zist Shimi and Pars Azmoon, Terran, Iran). The serum alternative complement activity (ACH50) was estimated according to the methodology of Yano (1992). Serum lysozyme activity was determined following the method of Ellis (1990), which a turbidimetric test is based on the lysis of *Micrococcus lysodeikticus* (Sigma) exposed to lysozyme in a 96-well plate.

Challenge test: After 8 weeks, randomly 30 fish from each group were challenged intraperitoneally with 0.1ml of live *Y. ruckeri* containing 1.2×10^{8} CFU ml (Akhlaghi & Sharif Yazdi 2008). Mortality was recorded up to 10 days.

Fish survival: At the end of the trial (after challenge), the survival rate (SR) was calculated according to standard procedures using the following formula:

SR= [number live fish/number initial fish]×100

Statistical analysis: All statistical analyses were performed using SPSS 17 software (SPSS Inc., Chicago, IL, USA). Firstly, the variance of data was analyzed using one-way ANOVA, and Duncan's multiple range test was then performed to determine significant differences among groups. Duncan test verified significant differences between the treatment replicates at a confidence level of 95% (P<0.05).

RESULTS

Immune responses

Lysozyme activity: According to Table 1, a significantly higher lysozyme activity was observed in the treatment groups, except the H4% group, compared with the control in the pre-challenge (P<0.05). Lysozyme activity after challenge was significantly higher in all treatment groups than in the control group (P<0.05).

Alternative complement activity (ACH50): The highest compliment activity was observed in the H0.5% in pre-challenge (P<0.05). After challenge, significantly higher complement activity was observed in the treatment groups (except in the H4% group) compared with the control (P<0.05), (Table1).

Biochemical parameters: Glucose levels were significantly lower in all treated groups than in the control (P<0.05) for all assay durations (per and post-challenge), (Table 2). Total protein and globulin levels did not increase in the treated groups compared with the control (P>0.05) (per and post-challenge), but maximum levels of total protein and globulin were recorded in the treated groups. Albumin content was significantly higher in fish fed the 0.5% *H. perforatum* diet (per-challenge) (P<0.05). There was no significant increase in other groups compared with the control.

Table 2. Biochemical indices of rainbow trout after feeding with different levels of *H. perforatum* for 8 weeks (per-challenge) and post-challenge with *Y. ruckeri.*

Period	Groups	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Glucose (g/dl)
Per-challenge	Control	4.02±0.74	2.02±0.34^{a}	1.99±0.41	96.56±1.91^{b}
	H0.5%	4.62±0.08	2.50±0.10^{b}	2.12±0.02	72.37±2.67^{a}
	H1%	4.39±0.08	2.24±0.10^{ab}	2.15±0.03	68.36±2.07^{a}
	H2%	4.35±0.08	2.35±0.13^{ab}	1.99±0.07	67.80±4.61^{a}
	H4%	4.31±0.15	2.35±0.20^{ab}	1.94±0.17	71.43±2.73^{a}
Post-challenge	Control	3.20±0.34	1.90±0.29^{a}	1.16±0.41	55.57±3.75^{b}
	H0.5%	3.50±0.12	2.24±0.13^{b}	1.26±0.04	28.76±2.12^{a}
	H1%	3.52±0.06	2.24±0.10^{b}	1.28±0.12	27.92±2.50^{a}
	H2%	3.24±0.11	2.21±0.12^{ab}	1.12±0.01	28.98±2.37^{a}
	H4%	3.18±0.03	2.18±0.11^{ab}	0.99±0.09	29.63±1.18^{a}

Data presented as mean±S.D. Data in the same columns with different superscript letters are significantly different (P<0.05).

Table 3. Hepatic enzymes of rainbow trout after feeding with different levels of *H. perforatum* for 8 weeks (per-challenge) and post-challenge with *Y. ruckeri.*

Period	Groups	AST (U/l)	ALT (U/l)	ALP (U/l)	LDH (U/l)
Per-challenge	Control	24.76±1.15^{ab}	3.13±0.72	817.33±36.54	1256.67±86.2^{a}
	H0.5%	20.54±0.93^{a}	2.86±0.66	666.1±36.59	1130.01±95.33^{a}
	H1%	20.90±1.23^{a}	2.33±0.80	746.67±42.37	1051.33±74.02^{a}
	H2%	37.33±0.86^{b}	3.06±0.68	674.33±40.21	1663.33±97.69^{b}
	H4%	65.33±1.65^{c}	3.16±0.82	819.02±37.22	2061.05±91.14^{c}
Post-challenge	Control	54.66±14.23^{ab}	5.06±0.42^{ab}	156.33±18.06	1899±112.2
	H0 .5%	19.03±12.59^{a}	3.00±0.65^{a}	119.67±17.23	1623±125.02
	H 1%	32.86±13.24^{ab}	3.06±0.31^{a}	117.33±15.06	2499±0.98
	H 2%	76.33±12.67^{b}	3.40±0.45^{a}	121.67±17.42	2559±118.23
	H 4%	77.24±13.20^{b}	6.63±0.44^{b}	126.69±17.08	2772±0.86

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase; Data represent as mean±S.D. Data in the same columns with different superscript letters are significantly different (P<0.05).

During the post-challenge period, albumin levels weresignificantly higher in the H0.5% and H1% groups than in the control (P<0.05), (Table 2).

Hepatic enzymes: The results showed that there were no significant changes in ALT and ALP in any of the treatment groups compared with the control after 8 weeks (P>0.05). The activities of AST and LDH were significantly increased in the treatment groups (AST in H4% group and LDH in H2% and H4% group) compared with the control and other treatment groups at the end of 8 weeks (P<0.05). Based on the results of after challenging, we found no significant differences in AST, ALT, ALP, and LDH levels between fish fed a food-enriched *H. perforatum* and control fish (P>0.05) (Table 3).

Fish survival: After the challenge, survival rates were higher in the H0.5% (45.39), H1% (71.33), and H2% (41.36) groups compared with the control (P<0.05). Moreover, the difference between the H4% (31.65) and control groups was not significant (Fig. 1).

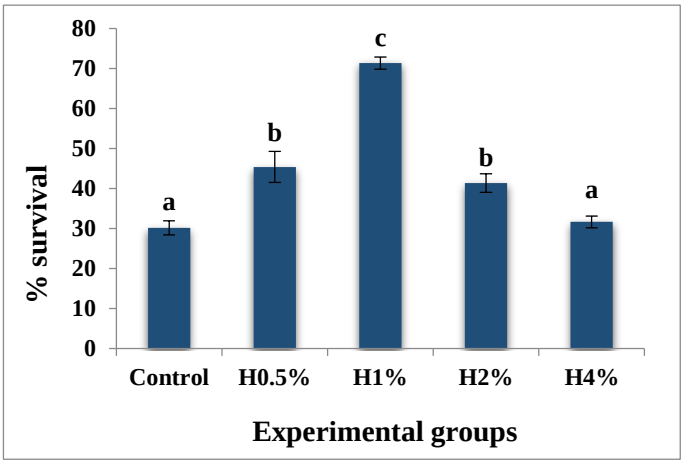

Fig.1. Survival of rainbow trout fed different levels of *H. perforatum* after challenging. Data represent the mean±S.D. Data in the same row with different superscript letters are significantly different (P<0.05).

DISCUSSION

Innate immunity is essential for fish survival, and immunostimulants help reduce disease-related mortality in aquaculture, particularly in the larval stage (Pothriaj et al. 2023). Several studies have demonstrated that immunostimulants are beneficial for enhancing and improving immunity and disease resistance in fish; increasing the immune response can also be an effective strategy for increasing fish performance (Saha et al. 2023; Nazeemashahul et al. 2023). This study illustrated that the inclusion of *H. perforatum* in the diet through immunostimulatory effects improves the health and immune system of rainbow trout. Based on the findings, lysozyme activity was significantly increased in all treatment groups except the H4% compared with the control. This is in agreement with Mehrabi & Firouzbakhsh (2020) who investigated the effects of the powder of two medicinal plants (*Aloe barbadensis* and *Urtica dioica*) on growth performance and immune responses in rainbow trout (0.5, 1, and 1.5% Aloe vera powder and 0.5, 1, and 1.5% of nettle powder). Based on their results, serum lysozyme and complement system activities increased with dietary supplementation, and the best results were found with 1.5% aloe vera and 0.5% nettle. In other studies, researchers demonstrated the effects of plants on increasing lysozyme activity and improving the immune system of rainbow trout (Awad et al. 2013; Haghighi & Rohany 2013; Adel et al. 2016).

Lysozyme is a fish defense element that causes lysis of bacteria and activation of the complement system (Magnadottir 2006). This is a strong antibacterial enzyme in the non-specific immune system that prevents the formation of bacterial colonies by digesting the bacterial cell membrane (Van Hai 2015). Therefore, an increase in serum lysozyme levels indicates an improvement in the fish's immune system, which increases resistance to infections. In addition, studies have shown that an increase in lysozyme levels is generally the result of an increase in the number of phagocyte cells (Sahu et al. 2007). According to Previous research, effective compounds of *H. perforatum* (flavonoids and tanen) can increase immune parameters (Jiang et al. 2012). These compounds protect the phagocytic cell membrane against free radicals and can improve the function of these cells through increased lysozyme and complement activity, thereby improving immune system function (Nazeemashahul et al. 2023).

Moreover, the results of this study indicated that the alternative complement activity (ACH50) was significantly higher in the treatment groups than in the control, especially in the H0.5% and H1% groups. Consistent with our findings, Farzollahi et al. (2019) reported that supplementation with combined levels of 1.5 % extract of *H. perforatum* and *Cichorium intybus* in diet of rainbow trout resulted in the highest levels of ACH50 activity. Contrary to our results, Azizi et al. (2020) reported that ACH50 and lysozyme levels in of rainbow trout fed 3% *H. perforatum* were not significantly different from those of the control group. Difference in the results of various studies can be due to differences in the amount of plant, effective components of plant, form of plant (powder, extract or oil), life stage, weight and age of the experimented fish, and experimental conditions.

In addition, this study demonstrate that a *H. perforatum* diet does not significantly increase total protein and globulin levels in rainbow trout. This finding is consistent with Nya and Austin (2011), who evaluated the effects of garlic (0.5g and 1g) on total protein levels in rainbow trout. Moreover, based on our results, albumin levels were significantly increased in the treatment groups (H0.5% per-challenge, H0.5% and H1% post-challenge) compared with the control. Other studies reported increased albumin levels in rainbow trout and common carp following diet supplementation with *Mentha piperita, Inula helenium, Tussilago farfara, Brassica nigra, Echinacea purpurea and Chelidoniume majus* (Mohamad & Abasali 2010; Adel et al. 2016). Albumin, the most abundant protein in vertebrate blood plasma, functions as a carrier for various nutrients, metabolites, and xenobiotics (peters 1995). Therefore, increased albumin levels may indicate improved fish health. In addition, the results of this study demonstrated that glucose levels was

significantly lower in the treatment groups than in the control. Consistent with our results, another researcher observed decreased glucose levels in their experiments after feeding fish with *magnifera indica* (Sahu et al. 2007) and *Ocimum basilicum* (Amirkhani & Firouzbakhsh 2015). Environmental stressors under culture conditions can cause disorders in the production of blood cells and decrease immunity in fish (Saha et al. 2023). *H. perforatum* has anti-stress characteristics that cause decreased stress in fish during the experimental period, leading to a decrease in glucose levels and improving the health of rainbow trout.

Based on the results, the levels of ALT and ALP enzymes were not significantly different between the treatment and control groups. This result indicates the lack of effect of *H. perforatum* on the levels of these enzymes, which can be considered a positive finding. The liver is involved in many metabolic processes, including protein synthesis and detoxification. In addition, this organ produces bile and certain hormones, and plays an important role in removing toxins from the body (Milkarizi et al. 2023). However, these processes are performed via the production of hepatic enzymes. Without these enzymes, these chemical processes would not proceed rapidly (Mohammadi et al. 2021). According to studies, the decrease or lack of increase in the levels of enzymes in fish plasma may be due to the effects of flavonoids and antioxidants in plants (Nazeemashahul et al. 2023) on the physiological function of cell membranes in different tissues, especially liver tissue (Teschke & Eickhoff 2015). In fact, the presence of flavonoids and antioxidants in plants may increase the antioxidant capacity of cells and the stability of cell membranes, thereby preventing intracellular enzymes from leaking into the blood (Banaei et al. 2015; Pothriaj et al. 2023). Although, in this study AST and LDH levels were significantly increased in the H2% and H4% groups. Therefore, the results illustrated that 2% and 4% *H. perforatum* had a negative impact on some hepatic enzymes (AST and LDH) in rainbow trout. Hepatic enzymes are normally found inside liver cells. When the liver is damaged, liver cells release enzymes into the blood, and elevated enzyme levels are markers of liver damage (Milkarizi et al. 2023). Moreover, most reports of the toxic effects of medicinal plants or their products have been attributed to hepatotoxicity (Teschke & Eickhoff 2015). The severity ranged from increased hepatic enzyme levels to liver necrosis. This may be related to liver dysfunction due to the presence of anti-nutrients in plants compounds (Teschke & Eickhoff 2015) and high plant dosages. Therefore, investigating the hepatotoxicity of medicinal plants is important for preventing the occurrence of such complications (Milkarizi et al. 2023). Similarly, complications occurred in rats that received 100 mg/kg *H. perforatum* extract (Vattikuti & Ciddi 2005). Moreover, Mohammadi et al. (2021) reported that the use of high concentrations (20 mg/kg body weight) of senile aqueous extract increased hepatic enzyme levels (GGT, ALP) and liver damage in mice. In another study, an increase in bile flow and alkaline phosphatase was observed after peppermint oil treatment in rats (Vo et al. 2003).

According to the results of the present study, the *H. perforatum* diet (Except 4%) significantly increased the survival rate of rainbow trout and resistance to *Y. ruckeri*. This may be due to the enhancement of the nonspecific immune system of fish by *H. perforatum*. This plant has a large amount of hypersin and flavonoids that have antioxidant, antibiotic, anti-stress, anti- inflammatory, and immunostimulatory effects (Tang et al. 1990; Raso et al. 2002; Winkler et al. 2004; Benedi et al. 2004; Silva et al. 2004). Consistent with our results, Heydari et al. (2020), who investigated effects of *Mentha longifolia* extract (0.1%, 0.2% and 0.3%) on blood (RBC and WBC counts, hematocrit, hemoglobin) and immune parameters (complement, lysozyme, neutrophil, respiratory burst, total protein, and albumin) and disease resistance against yersiniosis in rainbow trout. The results of their study indicated that the use of *M. longifolia* hydroalcoholic extract in rainbow trout can be effective for the regulation of immunity, expression of immune-related genes, and increased mucosal immunity against *Y. ruckeri.* In addition, Citarasu et al. (2010) reported

that flavones and flavonoids in plants can improve leukocyte production or stimulate their proliferation, thereby increasing fish immunity. In other words, some components involved in the nonspecific immune system of fish are strengthened (Zou et al. 2004; Pothriaj et al. 2023). The limitations of this study include the lack of histopathological evaluation of liver tissue and the expression of immune-related genes. Therefore, histopathological examinations and the expression of immune-related genes should be considered in future studies.

CONCLUSIONS

Rainbow trout is an economically important species in the aquaculture industry, providing nutrition and livelihoods to the people. Therefore, reducing mortality during cultivation and increasing fish production are essential. This study demonstrated that *H. perforatum* particularly at 0.5% and 1% doses, as an immunotimolator can improve immune system function in rainbow trout. Therefore, the mechanism of action of this plant is dose-dependent. Moreover, Aghili et al. (2014) have exhibited evidences about the mechanism of action of *H. perforatum* is a dose-dependent influence on the immune system of rats, and our result is in line with this finding. In addition, the results of the present study illustrated that after pathogenicity in fish fed *H. perforatum*, complications and mortality rates decreased, whereas survival rates increased, which could be due to the antibiotic and anti-inflammatory characteristics of this plant. In conclusion, the use of *H. perforatum* (0.5% and 1%) in the diet of rainbow trout has a positive impact on the immune status and health of this species that extremely led to enhanced production efficiency in aquaculture.

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مقاله کامل

اثرات پودر گیاه علف چای (*Hypericum perforatum*) بر پاسخ‌های ایمنی، سطح آنزیم‌های کبدی و مقاومت در برابر *Yersinia ruckeri* در ماهی قزل آلای رنگین کمان

نجمه طبسی‌نژاد[1]،فرید فیروزبخش*[1]، خسرو جانی خلیلی[1]

[1]گروه شیلات، دانشکده علوم دامی و شیلات، دانشگاه علوم کشاورزی و منابع طبیعی ساری، ساری، ایران.

چکیده: محرک‌های ایمنی نقش مهمی در فعال کردن مکانیسم‌های دفاعی غیراختصاصی ماهیان در برابر عوامل بیماری‌زا دارند و برای کنترل بیماری‌های ماهی ارزشمند هستند. هدف از انجام مطالعه حاضر، ارزیابی اثر پودر گیاه علف‌چای (*Hypericum perforatum*) بر پاسخ‌های ایمنی، سطح آنزیم‌های کبدی، و مقاومت در برابر باکتری *Yersinia ruckeri* در ماهی قزل‌آلای رنگین‌کمان (*Oncorhynchus mykiss*) بود. ماهی‌ها با میانگین وزنی ۱±۵ گرم به‌مدت ۸ هفته با جیره غذایی حاوی صفر درصد (کنترل)، ۰/۵ درصد (H0.5%)، ۱ درصد (H1%)، ۲ درصد (H2%)، و ۴ درصد (H4%) پودر گیاه *H. perforatum* غذادهی شدند. در پایان ۸ هفته، باکتری *Y. ruckeri* به‌صورت داخل صفاقی به ماهیان (از هر گروه ۳۰ عدد) تزریق شد و میزان مرگ و میر به‌مدت ۱۰ روز به ثبت رسید. به‌علاوه، پارامترهای ایمنی (لایزوزیم و فعالیت کمپلمان)، بیوشیمیایی (گلوکز، پروتئین تام، آلبومین و گلوبولین)، و سطح آنزیم‌های کبدی (LDH, AST, ALT, ALP) قبل و بعد از بیماری‌زایی با باکتری مورد ارزیابی قرار گرفت. نتایج نشان داد که *H. perforatum* باعث افزایش معنی‌دار سطوح لایزوزیم، کمپلمان و آلبومین و کاهش میزان گلوکز (قبل و بعد از چلنج با باکتری) در ماهیان شد ($P<0/05$). از طرفی، سطوح آنزیم‌های AST و LDH در گروه‌های H2% و H4% نسبت به گروه کنترل افزایش معنی‌داری نشان داد ($P<0/05$). همچنین بعد از پایان دوره، بالاترین نرخ بقاء در گروه‌های H0.5%، H1%، و H2% نسبت به گروه کنترل مشاهده شد ($P<0/05$). نتاج این مطالعه نشان می‌دهد که تغذیه ماهی قزل‌آلای رنگین‌کمان با دوزهای پایین گیاه علف چای (۰/۵ و ۱ درصد) باعث بهبود کارایی سیستم ایمنی و افزایش نرخ بازماندگی در دوره چالش با باکتری *Y. ruckeri* خواهد شد. بنابراین، این گیاه می‌تواند با کاهش مرگ و میر و افزایش سلامت ماهی قزل‌آلای رنگین‌کمان، موجب افزایش تولید و بهره‌وری در آبزی‌پروری گردد.

کلمات‌کلیدی: گیاهان دارویی، لیزوزیم، کمپلمان، یرسینیوزیس، علف‌چای، آبزی‌پروری