Growth performance and haematological and immunological indices of rainbow trout (*Oncorhynchus mykiss*) fingerlings supplemented with dietary *Ferulago angulata* (Schlecht) Boiss

Somayeh Bohlouli[1], Ehsan Sadeghi[2]

[1]Department of Veterinary Medicine, Faculty of Agriculture,
Kermanshah Branch, Islamic Azad University, Kermanshah, Iran
[2] Kermanshah University of Medical Sciences, Research Center for Environmental Determinants of Health (RCEDH), Kermanshah, Iran

Received November 10, 2015
Accepted September 8, 2016

Abstract

In this study, the effects of *Ferulago angulata* extract on the growth, haematological, and immunological indices of rainbow trout (*Oncorhynchus mykiss*) fingerlings were evaluated. Basal diet was supplemented with 0 (control), 0.5, 1, and 2 $g \cdot kg^{-1}$ *F. angulata* and was randomly allocated to experimental fish of an initial average weight of 7.45 ± 0.02 g. After 8 weeks of experiment, the fish supplemented with *F. angulata* extract showed increased but non-significant ($P > 0.05$) growth performance. No significant differences were found between trial control groups in haematological indices such as red blood cell count, haematocrit, and haemoglobin, but there was a significant increase in white blood cells, lymphocytes, neutrophils and monocytes in the *F. angulata* extract groups ($P < 0.05$). Also, there were significant differences between the fish supplemented with dietary *F. angulata* extract and the control group regarding immunological indices, including immunoglobulin M, lysozyme, and classical and alternative complement pathway ($P < 0.05$). These findings suggest that the administration of *F. angulata* extract has a positive effect on the immunological indices and the immune system activity in rainbow trout fingerling.

Fish, herbal extract, blood indices, immune system

Aquaculture industry has one of the most important roles in food production in most countries. One of the best commercial fish in the aquaculture industry is rainbow trout (*Oncorhynchus mykiss*), which has a valuable share in the human food supply. Rainbow trout is grown in many countries, and also in Iran (Talebi et al. 2013). Rainbow trout farming faces many problems and stressors such as pathogens, overcrowding, poor water quality and low oxygen concentration. Accumulation of metals, especially arsenic is an important stressor for rainbow trout during the farming period (Celechovska et al. 2011). Nitrite concentrations may cause physiological changes in rainbow trout, e.g. in the haematological and biochemical indices (Zuskova et al. 2013).

These indices are high risk and weaken the immune system, increasing disease incidence (Shalaby et al. 2006). Improving the immune system of the fish, especially in valuable species such as rainbow trout is the most important principle of fish farming. Food additives, growth stimulants and immunostimulants, including chemical agents, nutritional factors, bacterial and probiotic components, and animal or plant extracts affect the immune system and body defences against disease agents (Magnadottir 2006). Increased use of antibiotics, growth stimulants and chemical compounds leads to increased resistance of microorganisms against antibiotics and drugs. Antibiotic resistance is one of the most fundamental problems in aquaculture industry (Ardo et al. 2008). Drug resistance, decreased meat quality and high cost lead to the use of natural

Address for correspondence:
Somayeh Bohlouli
Department of Veterinary Medicine
Faculty of Agriculture, Kermanshah Branch
Islamic Azad University, Kermanshah, Iran

Phone +98 9113 232 385
E-mail: sbohloli@yahoo.com
http://actavet.vfu.cz/

stimulants in fish farming, especially herbal and traditional plants (Anilachalam et al. 2010). Nowadays, the use of natural plants as growth and immune stimulant factors along with their compounds such as essential oils and herbal extracts has improved the non-specific immune system in fish farming. Using traditional and medicinal plants as safety stimulants is a suitable alternative to synthetic antibiotics, chemical growth agents and immune stimulants. Immune stimulants, especially herbal medicine improve the immune system and increase the host's resistance to disease via increasing the number of white blood cells and production of antibodies (Rao et al. 2006). *Ferulago angulata* (known as Chavir in Iran) belongs to the *Apiaceae* family. It grows in the west of Iran in the mountains of Kermanshah and is used as an important herbal medicine among the indigenous people in the west of Iran (Khan Ahmadi et al. 2011). Anti-oxidant, antibacterial, and antifungal effects of *F. angulata* have been reported (Heidari et al. 2014). Several active components have been identified by analysing different parts of ferulago with ethanol. The most important compounds are cis-ocimene, α-pinene, β-germacrene, σ-terpinene, trans-β-ocimene, germacrene D, limonene, bornyl acetate, myrcene, camphene, allo-neo-ocimene, β-pinene, bicyclogermacrene and sophrosyne in ethanolic extract of *F. angulata* (Sodeifian et al. 2011). Cis-ocimene and α-pinene are the most important active compounds of *F. angulata* essential oil (Darderafshi et al. 2014). The phenolic compounds of the plant are antioxidant agents which can inhibit free radicals, so they can be effective in preventing many oxidative diseases such as cancer. Also, these compounds have antibacterial and antifungal effects (Hosseini et al. 2012). This study was carried out to evaluate the effects of *F. angulata* (Schlecht) Boiss on the growth indices and haematological and immunological indices of rainbow trout (*Oncorhynchus mykiss*) fingerlings.

Materials and Methods

Preparation of herbal extracts

Ferulago angulata (Schlecht) Boiss (Chavir) was collected from the Kermanshah Dalahoo Mountains in western Iran, in late spring and was identified by the Herbarium of the Islamic Azad University, Kermanshah Branch. The whole plant (stem, leaf, and flower) was dried and powdered. The powdered plant (100 g) was kept in 500 ml of 70% ethanol for 48 h at room temperature. The macerated plant extracted by percolation method was removed from the percolator and filtered by Whatman filter paper, Grade: 4, (20 – 25 µm). It was then dried under reduced pressure at 37 °C by a rotator evaporator (Zare Shahneh et al. 2013).

Diet preparation

A commercial and basal diet for *Oncorhynchus mykiss* was purchased from Beyza Feed Mill (BFM), Beyza, Iran. The main components of the diet, including 484 $g \cdot kg^{-1}$ protein, 141 $g \cdot kg^{-1}$ fat, 172 $g \cdot kg^{-1}$carbohydrate and 3,460 $kcal \cdot kg^{-1}$ diet were supplemented with 0.5, 1 and 2 $g \cdot kg^{-1}$ *F. angulata* extract in the experimental group and 0% *F. angulata* extract in the control group. The basal diet and *F. angulata* extract were mixed by mixer and made into pellets. The pellets were air-dried at room temperature and broken into tiny pieces. Then the diet pellets were packed and stored in air-tight containers at refrigerator temperature (4 °C) until used. All groups were fed for 8 weeks (Bohlouli et al. 2016). Each diet was randomly allocated to triplicate groups of fish with the initial average weight of 7.45 ± 0.02 g.

Experimental design

A total of 300 rainbow trout fingerlings with average body weight of 7.45 ± 0.02 g were randomly allocated to 12 tanks, 25 fish in each tank containing 200 l water and with a flow-through system. Water conditions, i.e. temperature 12 ± 0.2 °C, pH = 7.4 and dissolved oxygen = 7.5 ± 0.92 $mg \cdot l^{-1}$ were measured and controlled every day during the 8 weeks of experiment. The fish were divided into four groups (control, 0.5, 1 and 2 $g \cdot kg^{-1}$ *F. angulata* extract) with three replicates for each experiment. The fish were hand-fed at 3–5% body weight four times a day at 07:00, 11:00, 15:00, and 19:00 h.

Estimation of growth indices

The feeding ratio was surveyed every two weeks after a 24 h starvation and batch weighing. At the end of experiment, the feeding was stopped and all of the fish were weighed after 24 h. All analyses were carried out on 15 fish in each group (5 samples from each replicate). The fish in all groups were anaesthetized with 2% 2-phenoxyethanol. The weight gain (WG), feed conversion ratio (FCR), and specific growth rate (SGR)

were calculated using the standard formula for indicators of growth performance and feed utilization (Bagni et al. 2005):

Weight gain = final weight – initial weight

Food conversion rate (FCR) = weight gain/feed intake

Specific growth rate (SGR) = 100 × [ln (final body weight) - ln (initial body weight)]/days

Sampling and serum collection

A total of 15 blood samples were collected from 15 fish in each group (5 samples from each replicate). Fish were anaesthetized with 2% 2-phenoxyethanol, and were bled from caudal vein. Blood was divided into two parts. Half of the blood samples were stored in heparinized tubes for haematological analysis. The other half of blood samples were kept at room temperature for 1 h without anticoagulant and then centrifuged (10 min at 3,500 *g*) to separate serum for measurement of immunological indices.

Haematological assay

For haematological analysis, the blood samples were diluted and stained with Natt-Herrick's solution. Red blood cell counts (RBC $\times 10^6/mm^3$) and total white blood cell counts (WBC $\times 10^3/mm^3$) were calculated after dilution with Natt-Herrick's staining solution by Neubauer haemocytometer. Haemoglobin (Hb) and haematocrit (HCT) were assayed by the photometric assay of cyanomethaemoglobin and the microhaematocrit method, respectively (Houston 1990). Blood was spread on the slides and Giemsa stained blood smears were conducted for differential count of WBC (neutrophils and monocytes) (Hrubec et al. 2001). The rest of haematological indices, including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were analysed by standard formulas (Ranzani-Paiva et al. 2004).

Immunological assay

Serum lysozyme content was determined by the lysis of *Micrococcus lysodeikticus* (lysozyme sensitive gram positive bacterium) (Sigma, St. Louis, MO, USA) according to the method of Demers and Bayne (1997). Hen egg white lysozyme (Sigma) in 0.1 M phosphate citrate buffer (pH = 5.8) was used as standard. Serum sample (1.75 µl) was situated into the plate wells. *Micrococcus lysodeikticus* suspension (75 $mg \cdot ml^{-1}$) was diluted in phosphate citrate buffer (Merck, Darmstadt, Germany) and added to each sample in the well. After fast mixing, the optical density change was measured by spectrophotometer (bio photometer Eppendorf) (every 30 s for 300 s at 450 nm at approximately 18–20 °C). IgM level was assayed by ELISA kit (Cusabio, China). For IgM determination, 96-well plates were coated with 100 ml serially diluted (1:200) serum samples, incubated overnight at 4 °C and washed three times with buffered Tween-20 PBS (50 mM sodium phosphate, pH = 7.4, containing 150 mM NaCl and 0.1% Tween-20). The wells were blocked for 2 h at room temperature with 5% skim milk and underwent three washes with Tween-20 PBS. Anti-fish serum (100 µl at a 1:2000 dilution) was added to each well and incubated for 1.5 h at 37 °C prior to rinsing with Tween-20 PBS.

Identical conditions were used for incubation with the secondary anti-mouse antibody. The plates were revealed by incubation (30 min, room temperature) in the dark with 100 ml of a 0.42 mM solution of o-phenylenediamine dihydrochloride (OPD) in 100 mM citrate/sodium acetate buffer, pH = 5.4, containing 0.03% hydrogen peroxide as a substrate. The reaction was stopped by adding 25 ml of 2 M H_2SO_4 per well. Absorbance of the wells was read at 490 nm. Negative controls consisted of samples without primary antibody. The mean absorbance of the negative controls for each plate was subtracted from the optical density at 490 nm (Yeh et al. 2008).

Alternative complement pathway.

Alternative complement activity (ACH_{50}) $units \cdot ml^{-1}$ was assayed by the method of Sunyer and Tort (1995) with modifications described previously. Sheep red blood cells (SRBCs) were used as target cells in the presence of gelatin veronal buffer (GVB, Sigma, St. Louis, MO, USA). Individual 20-µl aliquots (2-fold dilutions) of a serially diluted serum with GVB-EGTA-Mg2+ buffer (10 mM ethyleneglycoltetraacetic acid and 10 mM $MgCl_2$ in GVB) as a complement source were mixed with 6 µl of SRBC suspension (4×108 $cells \cdot ml^{-1}$), and the mixture was incubated at 21 °C, pH 7.2 for 2 h. The haemolytic reaction was stopped by adding 200 µl of GVB containing 10 mM EDTA. The mixtures were centrifuged at 1,600 × *g* for 10 min at 4 °C. The optical density (OD) of the supernatants was measured at 414 nm using an enzyme-linked immunosorbent assay (ELISA) reader (A). The reactions were supplemented with 6 µl EDTA-GVB, 20 µl EDTA-GVB, and 220 µl distilled water to replace the SRBC suspension, the diluted serum, and the diluted serum + EDTA-GVB buffer, respectively, as the SRBC blank (B), serum blank (C), and 100% heamolysis sample (D). The degree of haemolysis (Y) was defined as Y= [A− (B+C)] (D−C) −1 and calculated, and the lysis curve for each specimen was obtained by plotting Y (l−Y) −1 against the volume of complements added on alog/log-scaled graph. The volume of serum complement producing 50% haemolysis (ACH_{50}) was determined, and the number of ACH_{50} $units \cdot ml^{-1}$ was calculated for each experimental group (Yeh et al. 2008).The activity of complement component 3 (C3) was determined by C3 kit (Zhejiang Elikan Biological Technology Co., Ltd, Wenzhou, Zhejiang, China) and that of complement component 4 (C4) was assayed by C4 kit (Zhejiang Elikan Biological Technology Co., Ltd. Wenzhou, Zhejiang, China) (He et al. 2009).

Statistical analysis

Data were expressed as mean ± SD (standard deviation). The means were analysed by one-way analysis of variance (ANOVA) test using SPSS 19; Chicago, IL, USA, and when significant difference was indicated, the means were tested using least significant difference (LSD) test to compare the means of treated groups against the control group. The level of $P < 0.05$ was accepted as significant.

Results

The results of growth indices are summarized in Table 1. According to the results, growth indices, including WG, FCR and SGR were increased in parallel with increasing *Ferulago angulata* extract concentration during the experiment, but no significant differences were observed in the treatment group compared with the control group ($P > 0.05$). The haematological indices are presented in Table 2. No significant differences were observed between treatment groups with *F. angulata* and the control group in terms of haematological indices, including RBC, Hb, HCT, MCV, MCH and MCHC ($P > 0.05$). The rest of haematological indices such as WBC, lymphocytes, neutrophils and monocytes were increased in *F. angulata* extract groups. There was a significant increase in WBC, lymphocytes, neutrophils and monocytes of rainbow trout fingerlings supplemented with the *F. angulata* extract compared to the control group ($P < 0.05$). The maximum level of WBC, neutrophils, lymphocytes and monocytes was seen in the *F. angulata* extract (2 $g \cdot kg^{-1}$), and the minimum level was seen in the control group (without the *F. angulata* extract). Table 3 shows the quantity of immunological indices containing IgM, lysozyme, ACH_{50}, C3 and C4 in the blood of rainbow trout fingerlings at the end of the experiment. IgM increased significantly in the fish supplemented with the *F. angulata* extract, so the maximum level was observed in 2 $g \cdot kg^{-1}$ ($P < 0.05$). Lysozyme increased in the fish supplemented with 2 $g \cdot kg^{-1}$ *F. angulata* extract ($P < 0.05$). Classical pathways and complement components 3 and 4 (C3 and C4) were increased by adding the *F. angulata* extract to diet ($P < 0.05$). Also, the increase of ACH_{50} in different doses of *F. angulata* extract showed a significant difference between experimental and control groups ($P < 0.05$).

Table 1. Growth indices of rainbow trout (*Oncorhynchus mykiss*) fingerlings fed diets supplemented with different concentrations of *Ferulago angulata* extract for 8 weeks.

Indicator	Concentration of dietary in *F. angulata* extract			
	0	0.5 $g \cdot kg^{-1}$	1 $g \cdot kg^{-1}$	2 $g \cdot kg^{-1}$
Initial weight (g)	7.45 ± 0.02^a	7.76 ± 0.13^a	7.94 ± 0.02^a	8.06 ± 0.04^a
Final weight (g)	17.25 ± 0.40^a	17.66 ± 0.33^a	17.96 ± 1.55^a	18.23 ± 0.47^a
WG (g)	9.8 ± 0.42^a	9.9 ± 0.20^a	10.02 ± 1.53^a	10.17 ± 0.43^a
FCR	0.92 ± 0.01^a	0.95 ± 0.02^a	0.97 ± 0.01^a	0.98 ± 0.04^a
SGR (% day -1)	1.63 ± 0.06^a	1.65 ± 0.01^a	1.67 ± 0.17^a	1.69 ± 0.05^a

Data are expressed as mean ± SD. No significant difference was observed between experimental groups. ($P > 0.05$)
WG - weight gain; FCR - food conversion rate; SGR - specific growth rate

Discussion

This study examined the effect of *Ferulago angulata* (Schlecht) Boiss extract-supplemented diet on rainbow trout fingerlings (*Oncorhynchus mykiss*) as an increasingly important fish species in aquaculture. The growth indices and FCR indicated no significant difference between the fish supplemented with the *F. angulata* extract and the

Table 2. Haematological indicators of rainbow trout fingerlings fed diets supplemented with different concentrations of *Ferulago angulata* extract for 8 weeks.

Indicator	Concentration of dietary *F. angulata* extract			
	0	0.5 g·kg^{-1}	1 g·kg^{-1}	2 g·kg^{-1}
RBC (× 10^6/mm ^{3})	0.91 ± 0.05^a	0.92 ± 0.03^a	0.91 ± 0.09^a	0.93 ± 0.03^a
Haemoglobin (g·dl^{-1})	6.87 ± 0.22^a	6.98 ± 0.46^a	7.17 ± 0.64^a	7.23± 0.15^a
Haematocrit (%)	33.33 ± 1.23^a	34.10 ± 1.65^a	33.73 ± 2.93^a	34.25 ± 2.10^a
MCV (fl)	366.26 ± 13.58^a	370.65 ± 25.42^a	370.65 ± 13.50^a	368.27 ± 30.15^a
MCH (pg)	75.49 ± 0.58^a	75.86 ± 3.79^a	78.79 ± 2.08^a	77.74± 6.93^a
MCHC (%)	20.61 ± 0.60^a	20.47 ± 0.21^a	21.25 ± 0.78^a	21.13 ± 0.31^a
Neutrophil (%)	28.80 ± 2.10^a	33.10 ± 2.00^{ab}	37.13 ± 3.04^{bc}	42.73 ± 2.56^c
Lymphocyte (%)	69.87 ± 3.06^a	71.67 ± 1.53^{ab}	74.42 ± 5.00^{bc}	79.36 ± 2.36^c
Monocyte (%)	01.77 ± 0.58^a	02.97 ± 0.58^{ab}	03.80 ± 1.00^{bc}	04.93 ± 0.58^c
WBC (× 10^3/mm ^{3})	9.86 ± 0.42^a	11.75 ± 0.78^{ab}	14.29 ± 0.90^{bc}	16.35 ± 1.37^c

Data are expressed as mean ± SD. Means in the same row with different superscripts are significantly different ($P < 0.05$).
RBC - red blood cells; MCV - mean corpuscular volume; MCH - mean corpuscular haemoglobin; MCHC - mean corpuscular haemoglobin concentrations; WBC - white blood cells

Table 3. Immunological indicators of rainbow trout fingerlings fed diets supplemented with different concentrations of *Ferulago angulata* extract for 8 weeks.

Indicator	Concentration of dietary *F. angulata* extract			
	0	0.5 g·kg^{-1}	1 g·kg^{-1}	2 g·kg^{-1}
IgM (mg/dl)	52.6 ± 12.887^a	83 ± 4.544^{ab}	87 ± 11.213^{ab}	90 ± 6.071^b
Lysozyme (U/ml)	24.8 ± 7.19^a	34.33 ± 1.386^{ab}	43 ± 1.071^{ab}	49.6 ± 1.121^b
C3 (mg/dl)	26.9 ± 8.234^a	39.17 ± 6.429^{ab}	55.5 ± 6.263^{ab}	59.7 ± 10.849^b
C4 (mg/dl	25.2 ± 8.325^a	37.2 ± 4.359^{ab}	47.9 ± 9.899^{bc}	62.8 ± 4.243^c
ACH_{50} (u/ml)	57.4 ± 9.072^a	94 ± 12.767^{ab}	104.5 ± 3.536^{bc}	107.1 ± 3.828^b

Data are expressed as mean ± SD. Means in the same row with different superscripts are significantly different ($P < 0.05$).
IgM - immunoglobulin M; C3 - complement components C3; C4 - complement components C4; ACH_{50} - alternative complement pathway

control group. Some natural herbs and herbal extracts have positive effects on growth indices (Immanuel et al. 2004; Sivaram et al. 2004), while others do not have growth stimulation effects (Dugenci et al. 2003). In rainbow trout fingerlings, body weight gain and growth performance were significantly higher in fish supplemented with *Echinacea purpurea* than in the control group (Bohlouli Oskoii et al. 2012). But there were no significant differences in growth indices, including WG, FCR and SGR, between trial groups supplemented with different concentrations of oak fruit extract in rainbow trout fingerlings (Bohlouli et al. 2016). In another study, the effect of *F. angulata* on growth performance was surveyed in broiler chickens, and *F. angulata* was reported to improve the growth performance in broiler chickens (Rostami et al. 2015). Contradictory results in growth indices are believed to be linked to the type of herbal species and animals. Based on our results, there were no significant differences in haematological indices, including RBC, Hb, HCT, MCV, MCH and MCHC in the fish supplemented with *F. angulata.* This

is in agreement with the results reported by the study of Rostami et al. (2015) who reported no significant differences for the haematological indices RBC, Hb, HCT, and RBC (MCV, MCH, and MCHC) in broiler chickens supplemented with *F. angulata* (Rostami et al. 2015). Other haematological indices including the WBC count, lymphocytes, neutrophils, and monocytes were increased significantly in the fish supplemented with the *F. angulata* extract. In broiler chickens treated with *F. angulata*, an increase in WBC count and lymphocytes was found but there were no significant differences (Rostami et al. 2015). The findings of WBC count and other defence cells in rainbow trout treated with *Echinacea purpurea* and Persian oak (*Quercus brantii*) were similar to the results of this study (Bohlouli Oskoii et al. 2012; Bohlouli et al. 2016). The WBCs provide protection against pathogens (Harikrishnan et al. 2012). In this study, *F. angulata* extract-supplemented diet improved the immunological indices in experimental groups compared to control group. Immunological indices were increased and improved parallel to the increase of *F. angulata* extract concentration. The IgM and serum lysozyme activity increased along with the increased doses of *F. angulata* extract. The secretion of humoral immune responsive proteins by cellular immune response was likely to increase IgM (Malik et al. 2007). Positive effects of *F. angulata* on humoral immune responses and the immune system have been shown in broiler chickens (Govahi et al. 2013). Serum lysozyme activity increased in rainbow trout fingerlings fed and treated with *Quercus brantii* extract (Bohlouli et al. 2016) and supplemented with *Aloe vera* extract (Haghighi et al. 2014). Some studies have reported various effects of herbal extracts on serum lysozyme activity (Ardo et al. 2008; Harikrishnan et al. 2011; Harikrishnan et al. 2012). Alternative complement pathway (ACH_{50}) and classical pathways (C3 and C4) increased in experimental groups supplemented with *F. angulata* extract. These immunological variables were reported in rainbow trout fingerlings supplemented with Persian oak (Bohlouli et al. 2016). An alternate complement system was found to be enhanced by traditional Korean herbs in *Paralichthys olivaceus* (Harikrishnan et al. 2012). Diet supplementation with herb oils (*Thymus vulgaris* and *Foeniculum vulgare*) can increase disease resistance. Thyme and fennel oils can improve the immune system against *Yersinia ruckeri* infection by increasing some biochemical indices and electrolytes in rainbow trout (Kucukgul Gulec et al. 2013).

Similar immunological results have been shown in broiler chickens supplemented with *F. angulata* extract (Govahi et al. 2013; Rostami et al. 2015). A number of phenolic compounds are found in *F. angulata*; they include aromatic groups such as flavonoids, isoflavonoids, phenols acid, and anthocyanins (Sodeifian et al. 2011). Phenolic compounds are active ingredients of a medicinal plant as an important antioxidant agent (Huda-Faujan et al. 2009). Phenolic compounds of *F. angulata* contain antioxidant and antibacterial factors, so *F. angulata* can inhibit many oxidative and bacterial diseases in rainbow trout fingerlings supplemented with this herb. These immune-enhancing effects may be related to phenolic compounds, flavonoids such as cis-ocimene and α-pinene, the active component of *F. angulata* (Schlecht) Boiss extract.

In conclusion, supplementation with *F. angulata* in our study increased and improved the defensive and immune system activity in rainbow trout fingerlings. Natural herbs and herbal extracts containing antioxidants and antimicrobial components can be used as immunostimulants in the aquaculture industry.

Acknowledgements

This study was supported by the Islamic Azad University, Kermanshah Branch with Grant no. 44-5.

References

Anilakumar KR, Sudarshana Krishna KR, Chandramohan G, Ilaiyaraja N, Khanum F, Bawa AS 2010: Effect of Aloe Vera gel some none-specific immunity of red seabream *Pagrus major*. Fish Sci **73**: 63-69

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

Bagni M, Romano N, Finola, MG, Abelli L, Scapigliati G, Tiscar PG 2005: Short- and long-term effects of a dietary yeast betaglucan (Macrogard) and alginic acid (Ergosan) preparation on immune response in sea bass (*Dicentrarchus labrax*). Fish Shellfish Immun **18**: 311–336

Bohlouli S, Ghaedi G, Heydari M, Rahmani A, Sadeghi E 2016: Effect of dietrary Persian oak (*Quercus brantii var.persica*) fruit extract on survival growth per foromance, hematological and immunological parameters in rainbow trout, *Oncorhynchus mykiss*, fingerlings. Aquacult Nutr **22**:745-751

Bohlouli Oskoii S, Tahmasebi Kohyani A, Parseh A, Salati AP, Sadeghi E 2012: Effects of dietary administration of *Echinacea purpurea* on growth indices and biochemical and hematological indices in rainbow trout (*Oncorhynchus mykiss*) fingerlings. Fish Physiol Biochem **38**: 1029-1034

Celechovska O, Harkabusova V, Macharackova B, Vitoulova E, Lavickova A 2011: Accumulation of arsenic during the growing period of rainbow trout (*Oncorhynchus mykiss*). Acta Vet Brno **80**: 219-225

Darderafshi MJ1, Bahrami Gh, Sadeghi E, Khanahmadi M, Mohammadi M, Mohammadi R 2014 :The effect of *Ferulago angulata* essential oil on *Staphylococcus aureus* during the manufacture and preservation of Iranian white cheese. Iranian J of Nutr Sci Food Tech **8**: 13-20

Demers NE, Bayne CJ 1997: the immediate effect of stress on hormones and plasma lysozyme in rainbow trout. Dev Comp Immunol **21**: 363-373

Dugenci SK, Arda N, Candan A, 2003: some medicinal plants as immune stimulant for fish. J Ethnopharmacol **88**: 99-106

Govahi R, Ghalamkari G, Toghyani M, Eghbal Saied S, Mohammadrezaei M, Shahryari M, Dehghani Abari A 2013: Effect of *Ferulago angulata* sub. *carduchorum* on total serum antioxidant activity and some of the humoral immune responses in broiler chicks. JHD **4**: 119-126

Haghighi M, Sharif Rohani M, Samadi M, Tavoli M, Eslami M, Yusefi R 2014: Study of effects *Aloe vera* extract supplemented feed on hematological and immunological indices of rainbow trout (*Oncorhynchus mykiss*). Int J Adv Biolog Biomed Res **2**: 2143-2154

Harikrishnan R, Heo J, Balasundaram C, Kim MC, Kim JS, Han YJ, Heo MS 2010: Effect of traditional Korean medicinal (TKM) trihedral extract on the innate immune system and disease resistance in *Paralichthys olivaceus* against *Uronema marinum*. Vet Parasitol **170**:1-7

Harikrishnan R, Kim JS, Kim MC, Balasundaram C, Heo MS 2011: *Lactuca indica* extract as feed additive enhances immunological parameters and disease resistance in *Epinephelus bruneus* to *Streptococcus iniae*. Aquaculture **318**: 43-47

Harikrishnan R, Kim DH, Hong SH, Mariappan P, Balasundaram C, Heo MS 2012: Non-specific immune response and disease resistance induced by *Siegesbeckia glabrescens* against *Vibrio parahaemolyticus* in *Epinephelus bruneus*. Fish Shellfish Immun **33**: 359-364

He S, Zhou Z, Liu Y, Shi P, Yao B, Ringo E, Yoon I 2009: Effects of dietary *Saccharomyces cerevisiae* fermentation product (DVAQUA) on growth performance, intestinal autochthonous bacterial community and non-specific immunity of hybrid tilapia (*Oreochromis niloticus* × *O. aureus*) cultured in cages. Aquaculture **294**: 99-107

Heidari S, Akrami H, Gharaei R, Jalili A, Mahdiuni H, Golezar E 2012: Anti-tumor activity of *Ferulago angulata* Boiss extract in gastric cancer cell line via induction of apoptosis. Iran J Pharm Res **13**: 1335-1345

Hosseini N, Akbari M, Ghafarzadegan R, Changizi Ashtiyani S, Shahmohammadi R 2012: Total phenol, antioxidant and antibacterial activity of the essential oil and extracts of *Ferulago angulata* ssp. J Med Plant **11**:80–89

Houston AH 1990: Blood and circulation. In: Schreck CB, Moyle PB (Eds): Methods in Fish Biology. American Fisheries Society, Bethesda, pp 273-335

Hrubec TC, Smith SA, Robertson JJ 2001: Age related in haematology and biochemistry of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). Vet Clin Pathol **30**: 8-15

Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS 2009: Antioxidant activity of plants methanolic extracts containing phenolic compounds. Afr J Biotech **8**: 484-489

Immanuel G, Vicncybai VC, Sivaram V, Palavesam A, Marian MP 2004: Effect of butanolic extracts from terrestrial herbs and seaweed on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaes indicus* juveniles. Aquaculture **236**: 53-65

Khan Ahmadi M, Shahrezaei F, Alizadeh A 2011: Isolation and Structural Elucidation of Two Flavonoids from *Ferulago angulata* (Schlecht) Boiss. Asian J Res Chem **11**: 1667-1670

Kucukgul Gulec A, Danabas D, Ural M, Seker E, Arslan A, Serdar O 2013: Effect of mixed use of thyme and fennel oils on biochemical properties and electrolytes in rainbow trout as a response to *Yersinia ruckeri* infection. Acta Vet Brno **82**: 297-302

Malik F, Singh J, Khajuria A, Suri KA, Satti NK, Singh S, Kaul MK, Kumar A, Bhatia A, Qazi GN 2007: A standardized root extract of *Withania somnifera* and its major constituent withanolide-A elicit humoral and cell mediated immune responses by up regulation of Th1-dominant polarization in BALB/c mice. Life Sci **80**: 1525-1538

Magnadottir B 2006: Innate immunity of fish (overview). Fish Shellfish Immun **20**: 137-151

Ranzani-Paiva MJT, Ishikawa CM, das Eiras AC, Da Silveira VR 2004: Effects of an experimental challenge with *Mycobacterium marinum* on the blood parameters of Nile tilapia, *Oreochromis niloticus* (Linnaeus, 1757). Braz Arch Biol Technol **6**: 945-953

Rao Y, Das BK, Iyotymayee P, Chakraborty R 2006: Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas hydrophila*. Fish Shellfish Immun **20**: 265-273

Rostami F, Hossein A Ghasemi, Taherpour K 2015: Effect of *Scrophularia striata* and *Ferulago angulata*, as alternatives to virginiamycin, on growth performance, intestinal microbial population, immune response, and blood constituents of broiler chickens. Poult Sci **94**: 2202-2209

Shalaby AM, Khattab YA, Abdel Rahman AM 2006: Effects of (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Ooreochromis niloticus*). J Venom Ani Toxins **12**: 172-201

Sivaram V, Babu MM, Immanul G, Murugadass S, Citarasu T, Petermarian M 2004: Growth and immunoresponse of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. Aquaculture **237**: 9-20

Sodeifian GH, Ansari K, Bamoniri A, Mirjalili B 2011: Study of chemichal composition of the essential oil of *Ferulago angulata* (Schlecht) Boiss from Iran using super critical fluid extraction and nano scale injection. Dig J Nanomat Biostruct **6**: 161-168

Sunyer JO, Tort L 1995: Natural hemolytic and bactericidal activities of sea bream *Sparus aurata* serum are affected by the alternative complement pathway. Vet Immunol Immunopathol **45**: 333-345

Talebi M, Khara H, Zoriehzahra J, Ghobadi S, Khodabandeh A, Mirrasooli E 2013: Study on effect of red bell pepper on growth, pigmentation and blood factors of rainbow trout (*Oncorhynchus mykiss*). World J Zoolog **8**: 17-23

Yeh SP, Chang CA, Chang CY, Liu CH, Cheng W 2008: Dietary sodium alginate administration affects fingerling growth and resistance to *Streptococcus* sp. and iridovirus, and juvenile non-specific immune responses of the orange-spotted grouper, *Epinephelus coioides*. Fish Shellfish Immun **25**: 19-27

Zare Shahneh F, Valiyari S, Azadmehr A, Hajiaghaee R, Bandehagh A, Baradaran B 2013: Cytotoxic activities of *Ferulago angulata* extract on human leukemia and lymphoma cells by induction of apoptosis. J Med Plants Res **7**: 677-682

Zuskova E, Machova J, Velisek J, Stara A, Svobodova Z, Kocour Kroupova H 2013: Recovery of rainbow trout (*Oncorhynchus mykiss*) after subchronic nitrite exposure. Acta Vet Brno **82**: 73-79