Effects of mushroom *(Lentinula edodes)* extract on growth performance, immune response and hemato-biochemical parameters of great sturgeon juvenile (*Huso huso* Linnaeus, 1754)

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Abstract

This research was carried out to survey the ffects of dietary of *Lentinula edodes* mushroom extract as a feed supplement on growth performance and survival rate, immune response, hematological and biochemical characteristics of beluga juvenile (*Huso huso*). Four hundred and fifty fish after acclimation with average weight of 40.45 ± 5.72 g were fed in a wholly randomized design in 5 treatments (basic diet containing 0% (control), 00.5%, 1%, 1.5% and 2% *L. edodes* mushroom extract) and 3 replicates. During the experiment (56 days), a total of eight samples were taken in 8 weeks. The mean of data were analyzed using one-way ANOVA and Duncan's post hoc test at 5% level. Results indicated that administration of 2% of mushroom extract for 8 weeks improved weight gain, specific growth rate and feed conversion ratio of beluga (P<0.05). Among the investigated hematological indices, Supplementing 2% *L. edodes* resulted in increased levels of the erythrocytes (RBC), hemoglobin (Hb), haematocrit (Hct) in comparison with the control group (P<0.05). At the end of the assay, there were significant decreased in some of the biochemical parameters (Cholesterol, alkaline phosphatase (ALP) and alanine aminotransferase (ALT)) in the treatments containing mashroom extract (P <0.05). Also the activity of serum lysozyme activity and superoxide dismutase (SOD) Among immune parameters studied were increased in group of 2% of *L. edodes* compared to other treatments (P <0.05). These results indicated that the addition of supplemental mashroom extracts, especially at 2% level to the beluga juveniles diet, were improved the growth performance, some hematological and biochemical parameters and immune response of beluga juvenile.

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Introduction

One of the most valuable fish species in the Caspian Sea is The beluga *(H. huso)* which is known as the largest species among caviar fish (Jalali, Hosseini, & Imanpour 2008).Great Sturgeon has special features such as rapid growth, market price, easy reproduction and high endurance against adverse environmental conditions , which makes it an appropriate species in the aquaculture industry (Mohseni, Ozorio, Pourkazemi, & Bai 2008; Abtahi, Yousefi, & Kenari 2013). The rearing of sturgeon has seen considerable advances in past years (Amlashi, Falahatkar, Sattari, & Gilani 2011). So, further reconnaissance of commercially available dietary additives to ameliorate performance and disease resistance of these fish is still highly demanded. Bans and restrictions of antibiotics as edible additives in fish breeding in many countries have resulted in the enhancement in studies on alternative dietary supplements such as medicinal herbs to augment the health and production of cultured fish. Phytogenics can exert multiple effects on organisms, including the betterment of feed palatability, efficiency and digestion, decrease of nitrogen excretion and improvement of intestine flora and health status (Harikrishnan, Balasundaram, & Heo 2011; Sheikhzadeh, Nofouzi, Delazar, & Oushani 2011). Meanwhile, other virtues of herbs, such as antioxidant and antiviral properties, or their effects on the immune system cannot be ignored. One such instance is *Lentinula edodes* mushroom extract. Fungus have been used as food for centuries all over the world due to their characteristic soft contexture and mild flavor (Wandati, Kenji, & Onguso 2013). They are documented as being good source of nutrients and bioactive compounds which are useful to the human body (Chang & Miles 2011). pharmaceutical mushroom, *L. edodes*, an edible fungus, that is also known as Shiitake mushroom was chosen for the present study. This mushroom has been attributed with many medical properties both in eastern and western medicine. On human health, the effects could range from diminish cholesterol, lowering blood pressure, reinforcement the immune system against diseases, containing viral ones, fighting tumors, and improving liver function (Bobek, Ginter, Kuniak, Babala, Jurcovicova, Ozdin & Cerven 1991; Mau, Lin, & Song 2002; Reguła & Siwulski 2007). This function of mushroom extract is due to compounds such as saponins, terpenoids, polyphenols and flavonoids (Wandati *et al*. 2013). Saponins have been reported to possess a vast range of pharmacological properties, including anti-inflammatory and anti-diabetic properties (Lee *et al*. 2012). Terpenoids (isoprenoids) are secondary metabolites with molecular structures containing carbon backbones made up of isoprene (Wandati *et al*. 2013). The compounds have been expressed to show a intensive range of pharmacological benefits that embrace anti-malarial, anti-inflammatory and anti-cancer among others (Thoppil & Bishayee 2011). Phenolic compound are antioxidant and exhibit a vast extensive of spectrum medicinal attributes (Mariappan,

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Vinayagam, & Durai 2015). The total polyphenols content show a positive correlation with flavonoids (Nijveldt, Van Nood, Van Hoorn, Boelens, Van Norren &Van Leeuwen 2001). Polyphenols from various natural sources has plants (Alberto, Rinsdahl, Canavosio, & Manca de Nadra 2006), have been reported to have a variety of biological effects, among antimicrobial activities. Flavonoids are synthesized by plants as a response to microbial infections and are recognized to have effective antimicrobial effects against a vast range of microorganisms (Recio, Rios, & Villar 1989). The mushrooms could improve health because of its chemical compounds which are: lentinan, eritadenine, and Lergothioneine (Smith, Rowan, & Sullivan 2002; Bernaś, Jaworska, & Lisiewska 2006). In addition the Shiitake mushroom has several anti-oxidants (selenium, uric acid, vitamin A, E, C) as well as vitamin D (Mau *et al*. 2002).

Several investigations have confirmed the positive effects of medicinal plants on great sturgeon (Binaii, Ghiasi, Farabi, Pourgholam, Fazli, Safari, Alavi, Taghavi, & Bankehsaz 2014; Gholipour Kanani, Nobahar, Kakoolaki, & Jafarian 2014; Akrami, Gharaei, Mansour & Galeshi 2015; Vahedi, Hasanpour, Akrami, & Chitsaz 2017), but there is no valid evidence based on the affect of mushroom extract on growth performance, innate immune response and hemato-biochemical parameters of beluga juvenile. Hence, the target of the current research evaluated effects of dietary supplementation of *L. edodes* mushroom extract on the immune response, growth performance and hemato-biochemical parameters of great sturgeon juvenile (*H. huso*) in the natural environmental conditions of a commercial farm.

Materials and Methods

Fish

450 Juvenile sturgeon (*H. huso*) weighing 40.45 ± 5.72 g were obtained from Sadd-e Voshmgir Fisheries complex (Golestan province,next to Caspian sea, Iran) and used for the experiments. The health status of fish was inspectedand observed immediately upon arrival in the ponds and with 14 day intervals there after based on standardized laboratory procedures (Austin & Austin 1989). The fish did not show any signs of disease during the acclimation period. Fish were randomly distributed into 5 groups following a totall randomized design, with each group consisting of 30 fish. These groups were triplicate utilizing the same design. Prior to the trials the fish were adapted to the conditions in the test ponds. Before the experiment was initiated, the fish were fed commercial sturgeon feed pellets for 8 weeks. The trials were carried out in 15 polypropylene tanks [30 fish per tank (2m × 2m × 0.5m)] with the flow rate set at 1.5 litres s^{-1}, water temperature 18-21°C and dissolved oxygen 6-8 ppm for 56 days. Adaptation to these tanks was performed for 10 days with a commercially pelleted diet from Faradaneh company products used for sturgeon 3-5% of the body weight twice per day during the acclimation period.

Preparation of *L. edodes* mushroom extract

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L. edodes was collected from a local market and the production process of *L. edodes* mushroom extracted in water was done according to the method described by (Yap & Ng 2001) and can be summarized by the following steps: 500 g of mushroom were dissolved in 1000 mL of water in glass thermos. The thermos were firmly covered with aluminium foil and kept for 24 h in 60–65 °C temperature of water bath. Mushroom extract was filtered through filter paper to remove particles. The final solution was lyophilized in dark bottles and kept at 4°C. For the trials, 0.5%, 1%, 1.5% and 2% concentrations of the lyophilized mushroom extract were added to the commercial sturgeon feed and then stored in the fridge until use.

Diet preparation

To prepare the diets, a commercial pellet diet (containing 48-50% protein, 12-16% lipid and 7-10% ash) for sturgeon fishes. This diet without mashroom extract was considered as control diet. The experimental diet was prepared utilizing the basal diet supplemented with 0.5%, 1%, 1.5% and 2% *L. edodes* mushroom extract. To prepare the diets, a commercial pellet diet was blended , then combined with the mushroom extract with water (100 mL of water / kg of diet) to form a paste, which passed through a meat grinder and were allowed to dry for 18 h at 45°C by air circulation and stored at 4°C until use (Razaghi Mansour, Akrami, Ghobadi, Denji, Ezatrahimi & Gharaei 2012; Akrami, Iri, Rostami, & Razaghi Mansour 2013). Juveniles were fed the experimental diet for 8 weeks in rate of 3-5 percen of the body weight per day, spread across two feeding times (07:00 and 19:00).

Growth performance

To regulate the feeding amount and estimate growth performance, all of fish from each tank were biometried and weighted every 15 days during the experiment, at least 24 h after the last feeding. At the end of the feeding trial, weight gain (WG%), specific growth rate (SGR; % ady^{-1}) and feed conversion ratio (FCR) were calculated based on the following formulas:

WG (%) = 100 × (Wt –W0) × 100 / W0 (Tacon 1990).

FCR = dry feed intake (g) / wet WG (g) (Hevrøy, Espe, Waagbø, Sandnes, Ruud & Hemri 2005).

SGR (% day^{-1}) = (Ln W_t - Ln W_0) × 100 / t (Hevrøy *et al*. 2005).

Survival rate = (N_t / N_0) × 100 (Ai, Mai, Tan, Xu, Duan, Ma, & Zhang 2006).

Here, W_t and W_0 are final and initial body weights (g), respectively, t is duration of experimental days, N_0 is the initial number of fish, and N_t is the final number of fish.

Blood sample collection and serum separation

At the end of trial, ten fish catch from each tank were sampled for blood. In order to provide sufficient blood for the subsequent assays fish were anesthetized by using clove oil (*Eugina caryophillata*), then by use of a syringe, 4ml

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blood samples removed from the caudal vein. For serum isolation, blood samples were put into non-heparinized tubes and left to clot for 12 h (at 4 °C), prior to centrifugation at 5000 g for 5 min in a clinical centrifuge. Isolated sera were stored at -20 °C until further analysis (Soleimani, Hoseinifar, Merrifield, Barati, & Abadi 2012).

Hematological levels

In order to study the hematological parameters, the blood samples were suspended in heparinized tube made from the blood of 10 fish in each group. To measure blood parameters, the following were performed:

1-Values of red blood cell (RBC) and white blood cell (WBC) counts were determined using a Neubaeur hemocytometer based on method of (Martins, Tavares-Dias, Fujimoto, Onaka, & Nomura 2004).

2-Hemoglobin levels (Hb) were determined by the cyano-methemoglobin spectrophotometry method (Biochorm Libra S12 , UK).

3- Hematocrit level were determined by method of (Schaperclaus, Kulow & Schreckenbach 1991). In this method Blood samples of fish were taken into heparinized capillary tubes. Duplicate samples were used from each fish. The tubes were then centrifuged (at 10.500 g for 5 min) in a hematocrit centrifuge. The percentage hematocrit value was measured by overlaying the tubes on a sliding scale hematocrit reader.

4- Differential leukocyte counts (monocyte, lymphocyte and neutrophil) was made in peripheral blood smears stained with May–Grunwald and Giemsa stains (Ghiasi, Mirzargar, Badakhshan, & Shamsi 2010).

Biochemical assays

Blood biochemical parameters were evaluated based on the following laboratory methods utilizing an auto - analyzer BT - 3000 (Biotecnica, Italy) and commercial kits (Pars azmoon, Tehran, Iran). Total protein and glucose levels (glucose oxidase) were measured by the Biuret and glucose oxidase methods, respectively (Asadi, Hallajian, Asadian, Shahriari, & Pourkabir 2009). Triglycerides were obtained lipase (lipase / GPO - PAP), cholesterol (cholesterol oxidase), albumin (Bromocresol Green method) (Borges, Scotti, Siqueira, Jurinitz, & Wassermann 2004). Globulin was calculated by subtracting albumin value from total plasma protein (Maqsood, Samoon, & Singh 2009) Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) enzymes were distinguished by colorimetrically and alkaline phosphatase (ALP) was determined using an enzymatic method (Borges *et al*. 2004).

Immune parameters assay

Lysozyme activity

The Lysozyme level in blood was determined by using a modified lysoplate base on the method described by (Siwicki, Anderson 1993) with slight. Modifications. Briefly, aliquots 2 ml of *Micrococcus lysodeikticu*s suspension (Sigma, USA) (0.375 mg ml^{-1}, 0.05 M sodium phosphate buffer, pH 6.2) and 50 µl of each

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serum sample were mixed properly for 15s and measured in a spectrophotometer at 450 nm (Biochorm Libra S12, UK). The optical density was immediately measured at intervals of 15, 30 and 270 s. A unit of lysozyme activity was defined as the amount of sample causing a reduction in absorbance of 0.001 min^{-1} and lysozyme activity was expressed as units (U) per minute.

Determination of serum Ig level

Procedure wherein the immunoglobulin is first precipitated out of solution with polyethylene glycol (10.000kDa).for this assay,100 µl of the plasma were mixed with an equal amount of 12% polyethylene glycol and in incubated for 2h under constant agitation at room temperature. After centrifugation at 5000 × g for 15 min,the supernatant was removed and the remaining concentration of protein determined by subtracting this figure from the total serum protein concentration (Siwicki, Anderson 1993).

Serum superoxide dismutase (SOD) activity

The activity of superoxide dismutase (SOD) was determined by RANSOD kit (RANdox Labratories Ltd. UK). Briefly, At first was collected beluga blood using EDTA as anticoagulant. In this method, xanthine and xanthine oxidase were used, and the radicals were able to react with 2- (4 - idophenyl) - 3 - (4 - nitrophenol) - 5 - phenyltrazolium chloride (INT) and produce a formazan red, measured at 505 nm (Biochorm Libra S12, UK). Due to existed of the enzyme SOD in the sample, the superoxide radicals was turned into hydrogen peroxide and oxygen and prevented the formation of red formazan. Activity was determined by the degree of prevention of this reaction. For measurment SOD: a 50 ml of RBC and disilled water (1:200 v v^{-1}, equivalent to approx 75 mg Hb) was utilized. Enzyme activity was computed as (U SOD / g Hb).

Alternative complement activity (ACH50)

Alternative complement activity was tased based on the procedure of (Ortuño, Cuesta, Rodríguez, Esteban, & Meseguer 2002). In this method (500µl) of test serum as complement source, serially diluted in Hank's buffer (HBSS), were added to 500µl of SRBC (final concentrations 10–0·078%). Then put samples in incubator at 22º C for 1 hour and were centrifuged 800 × g for 5 min at 4ºC to remove non-lysed RBC. The relative hemoglobin content of the supernatants was assessed by measuring their optical density at 540 nm in a spectrophotometer (Biochorm Libra S12, UK). The values of maximum (100%) and minimum (spontaneous) hemolysis were obtained by adding 500 l of distilled water or HBSS to 500 l samples of SRBC, respectively. The degree of haemolysis (Y) was estimated and the lysis curve for each specimen was obtained by plotting Y / (1-Y) against the volume of serum added (ml) on a log–log scaled graph. The volume of serum producing 50% haemolysis (ACH50) was determined and the number of ACH50 units ml^{-1} was obtained for each experimental group.

Respiratory burst activity

Respiratory burst activity of neutrophils was quantified utilizing the reduction of nitroblue

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tetrazolium (NBT) to formazon as a measure of superoxide anion (O_2^-) production. This test was done by the method of (Secombes 1990) as modified by (Stasiack & Bauman 1996).The blood was collected by piercing the caudal peduncle in a test tube containing 2.7% EDTA as anticoagulant. Fifty microlitres of blood was placed into the wells of 'U' bottom microtitre plates and incubated at 37°C for 1 h to facilitate adhesion of cells. Then the supernatant was removed and the adhered wells were washed three times in PBS. In this step, 50 ml of 0.2% NBT was added and resulting solution was incubated for a further hour. The cells were then fixed with 100% methanol for 2-3 min and again washed thrice with 30% methanol. The plates were then air dried. Sixty microlitres 2 N potassium hydroxide and 70 ml dimethyl sulphoxide were added into per well to dissolve the formazon blue precipitate formed. The OD of the turquoise blue solution was then read in an ELISA reader at 540 nm (Elisa Reader, SCO GmbH Reader MPR1, Germany).

Statistical analysis

Values were expressed as Mean ± standard diviation (SD). The immune response, stress resistance, digestive enzyme activities and growth performance were tested using one-way ANOVA and Duncan's multiple range test was used for comparison of the average values at the 5% level of significance utilizig software SPSS (Version 16.0).

Results

growth performance

The growth performance of great surgeon fed diets supplemented with varying levels of dietary *L. edodes* mushroom extract is presented in Table 1. Compared to the control treatment, fishes fed 2.0 g *L. edodes* mushroom extract kg^{-1} diet displayed improved ($P<0.05$) growth performance, containing weight gain (g kg^{-1}), SGR and FCR. There were no remarkable differences in survival rate among experimental and control ($P>0.05$).

Table 1. Growth performance and feed utilization of great sturgeon juvenile fed with diets containing different levels of *L. edodes* for 8 weeks.

Parameters	Control	0.5	1	1.5	2
Weight gain %	618. 3 ± 23.9 ^{c}	632.4 ± 35.3 ^{c}	662.23 ± 46.2 ^{b}	689.2 ± 39.1 ^{b}	748.1 ± 52.9 ^{a}
SGR (%/day)	3.43 ± 0.11 ^{b}	3.81 ± 0.31 ^{b}	3.95 ± 0.20^{ab}	4.00 ± 0.16 ^{ab}	4.11 ± 0.22^{a}
FCR	0.98 ± 0.04 ^{a}	0.91 ± 0.07 ^{b}	0.89 ± 0.05 ^{b}	0.82 ± 0.02 ^{b}	0.75 ± 0.16^{c}
Survival (%)	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}	100 ^{a}

Data expressed as mean ± SD, (n=30). Values in the same column sharing the same superscript letter are not significantly different (P>0.05).

Biochemical assays

Biochemical analysis was carried out in following sequence

The results of blood serum biochemical test of great sturgeon juveniles fed with *L. edodes* mushroom such as Cholestrol, ALT, ALP, Triglycerid, Glucose, Total Lipid,Total protein,

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Albumin, Globulin, LDH and AST are given in Table 2. The lowest Cholestrol and the highest ALT and ALP levels was observed in 2% *L. edodes* extract group, which was differ significantly from control group (P<0.05). Glucose, Triglycerid, Total Lipid, Total protein, Albumin, Globulin, LDH and AST activities among the various group of beluga fed *L. edodes* mushroom extract showed no significant differences (P>0.05). It can be observed that in mostly the best results were obtained in treatment containing 2% *L. edodes* extract supplemented diet.

Table 2. Blood serum biochemical parameters of beluga juveniles fed with *L. edodes* mushroom (% feed) added diet at different levels for 8 weeks

parameter	Control	0.5%	1%	1.5%	2%
Glucose (mg l^{-1})	64.00 ± 2.10^{a}	63.12 ±4.03^{a}	61.00 ±3.14^{a}	60.21 ± 3.09^{a}	59.30 ± 5.00^{a}
Total Protein (mg l^{-1})	1.28 ± 0.09^{a}	1.75 ± 0.47^{ab}	1.24 ± 0.19^{a}	1.75 ± 0.21^{a}	1.11 ± 0.09^{a}
Cholestrol (mg dl^{-1})	98.6 ± 17.22^{a}	85.12 ± 13.10^{b}	81.19 ± 13.02^{c}	65.47 ± 11.12^{d}	45.36 ± 9.24^{e}
Albumin (mg l^{-1})	0.56 ± 0.12^{a}	0.53 ± 0.31^{a}	0.49 ± 0.15^{a}	0.60 ± 0.24^{a}	0.59 ± 0.25^{a}
Globulin (mg l^{-1})	0.53 ± 0.01^{a}	0.6 ± 0.11^{a}	0.63± 0.05^{a}	0.68 ± 0.09^{a}	0.54 ± 0.08^{a}
AST (IU dl^{-1})	440.65 ± 55.20^{a}	442.66 ± 51.67^{a}	446.83± 43.84^{a}	449.32 ± 45.20^{a}	450.40 ± 48.18^{a}
ALT (IU dl^{-1})	45.16 ± 2.9^{a}	44.86 ± 3.3^{a}	42.68 ± 3.1^{a}	44.27 ± 3.7^{a}	37.09 ± 3.6^{b}
ALP (IU dl^{-1})	568.20 ± 45.60^{a}	581.50 ± 52.36^{a}	584.74 ± 45.19^{a}	571.20 ± 59.12^{a}	542.50 ± 65.20^{b}
LDH (IU dl^{-1})	587.23 ± 44.11^{a}	570.50 ± 34.17^{a}	581.80 ± 42.21^{a}	575.19 ± 39.11^{a}	590.11 ± 45.64^{a}
Triglycerid (mg dl^{-1})	483.13 ± 31.18^{a}	485.23 ± 39.11^{a}	480.11 ± 25.17^{a}	475.15 ± 26.20^{a}	470.25 ± 26.09^{a}
Total Lipid (mg dl^{-1})	415.14 ± 27.23^{a}	398.45 ± 23.25^{a}	401.84 ± 25.60^{a}	394.19 ± 34.20^{a}	390.53 ± 21.02^{a}

Data are represented as mean ± SD. Values in the same rows sharing the same superscript letter are not significantly different (P>0.05).

Haematological parameters

The blood RBC ,WBC, Hb, Hct, Monocyte, Lynphocyte and Neutrophil counts of different groups are shown in Table 3. The hematocryte percentage, haemoglobin and RBC counts, indices significant interaction (P<0.05) among the treatments and control. In opposite there were no significant different between WBC, monocyte , Lymphocyte and Neutrophil that fed *L. edodes* mushroom extract on compared with the control group (P>0.05).

Table 3. Effects of dietary *L. edodes* mushroom (% feed) on Hematological parameters of beluga juveniles for 8 week using a one-way ANOVA (n =3 tank per treatment group with 15 fish per tank)

Parameter	Control	0.5%	1%	1.5%	2%
RBC (10^{6} $ml^{-1)}$	0.10 ±0.02^{c}	0.11 ±0.03^{c}	0.13 ±0.05^{ab}	0.15 ±0. 04^{b}	0.18 ±0.05^{a}
WBC (10^{3} $ml^{-1)}$)	10.8 ±0.9^{a}	10.20 ±1.8^{a}	11.4 ± 1.3^{a}	11.7 ± 0.3^{a}	11.43 ± 1.7^{a}
Hb (g dl^{-1})	3.9 ± 0.42^{b}	4.7 ± 0.58^{ab}	4.9 ± 0.82^{ab}	5.1 ± 0.63^{ab}	5.5 ± 0.94^{a}
Hct (%)	33.2 ± 1.12^{b}	35.2 ± 1.4^{b}	36.1 ± 1.7^{b}	35.9 ± 0.9^{b}	39.1 ± 1.2^{a}
Monocyte (%)	10.9 ± 0.9^{a}	12.1 ± 0.5^{a}	13.3 ± 1.1^{a}	11.3 ± 0.8^{a}	11.9 ± 1.3^{a}
Lymphocyte (%)	63.1 ± 1.2^{a}	62.2 ± 3.3^{a}	64.2 ± 1.4^{a}	65.2 ± 2.4^{a}	67.1 ± 2.11^{a}
Neutrophil (%)	21.4 ± 2.12^{a}	19.8 ± 1.8^{a}	20.3± 3.5^{a}	20.7± 1.5^{a}	22.1± 3.5^{a}

Data are represented as mean ± SE. Values of means in columns were significantly different (P<0.05).

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Innate immune parameters

Innate immune responses of beluga juvenile fed on different levels of dietary *L. edodes* mushroom extract are shown in Table 4**.** There was a remarkable increase (P<0.05) in the lysozyme activity and SOD between treatment compare with the control group. But, in the same time (8 weeks), according to statistical analysis of data, there were no significant differences (P>0.05) of serum total immunoglobulin (Ig) and Alternative complement activity (ACH50). The highest Respiratory burst activity was observed in fish in control group and the lowest was observed in fish fed on 2% *L. edodes* mushroom extract.

Table 4. Effects of dietary *L. edodes* mushroom extract on Innate immune parameters of beluga juveniles for 8 weeks

Parameter	Control	0.5%	1%	1.5%	2%
lysozyme	23.1 ± 1.15^{b}	26.7 ± 2.17^{b}	27.3 ± 3.15^{b}	29.8 ± 1.18^{b}	36.4 ± 2.12^{a}
SOD	37.4 ± 3.07^{b}	35.3 ± 3.41^{b}	39.6 ± 2.41^{b}	41.8 ± 3.27^{b}	47.5 ± 4.02^{a}
Ig	32.7 ± 2.25^{a}	33.1 ± 3.07^{a}	36.7 ± 4.05^{ab}	37.8 ± 2.31^{ab}	38.1 ± 3.41^{ab}
ACH50	140.4 ± 14.24^{a}	145.3 ± 12.52^{a}	142.6 ± 13.35^{a}	151.8 ± 14.08^{a}	148.5 ± 13.52^{a}
Respiratory burst activity (OD630)	1390.4 ± 145.12^{a}	1421.8 ± 121.41^{a}	1434.2 ± 155.35^{ab}	1428.5 ± 138.53^{ab}	1441.7 ± 148.54^{ab}

Values are expressed as mean ± SE,. Mean values bearing different superscripts at the different stage were statistically significant(P<0.05).

Discussion

This essay was carried out, to find the effect of *L. edodes* mushroom extract which was added to the commercial feed as increment of growth performance , hematological levels , immune response and some biochemical parameters improved. According to the results of the study, feeding the beluga juvenile fish with 2% *L. edodes* supplemented diet was significantly better than other treatments in growth performance, some biochemical, hematological and immunological parameters. In the present study, we found that feeding of fish with 2% *L. edodes* significantly improved SGR and FCR (P<0.05) in beluga juvenile which means that the mushroom diet acted as an appetiser. The changes in growth and feed intake suggest that dietary mushroom may play a role in changing the enzyme activity related to digestion that consequently improves the feed digestibility and nutrient absorption. Alike to our results, research the improvement of growth performance and feed proficiency were reported in great sturgeon after feeding ginger *(Zingiber officinale)* (Vahedi *et al*. 2017) and nettle *(Urtica dioica)* (Binaii *et al*. 2014). Furthermore the beluga juvenile fed onion powder onion (*Allium cepa*) significantly showed higher growth performance compared with those of the control group (Akrami *et al*. 2015). Similarly Vahedi *et al*. (2017) observed that the supplementation of diet with ginger extract significantly increased beluga juvenile growth performance and Gholipour kanani *et al*. (2014)) showed that the diet containing *Z.*

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officinale powder has increasingly affected the growth performance of beluga. From previous studies, it has been suggested that improvement growth performance might be due to chemical compounds such as lentinan, eritadenine, L-ergotioneine (Bernaś *et al*. 2006) and several anti-oxidants (selenium,uric acid and vitamin A,C,E) as well as vitamin D (Mau *et al*. 2002) which found in mushroom. In agreement with result of our investigation the affects of any food supplemention added to the fish diet to improve its performance, will be different under the influance fish species, size, added dose, fish nutritional/physiological status, and/or milieu culturing conditions.

hemato-biochemical parameters

Haematological indices have been used in several studies to specify the nutritional and or pathological condition of an animal in response to various diets (Akpodiete & Ologbodo 1998; Jahanbakhshi, Imanpoor, Taghizadeh, & Shabani 2013), and are considered as indicators of animal health status. It is reported that all feed supplemented materials, couldn't have necessarily significant effects on hematological parameters including RBC, HB and PCV, and for assess their effective performance, it must consider performance time, concentration, method of administration and physiological condition of fish (Vahedi *et al*. 2017). Although use of herbal supplements have a positive effects on some hemato-biochemicals factors, but there is dependented to dose-diets, so that more or less than the optimum levels can have an inhibitory or unaffected effect (Awad, Austin, & Lyndon 2013).

The present study revealed that the RBC of beluga received 2% mushroom extract was influenced positively during the experimental trial. Similar result was reported by Binaii *et al*. (2014), who obtained that there was remarkable enhancement in RBC for sturgeon fed diet nettle in comparison with the control. Unlike this investigation, the study on *H. huso* (Gholipour kanani *et al*. 2014; Akrami *et al*. 2015b) and Vahedi *et al*. (2017) demonstrated erythrocytes remained impressed after feeding pharmaceuticalherbs. These various results may have been caused by the discrepancy effects of medicinal plants or different immune system responses per fish.. In the current research, the haemogolobin content in the treated groups was remarkably upper than the control, which illustrates that oxygen supply increases consequently, reflecting beneficial health effect on fish. The result of this study is parallel with the finding of Binaii *et al*. (2014) who obtained Hb was affected in great sturgeon juvenile fed 12% nettle. Conversely, Gholipour kanani *et al*. (2014) and Akrami *et al*. (2015b) found that the level of Hb were not significantly effected by ginger and onion in the basal diet of juvenile beluga, respectively. According to the present study, the Hct% in the blood of fish fed with 2% mushroom extract supplemented diets significantly increased in comparison with those fed the control diet and demonstrated the positive health properties on fish. Similar effect were observed in beluga fed diet supplementation with herbal plants (Binaii *et al*. 2014; Gholipour kanani *et al*. 2014; Akrami *et al*. 2015b). On the contrary, result obtained by Vahedi *et al*. (2017) showed that the

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supplement of ginger extract did not affect the amount Hct of sturgeon fish. Different effects of herbal plant and immune system reactions in distinct species of fish against these compounds can be attributed to these different results. In our study, the number of white blood cells and differential leukocyte counts (monocyte, lymphocyte and neutrophil) had not considerable change ($P > 0.05$) between treatment groups with compared to the control group. White blood cells (WBC) are body defender against diseases and in case of infection in the body will be increased (Abdelmalek, Driss, Kallel, Guargouri, Missaoui, Chaabouni, Ayadi & Bougatef. 2015). Compatible to our study Vahedi *et al*. (2017) were recorded these blood cells were not significantly affected by ginger extract in diet of *H. huso*. Our results coincides with the investigation of study which demonstrated that Zinger Supplement in eight weeks had no remarkable effect on white blood cell levels of great sturgeon (Gholipour kanani *et al*. 2014). On the other hand Binaii *et al.* (2014) and Akrami *et al.* (2015b) reported that the addition of nettle (*Urtica dioica*) and onion powder considerably increased the number of white blood cells in the beluga, respectively on the end of trial.

Biochemistry

Biochemical assessment is used as a health indicator in animals. Alteration of blood biochemical contents must have changed in physiological characteristics. The result of present study revealed that, there were no significant differences in glucose among treatments, although the goup tested with 2% mushroom extract had a lower glucose. The results of current study are parallel with finding of Binaii *et al.* (2014) and Vahedi *et al.* (2016) stated that glucose levels did not change with the use of diet containing nettle and ginger, respectively. These results are disagreement with the Akrami *et al*. (2015) results, who founded by onion supplementation that noted diminished glucose after feedings with onion powder diet in *H. huso*. According to some scholars, serum total proteins is recognized as the most important index of nutritional and the health status of fish (Patriche, Patriche & Tenciu 2009). In our investigatin, total protein of juvenile great sturgron was not altetnation after feeding with different doses of mushroom extract at the end of the eight week which is in line with the prior work of Vahedi *et al*. 2017 who noted non-significant different in total protein. Netevertheless the use of ginger flour as supplemented diet could cause the enhancement of total protein in juvenile *H. huso* (Gholipour Kanani *et al.* 2014). Prior research by Binaii *et al*. (2014) have illustrated that supplementation with 12 percent *U. dioica* remarkably enhancement the total serum protein of great sturgeon, whrease use 1% of onion powder in the beluga diet effected remarkably reduced the total protein content compared to the control group. (Akrami *et al*. 2015b). The bioactive compounds found in mushroom directly affect human health by activating immune mechanism (Regula & Siwulski, 2007). The bioactive compounds found in mushroom straightly affect human safty via activating immune mechanism. Total

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polyphenols were the major naturally occurring antioxidant ingredients found in the methanolic extracts and suggests a Cholesterol-lowering potential (Mau *et al*. 2002). In the present study, cholestrol concentration was strongly affected by feeding sturgeon with mushroom extract. These results are agree with those of Akrami *et al*. (2015b) and Vahedi *et al* (2017) in beluga treated with onion and ginger, respectively (Akrami *et al*. 2015b; Vahedi *et al*. 2017). Moreover, mushroom extract did not remarkably affect on triglyceride and lipid in comparison with the control. However, the lowest value of triglyceride and lipid were observed in 2% mushroom extract treatment. The reduction of triglycerides could depend on saponin exist in fungus as described in (Lee, Lim, Kang, Min, Son, Lee, Park, Ngo, Tran, Lim & Hwang 2012). Conversely, Akrami *et al*. (2015b) demonstrated that the use of 1% onion powder in beluga diet decreased the amount of triglyceride significantly in comparison with the control. Furthermore, Binaii *et al*. (2014) in their study, did not observed any change the level of cholesterol and triglyceride in their fourth week after consumption of nettle supplement, while in the eighth week, treatments fed 6% and 12% nettle in the diet remarkably decreased against other groups. AST, ALT, LDH and ALP enzymes are known as indexs ndices of liver damage (Bhardwaj, Srivastava, Kapoor & Srivastava 2010). In this study AST, LDH of juvenile sturgeon were not influenced by different level of mushroom extract, wherease levels of ALT and ALP in the serum are affected remarkably. It seems that bioactive compounds polyphenols, flavonoids and saponins found in mushroom prevent lipid peroxidation of cell membranes and intercept the release of mentioned enzymes into the plasma (Bobek *et al*. 1991). Conversely this investigation, result was obtained by Akrami *et al*. (2015b) which reported that AST and LDH levels illustrated a considerable decrease in great surgeon juvenile fed diet with 1% onion in comparison with the control, while ALT and ALP levels were not impressed. Gholipour kanani *et al*. (2014) and Binaii *et al*. (2014) who revealed that there were no remarkably decreased ALT, ALP and AST in *H. huso* fed diet ginger and nettle in comparison with the control. The serum lysozyme activity is considered as a defense barrier against bacterial pathogens thus resulting in the reduction of disease (Misra & Fridovich 1972). In current investigation, lysozyme activity of *H. hus*o was also enhanced by increasing the concentration of the mushroom extract in the supplemented diets in comparison with the control group. The enhunced activity of lysozyme due to the role of mushroom extract supplemented diet in enhancing the non-specific immune response in great sturgeon. This result is consistence with earlier studies of (Baba, Uluköy & Öntaş 2015) who reported that the feeding 2% mushroom extract increased lysozyme activity of *Oncorhynchus mykiss*. Also, elevated lysozyme activity have been reported in beluga after supplementing diets with 1% onion powder (Akrami *et al*. 2015b). However, On the contrary, this investigatin, lysozyme activity was not impressed in juvenile great sturgeon fed with ginger (Gholipour kanani *et al*. 2014). Superoxide dismutase (SOD) as an important

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anti-oxidation enzyme widely exists in tissues of aerobic and anaerobic organisms. In the current essay, fish fed diets supplemented with 2% *L. edodes* mushroom extract showed remarkably higher SOD activities when compared to the other experimental diet. The enhancement activity of SOD in the serum supported the role of mushroom extract supplemented diet in enhancing the non-specific immune response in beluga. This result is in agreement with those obtained by Akrami *et al*. (2015b) who found that an enhancement in beluga SOD enzyme after feeding with onion powder. Conversely, Vahedi *et al.* (2017) found that there was an increase in the SOD activity in beluga treated with ginger extract. Moreover, Lv, Zhou, Rudeaux, & Respondek (2007) reported that diet supplemented with herbal immune regulation mixture (HIRM) extract did not increase SOD activity of common carp (*Cyprinus carpio*).This possibly attributed to be that each pharmaceutic plant a particular district of the host immune system or that the time era for induction of immune response by medicine plant differs with respect to type of immune parameter. Immunoglobulins are one of the major molecules in humoral immunity of vertebrates. In our experiment, mushroom extract had no considerable on a serum total immunoglobulin in beluga fed diet in comparison with the control group. Baba *et al*. (2015) found that significant enhancement in serum total immunoglobulin of rainbow trout (*O. mykiss*) fed with 2% *L. edodes* mushroom extract supplemented diet. Prior studies have demonstrated a considerable individual variation in serum IgM levels between fish. Some changes might be related to size (Magnadóttir *et al*. 1999a; Picchietti, Scapigliati, Fanelli Barbato, Canese, Mastrolla, Mazzini & Abelli 2001) environmental conditions (Klesius, 1990; Magnadóttir *et al*. 1999b) or disease status (Magnadóttir *et al*. 1995; Nielsen 1999). Unlike this study, some researchers reported an enhancement of the IgM level after using medicine plants in *H. huso* (Binaii *et al*., 2014; Akrami *et al*. 2015b; Vahedi *et al.* 2017). The complement system is an vital and effective part of the innate immune system. The current study demonstrated that supplementation of diet with different levels of mushroom extract did not remarkably affect on complement activity (ACH50) in plasma great sturgeon juveniles. Conversely to this results enhancement in the total complement activity were reported in fish fed with a diet enriched with Pomegranate, Pyrethrum and Prickly ash extract (Harikrishnan *et al.* 2011), *S. marianum* (Ahmadi, Banaee, Vosoghei, Mirvaghefei & Ataeimehr 2012) and *Nasturium nasturtium* extracts (Asadi, Mirvaghefei, Nematollahi, Banaee & Ahmadi 2012). The manufactures of oxygen free radicals by neutrophils via the respiratory burst are momentous events in bactericidal pathways in fish (Sharp & Secombes, 1993) the first product released from the respiratory burst is anion superoxide; hence, an accurate evaluation of this activity can be via O^{-}_{2} measurement (Secombes, 1990). The results of the curret investigation demonstrated that there were no considerable differences in respiratory burst activity among treatment, although the group tested with 2% *L. edodes* mushroom extract had higher respiratory burst

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activity. This is in disagreement with the study of Binaii *et al*. (2014) and Akrami *et al* (2015b), which noticed greater respiratory burst activity in great sturgeon administred with dietary nettle and onion after feeding for 8 weeks.

Based on the results, it could be inferred that feeding beluga with 2% *L. edodes* mushroom extract improved growth performance and hemato-immunological indices.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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تأثیر عصاره قارچ (*Lentinula edodes*) بر عملکرد رشد، پاسخ ایمنی و فراسنجه‌های خونی - بیوشیمیایی در فیل ماهی جوان (*Huso huso* Linnaeus 1754)

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چکیده

هدف از این مطالعه، بررسی اثرات رژیم غذایی حاوی مکمل عصاره قارچ (*Lentinula edodes*) بر عملکرد رشد و نرخ بقاء، پاسخ ایمنی، مشخصه‌های خونی و بیوشیمیایی فیل ماهی جوان بود. چهارصد و پنجاه ماهی پس از طی دوره سازگاری با وزن متوسط ۴/۵۷ ± ۴۰/۴۵ گرم در یک طرح کاملاً تصادفی در ۵ تیمار (رژیم غذایی پایه حاوی: ۰٪ (کنترل)، ۰/۵٪، ۱٪، ۱/۵٪ و ۲٪ عصاره قارچ) و ۳ تکرار تغذیه شدند. در طول آزمایش (۵۶ روز)، در مجموع هشت نمونه‌گیری در ۸ هفته انجام شد. میانگین داده‌ها با استفاده از آنالیز واریانس یک‌طرفه و تست تعقیبی دانکن در سطح ٪۵ مورد بررسی قرار گرفت. نتایج نشان داد که مصرف ۲ درصد عصاره قارچ در طول ۸ هفته باعث بهبود درصد افزایش وزن، نرخ رشد ویژه و ضریب تبدیل غذایی در فیل ماهی جوان شده است ($P<0/05$) شد. در بین شاخص‌های هماتولوژی بررسی شده، مکمل ۲٪ قارچ منجر به افزایش سطوح گلبول قرمز، هموگلوبین و هماتوکریت در مقایسه با گروه شاهد گردید ($P<0/05$). در پایان آزمایش در تیمارهای حاوی عصاره قارچ در میزان بعضی از پارامترهای بیوشیمیایی خون (کلسترول، آلکالین فسفاتاز و آلانین آمینو ترانسفراز) کاهش معنی‌داری مشاهده شد ($P <0/05$). فعالیت آنزیم‌های لیزوزیم سرم و سوپر اکسید دیسموتاز در بین شاخص‌های ایمنی مورد مطالعه نیز در تیمار ۲٪ قارچ نسبت به سایر گروه‌ها افزایش نشان داد ($P <0/05$). این نتایج بیان داشت که افزودن مکمل عصاره قارچ *L. edodes* بویژه در سطح ۲٪ به رژیم غذایی فیل ماهی جوان عملکرد رشد، بسیاری از فراسنجه های خونی، بیوشیمیایی و پاسخ ایمنی در ماهی مذکور را بهبود بخشید.

کلمات کلیدی: هماتو - ایمونولوژیکال، رشد، فیل ماهی، عصاره قارچ.

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