Effect of garlic peel on haematological, biochemical and digestive enzyme activity in beluga juvenile (*Huso huso*)

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Abstract

Our study was concentrated on the effect of garlic peel on haematological parameters, biochemistry and digestive enzymes on beluga juvenile (*Huso huso*) with mean body weight of 18. 41 ± 0. 89 g after the feeding trial for 90 days. Garlic peel powder added with 0%, %0.5, %1, %1.5 and %2 of feed to basic sturgeon diet (49% protein and 14% lipid). ultimately, haematological parameters, biochemistry, digestive enzyme activity were evaluated. Results showed that the hemoglobin (Hb) was remarkably greater in the treatment fed garlic peel diet in comparison with the control ($p<0.05$), while others blood indices did not significant differences ($p>0.05$) between juveniles fed control and garlic peel supplementation diets. The group fed 1.5% garlic peel showed highly significant different in total protein ($p<0.05$). However, Glucose, triglyceride, total lipid, cholesterol, ALP and ALT were significantly decrease in the juvenile fish fed by the 1.5% garlic peel diet compare to the control ($p<0.05$).The fish treated with 1.5% garlic peel exhibited significantly increase in amylase, protease and lipase compare to the control ($p<0.05$), but trypsin, chymotrypsin and pepsin were not affected ($p>0.05$). These results indicated that garlic peel powder in 1.5% level, improved blood indices and digestive enzymes activity of beluga sturgeon.

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Introduction

Sturgeons are valuable species, which are known as endangered fish species (Safarpour-Amlashi, Falahatkar, Sattari, Tolouei & Gilani 2011). Caviar fish rearing sounds in progress in last decade in order to moderate pressure on sturgeon habitats in north water of Iran

(Pourkazemi 2006). Great sturgeon, *Huso huso*, is an imperative aquaculture species in Urasia area, Japan and Iran. Sturgeon fishis appropriate species for aquaculture due to its yield value (Mohseni, Pourkazemi, Bahmani, Pourali & Sajjadi 2007). In last decades, so many researchers showed their interest to make some studies on the field of herbal medicines to be used in aquaculture. These herbals could be as alternatives for antibiotics made some pathogens being resistant to them.. (Hoseinifar, Mirvaghefi & Merrifield 2011). They are environmental friendly and have not shown any harmful side effects for animals using them (Talpur, Ikhwanuddin, Ambok & Bolong 2013). Garlic skins are contains pectin (27 %), combined rhamnose (1.42 %) and galactose (5.6 %). There are no a few evidences concerning the effect of garlic crusts on health of animals. It could be due to the feeding behavior of human being were not interested to eat some un-edible parts of garlic such as skins. There are a few evidences on the structure of garlic crusts which included some chemicals containing pectin (Abdel-Fattah & Khaireldin 1970; Abdel-Fattah & Khaireldin 1972; Alexander & Sulebele 1973). Also, Schmidtlein Schmidtlein & Herrmann (1975) reported the enzymatic hydrolysate of this crusts included p-coumaric acid, ferulic acid, and sinapic acid. Ifesan Ifesan & Fadipe (2014) confirmed that garlic skins ethanol extract presented either antioxidant and antimicrobial characteristics like garlic bulb which may be described that the bioactive compounds demonstrate in the garlic bulb are likely to be available in the peel. The bioactive antioxidants of garlic peel are N trans -Coumaroyloctopamine, N- trans -feruloyloctopamine, guaiacylglycerol-ß -ferulic acid ether, and guaiacylglycerol- ß -caffeic acid have been determined as trans -coumaric acid and trans -ferulic acid. Huge quantities of garlic are consumed all over the world for flavoring various types of food and their outer layers not been utilized and discarded as waste. Earlier studies are more focused on the utilization of garlic pulp and its extracts in fish (Harada,1990). Few information is available on the effects of garlic peel in aquaculture (Thanikachalam, Kasi & Rathinam 2010; Chitsaz & Akrami, 2015) .Hence the present study is aimed to determine the effect of garlic peel in biochemical and haematological and digestive enzyme parameters of juvenile beluga.

Materials and Methods

Fish and their maintenance

Beluga fingerlings were prepared by contribution of Shahid Marjani Sturgeon Fish Propagation and Cultivation Centre (Golestan, Iran). The fish were acclimatized for three weeks in the two thousand liter tanks filled with Gorganrood River water. Water temperature in the tanks was approximately 19 ± 1.1°C. The fish before starting the experiment were fed with a commercial feed FFT_1 from Faradaneh Co (Sharekord, Iran) three times a day during acclimatization period.

Plant material and feed formulation

The plant material, *Allium sativum* was purchased at local market (Gorgan, Iran). The peels were separated from the garlic bulbs. The

peels were washed thoroughly and oven dried at 50 ºC. They were then grinded into well powder using lab blender. Then the powder was incorporated into fish feed at a rate of 0 (control), 0.5%, 1% ,1.5% and 2% incorporated into a control diet (containing 49% protein,14% lipid, 8% ash) as experimental diets. Purchased feed was crinkled, mixed with the sufficient concentration of garlic crust and water to prepare the diets based on the treatments and allowed to be dried for 18 h at 45 ºC (Akrami, Nasritajan, Jahedi, Jahedi, Razeghi Mansour & Jafarpour 2015a; Akrami ,Gharaei, Razeghi Mansour & Galeshi 2015b). The handmade diet was kept in plastic bags at refrigerator (4°C) until animal consumption. The control diet was prepared by adding only water with no any crust power.

Experimental design

After 2 weeks of acclimatization period, one hundred and fifty fingerling with average weight of 18. 41 ± 0. 89 g were randomly distributed among 15 tanks, with 10 fish a tank, in triplicates. well aeration was injected to water of the tanks. The fish were fed with the finalized diet of 2 - 5% of the average body weight per day (three times daily at 08:00, 14:00 and 20:00) for 90 days (Akrami, Nasritajan, Jahedi, Jahedi, Razeghi Mansour & Jafarpour 2015a). Water temperature, dissolved oxygen and pH were adjusted at 19.2 ± 1.1ºC, 7.2 ± 0.57 mg L^{-1} and 7.15 ± 0.2, respectively.

Blood sample collection

After the experiment ended, 6 sturgeons were randomly sampled each tank.from each fish, Four ml of blood was gathered through the caudal vein.. Heparinized and non-heparinized tubes were then received the drawn samples to perform the haematological and biochemical assesses, respectively. Serum samples were centrifuged at 4,500 g for 10 min and kept at −20 ˚C until the analysis would be begun.

Blood factors assays

A Neubaur haemocytometer was used to assess the measures of Red blood cells (RBC) and white blood cells (WBC) pursuant to Martins *et al*. (2001). other factors including Haemoglobin (Hb) (Collier , 1944); haematocrit (Hct) (Goldenfarb Goldenfarb, Bowyer, Hall & Brosious, 1971); and differential white blood cell counts were examined. The latter was carried out through panchromatically-stained smears (Klontz, 1994). Blood smears was stained through the Giemsa method to measure the Differential leukocyte counts (neutrophil, lymphocyte, monocyte and eosinophil) using a light microscope. The samples prepared from heparinized tube were dried at room temperature, fixed in 96 % ethanol for 30 min and stained using Giemsa solution. (Ghiasi, Mirzargar, Badakhshan & Shamsi 2010). Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH),triglyceride, cholesterol, glucose, total protein and albumin content were examined calorimetrically using commercial kits (ZiestChem diagnostics, Tehran, Iran) (Fazlolahzadeh, Keramati, Nazifi, Shirin&Seifi 2011). Globulin was calculated by subtracting albumin from total protein of plasma.

Assay of digestive enzymes

From each replicate three sturgeons and a total of 12 fish from per treatment were randomly selected euthanized with clove powder (200 mg L^{-1}), dissected for collecting the whole digestive tract. They were homogenized in 100 mM Tris–HCl buffer with 0.1 mM EDTA (Ethylenediaminetetraacetic acid) and 0.1 % triton X-100 at 9:1 ratio (pH 7.8) in an electric homogenizer (Heidolph, Instruments Switzerland). Every steps were carried out on ice. The homogenate were centrifuged at 25,000 g for 20 min at 4˚C, supernatant gathered, afterward stored at -80˚C for subsequant analysis. Total protease activity was investigated at 25˚C using 1 % (w/v) casein (Sigma, USA) as asubstrate in 0.2 M phosphate buffer at pH 7.0 (Walter, 1984). Pepsin was calculated at 37˚C utilizing 2 % hemoglobin in 0.06 N HCl as a substrate (Zambonino & Cahu, 1994). Tyrosin was utilized as a standard, and one unit of proteolytic activity and pepsin was defined as the quantity of enzyme needed for the organization of 1 mg of tyrosin per min. Persuant to study of Langlois, Corring & Fevrier (1987), Amylase activity was determined using 0.3 % soluble starch as substrate dissolved in NaH_2PO_4 buffer (pH 7.4). Amylase activity (U) was defined as the mg of starch hydrolyzed during 30 minute per mL homogenate at 37˚C. Based on information from previous study of Iijima, Tanaka & Ota in 1998, Lipase activity was determined for 15 min at 30˚C utilizing p-nitrophenol myristate as substrate that is dissolved in 0.25 M Tris–HCl (pH 9.0). One unit of lipase activity (U mL^{-1}) was specified as the lmol of substrate hydrolyzed per minute in 30˚C per mL homogenate. Alkaline phosphatase activity was calculated at 37˚C using 4-nitrophenyl phosphate (PNPP) as substrate dissolved in 30 mM $NaHCO_3$ buffer (pH 9.8) (Bessey, Lowry& Brock 1946). One unit of enzyme was defined of lmol hydrolyzed PNPP per min at 37˚C. Total protein concentration of blood in the homogenate was measured pursuant to Bradford method (1976) utilizing bovine serum albumin as standard. The particular activity of calculated enzymes was expressed as unit enzyme activity per mg protein (U mg^{-1} protein).

Statistical methods

All the data of this study were examined to one-way analysis of variance (ANOVA) utilizing the statistical software program SPSS version 16.0 (SPSSInc., IL, USA). Duncan's post-hoc test was used to compare the averages of data at significance level of $p<0.05$.

Results

Haematological parameters

The blood RBC ,WBC, Hb, Hct, monocyte, lymphocyte, neutrophil and eosinophil counts of different groups are shown in Table 1. The haemoglobin indices significant different ($p<0.05$) among the treatments and control group. There were no significant different between RBC,WBC, haematocryte percentage, monocyte , lymphocyte , neutrophil and eosinophil counts that fed garlic peel on compared with the control group ($p>0.05$).

Table 1. Effects of dietary garlic peel (% feed) on Haematological parameters of beluga juveniles for 90 days using a one-way ANOVA

parameter	control	0.5%	1%	1.5%	2%
RBC(10^6 ml^{-1})	1.07 ± 0.01^a	1.09 ± 0.01^a	1.08 ± 0.01^a	1.12 ± 0.01^a	1.07 ± 0.01^a
WBC(10^3 ml^{-1})	21.69 ± 1.94^a	22.92 ± 1.58^a	22.49 ± 1.31^a	21.77 ± 1.61^a	22.48 ± 1.73^a
Hb (g/dl)	5.71 ± 0.92^b	6.18 ± 0.3^{ab}	6.5 ± 0.29^a	6.21 ± 0.35^{ab}	6.11 ± 0.17^{ab}
Hct%	24.46 ± 3.57^a	24.13 ± 2.85^a	21.76 ± 2.82^a	24.53 ± 2.57^a	23.13 ± 2.37^a
Monocyte (%)	1.66 ± 0.51^a	1.50 ± 0.54^a	1.69 ± 0.38^a	1.61 ± 0.12^a	1.58 ± 0.42^a
Lymphocyte(%)	73.16 ± 6.55^a	75.83 ± 1.9^a	73.50 ± 8.52^a	73.00 ± 1.23^a	73.42 ± 1.43^a
Neutrophil (%)	13.00 ± 1.89^a	14.33 ± 1.45^a	14.00 ± 1.32^a	12.00 ± 1.46^a	13.22 ± 1.54^a
Eosinophil(%)	12.16 ± 1.65^a	8.33 ± 1.52^a	10.83 ± 1.79^a	13.33 ± 1.22^a	11.62 ± 1.31^a

Biochemical assays

The results of blood serum biochemical test of juveniles fish fed with garlic peel are shown in Table 2. There was a significant increase ($p<0.05$) in the Total protein between treatment in comparison with the control group and in other treated groups, glucose, cholestrol, triglycerid, ALT, ALP and total lipid level reduced significantly ($p<0.05$) when in comparison with control group. But, in the same time (90 days), based on statistical analysis of data, there were no significant differences ($p>0.05$) of AST, LDH, Albumin, Globulin and Albumin / Globulin.

Table 2. Blood serum biochemical parameters of *Huso huso* juveniles fed with garlic peel (% feed) added diet at different levels for 90 days.

parametr	control	0.5%	1%	1.5%	2%
Glucose (mg l^{-1})	95.28 ± 2.06^a	92.33 ± 2.03^a	89.46 ± 2.16^{ab}	84.12 ± 1.48^b	88.31 ± 1.26^{ab}
Total Protein (mg l^{-1})	1.96 ± 0.25^a	2.68 ± 0.47^a	2.86 ± 0.19^{ab}	3.03 ± 0.21^b	2.23 ± 0.48^a
Cholestrol (mg dl^{-1})	104.50 ± 1.88^a	88.33 ± 2.20^{ab}	98.40 ± 1.72^a	72.50 ± 1.89^b	90.21 ± 1.1^{ab}
Albumin (mg l^{-1})	2.04 ± 0.19^a	2.10 ± 0.4^a	2.30 ± 0.01^a	1.96 ± 0.22^a	2.28 ± 0.21^a
Globulin (mg l^{-1})	0.94 ± 0.01^a	0.83 ± 0.19^a	0.93 ± 0.18^a	1.00 ± 0.27^a	0.88 ± 0.22^a
Albumin/ Globulin	2.17 ± 0.21^a	2.53 ± 0.76^a	2.47 ± 0.43^a	1.96 ± 0.75^a	2.59 ± 0.65^a
Triglycerid (mg dl^{-1})	303.16 ± 1.19^a	301.50 ± 4.20^a	311.16 ± 2.59^a	253.00 ± 3.44^b	298.1 ± 32.59^{ab}
Total Lipid (mg dl^{-1})	407.66 ± 2.55^a	391.83 ± 3.30^a	402.60 ± 1.30^a	355.50 ± 2.11^c	381.23 ± 2.42^b
(AST) (IU dl^{-1})	305.5 ± 6.16^a	298.66 ± 5.76^a	287.83 ± 6.82^a	281.50 ± 5.57^a	291.50 ± 4.81^a
(ALT) (IU dl^{-1})	6.16 ± 1.78^a	4.06 ± 1.34^b	4.66 ± 1.28^{ab}	4.00 ± 1.58^b	3.42 ± 1.25^b
(ALP) (IU dl^{-1})	62.83 ± 5.61^a	50.0 ± 8.36^b	56.33 ± 6.63^{ab}	50.12 ± 9.91^b	50.00 ± 8.65^b
(LDH) (IU dl^{-1})	1995.83 ± 1.36^a	1985.60 ± 1.48^a	1996.16 ± 1.31^a	1901.40 ± 2.26^a	1902.71 ± 2.15^a

Digestive enzyme activit

The total protease, pepsin, amylase, lipase , trypsin and chymotrypsin activity levels, pursuant to the experimental treatments, are shown in figure1-6. At the completion of the experiment, the results indicated that the fish fed garlic peel diet had significantly increased ($p<0.05$) total protease, lipase and amylase activity. More ever, the results illustrated that feeding fish with experimental treatments had no statistically significant difference effect on pepsin,trypsin and chymotrypsin activity between the treatments ($p>0.05$). The utmost values of enzymes were found in the 1.5% treatment, although they were not remarkably different with the control.

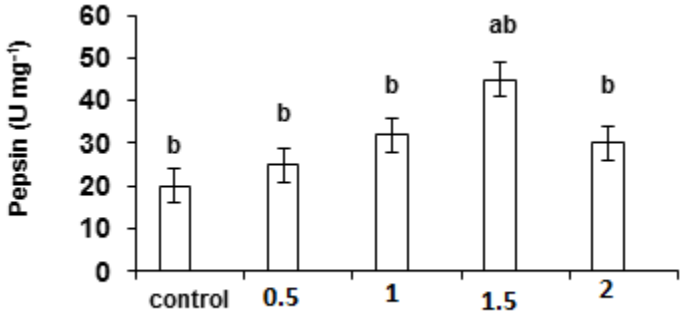

Figure 1. Total pepsin enzyme activities in beluga juvenile fed experimental diets for 90 days. values are averages ± SD. Different superscript shows difference at $p<0.05$.

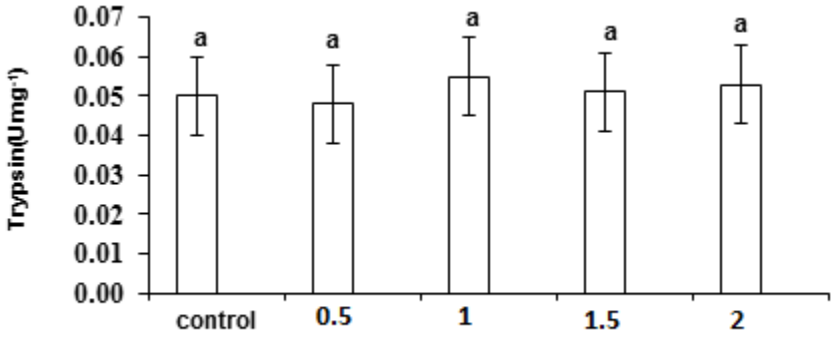

Figure2. Total trypsin enzyme activities in beluga juvenile fed experimental diets for 90 days. values are averages ± SD. Different superscript shows difference at $p<0.05$.

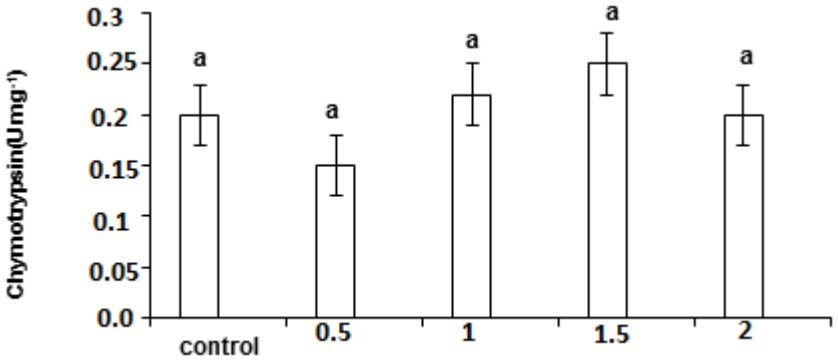

Figure 3. Total Chimotrypsin enzyme activities in beluga juvenile fed experimental diets for 90 days. values are averages ± SD. Different superscript shows difference at $p<0.05$.

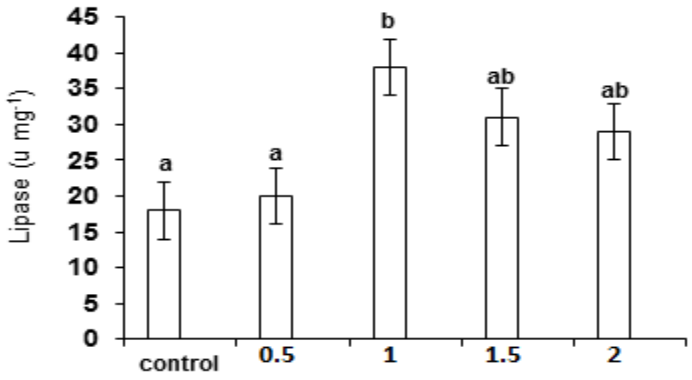

Figure 4. Total Lipase enzyme activities in beluga juvenile fed experimental diets for 90 days. values are averages ± SD. Different superscript shows difference at p<0.05.

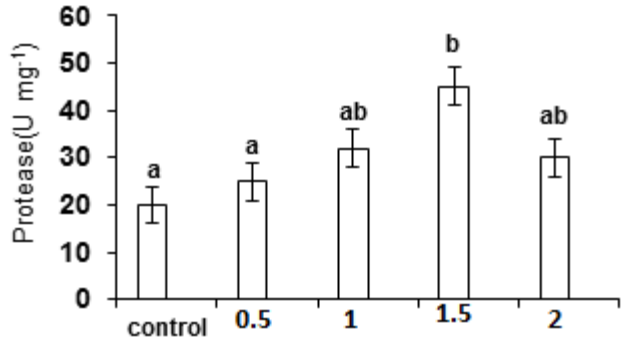

Figure 5. Total Protease enzyme activities in beluga juvenile fed experimental diets for 90 days. values are averages ± SD. Different superscript shows difference at p<0.05.

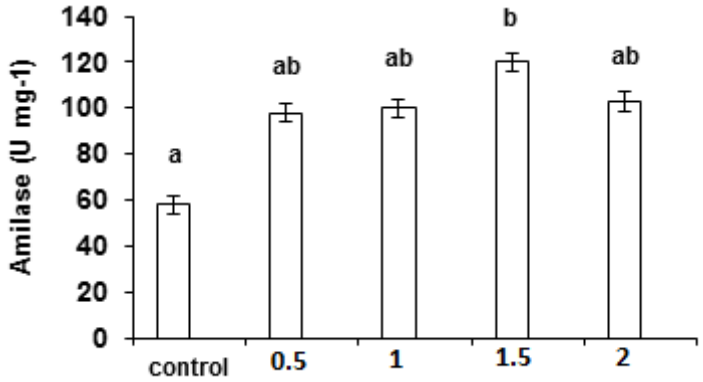

Figure 6. Total Amylase enzyme activities in beluga juvenile fed experimental diets for 90 days. values are averages ± SD. Different superscript shows difference at p<0.05.

Discussion

In the present study was evaluated on the effect of garlic peel in order to assess its role in haematological and biochemical digestive enzyme in *Huso huso* , which was selected as a fish model due to its importance in commercial sturgeon aquaculture. Haematological indicators are a criterion and reflectance of the effects of dietary treatments on the animal in terms of the kind, quality and amounts of the feed ingested and were accessible for the animal to meet its physiological, biochemical and metabolic necessities (Ewuola *et al.*, 2004). The WBC (leucocytes) are well known as one of the first defensive lines of the body that infection causes increase sharply their number. So many investigations have been done on proving the effectiveness of medicinal plants as immunostimulants and a factor increaseing the number of total WBC. (Jian and Wu, 2003). In our experiment, leucocytes count increased in the treatment fed garlic peel diet in comparison with the control group, although, this increase was no statistically significant. The incrumentleucocytes number following feeding of garlic peel diet demonstrates the immunostimulatory effects and anti-infection properties of garlic skin which this finding supports previous study of Thanikachalam *et al.*, (2010) who has shown that WBC count significantly increased in African catfish (*Clarias gariepinus*) following 20 day garlic peel post feeding. Similarly Gholipour kanani *et al.* (2014), determined that there was no significant difference in WBC for *H.huso* fed diet including ginger on 8 weeks. On the contrary, Binaii, Ghiasi, Farabi, Pourgholam, Fazli, Safari, Alavi, Taghavi & Bankehsaz (2014) and Akrami, Gharaei, Razeghi Mansour & Galeshi (2015b) reported that the supplementation of diets with nettle (*Urtica dioica*) and onion powder remarkably increased *H. huso* juvenile leucocytes, respectively.The haemoglobin (Hb) content in the blood plays a vital role and acts as carrier element.of oxygen to body tissues. In the current study, the haemogolobin content was significantly greater in 1% garlic peel than those the fed other diets, which illustrates that oxygen supply increases consequently, reflecting advantageous health effect on fish. This is in line with the work Binaii *et al.* (2014) who reported significant increase in haemoglobin level in *H.huso* fed with diet incorporated with nettle in comparison with the control group Conversely, GholipourKanani, Nobahar, Kakoolaki & Jafarian (2014) and Akrami *et al.* (2015b), found that the level of Hb were not affected by ginger and onion powder in the basal diet of juvenile *H.huso*. The current study revealed that administering garlic peel through fish feed had no significant difference in RBC(erythrocyte) compared to control ($p>0.05$). Similar result was reported by Gholipour kanani *et al* in 2014, who obtained that there was no significant difference in terms of RBC after feeding *H.huso* with diet ginger in comparision with the control.. Unlike this study, the erythrocyte count increased with the administration of garlic peel in African catfish (Thanikachalam, Kasi &Rathinam 2010). Moreover, dietary

inclusion of various source of additives (nettle and onion powder) increased RBC value in *H.huso* juvenile (Binaii *et al.,* 2014; Akrami *et al.*, 2015b). The cause of these various results might be attributed to difference in the affect of medicinal plant and immune system reaction (Binaii *et al.*, 2014). In this study Hct % level did not be affected by different levels of garlic peel. These results are in disagreement with previous studies, has been reported that immunostimulant herbal plant (nettle, ginger and onion powder) could increase Hct% in *H.huso* (Gholipour kanani *et al.,* 2014; Binaii *et al.*, 2014; Akrami *et al.*, 2015b). Sakai (1999) proposed that immunostimulants could decrease the fish loss in aquaculture due to by some diseases; Furthermore, consideration for the timing, dosages, method of administration, and the physiological condition of fish for the effective use of immunostimulants are very essential.

Blood cells containing lymphocytes, monocytes and neutrophils increased during severe infections caused by pathogens and straightly attack and destroy pathogenic microorganisms and another externaltoxic material. The findings of this study showed that these blood cells were not remarkably affected by garlic peel diet. This result coincide with the investigation of Gholipour kanani *et al* (2014) and Akrami *et al* (2015b), who offered that lymphocytes, neutrophils, monocyte and eosinophil levels were not offered affected by ginger and onion powder diet in great sturgeon respectively. The bioactive compounds are attributes and suggests a role in the inhibition of infections and activating immune mechanism(Ifesan 2014).

The present study results indicated that, glucose of the sturgeon blood was remarkably affected by the experimental diets. The results of this research are compatible with finding of Akrami *et al.* (2015b) noted reduced glucose after feeding with onion powder diet great sturgeon. These results are disagreement with finding of Binaii *et al.* (2014) that received glucose was not affected in *H. huso* juvenile fed nettle.

In the present study, the cholesterol and triglyceride were significantly decreased in treated fish group over the control which is in line with the Akrami *et al.* (2015b) who reported that utilization of 1% onion powder in diet of *H.huso* made a significant decrement on triglyceride and cholestrol when compared with control diet. Binaii *et al*. (2014) observed that the adition of garlic crust in week 4 there were no affect the cholestrol and triglyceride levels in treated fish. while they were remarkably decreased in beluga fed on dietary 6% and 12% nettle in comparison with the other group on week 8. This discrepancy could be caused by different in the effect of herbal plant in the form of extract and/or dried powder. Some researchers claim that the most importantly index of the biochemical nutritional and health situation of the fish is serum total proteins (Patriche *et al.*, 2009). In the present study, total protein had significant increase in 1.5% compare to the juvenile beluga after feeding with different doses of garlic crust and control group. Similarly, Thanikachalam *et al.*, (2010) showed that the dietary containing the garlic

skin had significant impact on protein of African catfish fingerling. The application of ginger powder as supplemented diet could cause the enhancementof total protein in juvenile *H.huso* (Gholipour kanani *et al,* 2014). Pursuant to Previous study of Binaii *et al.* (2014) utilization of supplementation with 12% nettle remarkably increased the total serum protein of great sturgeon, whrease consumption of 1% onion powder in diet of *H.huso* caused a significant reduce on total protein when compared with control group (Akrami *et al.,* 2015b).

It has been recognised that albumin and globulin are essential elements for preserving a healthy immune system (Jha, Pal, Sahu, Kumar& Mukherjee 2007; Nya and Austin, 2009). This paper show that albumin and globulin had no remarkably difference in fish fed diet including garlic peel in comparison with the control. This result is accord with finding of Binaii *et al.* (2014) that obtained albumin level was not affected in great sturgeon juvenile fed nettle. On the contrary Gholipour kanani *et al.* (2014) reported that, globulin remarkably increased in serum in *H.huso* fed diet ginger. However, Akrami *et al.* (2015b) found that albumin and globulin levels were lower in *bluga* fed on dietary onion powder in comparison with the control. On the contrary, Thanikachalam *et al.,* (2010) reporterd enhanced serum albumin and globulin in African catfish fingerling fed with all the dosages of garlic peel when compared to control group.

AST, ALT, LDH and ALP enzymes are utilized as indicators of liver damage. High levels may indicate degeneration, necrosis, and destruction of the liver becouse of cellular harm. (Bhardwaj, Strickland, Ahmad, Atanesyan, West & Lloyd 2009). LDH and AST revealed a non-significant difference in all beluga fed diet containing garlic peel in comparison with the control group that demonstrated the consumption of garlic peel did not seem to induce liver toxicity in fish. In this work, in fish fed diets including garlic peel remarkably decreased ALT and ALP activity for 8-week in comparison with the control. It could be inferred that utilize the garlic peel due to the presence of bioactive compounds prevented fish from infection by triggering immune system and its consumption might prevent lipid peroxidation of cell membranes and prohibit the release of mentioned enzymes into the plasma. Gholipour kanani *et al* (2014) and Binaii *et al.* (2014) who reported that there were no statistically significant difference in ALT, ALP and AST in great sturgeon fed diet ginger and nettle in comparison withthe control. Akrami *et al.* (2015b) also reported that AST and LDH levels showed a remarkably reduce in *Huso huso* juvenile fed diet with 1% onion in comparison with the control group and 0.5% onion powder diet, while ALT and ALP levels were not impressed.

Digestion is a key process in animal metabolism since it determines the availability of nutrients needed for all biological functions and a principal tool in studying the nutritional condition and adaptation of the organism to dietary change (Gisbert, Giménez, Fernández, Kotzamanis & Estévez, 2009).The flavor imparted by herbs and herbal products added in

fish diet changed the eating patterns, increased feed consumption and stimulated digestion by increasing the secretion of saliva, various digestive enzymes, bile, pancreatic enzymes activity and mucus in fishes (Lee & Gao, 2012; Platel, Rao, Saraswah & Srinivasan 2002). It has been shown that herbs stimulated the secretion of pancreatic enzymes, important factors in nutrient digestion and assimilation (Frankic, 2009). In the current study, juvenile beluga fed with garlic peel incorporated diet showed the increased activity of digestive enzymes such as amylase, lipase and protease which enhanced digestion and absorption of nutrients essential for fish growth. The results of this study is compatible with finding of Shubha Ratna Shakya (2017) who obtained digestive enzymes increased in *Catla catla* fed with *Cynodon dactylon*. Similarly, improved amylase activity under various herbal additives has been reported by many researchers (Sankar, Philip & Philip 2017). Trypsin activity is considered as a nutritional condition indicator of fish and its secretion is consistent with the pancreas activity (Sunde, Taranger & Rungruangsak-Torrissen 2001). Recent studies showed that protease-based digestion was considered as an important component for carnivorous fishes (Rungraungsak-Torrissen, Sunde, Berg, Nordgarden, Fjelldal & Oppedal 2008). The result of this research showed that there were no significant difference in trypsin activity among treatment, although the group tested with 1% garlic peel had higher trypsin. Plants and food additives increase absorption of essential nutrients leading to better growth of organisms (Windisch, Schedle, Plitzner & Kroismayer 2008). In the current study, variant concentrations of garlic peel did not remarkably affect on pepsin and chymotrypsin. This offers that the increased activity of the digestive enzymes in the beluga induced by extrinsic plant strains has an innate limit. The general conclusion prepared from the present study is that the medicinal plant plays a vital role in digestive enzyme activity.

Conclusion

Garlic peel could improve some haematobiochemical and digestive enzyme in beluga. Further research is essential to clarify the action mechanism of garlic peel, also the adequate inclusion dose and feeding course in *Huso huso*.

Conflict of interests

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

References

Abdel-Fattah A. F., Khaireldin A. (1970) Pectin of garlic skins. U. A. R. J. Chem. 13, 27-37.

Abdel-Fattah A. F., Edrees M. (1972) A study on the composition of garlic skins and the structural features of the isolated pectic acid. *Journal of the Science of Food and Agriculture* 23, 871-877.

Alexander M. M., Sulebele G.A. (1973) Pectic substances in onion and garlic skins. *Journal of the Science of Food and Agriculture* 24, 611-615.

Akrami R., Nasritajan M., Jahedi A., Jahedi M., Razeghi Mansour M., Jafarpour S.A. (2015a) Effects of dietary Synbiotic on growth, survival,lactobacillus bacterial, blood indices and immunity of beluga (Huso huso) juvenile. *Aquaculture Nutrition* 21(6), 952–959.

Akrami R., Gharaei A., Razeghi Mansour M. Galeshi A. (2015b) Effects of dietary onion (Allium cepa) powder on growth, innate immune response and haemato-biochemical parameters of beluga (Huso huso) juvenile. *Fish and Shellfish Immunology* 45 (2), 828–834.

Bessey OA, Lowry OH., Brock MJ. (1946) Rapid coloric method for determination of alkaline phosphatase in five cubic millimeters of serum. *Journal* of *Biological Chemistry* 164, 321–329.

Bhardwaj N., Strickland AD., Ahmad F., Atanesyan L., West K., Lloyd DM. (2009) A comparative histological evaluation of the ablations produced by microwave, cryotherapy and radiofrequency in the liver. *Pathology* 41 (2), 168–172.

Binaii M., Ghiasi M., Farabi S.M., Pourgholam R., Fazli H., Safari R., Alavi S.E., Taghavi M.J., Bankehsaz Z. (2014) Biochemical and haemato-immunological parameters in juvenile beluga (Huso huso) following the diet supplemented with nettle (Urtica dioica). *Fish Shellfish Immunology* 36 (1), 46- 51.

Bradford M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72, 248–254.

Chitsaz H., Akrami R. (2016) Effect of garlic peel on metabolic enzymes activity in common carp (Cyprinus carpio) The first national conference of aromatic and medicinal herbs Gonbad kavous university.

Collier H.B. (1944) The standardization of blood hemoglobin determinations. *Canadian Medical Association Journal* 50, 550–552.

Ewuola E.o., Folayan o.A., Gbore F.A., Adebunmi Akanji R.A., Ogunlade J.T., Adeneye J.A. (2004) Physiological response of growing West African dwarf goats fed groundnut shell-based diets as the concentrate supplements. *Bowen Journal of Agriculture* 1(1), 61-69.

Fazlolahzadeh F., Keramati K., Nazifi S., Shirin S., Seifi S. (2011) Effect of garlic (Allium sativum) on haematological parameters and plasma activities of ALT and AST of Rainbow trout (Oncorhynchus mykiss) in temperature stress. *Australian Journal of Basic and Applied Sciences* 5 (9), 84-90.

Frankic T., Voljc M., Salobir J., Rezar V.(2009) Use of herbs and spices and their extracts in animal nutrition**.** *Acta agriculturae Slovenica.* 94(2), 95-102.

Ghiasi F., Mirzargar SS., Badakhshan H., Shamsi S. (2010) Effects of low concentration of cadmium on the level of lysozyme in serum, leukocyte count and phgocytic index in Cyprinus carpio under the wintering conditions. *Journal of fisheries and Aquatic Science* 5(2), 113-119.

Gholipour Kanani H., Nobahar Z., Kakoolaki Sh., Jafarian H. (2014) Effect of ginger- and garlic-supplemented diet on growth performance, some haematological parameters and immune responses in jvenile Huso huso. *Fish physiology and biochemistry* 40, 481–490.

Gisbert E., Giménez G., Fernández I., Kotzamanis Y. Estévez A. (2009) Development of digestive enzymes in common dentex, Dentex dentex during early ontogeny. *Aquaculture*, 287, 381-387.

Goldenfarb P.B., Bowyer F.P., Hall E., Brosious E. (1971) Reproducibility in the haematology laboratory: the microhaematocrit determination. *American Journal of Clinical Pathology* 56(1), 35–39.

Harada K. (1990) Attraction activities of spices for oriental weather fish and yellowtail. *Bulletin of the Japanese Society for the Science of Fish* 56, 2029-2033.

Hassanpour M. (2015) Effect of Dietary Ginger (Zingiber officinale) Extract on Growth, Biochemical and Immunological Parameters in Juvenile Huso huso, MSc. Thesis, Khazar Institute of Higher Education (Nonprofit- Nongovernment), Mahmoudabad, Iran pp:124.

Hoseinifar S.H., Mirvaghefi A., Merrifield D.L., (2011**)** The effects of dietary inactive brewer's yeast Saccharomyces cerevisiae var. ellipsoideus on the growth, physiological responses and gut microbiota of juvenile beluga (Huso huso). *Aquaculture nutrition* 25, 354-362.

Ifesan B. O. T., Fadipe E. A., (2014**)** Investigation of Antioxidant and Antimicrobial Properties of Garlic Peel Extract (Allium sativum) and Its Use as Natural Food Additive in Cooked Beef**.** *Journal of Scientific Research and Reports*. 3(5), 1-15.

Iijima N., Tanaka S., Ota Y., (1998) Purification and characterization of bile salt–activated lipase from the hepatopancreas of red sea bream, Pagrus major. *Fish Physiology and Biochemistry*, 18, 59–69.

Jha AK., Pal A.K., Sahu N.P., Kumar S. Mukherjee S.C. (2007) Haematoimmunological responses to dietary yeast RNA,w-3 fatty acid and b-carotene in Catla catla juveniles. *Fish & Shellfish Immunology* 23, 917–927.

Jian J., Wu Z. (2003) Effects of traditional Chinese medicine on nonspecific immunity and disease resistance of large yellow croaker (Pseudosciaena crocea). *Aquaculture*, 218, 1–9.

Klontz G.W. (1994) Fish haematology. *Techniques in Fish Immunology* 3, 121–132.

Langlois A., Corring T., Fevrier C. (1987) Effects of wheat bran on exocrine pancreas secretion in the pig. *Reproduction Nutrition Developpement* 27(5), 929–939.

Lee JY., Gao Y.(2012) Review of the application of garlic, Allium sativum, in aquaculture**.** *Journal of the World Aquaculture Society* 43(4), 447-458.

Martin-Kleiner I., Flegar-Mestric Z., Zadro R., Breljak D., Stanovic Janda S., Stojkovic R., Marusic M., Radacic M& Boranic M. (2001) The effect of the zeolite clinoptilolite on serum chemistry and hematopoiesis in mice. *Food and Chemical Toxicology* 39(7), 717-27.

Mohseni M., Pourkazemi M., Bahmani M., Pourali H.R., Sajjadi M.M. (2007) Effect of different dietary protein to energy ratios (P/E) on growth performance and body composition of farmed persian sturgeon (Acipenser persicus). *Iranian scientific fisheries journal* 16(1).

Nya E.J. Austin B. (2009) Use of dietary ginger, Zingiber officinale Roscoe as an immunostimulant to control Aeromonas hydrophila infections in rainbow trout, Oncorhynchus mykiss (walbaum). *Journal of Fish. Diseases* 32(11), 971-977.

Patriche T., Patriche N., Tenciu M. (2009), Cyprinids total blood proteins determination, *Scientific Papers Animal Science and Biotechnologies* 42(2), 95-101, Timişoara.

Platel K., Rao A., Saraswah G., Srinivasan K. (2002) Digestive stimulant action of three indian spice mixes in experimental rats. Die Nahrung. 46, 394-398.

Pourkazemi M., (2006) Caspian Sea sturgeon conservation and fisheries: Past, Present and Future. *Journal of Applied Ichthyology* 22, 12-16.

Rungraungsak-Torrissen K., Sunde J., Berg A.E., Nordgarden U., Fjelldal P.G. Oppedal F. (2008) Digestive efficiency, free amino acid pools and quality of growth performance in Atlantic salmon (Salmo salar L.) affected by light and vaccine type. *Fish Physiology and Biochemistry* 35 (2), 255-272.

Safarpour Amlashi A., Falahatkar B., Sattari M., Tolouei Gilani MH. (2011) Effect of di-etary vitamin E on growth, muscle composition, haematological and immu-nological parameters of sub-yearling beluga Huso huso L. *Fish & Shellfish Immunology* 30, 807-14.

Sakai M. 1999. Current research status of fish immunostimulants. *Aquaculture* 172, 63–92.

Sankar H., Philip B., Philip R. (2017) Effect of probiotics on digestive enzyme activities and growth of cichlids, Etroplus suratenis (Pearl spot) and Oreochromis mossambicus (Tilapia). *Aquaculture Nutririon Journal* 23, 852-864.

Schmidtlein H., Herrmann K. (1975) On the phenolic acids of vegetables. IV. Hydroxycinnamic acids and hydroxybenzoic acids of vegetables and potatoes. Z. Lebensm. Unters.-Forsch 159, 255-263.

Shubha Ratna Sh.(2017) Effect of Herbs and Herbal Products Feed Supplements on Growth in Fishes: A Review. *Nepal Journal of Biotechnology* 5(1), 58-63.

Sunde J., Taranger G.L., Rungruangsak-Torrissen K. (2001) Digestive protease activities and free amino acids in white muscle as indicators for feed conversion efficiency and growth rate in Atlantic salmon (Salmo salar L.) *Fish Physiology and Biochemistry* 25, 335–345.

Talpur A.D., Ikhwanuddin M., Ambok Bolong A. (2013) Nutritional effects on ginger (Zingiber officinal Roscoe) on immune response of Asian sea bass (Lates calcarifer) and disease resistance against Vibrio harveyi. *Aquaculture* 46, 400–401.

Thanikachalam K., Kasi M., Rathinam X. (2010) Effect of garlic peel on growth, haematological parameters and disease resistance against Aeromonas hydrophila in African catfish Clarias gariepinus (Bloch) fingerlings. *Asian Pacific Journal of Tropical Medicine*. 614-618.

Tras Brown K.M. (2007) Fish applied pharmacology, translated by: Fatemi M., Mirzargar s. Tehran University Press. first publish.

Vahedi AH., Hasanpour M., Akrami R., Chitsaz H. (2017) Effect Of Dietary Supplementation With Ginger (Zingiber officinale) Extract On Growth, Biochemical And Hemato-immunological Parameters In Juvenile Beluga (Huso Huso), *Iranian journal of aquatic animal health* 3(1), 26-46.

Vaseeharan B., Thaya R. (2013) Medicinal plant derivatives as immunostimulants. an alternative to chemotherapeutics and antibiotics in aquaculture. *Aquaculture International 22*(3), 1079-1091.

Walter H. (1984) Proteinases methods with hemoglobin, casein and azocoll as substrates. In Bergmeyer HU (ed) *Methods of enzymatic analysis*, vol V. Verlag Chemie, Weinheim, pp 270–277.

Wilson A., Demmig-Adams B. (2007) Antioxidant, anti-inflammatory, and antimicrobial properties of garlic and onions, *Nutrition and Food Science*, 27 (3).

Windisch W., Schedle K., Plitzner C., Kroismayer A. (2008) Use of phytogenetic products as feed additives for swine and poultry. *Journal of Animal Science* 86 (Supplement): E140–E148.

Yano T. (1992) Assay of hemolytic complement activity. In: J.S. Stolen, T.C. Fletcher, D.P. Anderson, S.C. Hattari, A.F. Rowley (Eds.). *Techniques in fish immunology*. SOS Publications, Fair Haven, New Jersey, USA. 2, 131-141.

Zambonino JL., Cahu CL. (1994) Influence of diet on pepsin and some pancreatic enzymes in sea bass (Dicentrarchus labrax) larvae. *Comparative Biochemistry and Physiology Part A: Physiology* 109, 209–212.

تأثیر جیره حاوی پوست سیر بر مشخصه‌های خونی، بیوشیمی و فعالیت آنزیم‌های دستگاه گوارش فیل ماهی جوان پرورشی (*Huso huso*)

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چکیده

تأثیر سطوح مختلف پوست سیر در جیره غذایی بر فراسنجه‌های خونی، بیوشیمی و فعالیت آنزیم‌های دستگاه گوارش فیل ماهی جوان پرورشی (*Huso huso*) پس از ۹۰ روز تغذیه مورد بررسی قرار گرفت. پودر پوست سیر در سطوح مختلف صفر، ۰/۵، ۱، ۱/۵ و ۲ درصد به جیره تجاری و پایه ماهی خاویاری حاوی ۳۳/۶۴٪ پروتئین خام و ۸/۳۹٪ چربی خام افزوده شد. در انتهای دوره پرورش، خونگیری از ساقه دمی ماهیان به ظاهر سالم انجام گرفت و مشخصه‌های خونی، بیوشیمی و فعالیت آنزیم‌های دستگاه گوارش فیل ماهی جوان پرورشی (*Huso huso*) مورد بررسی قرار گرفت. افزایش معنی‌داری در میزان هموگلوبین در ماهیان تغذیه شده با سطح ۱٪ پودر پوست سیر بدست آمد($p<۰/۰۵$) ولی در سایر شاخص‌های هماتولوژی تفاوتی بین تیمارها مشاهده نگردید. ($p>۰۵$). همچنین در مطالعه شاخص‌های بیوشیمی سرم فیل ماهیان تغذیه شده با سطح ۱/۵٪ پوست سیر تفاوت معنی‌داری در مقادیرگلوکز، پروتئین تام ، کلسترول، تری گلیسرید، چربی تام، ALP و ALT مشاهده شد($p<۰/۰۵$)، اما در مقدارآلبومین،گلوبولین، نسبت آلبومین به گلوبولین، AST و LDH تفاوت معنی‌داری بین برخی تیمارهای آزمایشی و گروه شاهد مشاهده نگردید. ($p>۰۵$). در مطالعه مقادیر آنزیمهای گوارشی فیل ماهیان پرورشی تغذیه شده با سطح ۱/۵٪ پوست سیر تفاوت، تیمارهای ۱٪ و ۱/۵٪ به ترتیب بر میزان لیپاز؛ آمیلاز و پروتئاز تاثیر معنی‌داری داشتند اما بر سایر آنزیم‌های گوارشی مورد مطالعه (پپسین، تریپسین و کیموتریپسین) تأثیری نداشتند. در مجموع نتایج بدست آمده از این تحقیق نشان داد افزودن پوست سیر به جیره ماهیان جوان خاویاری پرورشی به ویژه در سطح در ۱/۵ درصد می‌تواند منجر به بهبود شاخص‌های ایمنی گردد.

کلمات کلیدی: پوست سیر، مشخصه‌های خونی، بیوشیمی، فعالیت آنزیم‌های گوارشی، فیل ماهی

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