Effect of dietary supplementation with ginger (*Zingiber officinale*) extract on growth, biochemical and hemato-immunological parameters in juvenile beluga (*Huso huso*)

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Abstract

The study was performed to examine the efficacy of ginger (*Zingiber officinale*) extract on growth performance, biochemical and hemato-immunological parameters in juvenile beluga (*Huso huso*). Fish were divided into 4 groups before fed diet for 8 weeks with 0.5%, 1% and 1.5% ginger extract and with unsupplemented commercial diet as the control. Results showed that there was a significant different in weight gain in fish fed ginger extract diet compared to the control (P<0.05). There were no significant differences in condition factor, feed conversion ratios, specific growth rate and survival between juveniles fed control and ginger extract supplementation (P>0.05). In addition, there were no significant differences of RBC, WBC counts, Hct, monocyte, lymphocyte, neutrophil, eosinophil, glucose, TPP, triglyceride, lipid and globulin levels between the treatment groups (P>0.05). Furthermore, alternative complement activity (ACH50), serum total immunoglobulin (Ig) and lysozyme activity were significantly increased in 1.5% ginger fed fish (P<0.05); however, it did not change the SOD activity, significantly (P>0.05). Therefore, the results suggest that by using 1.5% this extract there will be an improvement in hemato-biochemical parameters and immune function of juvenile beluga.

Keywords: ginger (*Zingiber officinale*) extract, growth, hemato-biochemical, immune response, *Huso huso*.

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Introduction

Beluga (*Huso huso*) is one of the most important species of sturgeon in the Caspian Sea, which is due to overfishing, habitat destruction and pollution of natural water is at risk. This fish is a suitable species for aquaculture in Iran (Akrami, Nasritajan, Jahedi , Jahedi, Razeghi Mansour & Jafarpour 2015a). The most important risks faced by fish farmers were reducing the survival rate of fish and the incidence of some diseases and pollution, especially in the early stages of life. Therefore, strengthening the immune system and the immune fish, especially in the economic value species of the basic needs of growers and researchers in this direction is the most important approaches (Shalaby, Khattab & Abdel Rahman 2006). Although the vaccination of fish is one of the most effective ways to control infectious diseases of fish, proper operation of commercial vaccines against some diseases of viral, bacterial or parasitic not been produced. One of the most effective methods of prevention and control of these diseases and infections is the use of various kinds immune stimulants. The immunostimulants enhance resistance to infectious disease by increasing the non-specific and specific immune mechanisms in fish and shellfish (Misra, Das, Mukherjee & Pattnaik 2006, Afsharnasab, Kakoolaki & Mohammadidost 2016). Recently, the use of herbal compound as immune stimulants for enhance non-specific immune system is commonly cultured fish (Rao, Das, Iyotymayee & Chakrabarti 2006, Kakoolaki, Akbary, Zorriehzahra, Salehi, Sepahdari, Afsharnasab, Mehrabi & Jadgal 2016). Immunostimulant plants or their by-products contain several phenolic, polyphenolic, alkaloid, quinone, terpenoid, lectine, and polypeptide compounds, many of which shown to be very effective alternatives to antibiotics, chemicals or synthetic compounds and vaccines. They also facilitate growth, anti-stress, environmentally friendly and antimicrobial properties in finfish and shrimp (Maqsood, Singh, Samoon & Munir 2011). The most important advantage of using immunostimulant plants in aquaculture is that they contain natural organic materials that do not cause any threat to fish health or to the

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environment or to human health (Talpur, Ikhwanuddin & Ambok Bolong 2013). Ginger is effective in the control of a range of bacterial, viral, fungal and parasitic diseases (Martins, Tavares-Dias, Fujimoto, Onaka & Nomura 2004). In addition, ginger is effective as an immunomodulatory agent in animals and fish and helps to reduce the losses caused by diseases in aquaculture (Nya & Austin 2009, Gholipour kanani, Nobahar, Kakoolaki & Jafarian 2014). Ginger rhizomes contain a number of active ingredients as ginger oil, gingerols, which can be converted to shogaols, zingerone and paradol (Chang, Liu, Wu, Chiang, Lian & Hsieh 2012). Several researches have reported the beneficial effects of herbal plants on beluga (Gholipour *et al*. 2014, Akrami, Gharaei, Razeghi Mansour & Galeshi 2015b) but there is no documented evidence about the effect of ginger extract on beluga. Therefore, the objectives of the present study evaluated the effects of different levels of ginger extract on the beluga juveniles concerning their growth, blood and biochemical profile as well as on the immune response of *Huso huso*.

Materials and Methods

Fish and rearing condition

Fingerlings of beluga were obtained from Shahid Marjani Sturgeon Fish Propagation and Cultivation Centre (Golestan, Iran) then transferred to Sadde-Voshmgir Cultivation center (Gorgan, Iran). After 3 weeks of acclimatization period, 120 fish with mean weight of 18.81 ± 0.89 g were randomly distributed among 12 tanks, with 10 fish in each, in triplicates per diet. Continuous aeration was provided to each tanks through air stone connected to a central air compressor. During the experimental period, water temperature, dissolved oxygen and pH were 22.2 ± 1.45 ^{O}C , 6.7 ± 0.87 mg L $^{-1}$ and 8.15 ± 0.3, respectively.

Feed and feeding

The fish were fed diet containing 33.64% protein, 8.39% lipid, 7.19% ash from Faradaneh Co (Sharekord, Iran). Ginger hydroalcoholic extract (*Zingiber officinale*) rhizomes were purchased from Essence Giah Co (Gorgan, Iran). The dried ginger rhizome was powdered in an

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electric blender. The extract was prepared with the standard method. To do this, dried ginger in 75% ethanol was percolated for 72 hours. Then, the slurry was filtered with Whattman No. 1 filter paper and centrifuged for 5 min at 5000 rpm. The filtrate obtained from ethanol using a rotary device, the excess solvent was separated from the extract. These crude extract was stored at 4ºC until use. The extract added to formulated fish diet in three different doses at a rate of 0.5%, 1% and 1.5% (v/w) as experimental diets. The control diet was prepared by adding only water and received no ginger extract. The fish were fed with the experimental diet for 8 weeks at the rate of 2 - 5% of the body weight daily according to the method of Akrami et al. (2015a).

Growth and feed efficiency parameters

biometry was calculated based on a 15-day sampling to adjust the feeding rate and estimate growth performance. At the end of the feeding trial, weight gain (WG g), specific growth rate (SGR %/day), condition factor (CF), feed conversion ratio (FCR) and survival rate were calculated according to the standard formula (Hevroy, Espe, Waagbo, Sandness, Rund & Hemer 2005).

Blood sample collection

At the end of the experiment, 6 fish were sampled randomly from each tank and about 4 ml of blood was drawn from the caudal vein, using a non-heparinized syringe. Then, blood samples were introduced to both heparinized and non-heparinized tubes in order to perform haematological and immunological studies, respectively. Serum samples were attained after centrifugation (4,500 g for 10 min) and stored at -20 ^{O}C until analysis. Red blood cells (RBC) and white blood cells (WBC) were counted using a Neubaur haemocytometer (Martins, Tavares-Dias, Fujimoto, Onaka & Nomura 2004). Additional parameters were determined: Haemoglobin (Hb) and haematocrit (Hct) according to Collier (1944) and differential white blood cell counts were obtained by preparing panchromatically-stained smears (Klontz 1994). Differential leukocyte counts (neutrophil, lymphocyte, monocyte and eosinophil) were determined using Giemsa

staining method of blood smears using a light microscope. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkalin phosphatase (ALP), lactate dehydrogenase (LDH), triglyceride, cholesterol, glucose, total protein and albumin content was determined colorimetrically using kits supplied by ZiestChem diagnostics, Tehran, Iran (Fazlolahzadeh, Keramati, Nazifi, Shirin & Seifi 2011). Globulin content was calculated by subtracting albumin content from serum total protein content.

Immune parameters assay

Alternative complement activity was assayed according to the procedure of Yano (1992) by using rabbit red blood cells (RaRBC). The volume of serum yielding 50% haemolysis was determined and used to calculate the complement activity of the sample (value of ACH50 is in percent). Lysozyme level was determined by turbidometric assay according to the method of Ellis (1990) with slight modifications. Aliquots (1.75 mL^{-1}) of *Micrococcus lysodeikticus* suspension (Sigma) (0.375 mg mL^{-1}, 0.05 M PBS, pH 6.2) were mixed with 250 μL^{-1} of each sample and the optical density was measured after 15 and 180 s by spectrophotometer (Biophotometer Eppendorf) at 670 nm. PBS was used as a blank and results were expressed according to amounts of lysozyme (µg) per 1 mg of sample calibrated using a standard curve determined with hens' egg white lysozyme (Sigma) in sterile sodium phosphate buffer.

Serum total immunoglobulin (Ig) level were determined according to the method described by Siwicki and Anderson (1993). Briefly, serum total protein content was measured using a micro protein determination method (C-690; Sigma), prior to and after precipitating down the immunoglobulin molecules, using a 12% solution of polyethylene glycol (Sigma). The difference in protein content represents the Ig content.

Serum Superoxide dismutase (SOD) activity was measured spectrophotochemically by the ferricytochrome C method using xanthine/xanthine oxidase as the source of superoxide radicals (Ai, Xu, Mai, Xu, Wang & Zhang 2011). The reaction mixture consisted of

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50 mM potassium phosphate buffer (pH 7.8), 0.1 mM EDTA, 0.1 mM xanthine, 0.013 mM cytochrome C and 0.024 IU mL^{-1} xanthine oxidase. The reaction was triggered after the addition of the xanthine oxidase. One activity unit was defined as the amount of enzyme necessary to produce 50% inhibition of the ferricytochrome C reduction rate that measured at 550 nm. Enzyme activity was expressed as units per ml serum (U mL^{-1}).

Statistical methods

Values for each parameter measured were expressed as mean ± SD. Statistical analysis was carried out using one-way analysis of variance by using SPSS (version 16) for Windows (SPSS, Chicago, IL). Differences between means were determined using Duncan's multiple test ($P<0.05$).

Results

Results showed that there was a significant in weight gain in fish fed ginger extract diet ($P<0.05$) compared to the control. There were no significant differences ($P>0.05$) in condition factor (CF), feed conversion ratio (FCR), specific growth rate (SGR) and survival between juveniles fed control and ginger extract supplementation (Table 1).

Table 1 Growth parameters of beluga juveniles fed with ginger extract (% feed) added diet at different levels for 8 weeks.

parameter	Control	0.5%	1%	1.5%
WG (g)	94.08 ± 6.47 [b]	107.01 ± 2.80 [a]	104.42 ± 2.80 [a]	110.87 ± 1.91 [a]
SGR (%/day)	3.03 ± 0.11 [a]	3.16 ± 0.01 [a]	3.13 ± 0.01 [a]	3.15 ± 0.01 [a]
FCR	0.92 ± 0.01 [a]	0.90 ± 0.01 [a]	0.89 ± 0.01 [a]	0.87 ± 0.01 [a]
CF(g cm^{-3})	0.45 ± 0.001 [a]	0.45 ± 0.01 [a]	0.46 ± 0.001 [a]	0.46 ± 0.001 [a]

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

Statistical analysis of data showed that there were no significant differences of erythrocyte (RBC) count, haematocrit, monocyte, lymphocyte, neutrophil, eosinophil and leucocyte (WBC) between the treatment groups ($P>0.05$), but hemoglobin had significant different between control group and the group fed 1% ginger extract (Table 2).

The glucose, total protein, Triglycerid, total lipid, albumin, globulin, AST, LDH and

albumin: globulin did not show any significant difference between treatments (P>0.05). Fish groups fed ginger extract diet at 0.5% and 1.5% feed had significantly lower ALT and ALP compared with the control group (P>0.05). The total cholesterol level showed decrease significant compared with the control (P<0.05) (Table 3).

Table 2 Haematological parameters of beluga juveniles fed with ginger extract (% feed) added diet at different levels for 8 weeks

parameter	Control	0.5%	1%	1.5%
RBC (10^6 ml^{-1})	1.07 ± 0.01 [a]	1.07 ± 0.01 [a]	1.05 ± 0.01[a]	1.07 ± 0.01 [a]
WBC (10^3 ml^{-1})	21.69 ±2.94[a]	22.78 ± 0.58 [a]	23.49 ± 1.31 [a]	22.77 ± 0.61 [a]
Hb (g dl^{-1})	5.71 ± 0.92 [b]	6.18 ± 0.3 [ab]	6.5 ± 0.29 [a]	6.08 ±0.35 [ab]
Hct (%)	24.46 ± 3.57 [a]	24.13 ± 2.85 [a]	21.76 ± 2.82 [a]	24.53 ±2.57 [a]
Monocyte (%)	1.66 ± 0.51 [a]	1.50 ± 0.54 [a]	1.66 ± 0.51[a]	1.66 ±0.51 [a]
Lymphocyte (%)	73.16 ± 6.55 [a]	75.83 ± 10.9 [a]	73.50 ± 8.52 [a]	73.00 ±7.23 [a]
Neutrophil (%)	13.0 ± 3.89 [a]	14.33 ± 4.45 [a]	14.0 ± 5.32 [a]	12.0 ± 3.46 [a]
Eosinophils (%)	12.16 ±3.65 [a]	8.33 ±7.52 [a]	10.83 ±6.79 [a]	13.33 ± 4.22 [a]

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

Table 3 Blood serum biochemical parameters of beluga juveniles fed with ginger extract (% feed) added diet at different levels for 8 weeks

parameter	Control	0.5%	1%	1.5
Glucose (mg l^{-1})	55.00 ± 3.16 [a]	62.33 ±17.03 [a]	60.00 ±6.26 [a]	55.00 ± 6.69 [a]
Total protein (mg l^{-1})	3.03 ± 0.25 [a]	2.93 ± 0.47 [a]	3.23 ± 0.19 [a]	2.96 ± 0.21 [a]
Cholestrol (mg dl^{-1})	104.50 ± 19.88 [a]	88.33 ± 20.20 [ab]	98.40 ± 15.72 [a]	72.50 ± 10.89 [b]
Albumin (mg l^{-1})	2.04 ± .0.19 [a]	2.10 ± 0.40 [a]	2.30 ± 0.01[a]	1.96 ± 0.22 [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]
Albumin: Globulin	2.17 ± 0.21 [a]	2.64 ± 0.76 [a]	2.53 ± 0.43 [a]	2.12 ± 0.75 [a]
AST (IU dl^{-1})	505.50 ± 68.16 [a]	458.66 ± 71.67 [a]	487.83± 16.82 [a]	441.50 ± 54.57[a]
ALT (IU dl^{-1})	47.16 ± 2.78 [a]	34.16 ± 9.34 [b]	41.66 ± 6.28 [ab]	38.00 ± 7.58[b]
ALP (IU dl^{-1})	620.83 ± 50.61 [a]	500.50 ± 98.36 [b]	563.33 ± 46.63 [ab]	504.00 ± 109.91 [b]
LDH (IU dl^{-1})	595.83 ± 98.36 [a]	585.60 ± 21.38 [a]	586.16 ± 13.31 [a]	609.40 ± 41.26 [a]

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Triglyceride (mg dl^{-1})	303.16 ± 17.19 [a]	303.50 ± 34.20 [a]	321.16 ± 32.59 [a]	283.00 ± 43.44 [a]
Total lipid (mg dl^{-1})	407.66 ± 20.55 [a]	391.83 ± 53.30 [a]	407.60 ± 17.30 [a]	355.50 ± 52.11 [a]

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

The result of immunological parameters showed that the SOD activity of beluga juvenile was not affected by different doses of ginger extract on 8-week when compared to the control (Fig. 1). Supplementing 1.5% ginger extract increased the Ig (Fig. 2) and lysozyme activity (Fig. 3) compared to the other groups. At the same time, the Ig and lysozyme activity showed a significant different in 1.5% ginger extract enriched diet feeding group compared to the 0.5% ginger extract (P<0.05). The Alternative complement activity (ACH50) percentage, in fish fed diet supplemented with 1.5% ginger extract showed increased compared with the other groups (P<0.05). The ACH50 percentage, in fish groups fed ginger extract diet feed at 0.5% and 1.5% was comparatively lower when compared with the control group (Fig. 4).

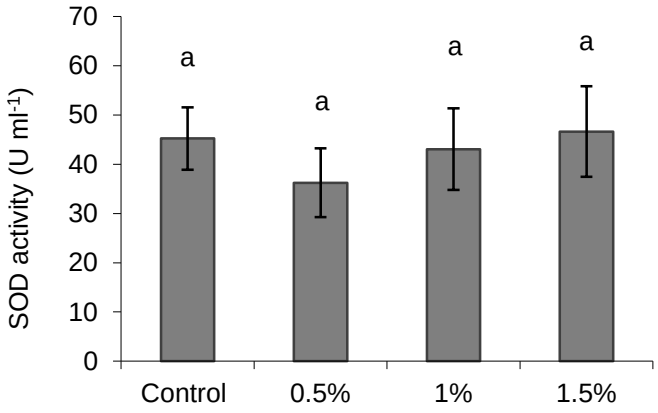

Figure 1 Serum Superoxide dismutase (SOD) activity, in the of beluga juveniles fed with dietary ginger extract at 0.5%, 1% and 1.5% levels. Values are expressed as mean±SD. Mean values bearing different superscripts at the different stage were statistically significant (P>0.05).

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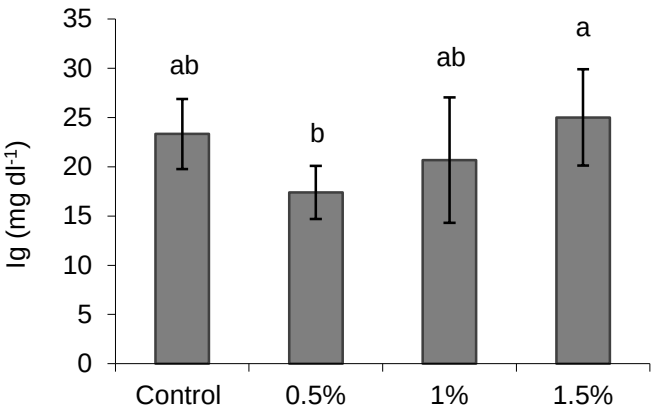

Figure 2 Serum total immunoglobulin (Ig) levels in beluga juveniles fed with dietary ginger extract at 0.5%, 1% and 1.5% levels. Values are expressed as mean ± SD. Mean values bearing different superscripts at the different stage were statistically significant (P<0.05).

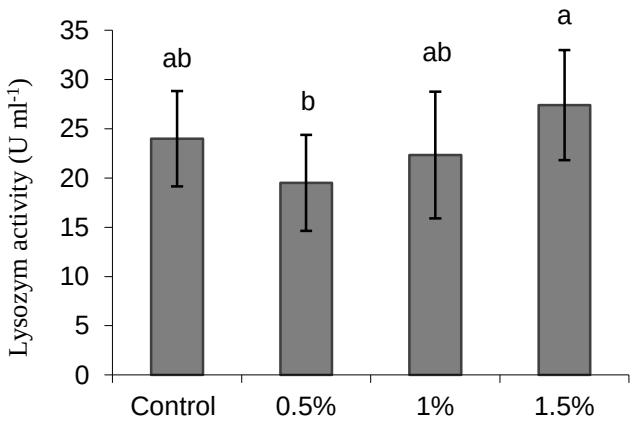

Figure 3 Serum lysozyme activity in the of beluga juveniles fed with dietary ginger extract at 0.5%, 1% and 1.5% levels. Values are expressed as mean±SD. Mean values bearing different superscripts at the different stage were statistically significant (P<0.05).

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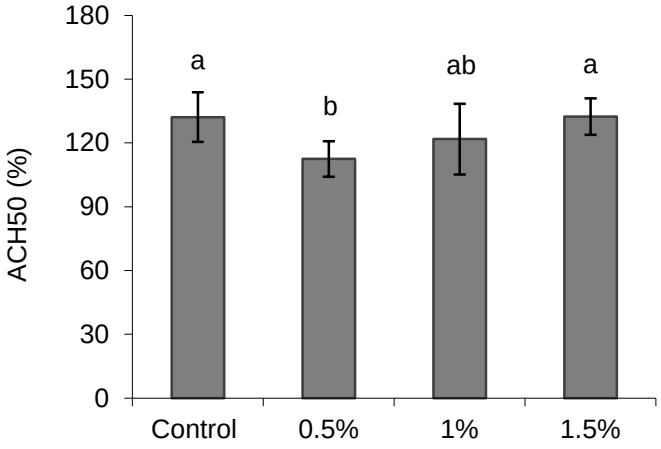

Figure 4 Alternative complement activity (ACH50), in the of beluga juveniles fed with dietary ginger extract at 0.5%, 1% and 1.5% levels. Values are expressed as mean±SD. Mean values bearing different superscripts at the different stage were statistically significant (P<0.05).

Discussion

The present study showed that ginger extract diet did not have significant effect on SGR, FCR and CF of beluga by feeding different doses compared with the control group, but increased weight gain significantly in comparison with the control group. However highest supplementation of ginger extract of 1.5% feed was most favourable for the growth of beluga. Moreover, FCR was improved which means that the ginger diet acted as an appetiser. Thus, digestibility increased and in turn the energetic benefits enhanced the growth. Similar to our results Gholipour kanani *et al.* (2014) observed that beluga fed diet containing ginger powder significantly increased growth performance. On the other hand, growth performance were affected by inclusion of the ginger in the diets of *Onchorhynchus mykiss* (Nya & Austin 2009), *Macrobrachium rosenbergii* (El-Desouky, El-Asely, Shaheen & Abbass 2012)**,** *Lates calcarifer* (Talpur *et al.* 2013) and *Penaeus monodon* (Venkatramalingam, Godwin & Citarasu 2007), in agreement with our result. The effects of dietary additives on fish performance may vary depending on fish species, size, the dose of the additive, fish nutritional/physiological status, and/or ambient culturing conditions.

In fish, blood parameters play an important role same as physiological indicator in

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prognosis any problem with fish. The WBC (leucocytes) serves as one of the first lines of body defense and their numbers increase sharply when infections arise. Many data were published to show that the herbal plant could be act as immunostimulants and increase the total WBC (Jian & Wu 2003). In our study, WBC increased in ginger extract group in comparison with control group, however, this increase was not significant. The increase WBC count following feeding of ginger extract diet demonstrates the immunostimulatory effects and anti-infection properties of ginger which is in line with the previous work of Gholipour kanani *et al*. (2014), who reported that there was no significant difference in WBC for beluga fed diet containing ginger on 8 weeks. Conversely, Binaii, Ghiasi, Farabi, Pourgholam, Fazli, Safari, Alavi, Taghavi, & Bankehsaz (2014) and Akrami *et al*. (2015b) reported that the supplementation of diets with nettle (*Urtica dioica*) and onion powder significantly increased beluga juvenile WBC, respectively on the end of trial. Significant increase in WBC of rainbow trout and Asian sea boss fed with ginger reported by Nya & Austin (2009), Haghighi & Sharif Rohani (2013) and Talpur *et al*. (2013). In the current study, the haemogolobin content was significantly higher in treated groups than the control, which demonstrates that oxygen supply increases consequently, reflecting beneficial health effect on fish. This is in agreement with Nya & Austin (2009) and Talpur *et al*. (2013) recorded enhanced haemogolobin content in rainbow trout and Asian sea bass after feeding with ginger diet. On the contrary, Gholipour kanani *et al*. (2014) found that the level of Hb was not affected by ginger in the basal diet of juvenile beluga. The present study revealed that administering ginger extract through fish feed had no significant difference in RBC compared with the control. Similar result was reported by Gholipour kanani *et al.* (2014), who obtained that there was no significant different in RBC for beluga fed diet ginger compared with the control. Unlike this study, the study on rainbow trout (Haghighi & Sharif Rohani 2013) showed RBC remained affected after feeding 1% powdered ginger. The reason for different results might be attributed to difference in the effect of herbal plant and immune system reaction. In this study Hct % level did not be affected by different levels of ginger extract. This condition trend was similar to the status of Hb and RBC values, which not changed in beluga fed diet supplemented ginger extract. These results are in disagreement with dose obtained by Nya & Austin (2009), Haghighi & Sharif Rohani (2013) , Talpur *et al.* (2013) and Gholipour kanani *et al.* (2014) who found that Hct% in fish fed with ginger diet improved significantly. The findings of blood cells including lymphocytes, monocytes and neutrophils in this study show that these blood cells were not significantly affected by ginger extract diet. This result coincide with the investigation of Gholipour

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kanani *et al.* (2014) who reported that blood cells levels were not significantly affected by ginger diet in beluga. Unlike this study, Nya & Austin (2009) and Talpur *et al.* (2013) reported that the administration of ginger increased these blood cells in rainbow trout and Asian sea bass.

Proximate composition of ginger includes alkaloids, flavonoids, polyphenols, saponin, steroids, tannin, fibre, carbohydrate, vitamins, carotenoids and minerals (Otunola, Oloyede, Oladiji, & Afolayan 2010). The bioactive compounds found in ginger directly affect fish health by activating immune mechanism. Polyphenols and flavonoids are recognised to have antioxidant properties and suggests a role in the prevention of infections and hypoglycaemic potential (Scalbert, Johnson & Saltmarsh 2005). Saponins have been demonstrated to have cholesterol-lowering effects, hypoglycaemic activity and antimicrobial properties to stop attacks by foreign pathogens and tannins have been reported to hasten the healing of wounds (Otunola *et al*. 2010). The result of present study revealed that, glucose of the fish was not significantly affected by the experimental diets. The results of this study are parallel with finding of Binaii *et al*. (2014) who obtained glucose was not affected in beluga juvenile fed nettle. These results are disagreement with by Talpur *et al.* (2013) and Akrami *et al.* (2015b) noted decreased glucose after feeding with ginger and onion powder diet in *L. calcarifer* and *H. huso* respectively. In the present work, the cholesterol was found low in treated fish group over the control which is in line with the earlier study of Talpur *et al.* (2013) and Akrami *et al*. (2015b) who obtained decreased cholesterol after feeding *L. calcarifer* and *H. huso* with ginger and onion powder diet respectively.

In our experiment, ginger extract had no significant difference on triglyceride and lipid compared with the control. However, the lowest value of triglyceride and lipid were observed in 1.5% ginger extract treatment. It could be explained that a bioactive compound saponin present in ginger is capable of improving hyperlipidemia (Talpur *et al*., 2013). on the contrary, Talpur *et al*. (2013) indicated that there was a decrease in plasma triglyceride and lipid levels in *L. calcarifer* after feeding with ginger diet. Moreover, Akrami *et al*. (2015b) reported that application of 1% onion powder in diet of *H. huso* made a significant decrease on triglyceride when compared with control group. Binaii *et al.* (2014) observed there were no change in the cholestrol and triglyceride levels between treatment and control group on week 4, whrease they were significantly decreased in *H. huso* fed on dietary 6% and 12% nettle compared to the other group on week 8.

Some authors claim that serum total proteins is the most importantly indicator of the biochemical nutritional and health status of the fish (Patriche, Patriche & Tenciu 2009). In the present study, total protein of juvenile beluga

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was not change after feeding with different doses of ginger extract at the end of sampling time. However, the use of ginger powder as supplemented diet can cause the increase of total protein in *L. calcrifer* (Talpur *et al*., 2013). and juvenile *H. huso* (Gholipour kanani *et al*., 2014). The reason for the discrepancy could be due to the different in the effect of herbal plant as extract and/or dried ginger powder. Previous study by Binaii *et al*. (2014) have revealed that supplementation with 12% nettle significantly increased the total serum protein of *H. huso*, whrease administration of 1% onion powder in diet of *H. huso* caused a significant decrease on total protein when compared with control group (Akrami *et al*., 2015b).

It has been recognised that albumin and globulin are vital elements for maintaining a healthy immune system (Jha, Pal, Sahu, Kumar, & Mukherjee 2007). This paper show that albumin and globulin had no significant difference in fish fed diet containing ginger extract when compared with the control. This is in agreement with finding of Binaii *et al*. (2014) who obtained albumin level was not affected in beluga juvenile fed nettle. Gholipour kanani *et al*. (2014) reported that, globulin significantly increased in serum, but no significant difference was found in albumin in *H. huso* fed diet ginger. Previous studies have reported increase in serum albumin and globulin with work relating to fish fed with ginger diet (Nya & Austin, 2009; Talpur *et al*. 2013). However, Akrami *et al*. (2015b) found that albumin and globulin levels were lower in *H. huso* fed on dietary onion powder compared with the control.

AST, ALT, LDH and ALP enzymes are used as indices of liver damage. Elevated levels may indicate degeneration, necrosis, and destruction of the liver due to cellular damage (Bhardwaj, Srivastava, Kapoor & Srivastava 2010). As differences in LDH and AST levels were not observed in the experimental groups, the consumption of ginger extract did not appear to induce liver toxicity in fish. In this study, all doses of ginger extract significantly decreased ALT and ALP activity compared to the control. It could be explained that the bioactive compounds polyphenols, flavonoids, tannins and saponins found in ginger prevented fish from infection by triggering immune system and its administration might prevent lipid peroxidation of cell membranes and inhibit the release of foresaid enzymes into the plasma. Gholipour kanani *et al*. (2014) and Binaii *et al*. (2014) who reported that there were no significant difference in ALT, ALP and AST in beluga fed diet ginger and nettle compared with the control group. Akrami *et al*. (2015b) also reported that AST and LDH levels showed a significant decrease in beluga juvenile fed diet with 1% onion compared to the control and 0.5% onion powder diet, while ALT and ALP levels were not influenced.

The serum lysozyme activity is considered as a defence barrier against bacterial

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pathogens thus resulting in the reduction of disease (Misra *et al*. 2006). In this study, lysozyme activitiy showed significant increase in 1.5% ginger extract group. The results of present study are consistent with those of Talpur *et al.* (2013) and Haghighi & Sharif Rohani (2013). They showed that dietary content of ginger significantly affected serum lysozyme activity in *L. calcrifer* and *O. mykiss* respectively. Moreover, elevated lysozyme activity have been reported in rainbow trout after supplementing diets with ginger (Talpur *et al*., 2013). Antache, Cristea, Grecu, Dediu, Creţu & Petrea (2014) observed that 1% *Zingiber officinale* increase lysozyme activity of *Oreochromis Niloticus*, but not statistically significant compared to control. Ginger administration in 2% concentration at *Epinephelus fuscoguttatus* species, led to an increased activity of lysozyme (Apines-Amar, Amar & Faisan 2013). However, unlike this study, lysozyme activity was not influenced in juvenile beluga fed with ginger (Gholipour kanani *et al*., 2013).

Teleost IgM resembles mammalian IgM in structure, physiological characteristics, soluble forms and membrane-bound forms. The soluble IgM forms which are present in the blood and other fluids play a role as an immune effector molecule (Ross, Wilson, Miller, Clem & Warr 1998). Our study showed increasing serum total immunoglobulin in fish fed with ginger extract in 1.5% group. This result coincide with the investigations of Binaii *et al*. (2014) and Akrami *et al*. (2015b) who reported enhanced levels of serum Ig level in beluga after feeding with nettle and onion powder respectively.

The complement system is an essential and effective part of the innate immune system. It can rapidly distinguish and opsonize bacteria for phagocytosis by specialized phagocytes or destroy them directly by membrane disorder (Ahmadi, Banaee, Vosoghi, Mirvaghefei, & Ataeimehr 2012). The increase in the complement activity (ACH50) observed in plasma of fish fed with diets enriched with 1.5% of ginger extract may help to identify and eliminate bacterial agents by phagocytosis. A possible mode of action of ginger is in immunostimulation as a result of its bioactive constituent, gingerol, which has been reported to induce the activity of interleukin-6. Also ginger has been regarded to have potent antioxidant properties, being an effective scavenger of superoxide radicals, and so has been proposed as a possible protective mechanism against autotoxicity and lethality (Gabor, Ichim & Suteu 2012). Increases in the total complement activity were reported in fish fed with a diet enriched with *Punica granatum*, *Chrysanthemum cinerariaefolium* and *Zanthoxylum schinifolium* extract (Harikrishnan, Kim, Ham, Heo, Balasundaram, Heo & Kim 2010), *S. marianum* (Ahmadi *et al*., 2012) and *Nasturium nasturtium* extracts (Asadi, Mirvaghefei, Nematollahi, Banaee & Ahmadi 2012).

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Superoxide dismutase (SOD) as an important anti-oxidation enzyme widely exists in tissues of aerobic and anaerobic creatures. SOD has been taken as an immune enzyme and a marker with several other related enzymes in evaluating shrimp immune function (Lin, Yeh, Li, Chen, Cheng & Chen 2009). In the present study, superoxide dismutase activity (SOD) activity was not significantly affected by dietary ginger extract supplementation. This is in agreement with the work of Yuan, Li, Chen, Sun, Wu, Gong, Tang, Shen & Han (2007) who noticed that there was no significant difference in SOD activity between the 0.5% and 1% herbal immune regulation mixture (HIRM) extract groups and the control group. These results contrary with the investigation of Akrami *et al*. (2015b) who reported enhanced level of serum SOD activity in *H. huso* treated with 1% onion powder After 8 weeks. This possibly attributed to be that each herbal plant a specific area of the host immune system or that the time course for induction of immune response by herbal plant differs with respect to type of immune parameter.

It is concluded that ginger extract supplemented diet at the concentration 1.5% relatively improved growth and hematological variables and remarkably enhanced immune function of juvenile beluga. Further research is needed to clarify the action mechanism of ginger extract, as well as the appropriate inclusion dose and feeding period in *H. huso*.

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تاثیر عصاره زنجبیل بر عملکرد رشد، مشخصه های خونی، بیوشیمی و ایمنی فیل ماهی پرورشی (*Huso huso*)

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چکیده

در این مطالعه تاثیر عصاره زنجبیل بر برخی پارامترهای رشد، شاخصهای بیوشیمی، خونی و ایمنی در فیل ماهی (*Huso huso*) جوان پرورشی بررسی شد. ماهیان با چهار جیره غذایی حاوی ۰، ۰/۵، ۱ و ۱/۵ درصد عصاره زنجبیل به مدت ۶۰ روز تغذیه شدند. نتایج حاکی از افزایش وزن معنی داری در ماهیان تغذیه شده با عصاره زنجبیل در مقایسه با گروه شاهد بود ($P<۰/۰۵$). تفاوت معنی داری در ضریب چاقی، ضریب تبدیل غذایی، نرخ رشد ویژه و بازماندگی بین تیمار حاوی عصاره زنجبیل و گروه شاهد مشاهده نشد ($P<۰/۰۵$). علاوه بر این، در تعداد کل گلبول های سفید و قرمز، درصد هماتوکریت، لنفوسیت، منوسیت، نوتروفیل، گلوکز، پروتئین، آلبومین، تری گلیسرید، چربی و گلبولین تفاوت معنی داری بین تیمار عصاره زنجبیل و گروه شاهد وجود نداشت ($P<۰/۰۵$). بیشترین میزان هموگلوبین و کمترین سطح کلسترول به ترتیب در تیمار ۱٪ و ۱/۵٪ عصاره زنجبیل بدست آمد. افزایش معنی داری در فعالیت کمپلمان سرم، فعالیت ایمونوگلبولین و فعالیت لیزوزیم سرم، در تیمار ۱/۵٪ عصاره زنجبیل بدست آمد ($P<۰/۰۵$) اگرچه در فعالیت سوپراکسیددیسموتاز تفاوت معنی داری بین تیمارهای آزمایشی و گروه شاهد بدست نیامد. در مجموع نتایج حاصل از این تحقیق نشان داد اضافه کردن ۱/۵٪ عصاره زنجبیل به جیره غذایی فیل ماهی جوان پرورشی باعث بهبود شاخص های خونی و بیوشیمی و تحریک سیستم ایمنی می شود.

کلمات کلیدی : عصاره زنجبیل، رشد، مشخصه های خونی، ایمنی، فیل ماهی

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