

Effect of purple coneflower (*Echinacea purpura*) and garlic (*Allium satvium*) as a supplemented dietary intake on some non-specific immune status, hematological parameters and growth performance in grower (*Huso huso*)

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Keywords

Echinacea purpurea; garlic; *Huso huso*; innate immunity

Abstract

The effects of orally administered purple cone flower *Echinacea purpura* and garlic (*Allium satvium*) on the non-specific immunity and growth condition of beluga weighing 1.15 ± 0.02 kg were evaluated for a period of 60 days. The three groups of fish were fed two diets containing 0.5% purple coneflower and 1% garlic and a control diet containing no supplemented herb. The growth performance was positively affected by both dietary herbal supplementations ($p < 0.05$). Among the humoral factors, serum alternative complement, lysozyme and respiratory burst activity increased significantly in all supplemented groups when compared to the control ($p < 0.05$). These findings demonstrate that dietary Echinacea and garlic can modulate some of the innate defense mechanisms, hematological and growth parameters in *Huso huso*.

Abbreviations

ACH50: Alternative Complement Pathway
 BWI: Body Weight Index
 CF: Condition Factor
 CL: Chemiluminescence
 FCR: Feed Conversion Ratio
 HEWL: Hen Egg White Lysozyme
 Hb: Haemoglobin concentration
 Hct: Haematocrit
 PCV: Packed Cell Volume
 RBC: Red Blood Cells
 SGR: Specific Growth Rate
 MCV: Mean Corpuscular Volume
 MCH : Mean Corpuscular Haemoglobin
 MCHC: Mean Corpuscular Haemoglobin Concentration
 WBC: White Blood Cell

Introduction

Great sturgeon (*Huso huso*) an ancient species of chondrosteian fishes from lower Jurassic period (Findeis, 1997) is an important fish species in the Caspian Sea and has been recently categorized as highly endangered species due to overfishing, degradation of natural habitat and environmental pollution (Gharaei et al., 2009; Tatina et al., 2010). Because of its fast growth capacities and high tolerance to adverse environmental condition, *Huso huso*, is a valuable species for artificial rearing and production (Sudagar et al., 2008; Yousefi et al., 2010).

Application of dietary supplements such as vitamins, prebiotics and immunostimulants have been assessed for their efficacies on beluga sturgeon in a few reports (Falahatkar et al. 2006; Akrami, et al. 2009; Jalali et al. 2009; Jafarian et al. 2010; Hosseinifar et al. 2011; Gholipour et al., 2013a), however, there is not enough information on the dietary effects of purple coneflower and garlic supplementation in this species.

Purple coneflower is one of the most important medical herbs (Bauer and Wagner 1991). It is widely used to treat common cold and other infectious disorders (Grimm and Muller 1999). Numerous studies assessed the health-improving properties of extracts derived from plants of the genus purple coneflower (Barrett 2003; Gholipour kanani et al. 2013 b). Purple coneflower consumption contributes to an increase of various cytokines, lymphocytes, and phagocytosis activity (Brauning et al. 1992; Sasagawa et al. 2006).

Garlic (*Allium sativum*) is a perennial plant in *Alliaceae* family with a perfect antiparasitic and antitumour properties (Damir and Davor 2004). In fish, garlic increases the welfare of fish, and it can help to control pathogens, especially bacteria and fungi (Sivam 2001; Corzo Martinez et al. 2007). There is no information on the oral consumption of these herbal plants in great sturgeon immunophysiology and growth behaviour.

The present study was conducted to determine the effect of dietary Echinacea and garlic on growth performance, hematological parameters and innate immune responses in endangered species of beluga sturgeon (*H. huso*).

Materials and methods

Fish and maintenance conditions

The study was conducted in Shahid Rajai Sturgeon Fish Propagation and Rearing Center of Sari, Sari, Iran. A total of 135 *Huso huso* (average weight of 1.15 ± 0.02 kg) were randomly distributed in nine 1000-L fiberglass tanks (each containing 15 fish). The fish were acclimatized for one week. The water quality parameters were 200 L/hour flow rate, 7.4-7.8 mg/L dissolved oxygen, pH 7.98 ± 0.2 ,

and temperature of 22.2 ± 2 °C. Purple coneflower and garlic were purchased from local supermarket, oven dried, and mixed with BioMar (France) formulated food.

Experimental diets

After acclimation, the fish fed 2% body weight three times a day (at 08:00, 16:00 and 24:00 hours) for 60 days using BioMar feed with protein (42.7%), lipid (13.5%), ash (12%), moisture (10.1%) and fiber (4.7%). The first group (45 fish in three replicates) was fed with 1% garlic supplemented diet, while the second group (45 fish in three replicates) were fed with 0.5% purple cone flower supplemented diet. The control group was also included. The trial length was 60 days.

Growth assessment

At the end of feeding trial, all fish were starved for 24 h and then weighed (body weight gain $\times 100$ / initial body weight), feed conversion ratio (dry feed fed/body weight gain) $\times 100$, specific growth rate (\ln final weight- \ln initial weight $\times 100$ / days), Body weight increase (final weight of fish- initial weight of fish) and condition factor (body weight/body length³ $\times 100$) were calculated according to Hung and Lutes (1989).

Blood sampling

Blood samples were obtained every 15 days from caudal vein after fish been anaesthetized with 80 ppm of clove oil (Gholipour Kanani et al. 2011). Serum samples were obtained from non-heparinized blood after centrifugation at 2500 rpm for 10 minute and stored at -70 °C until analyzed for alternative complement and lysozyme activity. The heparinized blood samples were used to measure hematological parameter and respiratory burst activity.

Hematological parameter

Red blood cells (RBC: 10^6 mm⁻³) and white blood cell (WBC: 10^3 mm⁻³) populations were counted manually by haemocytometry, using Neubauer haemocytometer (give original reference such Klontz 1994). Haemoglobin concentration (Hb: g/dL) was measured spectrophotometrically at 540 nm with cyanmethemoglobin method. Haematocrit (Hct: %) was measured with microcentrifuge method, using standard heparinized microhaematocrit capillary tubes (75 mm at 10000 rpm for 10 min). The derived erythrocytes of mean corpuscular volume (MCV: fL), mean corpuscular haemoglobin (MCH: pg) and mean corpuscular haemoglobin concentration (MCHC: %) were calculated give the original reference .

Respiratory burst (chemiluminescence assay)

The oxidative burst produced by leukocytes from the blood samples was performed only on day 60 by chemiluminescence (CL; measurement of light emission) assay,

using an automated system for CL analysis (Berthold detection system, USA) according to the methods described by Koshbavar-Rostami et al (2006). To determine optimal dilution of blood samples to obtain a maximum CL peak, a preliminary test was performed using serial dilution of normal fish blood sample prepared in working Hanks balanced salt solution (WHBSS, 53.2 g NaCl, 2.6 g KCl, 0.6 ml glucose, 0.4 g KH₂PO₄, 0.32 g Na₂HPO₄ dissolved in 100 ml distilled water). The 1:10 dilution of blood was chosen due to its ability to give maximum peak value. Luminol (5-amino-2,3-dihydro-1,4-pyrazolinedione, Sigma) was used and stock luminol solution containing 0.78 g KOH, 0.618 g boric acid, luminol 0.014 g, distilled water 10 ml (prepared just before use) was diluted for use. To determine if varying dilutions of luminol had an effect on the CL response, 1:10, 1:100 and 1:1000. The stock solution was found to enhance peak values. Each test vial contained 100 µL luminol and 200 µL diluted blood samples without zymosan. Measurements were made at 12.8 min intervals for 90 min).

Alternative complement pathway (ACH50)

Alternative complement pathway activity was assayed according to Matsuyama et al. (1988). Sheep red blood cells were washed twice with Alsever's solution and stored at 4 °C. Ethylene diamine tetra acetic acid (EDTA; Sigma) and ethylene glycol bis (β-amino ethyl ether)-N, N, N, N-tetra acetic acid, (EGTA; Sigma) were used. The buffers used in this experiment, together with their abbreviations, were: 10 EGTA-Mg-GVB, veronal-buffered saline containing 10 mM EGTA, 10 mM MgCl₂ and 0.1% gelatin (pH = 7.8); 10 mM EDTA-GVB: veronal-buffered saline containing 10 mM EDTA and 0.1% gelatine (µ= 0.15, pH = 7.5). Briefly, 0.5 ml of serially 8-fold diluted *Huso huso* serum in EGTA-Mg-GVB was placed in a set of test tubes and 0.2 mL of sheep red blood cells suspension (2×10⁶ cells /mL) was added. This mixture was incubated at 15 °C for 90 min. Addition of 2.8 mL of 10 mM EDTA-GVB buffer stopped the haemolytic reaction. After centrifugation, the value y (percentage haemolysis 10⁻²) was calculated from the optical density (OD) at 414 nm of the supernatant with spec-

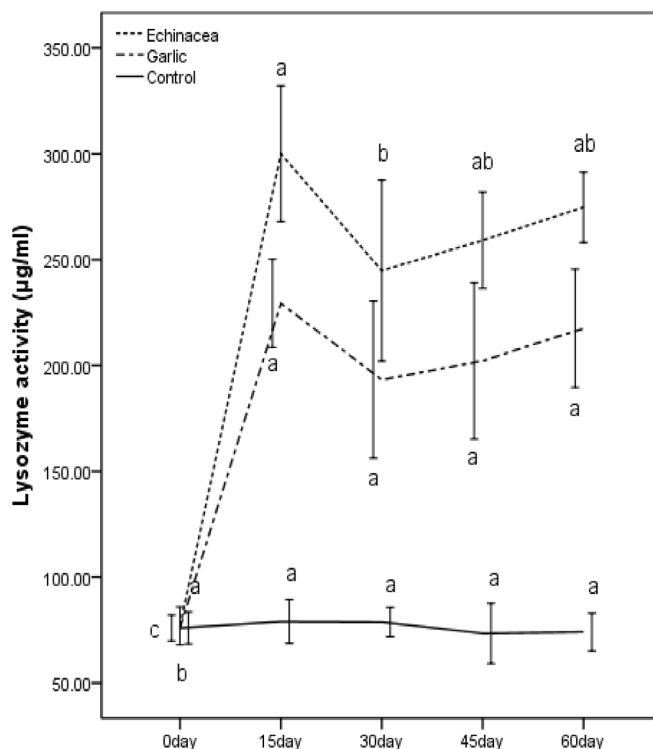


Figure 1 Lysozyme activity (Mean±SEM) in Beluga fed with 0.5% (Echinacea purpurea) and 1.0% (Garlic) dieted Kirschner wires and tension band in postoperative 6th weeks.

trophotometer. The value $y(1-y)^{-1}$ and the reciprocal of the serum dilution were plotted on log-log graph paper and the ACH 50 (units /mL) and the reciprocal dilution giving 50% haemolysis ($y(1-y)^{-1}=1$) were read from the graph.

Lysozyme assay

Lysozyme activity was measured with the turbidimetric method described by Eliss (1990) with slight modification. Suspension of 175 µL lyophilized *Micrococcus lysodeikticus* (Sigma M 3770) (0.2 mg/mL as the substrate in 0.1 M sodium acetate buffer adjusted to pH 5.5) was added to previously dispensed test serum (25 mL of each fish) in a 96-well U-bottom microtitre plate and initial OD was taken at 450 nm immediately. The final OD was taken 1 h after incubation (Sahoo et al., 2005) at 24 °C. A standard

Table 1 Effect of dietary purple coneflower (0.5%) and garlic (1%) on growth performance and weight gain of beluga

Factors	Control	Purple coneflower	Garlic
Final weight (kg fish ⁻¹)	1.36 ± 0.3 ^b	1.58 ± 0.3 ^a	1.61 ± 0.3 ^a
FCR (%)	6.96 ± 0.27 ^a	4.72 ± 0.13 ^b	4.60 ± 0.13 ^b
SGR (% day ⁻¹)	0.398 ± 0.02 ^b	0.492 ± 0.01 ^a	0.497 ± 0.01 ^a
Body weight index (%)	27.74 ± 2.33 ^b	34.72 ± 1.64 ^a	35.11 ± 1.52 ^a
CF (%)	0.41 ± 0.01 ^b	0.43 ± 0.007 ^a	0.42 ± 0.006 ^a

Data are presented as mean ± SEM, N=45. Data in same row with different superscripts are significant different ($p < 0.05$)

curve was prepared using lyophilized hen egg white lysozyme (HEWL; Sigma, USA). Serum lysozyme values were expressed as $\mu\text{g/ml}$ equivalent to HEWL activity.

Statistical analysis

One way analysis of variance (ANOVA; SYSTAT 16.0 software, SPSS) with bonferoni as post hoc was used to determine the significant between the treatments. Data were presented as mean \pm SE. The confidence level of 95 %, ($p < 0.05$) was used for data analysis.

Results

Growth performance

The growth performances of beluga fed the experimental diets are displayed in Table 1. Dietary supplementation of purple coneflower and garlic significantly enhanced final weight gain, specific growth rate, and body weight, and decreased food conversion ratio compared to fish fed the basal diet ($p < 0.05$). The final weight in treated fish with purple coneflower and garlic were respectively 1.58 ± 0.3 and 1.61 ± 0.3 kg. Weight gain fed with garlic was almost 220-250 g higher than the control group after 2 month feeding. No significant difference was seen in final weight between the two treatments ($p > 0.05$). The lowest FCR were obtained from fish treated with garlic (4.60 ± 0.13 and purple coneflower (4.72 ± 0.13). SGI, BWI and CF were significantly lower in control when compared to garlic and purple coneflower treatments ($p < 0.05$).

Hematological parameters

Hematological parameters are shown in Table 2 and 3. The Hb, HCT, MCV, MCH and MCHC increased significantly in both purple coneflower and garlic treatments compared to control group ($p < 0.05$). In addition, of RBC level in fish receiving both purple coneflower and garlic was insignificantly higher than control group ($p > 0.05$). Significantly higher levels of hemoglobin and hematocrit were seen in both purple coneflower and garlic group on 30 days of feeding ($p < 0.05$).

The results of white blood cells, lymphocytes, monocytes, neutrophils, and basophils are summarized in Table 4. Significant increase was seen in levels of WBC and lymphocyte in both *Echinacea purpurea* and garlic treatments on day 60 of experiment compared to control ($p < 0.05$).

Lysozyme activity

The results of Lysozyme activity is shown in Figure 1. Lysozyme activity reached its highest level on day 15 in both garlic and purple coneflower during the experiment ($p < 0.05$).

ACH50 activity

ACH50 activity is presented in Figure 2. Alternative

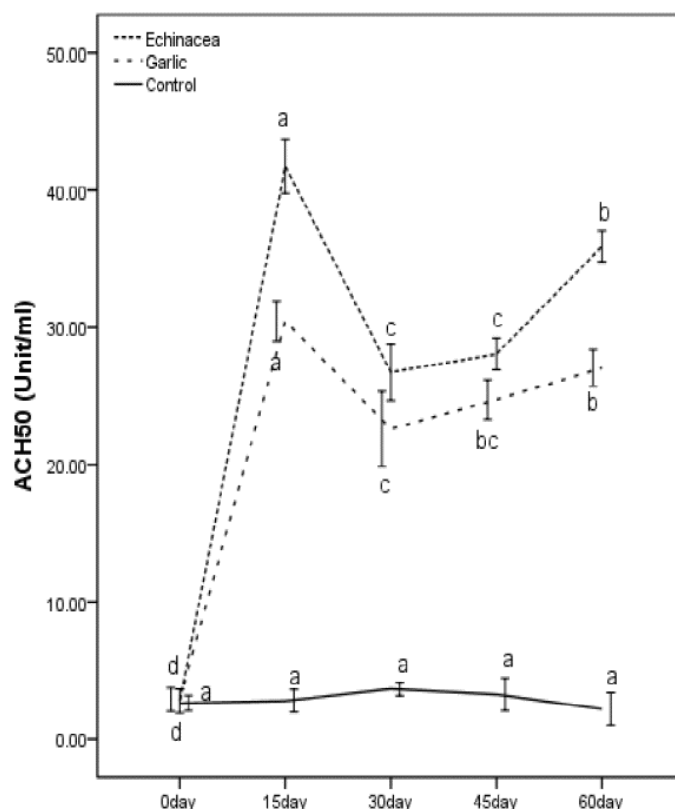


Figure 2
ACH50 activity (mean \pm SE) in Beluga fed with 0.5% (*Echinacea purpurea*) and 1.0% (Garlic) diet

complement activity had the highest level on day 15 during the trial.

Respiratory burst activity (chemiluminescence response)

Respiratory burst activity is shown in Figure 3. The results revealed that both garlic and purple coneflower had higher levels than control group at the end of experiment.

Discussion

Fish growth and disease resistance are two primary concerns in aquaculture. In last decades medicinal herbs as immunostimulants have been used to enhance nonspecific and specific defense mechanisms in order to increase resistance to disease (Chakrabarti and Rao 2006; Harikashram et al. 2011).

In the present study, feeding of great sturgeon with purple coneflower and garlic supplemented in the feed resulted in enhancing of weight gain, SGR, FCR, BWI and CF. In a previous study we found similar results when juvenile great sturgeon was fed with garlic at 1.% for 60 days at water temperature 25 °C (Gholipour et al., 2013a). Various extracts from herbs and spices are reported to improve animal performance by stimulating action on gut secretions or by having a direct bactericidal effect on gut microflora and furthermore the herbals active principles in the diets induce the secretion of the digestive enzyme

Table 2Hematological parameters of beluga fed with *Echinacea purpurea* (E) at 0.5% and garlic (G) at 1% and control (C).

Factors		Time of sample collection (day)				
		0	15	30	45	60
RBC _s (mm ³ 10 ⁻⁶)	E	2.3±0.1 ^a	2.3±0.1 ^a	2.4±0.07 ^a	2.4±0.1 ^a	2.7±0.06 ^a
	G	2.5±0.06 ^a	2.3±0.07 ^a	2.5±0.09 ^a	2.6±0.07 ^a	2.7±0.07 ^a
	C	2.4±0.03 ^a	2.3±0.05 ^a	2.4±0.08 ^a	2.5±0.03 ^a	2.6±0.08 ^a
Hb (gr dl ⁻¹)	E	3.1±0.2 ^b	5.4±0.1 ^a	5.8±0.3 ^a	5.1±0.2 ^a	4.1±0.3 ^a
	G	3.2±0.1 ^b	5.6±0.1 ^a	6.2±0.2 ^a	4.1±0.06 ^b	3.9±0.01 ^b
	C	3.3±0.1 ^a	4.3±0.1 ^a	4.7±0.2 ^a	4.0±0.3 ^a	3.6±0.1 ^a
HCT (%)	E	16.8±0.7 ^c	23±0.9 ^{ab}	23.4±0.9 ^a	20.5±0.9 ^{abc}	17.6±0.9 ^{bc}
	G	16.5±0.6 ^b	23.2±0.6 ^a	24.8±0.9 ^a	15.5±0.6 ^b	17.5±0.4 ^b
	C	16.8±0.5 ^a	15.4±0.4 ^a	16.5±0.1 ^a	16.6±0.5 ^a	15.6±0.1 ^a
MCV (fl)	E	71±2.3 ^b	101.5±2.6 ^a	94.8±2.1 ^a	87.6±2.1 ^a	69.2±2.4 ^b
	G	66±2.5 ^b	101.6±2.8 ^a	93.4±2.1 ^a	70.4±2.5 ^b	63.2±2.5 ^b
	C	66.7±1.6 ^a	70.3±2.1 ^a	68.8±1.2 ^a	65.1±2.1 ^a	63.2±1.8 ^a
MCH (pg)	E	15.7±0.9 ^c	23.4±0.7 ^{ab}	24.3±0.8 ^a	20.3±0.7 ^{bc}	18.4±0.8 ^c
	G	12.8±0.7 ^b	23.5±0.5 ^a	23.2±0.8 ^a	18.6±0.4 ^b	13.8±0.9 ^b
	C	13.2±0.7 ^a	14.8±0.6 ^a	13.8±0.4 ^a	14.4±0.3 ^a	13.6±0.6 ^a
MCHC (%)	E	19±0.5 ^b	23.2±0.1 ^{ab}	24.1±0.3 ^{ab}	25.8±0.2 ^a	24.1±0.3 ^a
	G	20.1±0.4 ^b	23.8±0.1 ^{ab}	26.4±0.2 ^a	22.3±0.06 ^{ab}	22.3±0.6 ^{ab}
	C	20.6±0.5 ^a	22.7±0.4 ^a	23.3±0.5 ^a	20.4±0.3 ^a	23.2±0.7 ^a

Data are presented as mean ± SEM, N=45. Data in same row with different superscripts are significant different ($p < 0.05$)**Table 3**Leucogram profile of beluga fed with *Echinacea purpurea* (E) at 0.5%, garlic (G) at 1% and control (C).

Factors		Time of sample collection (day)				
		0	15	30	45	60
WBC (10 ³ mm ⁻³)	E	14.3 ± 0.08 ^b	12.0 ± 0.14 ^b	27.1 ± 0.15 ^b	18.0 ± 0.5 ^a	18.6 ± 0.5 ^a
	G	14.0 ± 0.08 ^c	19.1 ± 0.09 ^c	20.0 ± 0.15 ^c	16.7 ± 0.2 ^b	18.4 ± 0.4 ^a
	C	14.1 ± 0.06 ^a	13.2 ± 0.04 ^a	13.1 ± 0.08 ^a	13.0 ± 0.09 ^a	13.0 ± 0.05 ^a
Lymphocyte (%)	E	60 ± 0.005 ^e	67 ± 0.004 ^d	75 ± 0.009 ^c	82 ± 0.007 ^b	88 ± 0.005 ^a
	G	60 ± 0.004 ^e	72 ± 0.009 ^d	87 ± 0.003 ^c	82 ± 0.004 ^b	89 ± 0.005 ^a
	C	61 ± 0.003 ^a	60 ± 0.004 ^a	62 ± 0.005 ^a	60 ± 0.001 ^a	61 ± 0.006 ^a
Monocyte (%)	E	22 ± 0.006 ^a	18 ± 0.005 ^b	14 ± 0.003 ^c	11 ± 0.002 ^d	8 ± 0.008 ^e
	G	22 ± 0.006 ^a	18 ± 0.006 ^b	13 ± 0.003 ^c	11 ± 0.002 ^d	6 ± 0.004 ^e
	C	24 ± 0.005 ^a	24 ± 0.002 ^a	20 ± 0.006 ^a	21 ± 0.005 ^a	22 ± 0.003 ^a
Neutrophil (%)	E	11 ± 0.004 ^a	6 ± 0.008 ^{ab}	6 ± 0.002 ^{ab}	4 ± 0.001 ^{ab}	3 ± 0.002 ^b
	G	10 ± 0.002 ^a	6 ± 0.004 ^b	4 ± 0.002 ^c	4 ± 0.002 ^c	3 ± 0.003 ^c
	C	11 ± 0.002 ^a	11 ± 0.006 ^a	10 ± 0.005 ^a	11 ± 0.008 ^a	11 ± 0.004 ^a
Basophil (%)	E	5 ± 0.002 ^a	3 ± 0.002 ^b	2 ± 0.001 ^c	1 ± 0.001 ^{cd}	1 ± 0.002 ^d
	G	5 ± 0.003 ^a	3 ± 0.006 ^b	3 ± 0.004 ^b	1 ± 0.002 ^{bc}	1 ± 0.002 ^c
	C	6 ± 0.003 ^a	5 ± 0.005 ^a	7 ± 0.001 ^a	8 ± 0.003 ^a	6 ± 0.004 ^a

Data are presented as mean ± SEM, N=45. Data in same row with different superscripts are significant different ($p < 0.05$)

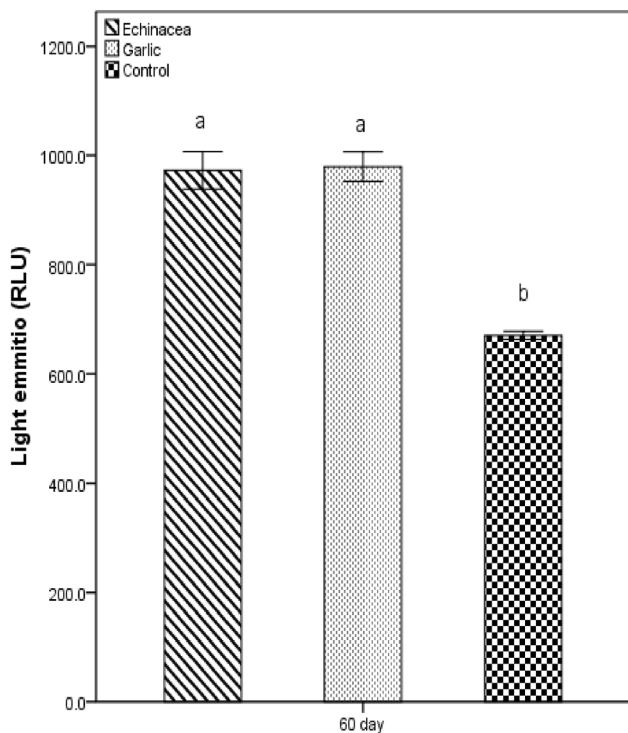


Figure 3
Respiratory burst activity (Relative Luminescence Units, RLU) in Beluga fed with 0.5% (Echinacea purpurea) and 1.0% (garlic) diet

and the growth promoter in herbs induced high protein synthesis (Citarasu, 2010). Many beneficial health properties of garlic are attributed to organosulphur compounds, particularly to thiosulfinates (Block 1992). Garlic contains allicin, which promotes the performance of intestinal flora, thereby improves digestion, with better utilization of energy leading to improved growth (Khalil et al. 2001). Supplementation of echinacea tends to improve feed conversion (Maass et al., 2005). Aly et al. (2008) observed a significant increase in the body weight gain and specific growth rates in tilapia fed echinacea supplemented diet at a rate of 0.25 ppt for 6 month. The same results regarding the effect of garlic on growth performance of juvenile *Huso huso* were shown by Gholipour et al. (2013a). Furthermore, similar benefits have been reported previously in several fish species (Shalaby et al. 2006; Salah et al. 2007; Thanikachalam et al. 2010; Mabrouk et al. 2011; Nya and Austin 2011; Bohlouli et al. 2011).

Mabrouk et al. (2011) reported enhanced weight gain and growth performance in Nile tilapia (*Oreochromis niloticus*) fed diets supplemented with 4% garlic compared to fish fed basal diets. The growth promoting influences of garlic have also been observed by Shalaby et al. (2006) in Nile tilapia where optimum growth, weight gain, SGR and FCR were obtained with a level of 3% dietary garlic inclusion. Nya and Austin (2011) also reported that 0.5-1% inclusion of garlic can elevate growth performance and optimum level of FCR in rainbow trout (*Oncorhynchus mykiss*) after 6 weeks feeding. Dietary inclusion of

1.5% garlic improved African catfish (*Clarias gariepinus*) growth parameters (Thanikachalam et al. 2010). Bohlouli et al. (2011) reported promoting effect of *Echinacea* (0.25-0.5%) on weight gain and growth performance in rainbow trout compared to control group. In addition, Salah et al. (2007) reported that 0.25 ppt *Echinacea* supplemented diet improved weight gain, SGR and FCR in Nile tilapia.

Improvement of weight gain is attributed to the enhancement of food intake after supplementation of garlic and Echinacea in diet (Przybilla and Wei 1998; Mabrouk et al. 2011; Shalaby et al. 2006; Salah et al. 2007; Thanikachalam et al. 2010; Bohlouli et al. 2011; Nya and Austin 2011). Allicin which is responsible for smell in garlic can stimulate the olfactory system of beluga which is located around the lip and can increase palatability of the diet for fish, as a consequence it improves food intake and weight gain (Thanikachalam et al. 2010; Mabrouk et al. 2011; Nya and Austin 2011). On the other hand, garlic can increase gastric acid in the stomach, which can improve the antibacterial activity in stomach and increase the appetite and food intake (Shalaby et al. 2006; Salah et al. 2007; Thanikachalam et al. 2010; Mabrouk et al. 2011; Nya and Austin 2011). Echinacea can improve growth performance and weight gain by improving digestive function (Salah et al. 2007; Bohlouli et al. 2011) which is in accordance with present study.

The employment of hematological techniques, including evaluation of erythrocyte count, hemoglobin, concentration, hematocrit value and leukocyte count, has provided valuable knowledge for fishery biologists in the assessment of fish health (Blaxhall 1972) and in monitoring stress responses (Soivio and Oikari 1976).

In this experiment, supplementation with Echinacea and garlic had positive influence on total white blood cell, lymphocytes, hematocrit, hemoglobin and mean corpuscular indices (MCV, MCH & MCHC) compared to the control group. Similar benefits have been reported previously in the other fish species fed dietary Echinacea and garlic (Shalaby et al. 2006; Ndong and Fall 2006; Salah et al. 2007; Salah et al. 2008; Salah and Mohamed 2010; Thanikachalam et al. 2010; Bohlouli et al. 2011; Nya and Austin 2011). Garlic has a positive effect on the transportation of respiratory gases; especially oxygen capacity of hemoglobin, so it can enhance MCH, Hb and PCV.

There are some studies revealing the effect of Echinacea herbal medicine on animals and a few of them are in fish species. Bauer et al. (1988) reported that phagocytosis in mice was enhanced after treatment with 0.1 ppm ethanolic extracts of Echinacea. Kuhn et al. (2005) reported an immune stimulating effect in sows by a repeated application of Echinacea every 5 days. In addition Cundell et al. (2003), found a significant increase of lymphocytes after one week in rats fed dried Echinacea preparations. Salah et al. (2007) reported significant increase in hematocrit and total leukocytes in Nile Tilapia fed 0.25 ppt Echinacea. Ad-

ditionally, Salah et al. (2008) announced that addition of 1% Echinacea can improve hematocrit and amount of leukocytes especially lymphocytes and eosinophil in Nile Tilapia. Bohlouli et al. (2011) reported the highest rate of white blood-cell and neutrophil percentage in the fish fed with 0.25-0.5% Echinacea. According to Shalaby et al. (2006) addition of 3% *Allium sativum* to tilapia diets increase hemoglobin, hematocrit and total leukocytes. Ndong and Fall (2006) reported that garlic at a concentration of 0.5 % for 2-4 week improved leukocyte count, and phagocytic index in juvenile hybrid tilapia. Thanikachalam et al. (2010) reported the enhancement of total leukocyte count and RBC in African cat fish fed diets supplemented with garlic (0.5-1.5). Nya and Austin (2011) also reported that 0.5-1% inclusion of garlic elevated total leukocyte count of rainbow trout after 2 week.

The active compounds of these herbs activate several components of the immune system, such as phagocytes, natural killer cells, T-lymphocytes, B-lymphocytes, complement, and lysozyme (Tang et al. 1997; Salah et al. 2010; Harikashram et al. 2011). Increase of phagocytic activity is a characteristic reaction associated with efficacy of Echinacea (Raa et al.1992; Barrett 2003). The antibacterial action of garlic depends on Allicin and Germanium which enhance natural kill cell and macrophage activity in experimental animals (Degruchy 1976; Aso et al. 1985;Ress et al.1993; Ankri and Mirelman 1999). Our findings corroborate previous researches (Sumiyoshi 1997; Oluwole 2001; Ozougwu 2011) on effects of garlic on WBC. Leucocyte play an important role in non-specific or innate immunity and their count can be considered as indicator of the health status of fish (Fazlolahzadeh et al. 2011). Consequently, significant improve of WBC is an indication of boosting of the immune system by *A. sativum*. Certainly, the proliferation rate and number of lymphocytes produced are critical for the magnitude and duration of protection against disease (Eggset et al. 1997). Various bioactive compounds have been found in garlic which is responsible to exhibit immunological properties of blood after oral uptake (Steiner and Li 2001; Amagase, 2006).

As a first line of defense, various peptides, such as lysozymes are present in serum where they prevent adherence and colonization by micro-organisms (Harikrishnan et al., 2011). Lysozyme is a fish defense element, which causes lysis of bacteria and activation of the complement system and phagocytes by acting as an opsonin (Magnadottir, 2006). In the present study, a significant increase in lysozyme activities was observed in the 0.5% Echinacea and 1% garlic supplemented diet compared to basal diet. Harikrishnan et al. (2011) observed significant enhancement of lysozyme activities in *Paralichthys olivaceus* fed 800 mg Echinacea/kg food on second and fourth week during their experiment. Likewise, Nya and Austin (2011) reported that fed diets supplemented with 0.5% of garlic tend to increase lysozyme activities in rainbow trout after 2 weeks. Also Salah et al. (2008) announced that adding 0.25 ppt of garlic im-

proves lysozyme activities in Nile Tilapia.

The bactericidal activity of complement has been well recognized as one of the key killing mechanisms of clearing pathogens. The complement system comprises a group of serum proteins and cell membrane receptors that function primarily to fight infection (Ellis 2001) which has been identified as a powerful non-specific defense mechanism, protecting fish against bacteria, fungi, viruses and parasites (Muller Eberhard 1988; Sunyer and Tort 1995; Tort et al.1996). As a result, higher complement levels could indicate better fish health (Amar et al. 2004). Few papers describe the effect of dietary herbal on complement activity in fish. Our findings indicated that alternative complement activity was influenced by inclusion of 0.5% Echinacea and 1% garlic in beluga diet. This is in line with the findings of Cuesta et al. (2005) which were on intraperitoneal administration of propolis in gilthead seabream and Amar et al. (2004) which demonstrated that oral administration of carotinoids can enhance complement activity in rainbow trout.

Phagocytosis has been recognized as an important cellular component of the innate immune system against invading microorganisms of fish (MacArthur and Fletcher 1985). It is well known that fish treated with immunostimulants show increased phagocytosis as well as respiratory burst activity (Sakai 1999). Our finding indicated that 0.5% Echinacea and 1% garlic improve respiratory burst activity in comparison with control group at the end of the experiment. Many investigators have reported enhanced bactericidal activity by the phagocytic cells of different fish species treated with immunostimulants (Jorgensen et al., 1993). Tabloid polysaccharides of Echinacea plant have a stimulatory effect of immune cells, so that increases the power of phagocytosis, chemotaxis and respiratory burst in macrophages and neutrophils (Wu et al., 2010). The moderator properties on immune system have been proven by caffeic acid compounds and alkalamides (Matthias et al., 2008; Millet et al., 2011). Nya and Austin (2011) reported improvement of respiratory burst activity in rainbow trout fed diet supplemented with 0.5% of garlic after 2 weeks. In addition, Salah et al. (2008) announced that adding 0.25 ppt of garlic improved respiratory burst activity in Nile Tilapia; all these findings are in accordance with present work.

To sum up, 0.5% Echinacea and 1% garlic can improve growth parameters, some immune responses and hematological parameters in *Huso huso*, which is economical and endangered species. However, animal strain, sex, time and way of consuming are factors that influence the effectiveness of these additives and more supplementary attempt such as bacterial and parasite challenge is needed to prove immunostimulatory effect of *Echinacea purpurea* and *Allium sativum*.

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