Iranian Journal of Fisheries Sciences 18(4) 830-846 2019
DOI: 10.22092/ijfs.2019.118227

# Effects of dietary Sel-Plex supplement on growth performance, hematological and immunological parameters in Siberian sturgeon (*Acipenser baerii* Brandt, 1869)

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Received: June 2017 Accepted: September 2017

**Abstract**

Selenium is one of the most essential trace elements in fishes. Therefore, determining the optimal level of selenium in the fish diet is one of the main concerns of researchers. This study investigated the effects of Sel-Plex (Selenium yeast) supplement on the hematological parameters and immunological parameters, and growth performance in Siberian Sturgeon, *Acipenser baerii*. The Siberian Sturgeon fed with diets supplemented with 0 (control), 5, 10 and 15 g $kg^{-1}$ Sel-Plex feed for 8 weeks. Growth performance, hematological parameters, total immunoglobulin, alternative complement activity (ACH50), C3 and C4 levels and lysozyme activity were measured at the end of the experiment. No significant differences have been observed ($p$>0.05) in weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR) and condition factors (CF) in the fish fed with different concentrations of Sel-Plex supplement when compared with the control group. The results showed that administration of 10 g Sel-Plex in this fish significantly increased erythrocyte, hemoglobin levels, hematocrit value, leukocytes, and lymphocytes counts. However, neutrophils of fish administrated with 10 and 15 g dietary Sel-Plex significantly decreased. The results revealed that Sel-Plex significantly increased total immunoglobulin, ACH50, C3, and lysozyme activities in the supplemented groups compared with the control. Plasma C4 levels in the fish fed with 5 and 15 g Sel-Plex were significantly higher than the control fish. The results of this study suggest that dietary Sel-Plex supplement at levels ≤15 g $kg^{-1}$ diet increased some hematological indices and improved the innate immune parameters of the Siberian Sturgeon, without affecting their growth performance.

**Keywords**: Sel-Plex, Siberian sturgeon, Growth performance, Immune parameters

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## Introduction

Trace elements play a critical role in biochemical and physiological processes in fish (Wang *et al*., 2015). Such processes include the formation of connective and skeletal tissues, production of hemoglobin and myoglobin, contributing to acid-base balance, transmission of nerves impulses, cell replication, gene transcription, and acting as cofactors for important enzymes and hormones in the body (Wang *et al*., 2015). Trace elements and vitamins improve the immune system by activating the T-cell-mediated immune responses and the adaptive immune response (Wintergerst *et al*., 2007). Maggini *et al*. (2007) suggest that adequate intakes of micronutrients can affect the physical barriers (skin and mucosa), cellular immunity, humoral immunity and antibody production.

Selenium, a trace element, is one of the most critical micronutrients in vertebrates (Hung, 2017; Nazari *et al*., 2017; Gholizadeh Zare Tavana *et al*., 2018). Some organic forms of selenium, such as seleno-cysteine are involved in the synthesis of seleno-proteins (Lu and Holmgren, 2009). Among vertebrates, fish have 30-37 types of seleno-proteins (Lobanov *et al*., 2009). Seleno-proteins are involved in the antioxidant defense system, thyroid activity, synthesis of other seleno-proteins, and selenium transfer (Kryukov *et al*., 2003). Selenium is necessary for reproduction and immune responses (Hoffmann and Berry, 2008). It is one of the main elements in glutathione peroxidase which is a key enzyme in protecting cell membrane lipids against oxidative damage in cellular and intracellular levels (De Riu *et al*., 2014). Selenium also acts as a cofactor in several vital enzymes including thioredoxin reductase, glutathione peroxidase and glutathione S-transferase (Zhang *et al*., 2008).

In fishes, Selenium is absorbed from the intestine, but the rate of selenium uptake depends on the physiological demands of the species. Previous studies have identified selenium as a trace element, required for normal body functions in fish, but toxic at elevated levels (Miller, 2006). The selenium requirements in fish are between 0.15-0.5 mg $kg^{-1}$ diets (Schwasz, 1995). Weiss and Hogan (2005) and Pacitti *et al*. (2016) found a relationship between selenium concentrations in blood and growth performance and immunological or hematological parameters. Selenium may increase the activity of natural killer cells (NK cells), increase T cell proliferation and vaccine efficacy in experimental animals (Broome *et al*., 2004). Moreover, the increase in lymphocyte proliferation and NK activity has been reported in humans after administering selenium (Broome *et al*., 2004). High levels of selenium in the diet (> 13-15 mg $kg^{-1}$) have been associated with impaired reproduction (Janz *et al*., 2010), suppressed immune system (Janz *et al*., 2010), oxidative stress (Miller, 2006), reduced growth, feed deficiency and increased mortality (Janz *et al*., 2010).

The physiological and toxicological studies in various fish species indicate

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the importance of chemical forms of selenium in diet or environment. There is a significant difference between organic and inorganic forms of selenium in their bioaccumulation and toxicity (Bakke *et al*., 2010; Janz *et al*., 2010). Selen-omethionine, an organic form of selenium, is the most common form of selenium in diets and often constitutes more significant than 80% of the total available Selenium (Young *et al*., 2010).

Sel-Plex is seleno-methionine that is biosynthesized by brewer's yeast, *Saccharomyces cerevisiae*. Compared with inorganic selenium, Sel-Plex is more digestible and better absorbed in tissues and lets the fish build nutrient reserves against periods of increased selenium demand without causing toxicity (Pacitti *et al*., 2015; Pacitti *et al*., 2016).

In our study, we investigated the effects of diets supplemented with organic selenium (Selenium yeast, Sel-Plex® supplement) on hematological and immunological parameters in juvenile Siberian sturgeon, *Acipenser baerii*. Sturgeons are among the most important commercial species in the world. Recently, cultivation of Siberian sturgeon, *A. baeri*, has increased in Western Europe (Gisbert and Williot, 2002). Cultivation of this species has also increased in Iran. Siberian sturgeon is highly compatible to rearing conditions and laboratory environments, which make it an appropriate biological model for physiological and nutritional studies (Hung, 2017).

## Materials and methods

Juvenile Siberian sturgeons, *A. baerii*, were used in the present study according to the National Ethical Framework for Animal Research in Iran (Mobasher *et al*., 2008). One hundred twenty Siberian sturgeons were obtained from the International Sturgeon Research Institute (Rasht, Gilan Province, Iran). Fish were randomly stocked into twelve 500-liter fiberglass tanks (10 fish per tank) and allowed to acclimate to the experimental environment in aerated tanks with 100% daily water exchange for two weeks before the experiment. Water temperature, pH and dissolved oxygen were maintained at 21 ± 2 ℃, 7.4 ±0.2, and 6±1 mg $L^{-1}$, respectively. During the acclimation period, fish were fed with pellets prepared according to commercial formulations obtained from Faradaneh Company, Shahrekord, Iran, according to the manufacturer's recommended rate. Dietary crude protein and lipid levels were formulated at 45±0.5 % and 15.75 ±0.3 % respectively (Table 1).

The formulated fish feed was enriched with organic selenium (Table 1). Organic Selenium was supplemented as Sel-Plex (Alltech Inc., Nicholasville, KY, USA) at 5, 10 and 15 g $kg^{-1}$ (equivalent to 5, 10 and 15 mg $kg^{-1}$ Selenomethionine, respectively) for a total of three treatments. Each supplemented diet was mixed in a mixer for 30 minutes and then homogenized into a paste by adding fish oil (20 mL $kg^{-1}$) and distilled water into the food mixer. Distilled water required for pelleting (20-40% of feed

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weight) was then added to the mixture and further homogenization. This mixture was passed through a meat grinder, producing strings, which were dried in an oven at 55°C for 12 h and then broken to produce 2-3 mm long pellets. The pellets were packed and stored at -20°C in a freezer. The control diet was prepared by the same process, although no supplement has been added.

**Table 1: Composition of experimental diets for Siberian sturgeon (*Acipenser baerii*)**

| **Ingredient** (%) | **Control** | **5 g kg$^{-1}$ Sel-Plex** | **10 g kg$^{-1}$ Sel-Plex** | **15 g kg$^{-1}$ Sel-Plex** |
|---|---|---|---|---|
| Commercial diet | 98 | 98 | 98 | 98 |
| Carboxy-methyl cellulose | 2 | 1.5 | 1 | 0.5 |
| Sel-Plex supplement | 0.0 | 0.5 | 1 | 1.5 |
| **Proximate composition (%)** | | | | |
| Crude protein (%) | 44.9±0.4 | 45±0.4 | 45±0.4 | 45.2±0.5 |
| Crude lipid (%) | 15.75±0.3 | 15.81±0.5 | 15.65±0.5 | 15.61±0.6 |
| Crude fiber (%) | 2.03±0.1 | 2.17±0.2 | 2.14±0.2 | 2.08±0.2 |
| Moisture (%) | 10.3±0.5 | 10.1±0.4 | 9.9±0.5 | 9.7±0.6 |
| Ash (%) | 7.5±0.6 | 7.9±0.7 | 8.4±0.7 | 9.4±1.3 |
| Gross energy (Kcal kg$^{-1}$) | 3320±93 | 3330±86 | 3330±76 | 3340±70 |

An 8 weeks feeding trial was conducted using 120 juvenile Siberian Sturgeons, with an average weight of 47.8±2.7 g and an average length of 26±1 cm (mean±SD). During the experimental period, fish were fed with sturgeon pellets with 0 (control), 5, 10 and 15 g kg$^{-1}$ Sel-Plex supplement at 3% of their body weight three times a day. Fish were weighed individually and measured every two weeks and group weights were employed to adjust feeding rates. At the end of the experiment, 12 fish per treatment were anesthetized with tricaine methane-sulfonate (MS-222) solution (500 ppm) and blood was collected from the caudal vein using 2 mL heparinized syringes. The collected blood was transferred into 2 mL microcentrifuge tubes. The blood sample was centrifuged for 15 min at 6000 g at 4 °C. Plasma samples were immediately stored at -25 °C prior to biochemical analysis.

The growth parameters such as weight gain, specific growth rate, feed conversion ratio and condition factors were calculated using the following formulas, on day 15 and 30 (Banaee *et al*., 2013).

$$\text{Weight gain} = \text{Final weight (g)} - \text{Initial weight (g)}$$

$$\text{Specific growth rate (SGR)} = \frac{\text{Ln (final body weight)} - \text{Ln (initial body weight)}}{\text{Experimental periods}} \times 100$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Condition factors (CFs)} = \frac{\text{Weight (g)}}{(\text{Length (cm)})^3} \times 100$$

The blood was immediately used to determine the number of red blood cells (RBC) and white blood cells (WBC) by using a hemocytometer at 400×magnification. Subsequently, blood was diluted to $10^{-2}$ and $10^{-3}$ in phosphate buffered saline (PBS), at pH

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7.2 (Ahmadi *et al*., 2014). Haematocrit (Hct) was determined by the microhematocrit method described by Ahmadi *et al*. (2014). Haemoglobin (Hb) concentration was measured, using cyanohemoglobin (Ahmadi *et al*., 2014). Blood smears were prepared according to Ahmadi *et al*. (2014) and Banaee *et al*., (2016) and examined at 400×magnification to differential white cell count.

The mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) were calculated according to the following formulas:

$$MCH = \frac{Hb \times 10}{RBC}; \quad MCV = \frac{Hct \times 10}{RBC}; \quad MCHC = \frac{Hb \times 100}{Hct}$$

Total immunoglobulin (Ig) was determined using Amar *et al*. (2000) method. Thus, 100 µL of plasma sample (100-fold dilutions in PBS) was mixed with an equal volume of 12% (v/v) solution of polyethylene glycol and incubated for 2 hours at room temperature which reduced the Ig. The Ig molecules were removed by centrifugation (5000 g at 4°C) and the protein content was determined like total protein determination by the Biuret reaction. This value was subtracted from the total protein level, which corresponds to the total Ig level and was expressed in mg $mL^{-1}$.

Alternative complement activity (ACH50) was evaluated following the procedure of Yano (1992) by using sheep red blood cells (ShRBC) (Bahar Afshan Research and Development Institute, Iran). ShRBC were washed and adjusted to $2\times10^8$ cell $mL^{-1}$ in ethylene glycol tetraacetic acid magnesium-gelatin veronal buffer (0.01 M). 100 µl of the ShRBC suspension was lysed with 3.4 mL of distilled water and the hemolysate was determined at 414 nm against distilled water to reach 100% lysis. The test plasma was diluted, and different volumes ranging from 0.1 to 0.25 mL were brought up to 0.25 mL total volume before being allowed to react with 0.1 mL of RaRBC in test tubes. After incubation at 20°C for 90 min with occasional shaking, 3.15 mL of a 0.9% (v/v) saline solution was added to each tube then centrifuged at 1600×G for 10 min at 4°C. The absorbance (A) of the supernatant was measured using a spectrophotometer at 414 nm. A lysis curve was obtained by plotting the percentage of haemolysis against the volume of plasma added. The volume of the plasma producing 50% haemolysis (ACH50) was determined and the number of ACH50 Units $ml^{-1}$ was obtained from each fish.

The immunoturbidimetric test (Pars Azmun, Iran) was adopted to determine the serum complement level. C3 and C4 in serum samples were mixed with the antibody provided by the kits, and then an antigen-antibody complex was produced. The optical density (OD) value was measured at 340 nm. Compared with the values of the standards from the kits, C3 and C4 contents were expressed in µg $ml^{-1}$ (Abdollahi *et al*., 2016).

The turbidimetric assay for lysozyme activity was carried out according to Lange *et al*. (2001) with minor modifications. Thus, plasma (50 µl)

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was added to 2 mL of a suspension of *Micrococcus luteus* (Actinobacteria: Micrococcaceae) (0.2 mg $ml^{-1}$) in a 0.05 M sodium phosphate buffer (pH 6.2). The reaction was carried out at 25°C and absorbance was measured at 570 nm after 0.5 min and 4.5 min by using a spectrophotometer. PBS was used as the blank. Hen's egg white lysozyme (Sigma) was used in sterile sodium phosphate Buffer (PBS) as a standard curve to determine the lysozyme activity of the samples. The specific activity (Units $mL^{-1}$ plasma) of lysozyme was determined.

The significant difference in the biochemical parameters of fish treated with different concentrations of Sel-Plex was examined using one-way ANOVA. Data were checked for normality (Kolmogorov-Smirnov test). Means were compared by Duncan's test and a $p<0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics 19. Data are presented as mean±SD.

## Results

There were no significant differences ($p>0.05$) in weight gain, specific growth rate, and feed conversion ratio and condition factors between fish which were fed with different concentrations of Sel-Plex supplement and the control group (Table 2).

**Table 2: growth performance of juvenile Siberian sturgeon (*Acipenser baerii*) fed 5, 10 and 15 g Sel-Plex supplement for 8 weeks.**

| Growth performance parameters | Control (0 g Sel-Plex supplement) | 5 g Sel-Plex supplement | 10 g Sel-Plex supplement | 15 g Sel-Plex supplement |
|---|---|---|---|---|
| Initial weight | 47.45±3.37[a] | 47.98±0.85[a] | 48.66±3.13[a] | 46.91±2.95[a] |
| Initial length | 26.6±1.2[a] | 26.1±0.5[a] | 26.5±0.7[a] | 25.9±0.9[a] |
| Final weight | 160.03±8.05[a] | 164.58±9.41[a] | 161.20±3.22[a] | 160.87±10.51[a] |
| Final length | 37.1±0.9[a] | 37.7±0.9[a] | 37.9±1.3a | 37.3±0.8[a] |
| WG (g) | 112.29±10.48[a] | 116.60±9.06[a] | 112.61±10.52[a] | 113.97±11.49[a] |
| SGR (%) | 2.16±0.20[a] | 2.20±0.09[a] | 2.14±0.16[a] | 2.17±0.17[a] |
| CF (%) | 0.32±0.2[a] | 0.31±0.02[a] | 0.30±0.02[a] | 0.31±0.01[a] |
| FCR | 1.26±0.13[a] | 1.21±0.10[a] | 1.25±0.13[a] | 1.24±0.12[a] |

Data are represented as mean±S.D. Different letters stand for statistically significant differences at $p<0.05$

Compared to the control group, fish fed with 10 g $kg^{-1}$ Sel-Plex supplement had significantly higher ($p<0.05$) erythrocyte, hemoglobin, and hematocrit values (Table 3). MCV and MCH indices were significantly ($p<0.05$) lower in fish fed with 10 g $kg^{-1}$ Sel-Plex supplement compared to the control group. The results indicated no significant changes in MCHC index or monocyte and eosinophil percentage in fish fed with Sel-Plex supplement (Table 3).

There was a significant increase in leukocytes and lymphocytes percentage in fish fed with 10 and 15 g $kg^{-1}$ Sel-Plex. The neutrophil percentage in fish fed with 10 and 15 g $kg^{-1}$ Sel-Plex was

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significantly ($p$<0.05) lower than the control group.

**Table 3: haematology parameters of juvenile Siberian sturgeon (*Acipenser baerii*) fed with Sel-Plex supplemented diet.**

| Hematological parameters | Treatments | | | |
|---|---|---|---|---|
| | Control (0 g Sel-Plex supplement) | 5 g Sel-Plex supplement | 10 g Sel-Plex supplement | 15 g Sel-Plex supplement |
| Red Blood Cells ($10^5$) | 7.66±0.34$^a$ | 7.70±0.41$^a$ | 8.69±0.31$^b$ | 7.35±0.21$^a$ |
| Hemoglobin (g $dL^{-1}$) | 6.78±0.54$^a$ | 6.66±0.32$^a$ | 7.34±0.19$^c$ | 6.34±0.17$^b$ |
| Hematocrit (%) | 35.00±1.12$^a$ | 34.80±1.20$^a$ | 37.60±1.05$^c$ | 33.00±0.65$^b$ |
| MCV (fl) | 45.7±0.90$^a$ | 45.2±0.90$^a$ | 43.3±0.80$^b$ | 44.9±0.70$^a$ |
| MCH (pg) | 88.4±4.00$^a$ | 86.5±1.00$^a$ | 84.5±1.00$^b$ | 86.3±1.00$^a$ |
| MCHC (g $dL^{-1}$) | 19.35±0.92$^a$ | 19.13±0.28$^a$ | 19.52±0.20$^a$ | 19.21±0.16$^a$ |
| White Blood Cells ($10^4$) | 14.82±0.26$^a$ | 14.68±0.25$^a$ | 15.18±0.51$^b$ | 15.14±0.26$^b$ |
| Lymphocyte (%) | 65.60±1.42$^a$ | 65.40±2.17$^a$ | 69.40±2.50$^b$ | 67.80±2.39$^a$ |
| Monocyte (%) | 4.60±1.08$^a$ | 4.80±0.79$^a$ | 4.20±0.79$^a$ | 4.60±0.52$^a$ |
| Neutrophils (%) | 29.40±0.82$^a$ | 29.60±1.42$^a$ | 26.00±2.36$^b$ | 27.00±1.45$^b$ |
| Eosinophils (%) | 0.40±0.25$^a$ | 0.20±0.21$^a$ | 0.40±0.25$^a$ | 0.6±0.41$^a$ |

Data are represented as mean±S.D. Different letters stand for statistically significant differences at $p$<0.05

There was a significant ($p$<0.05) difference in immunological parameters for all treatment groups in fish fed with Sel-Plex supplement as compared to the control group (Figs. 1-5). In fish fed diets with Sel-Plex supplement, immunoglobulin was significantly ($p$<0.05) increased compared to the control group (Fig. 1).

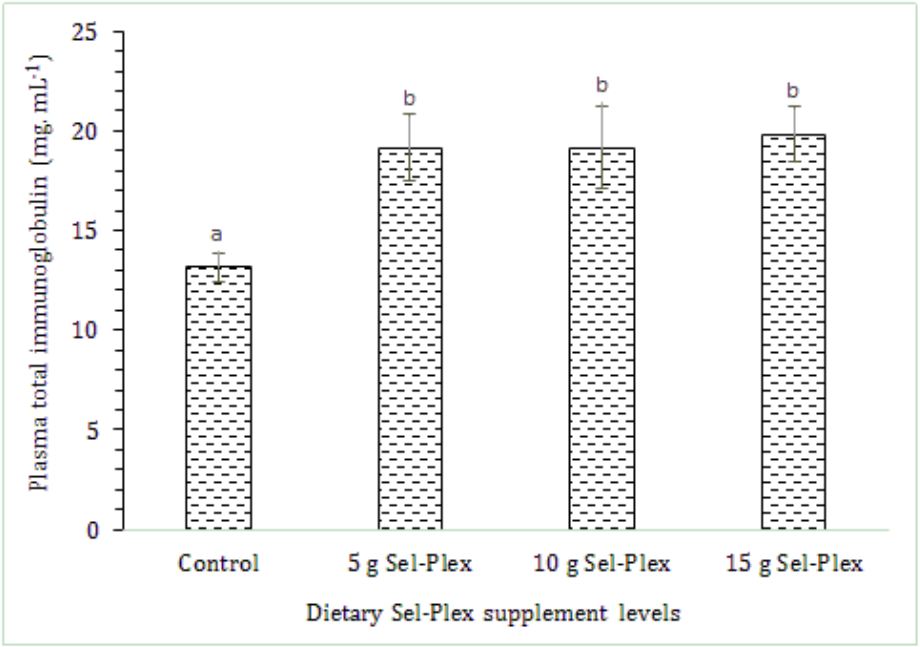



**Figure 1: Plasma total immunoglobulin levels of *Acipenser baerii* fed with diets supplemented with Sel-Plex. Data are presented as mean±S.D. Different letters stand for statistically significant differences at $p$ <0.05.**

Alternative complement pathway (ACH50) significantly increased with increasing Sel-Plex in diet of juvenile Siberian sturgeon (Fig. 2). We observed

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increased C3 activity with dietary inclusion of Sel-Plex supplement and it was the highest at 5 g $kg^{-1}$ level ($p$<0.05) (Fig. 3). In juvenile Siberian sturgeon, C4 was significantly ($p$<0.05) the highest in fish fed with 5 and 15 g $kg^{-1}$ Sel-Plex (Fig. 4).

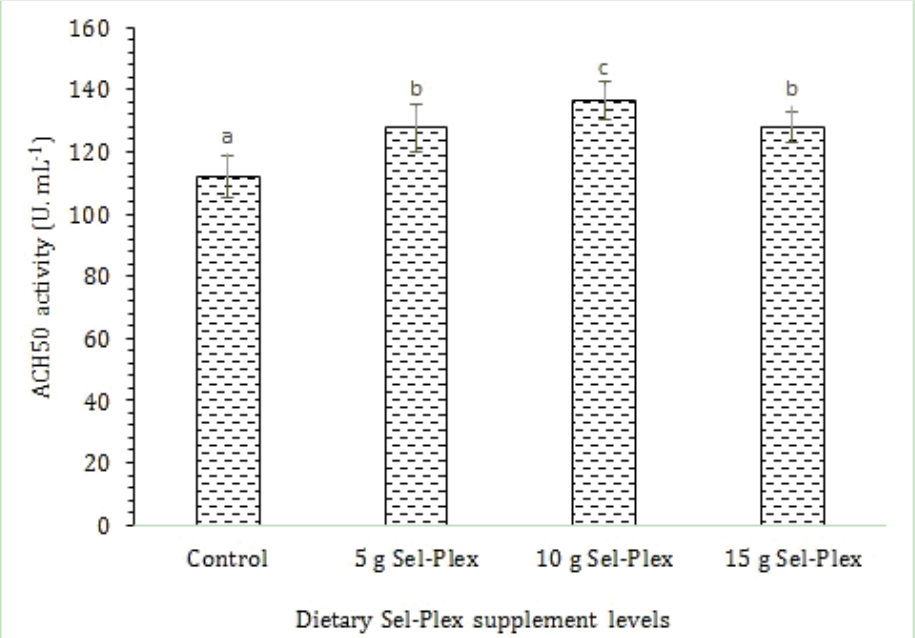



**Figure 2: Plasma total complement (ACH50) activity of *Acipenser baerii* fed with Sel-Plex supplemented diet. Data are presented as mean±S.D. Different letters stand for statistically significant differences at $p$<0.05.**

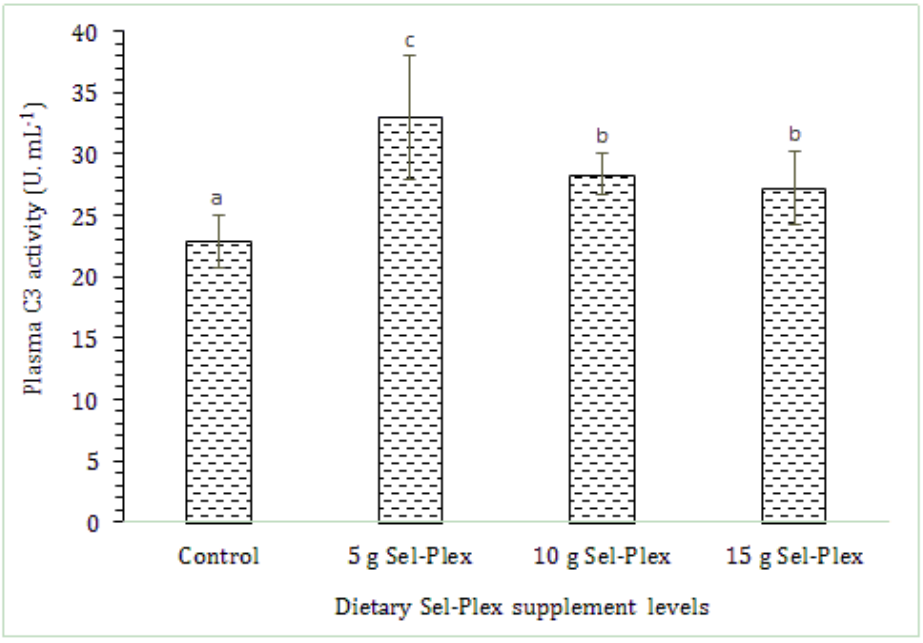



**Figure 3: Plasma C3 activity of *Acipenser baerii* fed with Sel-Plex supplemented diet. Data are presented as mean±S.D. Different letters stand for statistically significant differences at $p$<0.05.**

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**Figure 4: Plasma C4 activity of *Acipenser baerii* fed with Sel-Plex supplemented diet. Data are presented as mean±S.D. Different letters stand for statistically significant differences at $p<0.05$.**

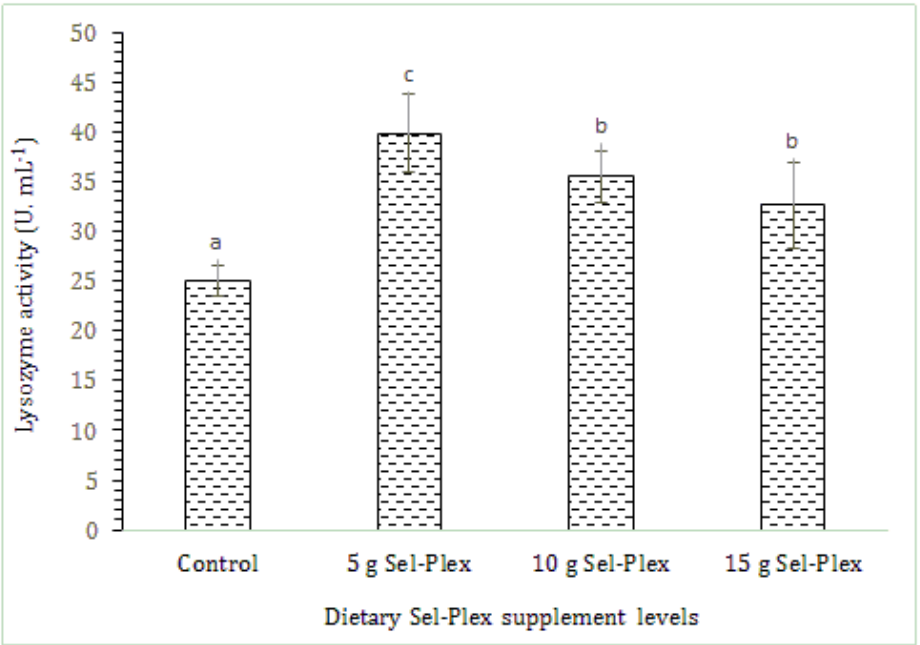



**Figure 5: Plasma lysozyme activity of *Acipenser baerii* fed with Sel-Plex supplemented diet. Data are presented as mean±S.D. Different letters stand for statistically significant differences at $p<0.05$.**

All diets containing Sel-Plex showed a significant increase in lysozyme activity compared to those fed only with the control diet ($p<0.05$) (Fig. 5).

## Discussion

Growth performance parameters of Siberian sturgeons fed with Sel-Plex-enriched diet were similar to growth performance of fish in the control

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group. Sel-Plex had no effects on weight gain, specific growth rate, feed conversion ratio and condition factors (Table 2). This is in agreement with the results of previous studies on the effects of selenium supplementation on rainbow trout (Rider *et al*., 2009; Pacitti *et al*., 2015). However, Ilham *et al*. (2016) found that Sel-Plex-supplemented diets improved the growth performance of juvenile yellowtail kingfish (*Seriola lalandi*).

Haematological parameters have provided valuable knowledge for monitoring fish health (Banaee *et al*., 2008). In the present study, the increase in erythrocytes, haematocrit and haemoglobin in the fish fed with 10 g $kg^{-1}$ dietary Sel-Plex could be attributed to increased erythrocytes in hematopoietic organs such as spleen and head kidney which play an important role in blood cells formation. Haratake *et al*. (2008) found that plasma selenium may be absorbed by the RBC through binding to hemoglobin. Compared with the control group, 10 g $kg^{-1}$ Sel-Plex in the diet significantly decreased the MCV and the MCH. However, Sel-Plex administration had no effects on the MCHC. Selenium can be absorbed by red blood cells through ion exchange and then binds to haemoglobin (Haratake *et al*., 2008). Reacting with GSSeSG (glutathione seleno-trisulfide), the haemoglobin bound selenium is produced in the RBC (Haratake *et al*., 2008). Then, the selenium which is bound to Hb may be released by a thiol exchange between glutathione (GSH) and Hb (Haratake *et al*., 2008). Therefore, the RBC count may remain normal, but measurable levels of the Hb will decrease. With a decrease in the Hb content, MCV and MCH indices decrease as well. However, with an increase in the Sel-Plex level in the diet (15 g $kg^{-1}$), no alterations are found in the blood indices. Furthermore, reduced MCV and MCH may be attributed to the increased number of new RBCs which are still small and have a lower hemoglobin level compared to the mature RBCs (Harvey, 2012).

An increase in the leukocyte count and lymphocyte percentage in the blood of the fish fed with 10 and 15 g $kg^{-1}$ Sel-Plex could be attributed to a higher lymphocyte proliferation. Zhuang *et al*. (2015) showed that selenium could affect the immune system through the upregulation of the interleukin (IL)-2 receptor α and β subunits on lymphocytes. Interleukins are involved in activating B cells, immunoglobulin synthesis and T-cell proliferation, differentiation of cytotoxic T lymphocytes, and production, activation and persistence of natural killer (NK) cell through the activation of cytokinins (Zhuang *et al*., 2015).

Although neutrophils have been decreased in the fish treated with 10 and 15 g $kg^{-1}$ Sel-Plex, Weiss and Hogan (2005) reported that dietary selenium did not significantly affect the percentage of neutrophils in domestic animals. In contrast, Ibeagha *et al*. (2009) demonstrated that the efficiency of neutrophils to kill or phagocytize pathogens was enhanced by adequate selenium status in domestic animals. Neutrophils are capable of destroying

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bacteria by producing superoxide-derived radicals. The continuous production of superoxide radicals depends on levels of selenium and glutathione peroxidase (GPx) in neutrophils. However, Arthur *et al*. (2003) reported neutrophils' dual pattern in removing pathogens in response to changes in dietary selenium levels. On the one hand, removal of pathogens by neutrophils increases significantly when selenium levels are low. On the other hand, an increase in selenium levels may not affect the removal of pathogens (Arthur *et al*., 2003). Our findings indicate that an increase in the dietary Sel-Plex decreases neutrophils percentage in blood. Effects of the Sel-Plex on the ability of neutrophils to eliminate pathogens are not evaluated in this study. However, the increase in neutrophils efficiency may compensate for their reduction.

Moreover, inclusion of Sel-Plex into the diet of fish had no effects on the percentage of monocytes and eosinophils.

Innate immunity and non-specific immune parameters could be a useful tool to evaluate the effects of selenium on the immune system of fish. As the most important component of humoral immunity in fish, immunoglobulins play a key role in identifying and eliminating pathogens such as bacteria and viruses. We found that immunoglobulin levels in the *A. baerii* fed with Sel-Plex supplement were significantly higher than immunoglobulin levels in the control group. Dietary selenium caused an increase in the synthesis rate of humoral immunoglobulins in breeding animals (Gelderman and Clapper, 2013; Hall *et al*., 2014). Increased total immunoglobulin levels in channel catfish which were fed with selenomethionine and selenium yeast supplement (Wang *et al*., 2015) agree with the results of the present study. Khan *et al*. (2008) reported similar results in broilers which were given selenium supplements, with or without vitamin E.

The complement system is one of the most important parts of the innate immune system in fish. A combination of factors such as diversity, high titer and activation of the complement system in different environmental conditions have made the complement system as one of the most effective parameters in the immune system of fish (Pushpa *et al*., 2014). As in mammals, three complement activation pathways are active in fish and fish protein complements are homologous to complement compounds in mammals in terms of structure and function (Pushpa *et al*., 2014). Thus, the increase in complement activity (ACH50) can help in recognizing and eliminating pathogens by phagocytosis (Ahmadi *et al*., 2012). A significant increase in alternative complement activity (ACH50) as well as an increase in C3 and C4 activities was observed in the fish after feeding with Sel-Plex for two months. The third component (C3) is a glycoprotein which is synthesized by hepatocytes and monocytes. C3 is one of the major proteins in the complement system in fish and usually acts as the

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substrate for both classical and alternative pathways (Pushpa *et al*., 2014). Interaction of opsonized cells with C3 receptors on phagocytes increases phagocytosis (Wang *et al*., 2015). C3 is a flexible molecule which interacts with other proteins of the complement system and therefore increases their performance (Wang *et al*., 2015). The fourth component (C4) is a glycoprotein which is synthesized by macrophages and monocytes. C4 plays a key role in the immunity and tolerance of animals. C4 deals with certain infections by connecting the recognition pathways to other effector proteins of the immune system (Boshra *et al*., 2004). C4 participates in eliminating certain pathogens. So, the increased activity of ACH50, C3 and C4 in plasma of the fish fed with Sel-Plex may indicate an increase in their synthesis rate in liver, macrophages and monocytes.

The results revealed a significant increase in lysozyme activity in all the treatment groups after feeding for two months, especially in the fish receiving 5 g $kg^{-1}$ Sel-Plex. Lysozymes have an antibacterial activity characterized by the ability to damage the cell wall of bacteria. Lysozyme is one of the non-specific factors in the immune system which is usually synthesized by leukocytes (Saurabh and Sahoo, 2008). The increased activity of lysozyme in the fish treated with Sel-Plex supplement may indicate stimulation of the non-specific immune system. Lysozyme has anti-inflammatory, anti-viral, and anti-bacterial properties and eliminates gram-negative and gram-positive bacteria (Saurabh and Sahoo, 2008).

This study aimed to investigate the effects of Sel-Plex on growth performance and immunological and hematological parameters in Siberian Sturgeon, *A. baerii*. In conclusion, dietary supplementation of Sel-Plex had no significant effect on growth performance but significantly increased specific hematological indices and improved the innate immune system of Siberian Sturgeon. Although the findings of this study show that Sel-Plex supplement may improve the immune system of fish, more research is needed to comprehend the immuno-stimulatory impact of this material fully.

**Acknowledgements**

This study was supported by grant from Behbahan Khatam Alanbia University of Technology. The authors are grateful to Maryam Banaie for proofreading the manuscript.

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