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Effects of selenium (Sel-Plex) supplement on blood biochemical parameters of juvenile Siberian sturgeon (*Acipenser baerii*)

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Abstract

Selenium (Se) and many of its compounds are among the most toxic micronutrients. Therefore, determining the optimal level of selenium in the diet of fish is one of the main concerns of researchers. The present study was designed to investigate the effects of Sel-Plex® (Selenium-enriched *Saccharomyces cerevisiae* yeast) on blood biochemical parameters in juvenile Siberian sturgeon, *Acipenser baerii*, as clinical biomarkers to evaluate the effects of selenium on the health of fish. In this study, fish were fed diets supplemented with 0 (control), 5, 10 and 15 g Sel-Plex per Kg feed for 8 weeks. Although a significant (p<0.05) decrease was observed in alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) activity in fish fed with 15 g Sel-Plex supplement, plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were significantly higher (p<0.05) than the control fish. The results showed that administration of 10 and 15 g Sel-Plex supplement in fish significantly (p<0.05) increased lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) activities. Administration of Sel-Plex significantly (p<0.05) decreased glucose, albumin, cholesterol and triglyceride concentrations. The results revealed that Sel-Plex significantly increased (p<0.05) globulin concentrations in the supplemented groups compared with the control group. Decreased total protein concentrations were only observed in fish fed with 5 g Sel-Plex. The results of this study show that diets containing high concentrations of Sel-Plex (10 g and 15 g) produced serious toxic effects, including changes in blood biochemical parameters.

Keywords: Siberian sturgeon, Biochemical parameters, Selenomethionine, Sel-Plex supplement

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Introduction

Selenium is one of the most important trace minerals in fishes, supporting multiple biochemical processes in the body (Hoffmann and Berry, 2008; De Riu *et al*., 2014). Selenium is available from various feed components. However, it is commonly added in formulated diets for cultured fish. As this need to selenium is partly met through the water and prey, selenium deficiency in fishes is rarely observed at feedlot periods (Wang *et al*., 2013). However, taking preventative measures to manage risks associated with poor selenium status may be beneficial.

Decreased growth performance (Hardy *et al*., 2010), increased rates of larval deformity, reduced rate of survival (Holm *et al*., 2005; de Rosemond *et al*., 2005), an impaired immune system (Hoffmann and Berry, 2008), dysfunction of endocrine glands (Behne *et al*., 2000), and antioxidant enzyme activity (Beckett and Arthur, 2005; De Riu *et al*., 2014) and imbalanced swimming and mortality are the main visible symptoms of selenium deficiency in the diet of fish (Wang *et al*., 2013). Furthermore, higher levels of selenium than what fish need are known to cause chronic toxicity in fish (Tashjian *et al*., 2006).

Various forms of selenium, including sodium selenate, sodium hydrogen selenite or sodium selenite are authorized to be added to foodstuff and dietary supplements, although selenomethionine is the main source of selenium, in an organic form in the diet of fish (Maher *et al*., 2010; Young et al., 2010; Nazari *et al*., 2017). Selenomethionine in the diet is absorbed by enterocytes and may accumulate in various tissues of fish (Hamilton, 2004; Bakke *et al*., 2010; Maher *et al*., 2010; Stewart *et al*., 2010). Selenium-enriched *Saccharomyces cerevisiae* yeast (Sel-Plex ®) contains high levels of selenomethionine (SeMet), and several experimental studies have established that SeMet and selenized yeast increase selenium levels in fishes (Godin *et al*., 2015). After it was authorized in 2006, the use of Sel-Plex increased in the diet of farmed species because it contained readily bioavailable sources of selenium. The half-life of Sel-Plex in animals is usually high (Pacitti *et al*., 2015), so bioaccumulation of selenium in various tissues can cause toxicity slightly above the threshold level. Due to a long half-life, there have been published concerns over the consumption of Sel-Plex.

The main objectives of this research were to determine the influence of dietary Sel-Plex on blood biochemical parameters and to see whether extra selenium in the diet could potentially be dangerous for fish health. Hence, this study was done to collect basic information on the effects of Sel-Plex on biochemical parameters of blood in juvenile Siberian sturgeon, *Acipenser baerii*. Siberian sturgeon, *A.baerii*, was selected for the present study because the family Acipenseridae is an important commercial species in terms of its meat and eggs (caviar). Many efforts have been made for breeding these fish in Iran (Yazdani Sadati *et al*., 2016). Therefore, research on

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nutritional requirements of sturgeon, especially trace minerals, seems essential.

Materials and methods

Fish husbandry

Juvenile Siberian sturgeon, *A. baerii*, were obtained from the International Sturgeon Research Institute (Rasht, Gilan Province, Iran), and used according to the National Ethical Framework for Animal Research in Iran (Mobasher *et al*., 2008). 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 a 100% water exchange rate/day for two weeks before the experiment. Water temperature, pH and dissolved oxygen were maintained at 21±2 °C, 7.4±2, and 6±1 mg L^{-1}, respectively. During the acclimation period, the fish were fed pellets prepared according to commercial formulations obtained from Faradaneh Company, Shahrekord, Iran, at the manufacturer's recommended rate. Dietary crude protein and lipid levels were formulated at 42 ± 2% and 14 ± 2% respectively (Table 1). All known nutritional requirements of Siberian sturgeon (NRC 2011) were met by the experimental feeds. The basal diet was frequently analyzed to contain 0.3 mg kg^{-1} selenium.

Diet preparation

The formulated fish feed was enriched with organic selenium. Organic Se 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. The amount of distilled water required for pelleting (20-40% of feed weight) was then added to the mixture and further homogenized. This mixture was passed through a meat grinder, producing string shapes, 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 was added.

Experimental design

An 8-week feeding trial was conducted using 120 juvenile Siberian sturgeons, *A. baerii*, with an average weight of 47.5 ±2.5 g and an average length of 26±1 cm (mean±SD). During the experimental period, fish were fed diets enriched with 0 (control), 5, 10 and 15 g kg^{-1} Sel-Plex supplement at 3% of their body weight three times a day for 8 weeks. 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 captured and anesthetized under clove solution (1:5,000). Anaesthetized fish were bled from the caudal artery/vein using 2-mL heparinized syringes. The collected blood was transferred into 2-mL micro-

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centrifuge tubes. The blood samples were centrifuged for 15 min at 6000 g (7319 rpm) at 4 °C. Plasma samples were immediately stored at -25 °C prior to biochemical analysis.

Blood biochemical parameters analysis

All blood biochemical parameters were determined using a UV-visible spectrophotometer (UNICO 2100) and standard biochemical reagents (Pars Azmun Company, Tehran, Iran). Each blood biochemical parameter was measured by a certain method. Total protein was measured at 540 nm by Biuret reaction (Johnson *et al*., 1999). Albumin assay is based on the dye-binding properties of plasma albumin with a bromocresol green. An increase in the blue-green color was measured at 630 nm (Johnson *et al*., 1999). Plasma globulin was measured based on the ratio of albumin to total protein (Johnson *et al*., 1999). Plasma glucose was measured by the glucose-oxidase method at 500 nm (Sacks, 1999), plasma cholesterol levels was measured by the CHOD-PAP enzymatic method at 510 nm and triglyceride levels was measured by GPO-PAP enzymatic method at 546 nm (Rifai *et al*., 1999). The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma was determined by NADPH consumption and its conversion to NAD^+ at 340 nm. Gamma-glutamyl transferase (GGT) activity is determined by a coupled enzyme assay in which GGT transfers the γ-glutamyl group from the substrate, L-γ-Glutamyl *p*-nitroanilide, liberating the chromogen, *p*-nitroanilide, at 418 nm proportional to the GGT activity. Lactate dehydrogenase (LDH) in plasma was determined based on the conversion of pyruvate to lactate at 340 nm, alkaline phosphatase (ALP) was measured based on the conversion of nitrophenol phosphate into nitrophenol and phosphate at 405 nm. Creatinine phosphokinase (CK) was measured based on the conversion of creatinine phosphate into creatinine at 340 nm and based on optical density (OD) absorption and the formula presented in the kits' manual (Moss and Henderson, 1999).

Data analysis

The significant differences found in the biochemical parameters of fish treated with different concentrations of Sel-Plex were 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 SPSS (IBM, 19) software. Data are presented as mean± SD.

Results

Fish fed the spiked diet of 15 g kg^{-1} Sel-Plex supplement for 8 weeks had significantly increased AST (Fig. 1) and ALT (Fig. 2) activities when compared to control group ($p<0.05$).

Activity LDH in plasma were significantly ($p<0.05$) increased in fish fed 10 and 15 g kg^{-1} Sel-Plex supplement when compared to the control group (Fig. 3).

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Activity of GGT in plasma was significantly (p<0.05) altered by dietary Sel-Plex supplement. However, in fish fed 10 g kg^{-1} Sel-Plex supplement, GGT activity was significantly increased when compared to the control group, while GGT activity was significantly decreased in fish fed 15 g kg-1 Sel-Plex supplement (Fig. 4).

ALP activity in plasma was significantly (p<0.05) decreased in fish fed 10 and 15 g kg^{-1} Sel-Plex supplement when compared to the control group (Fig. 5).

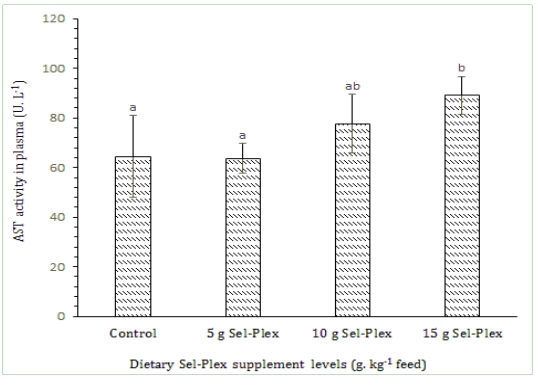

Figure 1: AST activity in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean ± S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

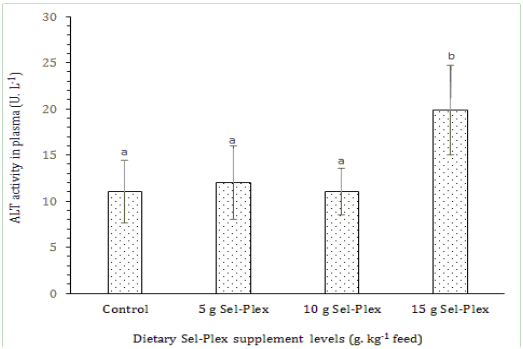

Figure 2: ALT activity in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean ± S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

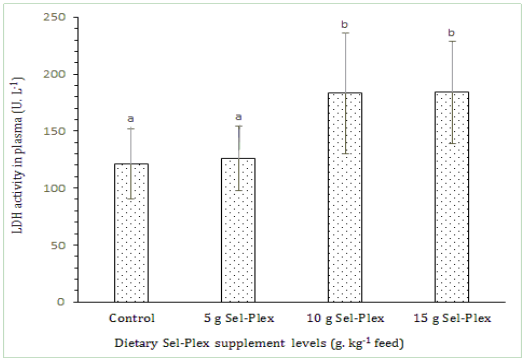

Figure 3: LDH activity in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean±S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

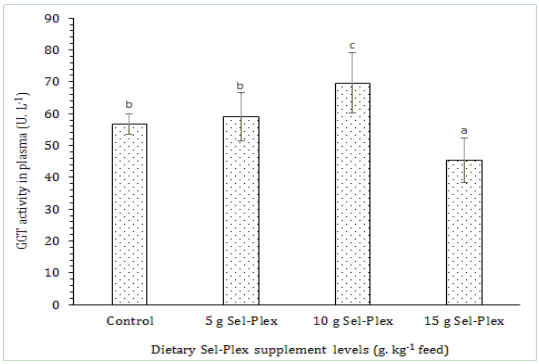

Figure 4: GGT activity in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean±S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

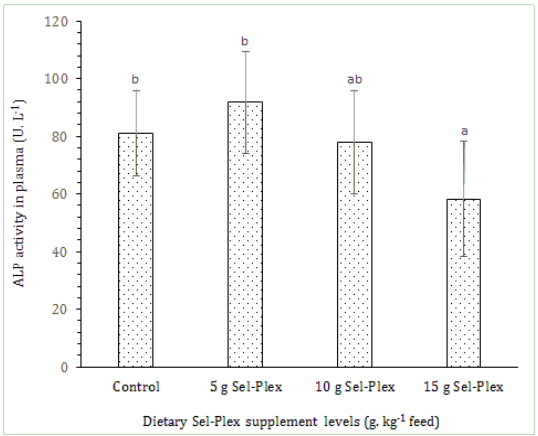

Figure 5: ALP activity in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean ± S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

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Results showed that activity of CPK in plasma were significantly (p<0.05) increased in fish fed 10 and 15 g kg^{-1} Sel-Plex supplement when compared to control group (Fig. 6).

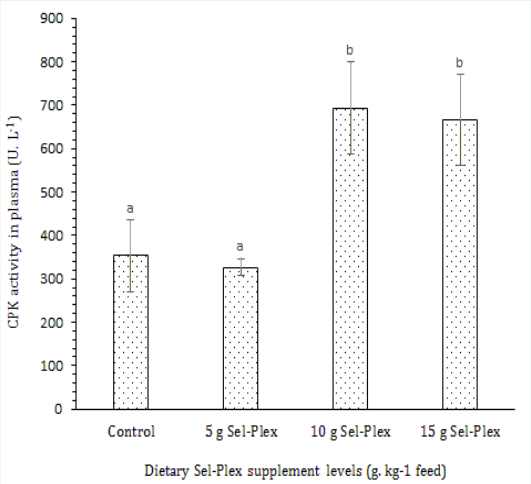

Figure 6: CPK activity in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean ± S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

A significant decrease was observed in blood glucose levels in fishes by oral feeding with Sel-Plex supplemented diets for 8 weeks (Fig. 7).

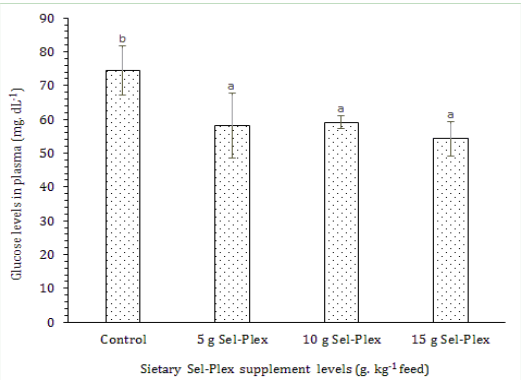

Figure 7: Glucose concentrations in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean ± S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

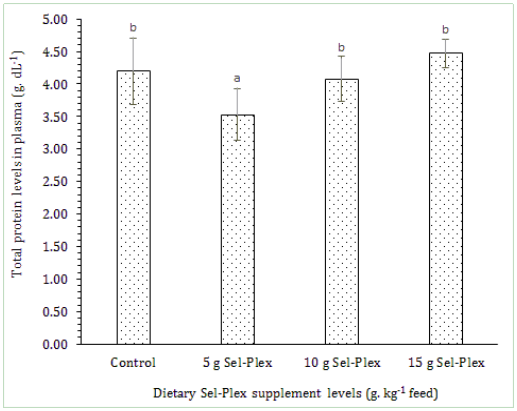

Figure 8: Total protein concentrations in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean ± S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

A significant (p<0.05) decrease in total protein concentration was observed in fish fed 5 g kg^{-1} Sel-Plex supplement when compared to control group (Fig. 8).

The concentration of plasma Albumin (Fig. 9) was significantly lower in all treatment groups than in the control fish (p<0.05).

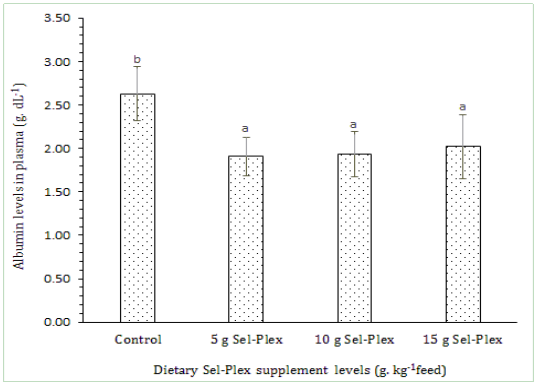

Figure 9: Albumin concentrations in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean±S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

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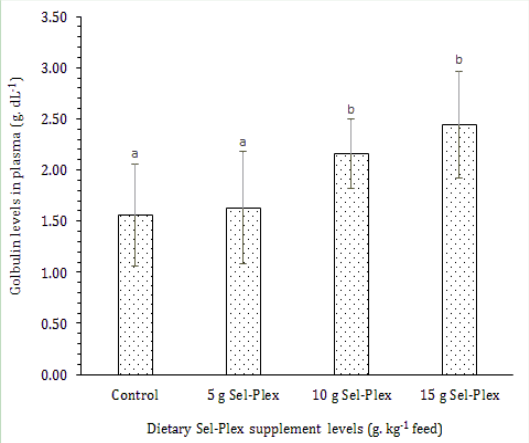

Figure 10: Globulin concentrations in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean±S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

Concentrations of globulins in plasma were significantly (p<0.05) increased in fish fed 10 and 15 g kg^{-1} Sel-Plex supplement when compared to the control group (Fig. 10).

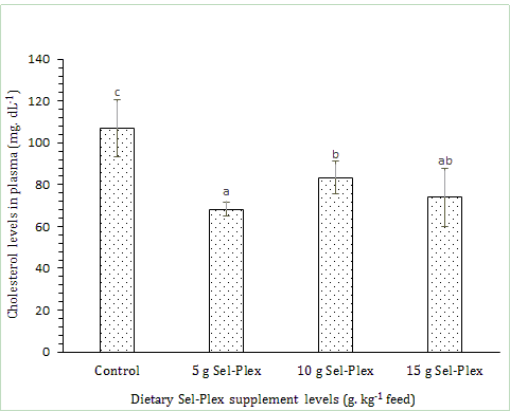

Figure 11: Cholesterol concentrations in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean±S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

The concentration of plasma cholesterol (Fig. 11), and triglycerides (Fig. 12) were significantly lower in all treatment groups than control fish (p<0.05).

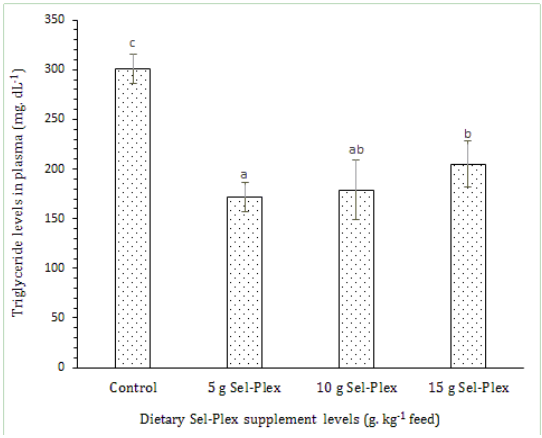

Figure 12: Triglyceride concentrations in plasma of *Acipenser baerii* fed with Sel-Plex supplemented diets. Data are presented as mean±S.D. Significant differences between values when compared with control groups were characterized by alphabet symbol (p<0.05).

Discussion

Quantitative measurement of blood biochemical parameters is the most common method of clinical diagnosis which can be used to detect gross abnormalities in the tissue of an experimental animal. Estimating glucose, total protein, albumin, globulin, cholesterol, triglycerides and enzymes concentration in plasma was carried out to see the effect of Sel-Plex on these biochemical parameters.

AST, ALT, LDH, ALP, GGT, and CPK are found in cells of different tissues, such as the heart, kidneys, liver, skeletal muscle, brain, erythrocytes, intestine and gills (Soleimany *et al*., 2016).

AST and ALT activities are important in cellular nitrogen metabolism, oxidation of amino acids, and liver gluconeogenesis (Murray *et al*., 2003). There were no noticeable changes in AST and ALT activates in the plasma of fish fed with 5 and 10 g Sel-Plex supplement. This is because

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selenium supplement in adequate levels can maintain the cellular membrane stability as a radical scavenger (Misra and Niyogi, 2009). Moreover selenium may affect the cellular glutathione peroxidase activity (Misra and Niyogi, 2009). In contrast, administration of 15 g Sel-Plex supplement significantly increased AST and ALT activities in plasma of fish. The observed increase in AST and ALT activities might be due to histopathological changes in various tissues especially necrosis of hepatocytes (Soleimany *et al*., 2016). The release of intercellular enzymes into the blood and their increased activity in plasma are the most important clinical signs in the diagnosis of damage to cell membranes (Murray *et al*., 2003).

LDH is an enzyme that participates in the anaerobic carbohydrate metabolism (Murray *et al*., 2003). Plasma LDH activity was not affected in fish fed with 5 g Sel-Plex compared to the control group. So, due to antiradical and antioxidant properties of selenomethionine (Sel-Plex), its administration at the lowest dose (5 g kg^{-1} Sel-Plex) might prevent lipid peroxidation of cell membranes and inhibit the release of the aforesaid enzymes into the plasma. Statistically, there was a significant difference in the plasma and LDH activities of fish fed with 10 and 15 g Sel-Plex compared to the control group. Increased activity of LDH in plasma indicated that Sel-Plex at 10 and 15 g concentrations caused oxidative damages to Siberian sturgeon hepatocytes, probably by inducing imbalance between cellular antioxidant capacity and reactive oxygen species (ROS) production (Misra and Niyogi, 2009). A significant increase in LDH activity in the plasma might signify a shift towards anaerobiosis, i.e. the pyruvate to lactate conversion is favored, suggesting that condition for pyruvate oxidation through Kerb's cycle is not suitable (Soleimany *et al*., 2016).

ALP plays a significant role in phosphate hydrolysis and in membrane transport and acts as a good bio-indicator of stress in biological systems (Murray *et al*., 2003). ALP activity was significantly decreased in plasma in fish fed diets containing 15 g Sel-Plex whereas its activity remains insignificantly near to that in the control group in fish fed with 5 and 10 mg Sel-Plex. Reduced activity of ALP may indicate the negative effect of selenium on the synthesis of the enzyme in the cells. In addition, increased rate of cell death may be caused by a decrease in ALP activity in fish fed with 15 g Sel-Plex.

GGT plays an important role in the gamma-glutamyl cycle, a pathway for the synthesis and degradation of glutathione and drug and xenobiotic detoxification (Murray *et al*., 2003). Although a significant increase in the activity of GGT was detected in plasma of fish fed with 10 mg Sel-Plex supplement at the end of the 60th day, the plasma GGT activity was significantly lower in fish treated with 15 mg. Kg^{-1} Sel-Plex than that in the control group ($p<0.05$). In the present study, increased levels of GGT may be due to hepatotoxicity which affects the

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permeability of the cell membrane and release of GGT into the blood. Both ALP and GGT increase in a damaged bile duct and injured hepatocytes. Reduced GGT activity may be attributed to the effect of Se on this enzyme's synthesis in cells.

The transfer of phosphate group from creatine phosphate to ATP in order to regenerate ATP is done by CPK (Murray *et al*., 2003). A significant increase was found in CPK activity in fish fed with 10 and 15 g Sel-Plex supplement. The increased activity of CPK may indicate a disorder in muscle fibres.

Reactive oxygen species production is known as a possible mechanism of selenium toxicity (Lavado *et al*., 2012). Tarze *et al*. (2007) found that selenium can have a negative effect on the cellular antioxidant capacity by oxidizing the intracellular levels of reduced glutathione (GSH).

A significant decrease was observed in glucose concentrations of plasma in fish fed with different concentrations of Sel-Plex. These results may be attributed to the hypoglycemic activity of Sel-Plex to activate glycogen synthesis in liver tissue. Chen *et al*. (2015) found that selenium can alleviate hyperglycemia by normalizing the activity of lactate dehydrogenase, glucose-6-phosphate dehydrogenase and glycogen phosphorylase and restoring the glycogen contents in liver tissue. Chen *et al*. (2015) believe that selenium has insulin-like properties and can regulate blood glucose concentrations in diabetic rats.

A significant decrease in plasma total protein was observed in fish which were orally dosed with Sel-Plex supplement at 5 mg per kg diet for 60 days. Although there was a significant decrease in plasma albumin concentrations of fish fed Sel-Plex, a significant increase was found in plasma globulin concentrations of fish fed with the diet containing different concentrations of Sel-Plex. The decrease in the plasma total protein concentrations could be correlated with severe damage to hepatocytes. However, Bozkurt *et al*. (2012) found that selenium supplementation had protective effects on protein concentrations in streptozotocin-induced diabetic rat.

The cholesterol synthesized in liver transfers to other tissues of the body through LDL, while HDL transports the cholesterol in peripheral tissues to liver. A significant decrease was found in plasma cholesterol concentrations of fish fed a diet enriched with Sel-Plex than cholesterol concentrations of fish fed a normal diet. So, the increased rates of high density lipoprotein synthesis (HDL) and excretion of cholesterol via the bile (Al-Quraishy *et al*., 2015; Su *et al*., 2015) decreases the cholesterol concentration in blood of the fish fed with Sel-Plex.

There was a significant decrease in triglyceride concentrations of plasma in fish fed with different concentrations of Sel-Plex. Nido *et al*. (2016) found that administration of selenium supplements can increase gene expression of enzymes involved in lipid metabolism

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and reduce blood cholesterol and triglyceride in mice fed a high-fat diet.

In this study, we demonstrated the effects of dietary Sel-Plex on certain blood biochemical parameters in Siberian sturgeon, *A. baerii*. Dietary selenium, as Sel-Plex at the concentration of 5 g for juvenile Siberian sturgeon fulfills the dietary requirements for selenoenzymes and proteins. Although selenium is an essential micronutrient to juvenile Siberian sturgeon, our results showed that by increasing the concentration of Sel-Plex in the diet (10 and 15 g Sel-Plex) adverse changes may appear in some blood biochemical parameters.

Acknowledgments

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