Effect of dietary selenium on growth performance, survival rate and biochemical-blood profile of farmed juvenile beluga (*Huso huso*)

Safabakhsh M.R.[1]; Mohseni M.[2]*; Bahri A.H.[1]; Mohammadizadeh F.[1]

Abstract

Beluga sturgeon (*Huso huso*), the largest freshwater fish, has attracted the attention of sturgeon culturists worldwide. The profitability of aquaculture beluga production mainly depends on physiologically suitable formulated diets. The objective of this research is to determine the optimal level of dietary selenium (Se) for beluga using the following parameters: growth performance traits (WG, SGR, PER, and FER), body proximate composition, and serum biochemical and immunological indices (glucose, total protein, Ig M, lysozyme, ALT and AST). To this aim beluga (n=315; with initial weight of 15.66±0.77 g) were fed with diets supplimented with sodium selenite (0.18, 5.43, 12.6, 24.3, 37.2, 71.4, and 144 mg kg^{-1}) for 10 weeks. Unexpectedly, all of the parameters exhibited considerable responses to the applied levels of Se. Growth performance indices displayed the highest values for the animals treated with diets containing 12.6 and 24.3 mg kg^{-1} Se, yet the lowest ones with 71.4 and 144 mg kg^{-1} (i.e., U-form response). Similar response was seen for crude lipid and protein content as well as for the activity of ALT and AST, whereas IgM and lysozyme did an anticline manner (i.e., the highest values in the midle Se levels). Moisture and ash contents and also serum total protein exhibited a Se-dose dependent increase. Based on the broken line regression model, optimal dietary Se requirement for juvenile beluga is about 18.2 mg kg^{-1}. Taken together this study extends our knowledge on one of the most essential trace elements and its optimal level for incorporating into beluga diet. It could also be a basic one in the sturgeon aquaculture industery.

Keywords: *Huso huso*, Selenium, Proximate composition, Biochemical indices

1-Department of Fisheries, College of Agriculture, Islamic Azad University Bandar Abbas, Bandar Abbas, Iran
2-International Sturgeon Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran
*Corresponding author's Email: mahmoudmohseni73@gmail.com

Introduction

Sturgeons are species of high worldwide biological and economical importance. These species belong to the order Acipenseriformes and family Acipenseridae with 27 species, which mainly live in temperate waters of the northern hemisphere. Over 80% of these species are assigned as endangered, vulnerable, or on the edge of extinction in the IUCN Red List because of anthropogenic manipulations such as overfishing, environmental pollution, and occupation of their natural spawning habitats (Peterson *et al.*, 2007; Falahatkar *et al.*, 2015; Hung, 2017). Hence, to augment and relieve high catch pressure on their natural populations as well as to produce human food, many countries are involved in artificial sturgeon production (Falahatkar *et al.*, 2015).

Beluga sturgeon (*Huso huso*), the largest freshwater fish, lives in the Black, Caspian, and Azov Seas, and its natural population in the Caspian Sea has been classified as a critically endangered species (Amirkolaie *et al.*, 2012; Hung, 2017). However, some features such as its fast growth, high adaptability to controlled conditions, and its expensive caviar have progressively increased the attraction of sturgeon culturists around the world (Abdolhay and Tahori, 2006; Mohseni *et al.*, 2008). Nutritionally, successful aquaculture production of Beluga relies on the basis of its nutrient requirements; that is, the profitability of aquaculture production mostly depends on a suitable formulated diet with constituents such as proteins, fats, vitamins, and minerals (Chebanov and Billard, 2001).

Selenium (Se) is a trace element essential for health and metabolic functions including development, fertility, reproduction, and immunity of both humans and animals, such as fish (Arshad *et al.*, 2011; Pappas and Zoidis, 2012; Nazari *et al.*, 2017). This metal is an important element in the structure of glutathione peroxidase (GSH-Px), and is necessary to protect the cell membrain structure and its function against oxidative stresses. The conjugated organic froms of Se (e.g. SeCys, SeMet) are present in proteins and enzymes which are involved in immunity enhancment, thyroid hormone production, genomic stability, muscle growth and other physiological functions (Kohrle *et al.*, 2005; Miezeliene *et al.*, 2011; Liu *et al.*, 2017). Along with these biological essentialities, however, due to the relatively higher toxicity of excessive dietary selenium than waterborne selenium (Hodson and Hilton, 1983), beyond the threshold levels for fish causes toxicity or body stress and irreversible organ damages such as growth reduction, lower feed efficiency, renal calcinosis and even mortality (Liu *et al.*, 2017). This means that, there is a narrow window between Se deficiency and toxicity for all animals, and it appears more important to investigate and carefully select the essential and optimum requirements of dietary Se for cultural fish (Cotter *et al.*, 2008). Despite the incresing attention regarding beluga rearing, little is known

about its nutritional Se requirements; therefore, the objective of this research is to determine the optimum nutritional Se for beluga following dietary exposure to different supplementation levels and on the basis of evaluating growth parameters and some of the blood biochemical indices.

Materials and methods

Fish and experimental diets

The study was carried out in the spring and summer of 2016. A total of 315 juvenile beluga with an initial weight of 15.66±0.77 g were obtained from the Shahid Beheshti Hatchery Center. The fish were assigned to seven groups with three replicates (15 per replicate) into 500 L-fiberglass tanks and aclimatized for two weeks. The tanks were supplied with flow through water (0.5 L min^{-1}) from the Sefidrud River in the north of Iran. For the dietary experiment, seven different Se spiked diets were formulated with different levels of selenate (Sigma, poole, UK) (0.18, 5.43, 12.6, 24.3, 37.2, 71.4, 144 mg kg^{-1}), and for simplification, hereafter the diets are denoted as control, Se_5, Se_{10}, Se_{20}, Se_{40}, Se_{80}, and Se_{160}, respectively. The basal purified diet (i.e., control diet) was constituted of fishmeal, wheat flour, gelatin, casein, dextrin, cellulose, corn starch, vitamin, mineral, and fish oil (Table 1). The experimental diets were prepared by grinding all dry ingredients in a hammer mill, weighed with a digital balance and then mixed for 20 min in a food mixer. While mixing, pre blended premix of fish oil was added, and then sodium selenite (Na_2SeO_3) solution was added to produce stiff dough. The wet dough was placed into a grinder and sieved using a mincing machine (Chega Co., Isfahan, Iran), and then dried via a ventilating oven at 40 °C. The dried diets were kept at -20 °C before the feeding trial. The approximate composition of the experimental diets is presented in Table 1.

Table1: Diet formulation and proximate analysis of the beluga basal diet (Mohseni *et al.,* 2014).

Ingredients	Level (%)
Casein	38
Gelatin	8
Fish meal	5
Wheat flour	11
Dextrin	7
Corn starch	11
Fish oil	12
Vitamin premix	2
Mineral premix	1
Alpha cellulose	5
Chemical analysis (g kg^{-1} in dry matter)	
Dry matter	92.11
Crude protein	41.92
Crude lipid	13.78
Ash	9.15

* United states Biochemical, Cleveland, OH, USA

▪Vitamin Mixture, Science Laboratories, Qazvin, Iran (composition per kg of mixture): vitamin A: 1,600,000 IU; vitamin D3: 400,000 IU; vitamin E: 40 g; vitamin K3: 2 g; vitamin B 1: 6 g; vitamin B2: 8 g; Pantothenic acid: 40 g; Niacin 12 g; vitamin B6: 4 g; Folic acid: 2 g; vitamin B12: 8 mg; vitamin C: 60 g; Biotin: 240 mg and Inositol: 20 g. 4. Mineral mixture, Science Laboratories, Qazvin, Iran (composition per kg of mixture); Iron sulfate: 6 g; Zinc sulfate: 10 g; Selenium: 20 mg; Cobalt sulphate: 100 mg; Copper sulfate: 600 mg; Manganese sulfate: 5 g; Iodine: 600 mg; Choline chloride: 6 g.

Feeding and biometry

The dietary test was performed for 10 weeks. During the trial, the fish were fed with the experimental diets three times daily (at 8 am, 14 pm and 20 pm). Feed leftovers and fish feces were removed from the tanks to prevent leaching Se into water. In addition, to determine fish intake, all the feces were collected with a mesh collector placed under the individual drain pipe of each tank (Mohseni and Sotudeh, 2013).The feeding trail was carried out under a 12 h: 12 h light: dark cycle condition, and water temperature, dissolved oxygen, total hardness, and pH were 16.5±0.3 °C, 7.35±0.21 mg L^{-1}, 315.2±12.3 mg L^{-1} and 7.5±0.3, respectively. To determine body weight and total length, biometry of the fish kept in each tank (n=15) was performed every 10 days.

Proximate chemical analysis

Proximate composition of the experimental diets and fish body were analysed according to AOAC (2005). Briefly, moisture was measured following oven-drying at 105 °C for 12 h, and ash through incineration in a muffle furnace at 600 °C for 6 hr. Crude protein (N×6.25) was measured by Kjeldahl method using an auto Kjeldahl system following acid digestion (Auto Analyser, unit 2300, Sweden). Lipid content was extracted by petroleum ether using a Soxhlet extraction apparatus (Soxtec 2050 FOSS Model, Switzerland).

Growth performance parameters

At the end of the feeding trial, body weight increase, specific growth rate (SGR), and survival rate were calculated as follows (Ai *et al.*, 2006):

- Weight gain (WG%)=100× (final body weight (g)- initial body weight (g) / (initial body weight)(g)/ number of days.

- Specific growth rate (SGR%) = 100 ×(final weight of fish(g)- initial weight of fish(g)) /days of feeding

- Feed efficiency ratio (FER) = (final wet weight (g) -initial wet weight (g))/ dry weight food consumed (g)

- Survival rate (%) = 100 × (initial number of fish - final number of fish)/ initial number of fish

Blood biochemical analysis

A total of 9 fish per treatment were selected for blood biochemical analysis. Blood samples were collected from the caudal vien into heparinized plastic syringes. The animals were not anesthetized to prevent possible influence of anesthetic substances on blood biochemical parameters (Torrecillas *et al.*, 2011). The blood samples were centrifuged (Labfuge-HeraeusSepatch- Germany) at 3000 rpm for 10 min and plasma was kept in a freezer (Hettich - Germany) at –80 °C until subsequent analysis. The concentration of serum glucose, total protein were measured using commercial kits (Pars Azmoon, Tehran, Iran). The enzymatic activity of alkaline phosphatase (ALT) and aspartate aminotransferase (AST) were determined using colorimetric method. Similarly, the glucose assay was performed by enzymatic and colorimetric methods (GOD-PAD). Brifely, a total volume of 10 µL of

serum was mixed with 1000μL of reagent and incubated for 20 min at room temperature, and then the absorbance was measured at 546 nm against the blank (10 μl of distilled water mixed with 1000 μl of reagent). The concentration was calculated using the following equation:

Glucose (mg dl^{-1})= sample B/calibrator B × calibrator concentration

The level of serum lysozyme was measured using 1.75 mL of lyophilized bacterial suspension of *Micrococcus lysodeikticus* (Sigma M-3770). Sodium phosphate buffer (0.05 M;0.375 mg per ml) was mixed with 250 μl of serum samples for 15 and 180 sec and the absorbance was measured using a spectrophotometer at 670 nm. Sodium phosphate buffer adjusted to pH 5.5 was used as the blank (Ellis, 1990). In addition, total IgM level was determined according to the published method by Alexander (Alexander and Ingram, 1992). Brifely, same volume (0.1 ml) of serum sample and 12.0% polyethylene glycol solution (Sigma) were mixed and incubated for 120 min, and then IgM molecules were precipitated following centrifuging at 5000 rpm at 4°C. The supernatant was diluted 30 times with 0.85% NaCl and the protein content was determined according to Bradford method. The difference between the protein values of untreated and polyethylene glycol treated samples was used to determine total IgM in the samples.

Statistical analysis
Statistical analysis was performed by SPSS software version 19.0 (SPSS, Chicago IL, USA). Data normality was assessed with Kolmogorov Smirnov test (KS-test; $p<0.05$), and also one-way ANOVA (i.e., Tukey's test) was applied to identify differences between treatments. Differences were considered significant at $p<0.05$ for all analyses. Data were expressed as mean±SD.

Results
Survival and morphology
During the experiment, some mortalities associated with dietary treatments were observed in only the experimental groups fed the diets conaining 37.2 and 144 mg kg^{-1} Se. No abnormal behavior or anomalous body shape was discerned in the experimental groups.

Growth performance and body composition
Significant differences in growth performance traits (WG, SGR, PER, and FER) were observed between the experimental groups (Table 2). With the increase in Se concentration, all of these parameters exhibited a similar pattern; as such, all of the growth parameters displayed an upward trend and a downward trend in response to the applied Se concentrations. The highest values of senelium were recorded for 12.6 and 24.3, whereas the lowest value for 144 mg kg^{-1} ($p<0.05$). The relationship between final body weight and dietary Se concentration was estimated based on the broken line regression model, which demonstrated that optimal Se requirement for maximizing final body weight of *H. huso* was 18.2 mg kg^{-1} diet (Fig. 1).

Table 2: Growth performance and feed utilization of the *Huso huso* fed with the difference diets at 70 days. Values are expressed as (Mean±SD, n=7) of three replicates.

Dietary Se (mg kg^{-1})	Growth performance parameters				
	Weight gain (WG)	Specific growth rate (SGR)	Protein efficiency rate (PER)	Feed efficiency rate (FER)	Survival rate (SR)
0.18	809.61±15.68[bcd]	3.15±0.024[bc]	1.76±0.05[ab]	70.74±1.96[c]	100[a]
5.43	886.35±31.40[ab]	3.16±0.045[bc]	1.78±0.063[ab]	69.78±2.53[c]	100[a]
12.6	907.09±46.72[a]	3.29±0.065[a]	1.81±0.034[a]	71.64±1.52[b]	100[a]
24.3	904.50±4.40[a]	3.29±0.007[a]	1.81±0.039[a]	72.09±1.37[b]	100[a]
37.2	849.37±39.58[abc]	3.21±0.006[ab]	1.67±0.02[bc]	65.13±0.75[a]	97.77±2.84[a]
71.4	790.47±16.62[cd]	3.12±0.03[bc]	1.54±0.04[c]	59.73±1.67[d]	100[a]
144	746.84±24.57[d]	3.05±0.04[c]	1.55±0.039[c]	61.57±1.56[d]	98.11±1.84[a]
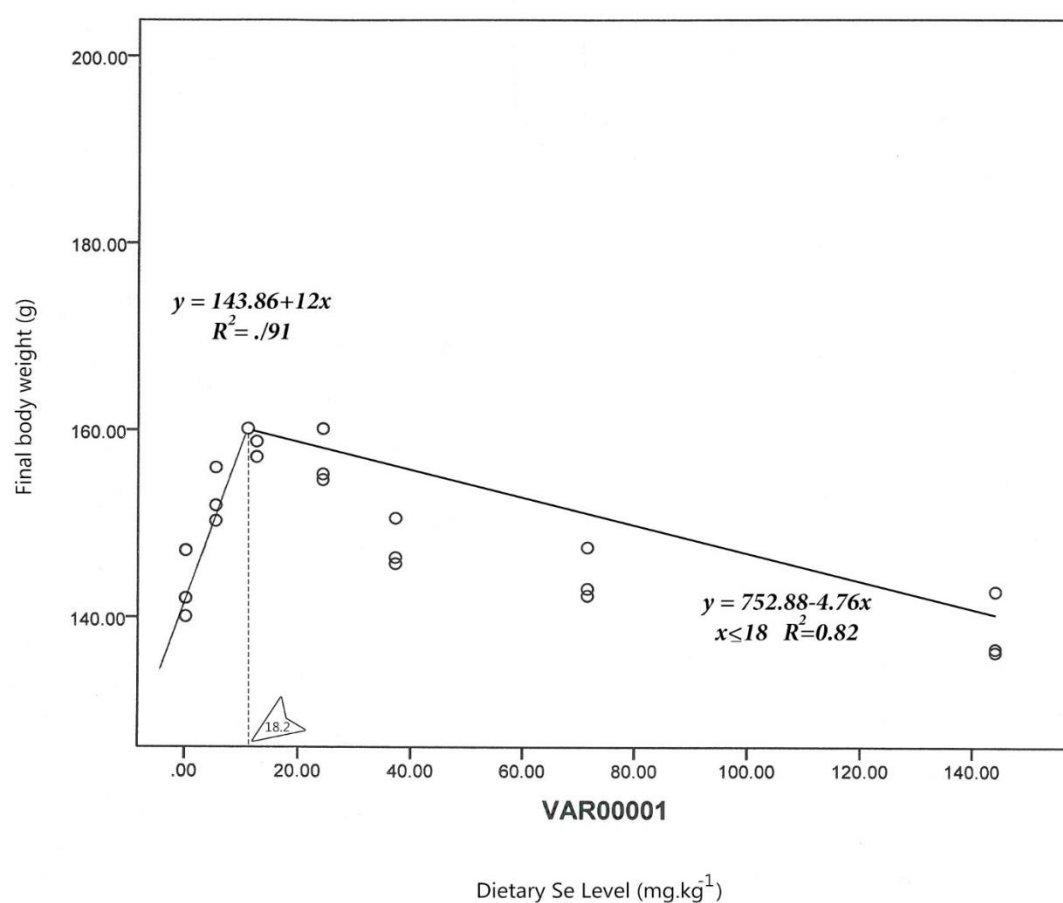
Figure 1: The relationship between final body weight and dietary se concentration based on the broken line regression model.

Proximate composition

As shown in Table 3, body composition of *H. huso* demonstrated significant changes following dietary exposure to different concentrations of Se (*p*<0.05) when compared to the control group. Moisture and ash contents exhibited a Se-dose dependent increase, with the highest percentage in the fish that intook 144 mg kg^{-1}. However, crude lipid and protein displayed an upward and downward trend in response to increasing Se levels; the highest and lowest values were observed in the

treatments receiving middle amounts (i.e., 12.6 and 24.3) and highest (144

mg kg^{-1}) amounts of Se, respectively.

Table 3: Proximate chemical analysis in juvenile *Huso huso* fed different levels of selenium for 10 weeks. Values are expressed as (Mean±SD, n=7).

Dietary Se (mg kg^{-1})	Parameters			
	Moisture (%)	Crude lipid(%)	Crude protein (%)	Ash (%)
0.18	73.93±1.10^c	7.20±0.01^c	1.76±0.08^{ab}	2.45±0.08^c
5.43	74.33±0.51^c	7.42±0.33^c	1.77±0.11^{ab}	2.44±0.10^c
12.6	74.40±0.75^c	7.75±0.05^c	1.81±0.06^a	2.61±0.02^{bc}
24.3	74.07±0.55^c	7.77±0.07^c	1.81±0.07^a	2.64±0.02^{bc}
37.2	75.18±1.44^c	8.42±0.09^a	1.67±0.03^{bc}	3.13±0.15^{ab}
71.4	77.63±0.76^b	6.92±0.12^b	1.54±0.07^c	3.62±0.71^a
144	79.50±0.65^a	6.21±0.30^b	1.54±0.06^c	3.48±0.26^a

Biochemical indices

The analyzed biochemical and immunological indices of serum in the experimental fish groups are presented in Table 4. The concentration of glucose as well as the activity of ALT and AST exhibited a U-form response with increasing the concentration of dietary Se, but IgM and lysozyme did an anticline manner. That is, the highest and lowest values were obtained in the middle Se concentrations (12.6, 24.3, and 37.2 mg kg^{-1}). In addition, the concentration of total protein showed a Se-dose dependent increase ($p>0.05$).

Table 4: Amounts of biochemical factors, immune and liver enzymes in *Huso huso* fed with different levels of selenium in the rearing period of 70 days. Values are expressed as (Mean±SD, n=7).

Dietary Se (mg kg^{-1})	Glucose (mg dl^{-1})	Total protein (g dl^{-1})	IgM (mg dl^{-1})	Lysozyme (U dl^{-1})	ALT (IU L^{-1})	AST (IU L^{-1})
0.18	59.57±0.33^b	2.69±0.2^b	29.78±1.32^c	78.63±0.20^b	9.44±0.51^b	173.90±3.36^b
5.43	58.52±0.25^b	2.97±0.08^b	31.50±0.43^c	80.57±0.36^b	8.51±0.35^{bc}	164.70±3.12^c
12.6	55.52±0.64^{bc}	2.84±0.12^b	44.41±1.53^a	92.13±0.46^a	7.72±0.39^d	160.30±2.30^c
24.3	55.35±0.31^{bc}	2.47±0.09^b	39.85±2.31^{ab}	91.57±0.15^a	8.95±0.18^{bc}	162.40±1.48^c
37.2	59.35±0.34^b	3.61±0.17^a	37.25±2.06^b	80.13±0.17^b	9.99±0.31^b	173.60±1.01^b
71.4	68.95±0.25^a	3.63±0.08^a	28.68±0.84^c	78.50±0.36^b	12.02±0.25^a	194.70±4.44^a
144	70.54±0.44^a	3.89±0.02^a	19.77±0.63^d	76.64±0.16^c	12.84±0.16^a	203.90±3.31^a

Discussion

In biological systems, Se is known as an essential trace mineral to play myriad roles such as maintaining normal growth, optimal immune system, biosynthesis of the enzyme glutathione peroxidase, and antioxidant against oxidative cellular injury (Lin

and shiau, 2007; Rider *et al*., 2009; Kumar *et al*., 2018). Although Se is available from various feed components, it is commonly added to formulated diets for cultured fish. The growth performance indices such as WG, SGR, and FCR could be used as the most basic parameters to assess the optimal and suboptimal levels of a dietary ingredient or element. In the present study, WG, SGR, PER, and FER demonestrated a similar response to dietary Se concentrations: significantly increased in the fish groups fed with 12.6 and 24.3 but decreased in response to 144 mg kg^{-1}. The results are consistent with previously reported studies. De Riu *et al.* (2014) who showed a significant reduction in WG of *Acipenser transmontanus* and *A. medirostris* following dietary exposure to more than 50 mg Se per kg diet. Tashjian *et al* (2006) declared a considerable growth reduction in *A. transmontanus* fed after intaking 41.7 mg Se kg^{-1} diet. A further supportive research suggested a threshold concentration of 11-20 µg Se g^{-1} diet for suitable growth in juvenile *H. huso*, and the authors' explanatory reason was high energy requirement for adaptation with chronic toxicity of Se in higher than the above mentioned range (Arshad *et al.,* 2011).

Body proximate composition seems to be a helpful indicator of the overall physiological status of fish (Ali *et al*., 2005). One unanticipated finding was that crude lipid and protein conciderably decreased in the experimetal group receiving the highest amiunt (144 mg kg^{-1}) of dietary Se. A possible explanation for this might be that high Se concentration induced physiological stress to *H. huso*, thereby decreasing the most important composition of their body. Support for this finding have come from studies of juvenile white sturgeon *A. transmontanus* (Tashjian *et al.* (2006) and Green sturgeon *A. medirostris* (De Riu *et al.* (2014) following intake of high amounts of dietborne Se.

Serum biochemichal indices are commonly evaluated to detect gross abnormalities in the body of experimental animals. This research measured the concentration of glucose, total protein, IgM level, as well as the activity of Lysozyme, ALT and AST. Glucose revealed a U-form response with increasing the amount of supplimentary Se. This finding is in agreement with that observed in Beluga (Mohseni and Sotudeh, 2013), African catfish (*Clarias garipinus*) (Abdel-Tawwab *et al.,* 2007) and rainbow trout, (*Oncorhynchus mykiss*) (Miller *et al.,* 2007) which showed low levels of glucose following dietary Se exposure. Total protein involves in physiological functions, such as osmoregulation, accumulation of antibodies and defense against xenobiotics, and mineral transportation. The observed increased concentration of this parameter in the highest Se level is suggested, while preliminary, engaging in immunity or transportation of the excess Se in cyrculating system. Lysozyme activity and IgM concentration are used to assess the influence of nutrients on the fish immunity system (Lin and Shiau, 2007; Puangkaew *et al*., 2009). These

two parameters were significantly increased in the fish fed with the diets containing 12.6 and 24.3 mg kg^{-1} Se as compared with the other treatments. Support for these observations have come from studies of hybrid striped bass (Cotter *et al*., 2008) and yellowtail king fish (*Seriola lalandi*) (Le and Fotedar (2014).

ALT and AST are clinically of high importance to assess the toxic effects of pollutants that cause tissue injury (Abdel-Tawwab, 2016) or to diagnose hepatopancreas dysfunction (Dadras *et al*., 2016). Being high in the cytoplasm of the liver cells, the enzymes pass through the cell membrane and enter the bloodstream during injuries (Tohidi *et al*., 2008). Since the applied higher concentrations of Se have not been used elsewhere in fish dietary regime, the observed significant increase in the activity of ALT and AST is difficult to explain, but it might be related to excessive accumulation of Se in the liver and in turn in hepatopancreas injuries.

According to these parameters, one of the more significant findings to emerge from this study is that dietborne Se in the range of 12.6 to 24.3 mg kg^{-1} demonstrated the best nutritional function. Nevertheless, the other diets with lower and higher concentrations of Se displayed unacceptable growth performance or biological functions. Taken together, this study extends our knowledge on one of the most essential trace elements and its optimal level for incorporating into beluga diet, and also could be a basic one in sturgeon aquaculture industry.

References
Abdel-Tawwab, M., Mousa, A.A. and Abbass, F.E., 2007. Growth performance and physiological response of African catfish, *Clarias gariepinus* (B.) fed organic selenium prior to the exposure to environmental copper toxicity. *Aquaculture*, 272, 335–345.

Abdel-Tawwab, M., 2016. Effect of feed availability on susceptibility of Nile tilapia, *Oreochromis nilotic* us (L.) to environmental zine toxicity: growth performance, bio chemical response, and zine bio accumulation. *Aquaculture*, 464, 309-315.

Abdolhay, H.A. and Tahori, H.B., 2006. Fingerling production and release for stock enhancement of sturgeon in the Southern Caspian Sea: An overview. *Journal of Applied Ichthyology*, 22(**s1**), 125-131.

Alexander, J.B. and Ingram, G.A., 1992. Non cellular nonspecific defense mechanisms of fish. *Annual Review of Fish Diseases*, 2, 249-279.

Ali, M., Iqbal, F., Salam, A., Iram, S. and Athar, M., 2005. Comparative study of body composition of different fish species from brackish water pond. *International Journal of*

Environmental Science and Technology, 2(**3**), 229–232.

Amirkolaie, A.K., Mahdavi, S. and Hosseini, S., 2012. Dietary fat content and feed supply influence growth and body composition in juvenile beluga sturgeon *(Huso huso)*. *Aquaculture International*, 20(**5**), 859-867.

Arshad, U., Takami, G.A., Sadeghi, M., Bai, S., Pourali, H.R. and Lee, S., 2011. Influence of dietary l-selenomethionine exposure on growth and survival of juvenile *Huso huso*. *Journal of Ichthyology,* 27, 761–765.

Association of Official Analytical Chemists (AOAC)., 2005. Official methods of analysis of official analytical chemists international. 18th ed. Association of Official Analytical Chemists, Arlington, VA. 62 P.

Chebanov, M. and Billard, R., 2001. The culture of sturgeon in Russia: production of juveniles for stocking and meat for human consumption. *Aquatic Living Resource*, 14, 375-381.

Cotter, P.A., Craig, S.R. and Mclean, E., 2008. Hyper accumulation of selenium in hybrid striped bass: a functional food for aquaculture. *Aquaculture Nutrition*, 14, 215–222.

Dadras, H., Hayatbakhsh, M.R., Shelton, W.L. and Golpour. A., 2016. Effects of dietary administration of rose hip and safflower on growth performance, haematological, biochemical parameters and innate immune response of Beluga, *Huso huso. Fish and Shellfish Immunology*, 59,109-114.

De Riu, N., Lee, J.W., Huang, S.Y., Moniello, G. and Hung, S.O., 2014. Effect of dietary selenomethionine on growth performance tissue burden, and histopathology in green and white sturgeon, *Aquatic Toxicology,* 148, 65-73.

Ellis, A.E., 1990. Lysozyme assays. In: J.S. Stolen; D.P. Fletcher; B.S. Anderson and W.B. Van Muiswinkel (eds). Techniques in fish immunology. SOS Publication, USA, pp.101-103.

Falahatkar, B., Soltani, M., Abtahi, B., Kalbassi, M.R. and Pourkazemi, M., 2015. The role of dietary L-ascorbyl-2-polyphosphate on the growth and physiological functions of beluga, *Huso huso* (Linnaeus, 1758). *Aquaculture Research*, 46(**12**), 3056-3069.

Hodson, P. and Hilton, J., 1983. The nutritional requirements and toxicity to fish of dietary and waterborne selenium. *Ecological Bulletins*, 335-340.

Hung, S.S., 2017. Recent advances in sturgeon nutrition. *Animal Nutrition*, 3(**3**), 191-204.

Kohrle, J., Jakob, F., contempre, B. and Dumont J.E., 2005. Selenium, the thyroid, and the endocrine system. *Endocrine Reviews*, 26, 944-984.

Kumar, N., Krishnani, K.K. and Singh, N.P., 2018. Comparative study of selenium and selenium nanoparticles with reference to acute toxicity, biochemical attributes, and histopathological response in fish.

Environmental Science and Pollution Research, 25(**9**), 8914 – 8927.

Le, K.T. and Fotedar, R., 2014. Immune responses to *Vibrio anguillarum* in yellowtail kingfish, *Seriola lalandi*, fed selenium supplementation. *Journal of the World Aquaculture Society*, 45(**2**), 138–148.

Lin, Y.H. and Shiau, S.Y., 2007. The effects of dietary selenium on the oxidative stress of grouper (*Epinephelus malabaricus*) fed high copper. *Aquaculture*, 267, 38–43.

Liu, G.X., Jiang, G.Z., Lu, K.L., Li, X.F., Zhou, M., Zhang, D.D. and Liu, W.B., 2017. Effects of dietary selenium on the growth, selenium status, antioxidant activities, muscle composition and meat quality of blunt snout bream, *Megalobrama amblycephala*. *Aquaculture Nutrition*, 23(**4**), 777-787.

Miezeliene, A., Alencikiene, E.G., Gruzauskas, R. and Barstys, T., 2011. The effect of dietary selenium supplementation on meat quality of broiler chickens. *Biotechnology, Agronomy and Society and Environment*, 15(**S1**), 61-69.

Miller, L.L., Wang, F., Palace, V.P. and Hontela, A., 2007. Effects of acute and sub chronic exposures to waterborne selenite on the physiological stress response and oxidative stress indicators in juvenile rainbow trout, *Aquatic Toxicology*, 83(**4**), 263-71.

Mohseni, M., Pourkazemi, M. and Bai, S.C., 2014. Effects of dietary inorganic copper on growth performance and immune responses of juvenile beluga, *Huso huso*. *Aquaculture Nutrition*, 20(**5**), 547-556.

Mohseni, M. and Sotudeh A.M., 2013. The effects of dietary selenium on the oxidative stress of beluga, Huso huso, fed high copper. *Iranian Scientific Fisheries Journal*, 21(**4**), 105-114.

Mohseni, M., Ozorio, R., Pourkazemi, M. and Bai, S., 2008. Effects of dietary L-carnitine supplements on growth and body composition in beluga sturgeon (*Huso huso*) juveniles. *Journal of Applied Ichthyology*, 24(**6**), 646-649.

Nazari, K., Shamsaie, M., Eila N., Kamali, A. and Sharifpour, I., 2017. The effects of different dietary levels of organic and inorganic selenium on some growth performance and proximate composition of juvenile rainbow trout (*Onchorhyncus mykiss*). *Iranian Scientific Fisheries Journal*, 16(**1**), 238-251.

Pappas, A.C. and zoidis, E., 2012. The Role of selenium in chicken physiology: New insights. In: chickens: physiology, diseases and farming practices.Kapur, I. and ISBN- 13: A. Mehra (Eds.). Nova Science Publishers New York, USA. 167 P.

Peterson, D.L., Vecsei, P. and Jennings, C.A., 2007. Ecology and biology of the lake sturgeon: a synthesis of current knowledge of a threatened North American *Acipenseridae*. *Reviews in Fish Biology and Fisheries*, 17(**1**), 59-76.

Puangkaew, J., Kiron, V., Somamoto, T., Okamoto, N., Satoh, S., Rider, S.A., Davies, S.J., Jha, A.N., Fisher, A.A., Knight, J. and Sweetman, J.W., 2009. Supra-nutritional dietary intake of selenite and selenium yeast in normal and stressed rainbow trout (*Oncorhynchus mykiss*): Implications on selenium status and health responses. *Aquaculture*, 295,282–291.

Tashjian, D.H., Teh, S.J., Sogomonyan, A. and Hung, S.S.O., 2006. Bioaccumulation and chronic toxicity of dietary L-eleno methionine in juvenile white sturgeon (*Acipenser transmontanus*). *Aquatic Toxicology*, 79, 401–409.

Tohidi, M., Harati, H. and Hadaegh, F., 2008. Association of liver enzymes with incident type 2 diabetes: A nested case control study in an Iranian population. Bio Med Central, BMC Endocrine Disorders 8(**1**), 5. Diabetes mellitus: complications and herapeutics. *Medical Science Monitor*, 12, 130-147.

Torrecillas, S., Makol, A., Caballero, M.J., Montero, D., Gines, R., Sweetman, J. and Izquierdo, M.S., 2011. Improved feed utilization, intestinal mucus production and immune parameters in sea bass (*Dicentra rchuslabrax*) fed mannan oligosaccharides (MOS). *Aquaculture Nutrition*. 17, 233-233.