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Research Article

Dietary organic copper and selenium interactions in juvenile beluga sturgeon (*Huso huso*): Effect on growth, oxidative stress, and immunity

Mohseni M.[1*], Sungchul C. Bai[2], Ozorio R.A.[3], Fadakar Masouleh F.[1]

1 International Sturgeon Research Institute, Iranian Fisheries Science Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Rasht, Iran
2 Department of Marine Bio-materials and Aquaculture/Feeds and Foods Nutrition Research Center (FFNRC), Pukyong National University, Busan 608-737, Rep of Korea
3 CIIMAR, Terminal de Cruzeiros do Porto de Leixões, Av. General Norton de Matos s/n, 4450-208, Matosinhos, Portugal
*Correspondence: mahmoudmohseni73@gmail.com

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Abstract

A 2×3 factorial design was used to assess the effects of dietary copper (Cu) and selenium (Se) on growth, oxidative stress, and immunity in Beluga sturgeon (*Huso huso*) juveniles fed optimal and high levels of Mintrex® copper and selenomethionine. Six experimental diets were formulated with two Cu levels (10 and 50 mg Cu kg^{-1}) and three Se levels (5, 11, and 55 mg Se kg^{-1}): $Cu_{10}Se_5$, $Cu_{10}Se_{11}$, $Cu_{10}Se_{55}$, $Cu_{50}Se_5$, $Cu_{50}Se_{11}$, and $Cu_{50}Se_{55}$. Each diet was fed to triplicate groups of 15 fish (initial weight 13.31±0.35 g) for 12 weeks. The fish fed with $Cu_{10}Se11$ diet exhibited the highest weight gain (1092.2±11.2%), significantly greater than those fed with $Cu_{10}Se_5$ (962.2±17.3%), $Cu_{10}Se_{55}$ (928.6±33.7%), and $Cu_{50}Se_{55}$ (721.1±46.6%) diets. The best feed conversion ratio was observed in $Cu_{10}Se_{11}$ (1.24±0.05), while the lowest value was in $Cu_{50}Se_{55}$ (1.81±0.16). The protein efficiency ratio was significantly high in $Cu_{10}Se11$ (1.87±0.02) and low in $Cu_{50}Se_{55}$ (1.28±0.16). The hepatic Cu and Se concentrations increased dose-dependently, reaching 38.12±2.4 µg g^{-1} and 11.68±0.8 µg g^{-1}, respectively, in $Cu_{50}Se_{55}$. The lowest thiobarbituric acid reactive substances (TBARS) value was recorded in $Cu_{10}Se11$ (5.83±0.56 U mg^{-1} protein), while the highest was in $Cu_{50}Se_{55}$ (11.8±0.56 U mg^{-1} protein). Antioxidant enzyme activities (CAT, GPx, and Cu-Zn SOD) were significantly reduced in $Cu_{50}Se_{55}$, whereas IgM (46.1±2.65 µg mL^{-1}) and lysozyme (17.7±0.95 U mL^{-1}) values were high in $Cu_{10}Se_{11}$, but they were markedly suppressed in $Cu_{50}Se_{55}$ (IgM: 21.4±1.09 µg mL^{-1}; lysozyme: 9.9±0.74 U mL^{-1}). Both IgM and lysozyme values were decreased with excess dietary Cu and Se and were negatively correlated with TBARS. These findings indicate that $Cu_{10}Se_{11}$, containing 10 mg kg^{-1}Cu and 11 mg kg^{-1} Se, optimally enhances growth, reduces oxidative stress, and strengthens immunity in Beluga juveniles, while excessive Cu and Se intake impairs antioxidant capacity and immune functions.

Introduction

Fish tissues are rich in polyunsaturated fatty acids (PUFAs), which makes them particularly susceptible to lipid peroxidation (Stéphan *et al*., 1995). Heavy metals are recognized as potent pro-oxidants that can accelerate the peroxidation of PUFAs (Halliwell and Gutteridge, 1993; Lin and Shiau, 2007). Copper (Cu) is an essential trace element for both humans and animals, playing a vital role in various biochemical processes (Lall, 2002; Tan *et al*., 2011). In Beluga sturgeon (*Huso huso*), Cu deficiency has been reported to inhibit growth and decrease hepatic copper-zinc superoxide dismutase (Cu-Zn SOD) activity (Mohseni *et al*., 2013).

Despite its essential role, Cu can be toxic when present at elevated concentrations. It is frequently added to fish feeds at levels exceeding dietary requirements (Maage, 1994). Excessive dietary Cu has been associated with growth suppression, increased mortality (Shiau and Ning, 2003), oxidative stress (Berntssen *et al*., 2000), and impaired immune function (Lundebye *et al*., 1999; Berntssen *et al*., 1999; Mohseni *et al*., 2013). In adult Beluga sturgeon, diets containing 320 mg Cu kg^{-1} resulted in reduced growth and feed efficiency, feed refusal, and elevated hepatic Cu accumulation (Mohseni *et al*., 2012).

Mintrex®Cu, a chelated form of copper, contains at least 17% Cu and 78% hydroxy methionine analog (HMTBa), enhancing its bioavailability compared to inorganic Cu sources. Moazenzadeh *et al*. (2020) reported that the optimal dietary Cu requirement for juvenile Siberian sturgeon (*Acipenser baerii*) ranges from 9.51 to 16.10 mg kg^{-1} of diet, based on growth performance and enzyme activity. Similarly, selenium (Se) supplementation has been shown to promote growth and improve feed utilization in juvenile Siberian sturgeon, suggesting its potential to alleviate mineral-induced stress (Foroshani *et al*., 2024). In pangasius (*Pangasianodon hypophthalmus*), dietary Cu at 8 mg kg^{-1} also enhanced immune-related gene expression while reducing oxidative and environmental stress (Kumar *et al*., 2024).

Selenium (Se), another essential trace mineral, has gained considerable attention in fish nutrition. Toxic concentrations of Se vary widely among species; rainbow trout, Chinook salmon, fathead minnow, striped bass, bluegill, and razorback sucker exhibit toxicity thresholds ranging from 2.4–70 mg Se kg^{-1} of feed and 47–472 µg L^{-1} in water (Hamilton, 2004). Excessive Se intake generally leads to reduced growth and survival, as well as histopathological damage (Lee *et al*., 2012). Selenium serves as a crucial component of glutathione peroxidase (GPx), an enzyme that protects cellular membranes from oxidative damage—a function first demonstrated by Rotruck *et al*. (1973) and later confirmed in numerous studies (Brigelius-Flohé and Maiorino, 2013; Steinbrenner and Sies, 2013). Furthermore, Se has been shown to protect against heavy metal toxicity in both mammals (Rana and Verma, 1997) and fish (Lin and Shiau, 2007), although this protective effect has not yet been investigated in Beluga sturgeon.

The Beluga sturgeon is one of the most commercially significant species in

freshwater and brackish-water aquaculture in Iran. Owing to its high economic value, it represents a promising candidate for the further expansion of aquaculture. However, information on the dietary requirements of most sturgeon species remains limited, and well-formulated diets have yet to be developed. Therefore, this study aimed to determine whether dietary Se supplementation can mitigate Cu-induced oxidative stress in Beluga sturgeon. Growth performance, feed utilization, and hematological and biochemical parameters, including plasma lysozyme activity and total immunoglobulin concentration, were assessed as indicators of non-specific immune responses.

Materials and methods

Experimental fish and feeding trial

Healthy Beluga sturgeon (*Huso huso*) were obtained from the International Sturgeon Research Institute in Guilan, Iran, and acclimated to the experimental conditions for two weeks before the feeding trial began. During acclimation, the fish were fed a basal diet without supplemental Cu or Se at satiation to prevent additional mineral intake and ensure a uniform nutritional background. At the start of the feeding trial, 270 uniformly sized fish were selected from the stock population and randomly distributed into six triplicate groups. Each 500-L circular fiberglass tank contained 15 fish (initial mean weight: 13.31±0.35 g) and represented one experimental unit, with three tanks assigned to each dietary treatment in a completely randomized design. The experimental tanks were supplied with flow-through water from the same source (Sefid-Rud River, Guilan) at a rate of 4.5±0.7 L min^{-1}. During the bioassay, the following water quality parameters were maintained: dissolved oxygen 7.33±0.15 mg L^{-1}, temperature 22.5±0.4°C, pH 7.2±0.2, alkalinity 156.5±3.8 mg L^{-1}, total hardness 360.3±15.4 mg L^{-1}, and NH_3 concentration 0.11±0.01 mg L^{-1}. All parameters were measured using test kits supplied by Shimi Sanat Vaheb Company. A natural photoperiod of approximately 10 hours of light and 14 hours of darkness was maintained throughout the experiment. Fish in each treatment group were fed their respective experimental diets to apparent satiation for 12 weeks. The feed was removed from the refrigerator one hour before feeding, weighed, and distributed among the tanks. Care was taken to ensure no uneaten feed remained to prevent Cu and Se leaching into the water. Feeding was conducted four times daily. Uneaten pellets were collected using mesh collectors placed beneath the drain pipes of each tank, then dried and weighed. Daily feed intake was calculated by subtracting the weight of uneaten feed from the total feed supplied. Every two weeks, fish from each tank were anesthetized, counted, and individually weighed. Fish were not fed on the day of weighing (12 hours before and after) to avoid including ingested feed in the weight measurements and to minimize handling stress (Mohseni *et al*., 2005).

Diet preparation

Six experimental diets were formulated to be isonitrogenous (43% crude protein) and isolipidic (14% crude lipid). The diets included two levels of Cu supplementation—10 mg Cu kg^{-1} (Cu10)

and 50 mg Cu kg^{-1} (Cu50)—and three levels of selenomethionine (SeMet; Fisher Scientific, Pittsburgh, PA, USA) at 5, 11, and 55 mg Se kg^{-1}. This produced a 2×3 factorial design (Mohseni *et al*., 2012; Safabakhsh *et al*., 2020) comprising six dietary treatments: $Cu_{10}Se_5$, $Cu_{10}Se_{11}$, $Cu_{10}Se_{55}$, $Cu_{50}Se_5$, $Cu_{50}Se_{11}$, and $Cu_{50}Se_{55}$. The formulation and proximate composition of the experimental diets, analyzed according to AOAC (2000) standards, are presented in Table 1.

Table 1: Formulation and chemical composition of the basal diet.

Ingredients (g Kg^{-1})	
Casein [1]	380
Gelatin[1]	80
Fish meal [2]	50
Wheat flour	110
Dextrin[1]	70
Corn starch[1]	110
Oil (Corn + Fish oil) [3]	120
Vitamin premix[4]	20
Mineral premixture (Se/Cu-free) [5]	10
Alphacellulose [1]	50
Dry matter %	90.8
Crude protein %	43.1
Crude lipid %	14.2
Gross energy (kJ g^{-1} diet)	19.79
Ash	7.3

[1]United States Biochemical, Cleveland, OH, USA.
[2]62% crude protein (Khazar Kilka Industrial Co., Guilan-Iran).
[3]Aras Oil Co. Ltd., Anzali, Iran (Lipid source is a 1:1 blend of fish oil and corn oil).
[4]Vitamin mixture (Contains as mg kg^{-1} diet): DL-alpha tocopherol acetate, 60 IU; DL-cholecalciferol, 3000 IU; Thiamin, 15 mg; Riboflavin, 30 mg; Pyridoxine, 15 mg; B12, 0.05 mg; Nicotinic acid, 175 mg; Folic acid, 5 mg; Ascorbic acid, 500 mg; Inositol, 1000 mg; Biotin, 2.5 mg; Calcium panthotenate, 50 mg; Choline chloride, 2000 mg.
[5]Mineral mixture (Se/ Cu free; as g kg^{-1} pre-mixture): Calcium carbonate (40% Ca), 2.15 g; Magnesium oxide (60% Mg), 1.24 g; Ferric citrate, 0.2 g; Potassium iodide (75% I), 0.4 mg; Zinc sulphate (36% Zn), 0.4 g; Manganese sulphate (33% Mn), 0.3 g; Dibasic calcium phosphate (20% Ca, 18% P), 5 g; Cobalt sulphate, 2 mg; Sodium selenite (30% Se), 3 mg; KCl, 0.9 g; NaCl, 0.4 g.

Vitamin-free casein (United States Biochemical, Cleveland, OH, USA) served as the primary protein source, supplemented with 5% anchovy fish meal. Lipid sources consisted of a 1:1 mixture of refined fish oil (Khazar Oil Co. Ltd., Anzali, Iran) and corn oil. Carbohydrate sources included wheat flour, dextrin, and corn starch (United States Biochemical, Cleveland, OH, USA). All diets were isoenergetic at 420 kcal 100 g^{-1} diet, following Mohseni *et al*. (2005). A premix containing Mintrex®Cu, a chelated form of Cu and SeMet, was incorporated into the diets.

Fish sampling

At the beginning and end of the feeding trial, the fish were fasted for 24 hours and anesthetized using clove powder at a concentration of 250 mg L^{-1} (Hassan *et al.,* 2021). After the 12-week feeding trial, the total number of fish, tank biomass, and feed intake were recorded. Growth and feed utilization were calculated following Hardy and Barrows (2002), including final weight (FW), weight gain (WG), specific growth rate (SGR), daily feed intake (DFI), feed conversion ratio (FCR), protein efficiency ratio (PER), and survival rate. The following equations were used:

WG (%)=(Final body weight – Initial body weight) ×100 / Initial body weight
SGR (% BW Day^{-1})=[(ln (Final body weight) – ln (Initial body weight)) / Trial duration (days)] × 100, in which IBW: Initial body weight and FBW: final body weight;
CF=(Body weight (g) / Fork $length^3$ (cm^3)) ×100
FCR=Total dry feed fed (g) / Total wet weight gain (g)
PER = Wet weight gain (g) / Protein intake (g)

After biometric measurements, five fish from each dietary group were randomly selected for blood collection. Blood was drawn from the caudal vessels using non-heparinized syringes. Samples were centrifuged at 3,000 g for 10 min at 4°C (Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) to separate the serum. The serum samples were stored at −80°C until used for biochemical analysis. Following blood collection, fish were immediately dissected on ice. The liver was carefully removed and weighed to calculate the hepatosomatic index (HSI) using the formula:
HSI = (Liver weight (g) / Body weight (g)) ×100

Liver samples were stored at −80 °C for subsequent determination of tissue Cu and Se concentrations.

Determination of proximate composition

Diets and white muscle samples (two fish per tank) were analyzed for proximate composition following standard procedures described by AOAC (2000). Moisture content was determined by oven-drying samples at 105°C for 24 hours. Crude protein (N×6.25) was measured using the Kjeldahl method after acid digestion with an auto-Kjeldahl system (LECO Instruments, St. Joseph, MI, USA). Crude fat was determined by ether extraction using a Soxhlet apparatus (64826 SUPELCO, Sigma-Aldrich, St. Louis, MO, USA). Crude ash content was obtained by combusting samples in a muffle furnace at 550°C for 3 hours and weighing the remaining residue.

Enzyme assay

The activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were determined following the method of Somi *et al*. (2009). For cytosolic enzyme analysis, liver samples were homogenized in a 1.15% KCl solution. Tissue SOD activity was measured spectrophotometrically based on the inhibition of superoxide-induced nicotinamide adenine dinucleotide (NADH) reduction, according to Paoletti *et al*. (1986). Hepatic and muscle GPx activities were analyzed following the protocol of Noguchi *et al*. (1973). Lipid peroxidation was assessed by quantifying thiobarbituric acid reactive substances (TBARS) as described by Uchiyama and Mihara (1978). Absorbance was measured at 530 nm, and TBARS concentrations were calculated by multiplying the optical density value by 5.2.

Cu and Se analysis

The concentrations of copper (Cu) and selenium (Se) in diets and tissues were measured according to AOAC (2000) protocols. Water samples were collected from the tank inlets at the beginning,

middle, and end of the trial. Each sample was filtered through a 0.22 µm syringe filter and analyzed for Cu and Se using a flame atomic absorption spectrophotometer (Perkin-Elmer 3300, Waltham, MA, USA) at the Centralized Analytical Laboratory, Pukyong National University (Republic of Korea). For tissue analysis (white muscle below the dorsal fin), approximately 2 g of sample was dried at 100°C for 24 hours, homogenized for 5 minutes, and digested with 2 mL of concentrated nitric acid and 0.5 mL of hydrogen peroxide in a microwave digestion system (CEM MDS 2100, Conquer Scientific, San Diego, CA, USA). The digests were then diluted to 25 mL with deionized water, filtered through a 0.22 µm membrane, and analyzed for Cu and Se concentrations using flame atomic absorption spectroscopy.

Statistical analysis

All statistical analyses were performed using SAS software version 9.1 (SAS Institute Inc., Cary, NC, USA). Data were tested for normality using the Kolmogorov–Smirnov test and for homogeneity of variances using Levene's test. A two-way ANOVA was applied to evaluate the effects of dietary copper and selenium, and Duncan's multiple range test was used for post-hoc comparisons when significant differences were detected. Results are expressed as mean±standard deviation (SD), and differences were considered statistically significant at $p<0.05$.

Results

The results for WG, SGR, FCR, PER, CF, HSI, and survival rate are presented in Table 2. WG, FCR, and PER were significantly influenced by dietary levels of Cu and Se ($p<0.05$). Fish fed the $Cu_{10}Se_{11}$ diet exhibited a significantly higher WG (1092.2±11.2%) compared to those fed $Cu_{10}Se_5$ (962.2±17.3%), $Cu_{10}Se_{55}$ (928.6±33.7%), and $Cu_{50}Se_{55}$ (721.1±46.6%). The FCR was significantly higher in fish fed $Cu_{50}Se_{55}$ (1.81±0.16) compared to other dietary groups, which ranged from 1.24±0.05 ($Cu_{10}Se_{11}$) to 1.42±0.03 ($Cu_{10}Se_5$).

Table 2: Effects of dietary copper and selenium on growth performance of juvenile beluga sturgeon (values are mean±SD, n=3).

Diet	Final weight (g)[1]	WG (%)[2]	FCR[3]	PER[4]	HSI (%)[5]	CF[6]	SGR (% d^{-1})[7]
$Cu_{10}Se_5$	136.7±4.2^{c}	962.2±17.3^{b}	1.42±0.03^{bc}	1.63±0.08^{bc}	2.59±0.07^{b}	0.58±0.01^{bc}	3.4±0.05^{b}
$Cu_{10}Se_{11}$	161.2±3.1^{a}	1092.2±11.2^{a}	1.24±0.05^{c}	1.87±0.02^{a}	3.19±0.25^{a}	0.76±0.08^{a}	3.5±0.01^{a}
$Cu_{10}Se_{55}$	136.9±3.0^{c}	928.6±33.7^{b}	1.46±0.24^{b}	1.59±0.01^{c}	2.87±0.28^{ab}	0.59±0.01^{bc}	3.3±0.09^{b}
$Cu_{50}Se_5$	152.3±2.8^{ab}	1024.3±28.3^{ab}	1.31±0.02^{bc}	1.76±0.01^{ab}	3.21±0.24^{a}	0.69±0.03^{ab}	3.5±0.02^{ab}
$Cu_{50}Se_{11}$	143.2±3.3^{bc}	1002.1±14.8^{ab}	1.32±0.11^{bc}	1.73±0.03^{abc}	3.33±0.35^{a}	0.59±0.01^{bc}	3.4±0.03^{ab}
$Cu_{50}Se_{55}$	110.8±8.9^{d}	721.1±46.6^{c}	1.81±0.16^{a}	1.28±0.16^{d}	2.21±0.32^{c}	0.55±0.03^{bc}	3.0±0.1^{c}

1.BW: final body weight; 2. WG: weight gain; 3. FCR: feed conversion ratio; 4. PER: protein efficiency ratio5. HSI: hepatosomatic index; 6. CF: condition factor; 7. SGR: specific growth rate; Values are mean ± SD (n = 3). Different superscript letters in each column indicate significant differences ($p<0.05$).

No significant change was found in FCR among fish fed $Cu_{10}Se_5$, $Cu_{10}Se_{55}$, $Cu_{50}Se_5$, and $Cu_{50}Se_{11}$ diets. The PER was highest in fish fed $Cu_{10}Se_{11}$ (1.87±0.02) and lowest in those fed $Cu_{50}Se_{55}$ (1.28±0.16). An interaction between Cu and Se was

observed. There was no fish mortality, except in the $Cu_{50}Se_5$ and $Cu_{50}Se_{55}$ groups, which experienced a 2% mortality rate (p>0.05). Dietary Cu content did not significantly affect HSI; however, Se content and the interaction between Se and Cu had a significant impact. Fish fed $Cu_{50}Se_{55}$ had a lower HSI (2.21±0.32%) compared to those on other diets, which ranged from 2.59±0.07% ($Cu_{10}Se_5$) to 3.33±0.35% ($Cu_{50}Se_{11}$). Significant interaction effects were observed between Cu and Se for CF, with fish fed $Cu_{10}Se_{11}$ showing a significantly higher CF (0.76±0.08) than those on other diets, which varied from 0.55±0.03 ($Cu_{50}Se_{55}$) to 0.59±0.03 ($Cu_{10}Se_{55}$ and $Cu_{50}Se_{11}$). Hepatic Cu content increased significantly from 24.11±1.20 to 40.41±1.99 µg/g tissue (p<0.05) with the rise in dietary Cu from 10 to 50 mg kg^{-1} diet (Table 3). Similarly, hepatic selenium (Se) content increased tenfold, from 1.27±0.17 to 11.06±0.88 µg/g, as dietary Se increased from 5 to 55 mg kg^{-1} (p<0.05).

Table 3: Effects of dietary copper and selenium on hepatic Cu and Se concentration (µg g^{-1} tissue) of juvenile beluga sturgeon (values are mean±SD, n=3).

Diet	Cu (µg g^{-1})	Se (µg g^{-1})
$Cu_{10}Se_5$	22.90 ± 1.16^b	1.39 ± 0.33^b
$Cu_{10}Se_{11}$	24.13 ± 2.06^b	1.73 ± 0.09^b
$Cu_{10}Se_{55}$	25.29 ± 1.57^b	10.44 ± 0.71^a
$Cu_{50}Se_5$	41.47 ± 2.81^a	1.15 ± 0.05^b
$Cu_{50}Se_{11}$	41.64 ± 2.34^a	1.97 ± 0.15^b
$Cu_{50}Se_{55}$	38.12 ± 2.41^a	11.68 ± 0.78^a

Different superscript letters in each column indicate significant differences (p<0.05).

Oxidative stress indicators are summarized in Table 4. Dietary Se content significantly influenced all oxidative stress indicators ($\boldsymbol{p}$<0.05). Fish fed a high Cu and Se level ($Cu_{50}Se_{55}$) exhibited the lowest activities of catalase (29.9±0.6 U/mg), GPx (109.5±2.95 µmol/mg protein/min), and copper-zinc superoxide dismutase (Cu-Zn SOD) (244.9±6.60 U/mg liver). Conversely, these fish exhibited the highest levels of thiobarbituric acid reactive substances (TBARS) (11.8±0.56 U/mg protein). Fish fed the $Cu_{10}Se_{11}$ and $Cu_{50}Se_5$ diets demonstrated the highest hepatic glutathione peroxidase (GPx) levels. Hepatic copper-zinc superoxide dismutase (Cu-Zn SOD) activity was significantly higher in fish fed the $Cu_{10}Se_{11}$, $Cu_{50}Se_5$, and $Cu_{50}Se_{11}$ diets.

Table 4: Effects of dietary copper and selenium on antioxidant enzyme activities and lipid peroxidation in beluga sturgeon (values are mean±SD, n=3).

Diet	CAT[2] (U mg^{-1} protein)	TBARS (nmol MDA mL^{-1})	GR[4] (U mg^{-1} protein)	GST[5] (U mg^{-1} protein)	GPx[6] (U mg^{-1} protein)	Cu-Zn SOD[7] (U mg^{-1} protein)
$Cu_{10}Se_5$	33.0 ± 0.65^b	7.58 ± 0.27^b	0.06 ± 0.01^d	269.7 ± 10.5^{ab}	116.7 ± 2.90^b	319.4 ± 7.17^b
$Cu_{10}Se_{11}$	35.3 ± 0.6^a	5.83 ± 0.56^d	0.09 ± 0.01^a	277.0 ± 11.53^a	129.9 ± 3.46^a	341.1 ± 6.85^a
$Cu_{10}Se_{55}$	33.9 ± 0.21^b	7.53 ± 0.68^b	0.06 ± 0.01^{cd}	281.2 ± 5.01^a	114.1 ± 3.12^{bc}	317.3 ± 6.39^b
$Cu_{50}Se_5$	35.8 ± 0.66^a	6.63 ± 1.22^{cd}	0.08 ± 0.01^{ab}	263.7 ± 4.04^b	126.6± 3.06^a	338.7 ± 6.47^a
$Cu_{50}Se_{11}$	35.6 ± 0.66^a	6.35 ± 0.71^{cd}	0.08 ± 0.01^{abc}	279.7 ± 3.06^a	122.1 ± 3.41^{ab}	335.5 ± 5.61^a
$Cu_{50}Se_{55}$	29.9 ± 0.6^c	11.8 ± 0.56^a	0.07 ± 0.02^{bcd}	269.3 ± 3.51^{ab}	109.5 ± 2.95^c	244.9 ± 6.60^c

CAT: catalase; GPx: glutathione peroxidase; SOD: superoxide dismutase; TBARS: thiobarbituric acid reactive substances. Different superscript letters in each column indicate significant differences (p<0.05).

Fish fed $Cu_{50}Se_5$ had the lowest glutathione S-transferase (GST) activity (263.7±4.04 µmol/mg protein/min), while those fed $Cu_{10}Se_{55}$ had the highest GST activity (281.2±5.01 µmol/mg protein/min). A significant interaction between Cu and Se was observed for all oxidative stress indicators except for GST and Cu-Zn SOD. Table 5 represents the effects of the supplemented diets on humoral innate immune parameters (Total immunoglobulin (IgM) and lysozyme). Lysozyme and IgM activity were the highest in beluga sturgeon fed the diets with $Cu_{10}Se_{11}$ and $Cu_{50}Se_{5,}$ and the lowest in fish fed the $Cu_{50}Se_{55}$ diets.

Pearson's correlation revealed significant negative associations between TBARS and both IgM (r= –0.77, p<0.01) and lysozyme (r= –0.75, p<0.01), while IgM and lysozyme were strongly positively correlated (r=0.98, p<0.001).

Table 5: Effects of dietary copper and selenium on serum immune parameters of beluga sturgeon (values are mean ± SD, n = 3).

Diet	IgM (µg mL^{-1})	Lysozyme (U mL^{-1})
$Cu_{10}Se_5$	31.4 ± 2.29^b	12.9 ± 0.78^b
$Cu_{10}Se_{11}$	46.1 ± 2.65^a	17.7 ± 0.95^a
$Cu_{10}Se_{55}$	30.3 ± 1.46^b	12.5 ± 1.04^b
$Cu_{50}Se_5$	41.4 ± 4.01^a	16.2 ± 0.64^a
$Cu_{50}Se_{11}$	38.8 ± 3.53^b	14.4 ± 0.31^b
$Cu_{50}Se_{55}$	21.4 ± 1.09^c	9.9 ± 0.74^c

IgM: immunoglobulin M. Different superscript letters in each column indicate significant differences (p<0.05).

Discussion

This study demonstrates that Beluga sturgeon are unable to obtain sufficient Cu and Se from an unsupplemented diet, emphasizing the necessity of dietary supplementation. The concentrations of Cu and Se in the rearing water remained within the natural ranges typically observed in the wild. While Se levels were below the detection limit, Cu concentrations ranged from 0.0073 to 0.0217 µg L^{-1} throughout the experimental period. Notably, no significant difference in Cu concentration was observed between tanks containing fish fed Cu-supplemented diets and those receiving the basal diet. The highest weight gain (WG) observed in this study is consistent with previous findings from our laboratory (Mohseni *et al*., 2008; Hassani *et al*., 2012), where WG ranged from 950% to 1200% in similarly sized Beluga sturgeon fed nutritionally adequate diets over 12 weeks. The dietary Cu and Se concentrations used in this experiment were based on established requirements—10–13 mg kg^{-1} Cu (Mohseni, 2012) and 11 mg kg^{-1} Se (Arshad, 2011). Growth performance was comparable between fish fed the Cu10Se11 diet and those receiving diets with either low or adequate Se levels, even when Cu was increased fivefold ($Cu_{50}Se_5$ and $Cu_{50}Se_{11}$). These results indicate that elevated dietary Cu (50 mg kg^{-1}) did not adversely affect growth. Tolerance to dietary Cu varies among fish species; for instance, Cu levels of 8 mg kg^{-1} and 16 mg kg^{-1} have been reported to reduce growth in tilapia and channel catfish, respectively (Gatlin and Wilson, 1986; Shiau and Ning, 2003). In contrast, Beluga sturgeon appear capable of tolerating much higher Cu concentrations—up to 160 mg kg^{-1}—without adverse growth effects (Mohseni *et al*., 2012). Similar resilience to elevated Cu and Se levels has been observed in other sturgeon species. White sturgeon

(*Acipenser transmontanus*) exhibited no significant reductions in growth or survival when fed diets containing high levels of SeMet (Tashjian and Hung, 2006; Tashjian *et al*., 2006). Likewise, both green and white sturgeon maintained normal growth performance on SeMet-enriched diets, despite considerable Se accumulation in their tissues (De Riu *et al*., 2014). Overall, sturgeon species appear to possess a relatively high tolerance to trace mineral imbalances. With respect to Se toxicity, Beluga sturgeon appear to be less sensitive than many other fish species. Fish exposed to 11–55 mg kg^{-1} Se (as SeMet) for 12 weeks exhibited a mean survival rate of 99.3±2.2%, whereas Chinook salmon showed a pronounced mortality at comparable Se levels (Cleveland *et al*., 1993). Similarly, white sturgeon fed diets containing 0.4–191.1 µg SeMet g^{-1} displayed no significant differences in mortality (Tashjian *et al*., 2006). In the present study, a 12-week exposure to SeMet and Mintrex®Cu did not affect survival rates; however, weight gain declined significantly in fish fed the $Cu_{50}Se_{55}$ diet, indicating that excessive levels of Cu and Se can adversely impact growth. Weight gain in fish fed the Cu10Se11 diet was comparable to those receiving the $Cu_{50}Se_5$ and $Cu_{50}Se_{11}$ diets but was significantly higher than in fish fed $Cu_{10}Se_5$, $Cu_{10}Se_{55}$, or $Cu_{50}Se_{55}$. Optimal levels of Cu and Se likely promote growth by enhancing the activity of enzymes involved in nutrient utilization (Zhong *et al*., 2023; Kayiira *et al*., 2024). In conditions of Se deficiency, Cu may partially compensate for Se-related physiological functions, reflecting similar mineral interactions observed in this study and in Atlantic salmon (*Salmo salar*) (Lorentzen *et al*., 1998). Elevated hepatic TBARS levels were detected in fish fed diets either deficient or excessive in Cu and Se, indicating reduced antioxidant enzyme activity. This effect was most evident in the $Cu_{50}Se_{55}$ group, which exhibited the highest TBARS concentrations and the lowest activities of catalase (CAT), superoxide dismutase (SOD), glutathione S-transferase (GST), and glutathione peroxidase (GPx). These enzymes play essential roles in neutralizing reactive oxygen species (ROS) and protecting cells from oxidative damage (Kelly and Havrilla, 1998). Similar trends have been documented in grouper (*Epinephelus spp.*) (Lin and Shiau, 2007; Lin *et al*., 2008) and rainbow trout (*Oncorhynchus mykiss*) (Handy, 2003), suggesting that excessive Cu and Se intake generally exacerbates oxidative stress. Non-specific immune responses, assessed through IgM concentration and lysozyme activity, declined with increasing dietary Cu and Se, showing a negative correlation with TBARS levels. While adequate amounts of these trace elements enhance immune function, excessive intake disrupts the balance between ROS generation and antioxidant defenses (Sumana *et al*., 2023; Kumar *et al*., 2024). Mechanistically, Se acts as an essential component of GPx, which catalyzes the reduction of hydrogen peroxide and lipid peroxides, whereas Cu serves as a cofactor for Cu/Zn SOD, an enzyme responsible for the dismutation of superoxide radicals. Fish fed the $Cu_{10}Se_{11}$ diet likely achieved optimal GPx and Cu-Zn SOD activity, thereby minimizing lipid peroxidation and supporting immune

performance. Conversely, excessive supplementation ($Cu_{50}Se_{55}$) may have overwhelmed the antioxidant defense system, resulting in ROS production beyond enzymatic capacity and leading to oxidative stress, impaired immunity, and reduced growth. These findings support a model in which Se and Cu function as complementary cofactors, where deviations from their optimal balance either constrain enzyme efficiency or intensify oxidative damage.

Conclusions

This study suggests that supplying Cu and Se at balanced, near-requirement levels (10 mg Cu kg^{-1} and 11 mg Se kg^{-1}) can improve growth, reduce oxidative stress, and support humoral immunity in juvenile Beluga sturgeon. By contrast, excessive supplementation (50 mg Cu kg^{-1} plus 55 mg Se kg^{-1}) appears to have harmful effects. While Cu may sometimes partially compensate for Se deficiency under certain conditions, the data do not point to a clear synergistic interaction; instead, Cu–Se interactions seem to be either compensatory or antagonistic, depending on the dietary concentration. Further studies are needed to track the activity of key antioxidant enzymes, such as GPx and Cu–Zn SOD, and to monitor redox status over longer feeding periods and in practical farm conditions, which would help refine dietary recommendations for this species.

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Conflicts of interest

The authors declare that they have no conflicts of interest relevant to this work.

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