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Antioxidant response versus selenium accumulation in the liver and kidney of the Siberian sturgeon (Acipenser baeri)

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ABSTRACT The aim of this study was to examine the effects of selenium on concentrations of metabolites and enzyme activities acting as antioxidant markers in liver and kidney of Siberian sturgeon Acipenser baeri. Sturgeons were fed selenium cysteine for 30 and 60 d at 1.25, 5, 20 mg Se kg1 . Selenium level in the control feed was 0.32 mg kg1 . Se concentration was measured in liver, kidney and muscle of every specimen. Sturgeon accumulated Se in tissues with a clear dose-response relationship and the highest Se concentration was recorded in liver. This outcome is lined up with the findings obtained on the antioxidant markers evaluated in both tissues, and in which a dose-response for several biomarkers was recorded in liver. The superoxide dismutase activity in Se-treated fish was generally induced, while catalase activity was lower in liver or unaltered in kidney. The concentrations of glutathione S-transferase, glutathione reductase and total glutathione responded differently for both tissues and were induced in a different way at both endpoints. No changes of glyoxalase I activity were noted for both Se-treated tissues, while for glyoxalase II enzyme in liver a dose-related pattern was found showing a reversible effect (decreased and increased counteractive response) only in the 5 mg kg1 group. Moreover, the highest Se concentrations did not cause marked changes in malondialdehyde levels of liver and kidney. The enhancement of glutathione peroxidase activity in Se-treated sturgeon might have prevented the lipid peroxidation in both tissues, providing to the Siberian sturgeon a great defense ability versus the prooxidant effect of selenium.

1. Introduction

Selenium is an essential nutrient for animal (Diplock and Hoekstra, 1976). Diet is the main font of selenium intake in fish. Selenocysteine (Se-Cys) and selenomethionine (SeMet) are the most abundant seleno compounds in fish diets. SeCys is the most relevant one being the aminoacid providing the catalytic site for the enzymatic selenoproteins (Stadtman, 1996). Vertebrates can synthesize SeCys but not SeMet being the latter generally metabolized through the trans-sulphuration pathways of the sulphur amino acids and then transformed into SeCys (Beilstein and Whanger, 1992). Recent field-based investigations have reported that selenium in fish is mainly recorded as selenomethionine (SeMet) and selenocystine (SeCys), with selenomethionine being more abundant with the increasing selenium exposure (Phibbs et al., 2011a, b). Selenomethionine, if not split in selenocysteine for the synthesis of selenoproteins, has a methionine similar structure and can be accumulated into tissues, such as muscle (Jacques, 2001). Selenium is a cofactor for several oxide-reductive enzymes reducing organic and inorganic peroxides in H2O and alcohols. The enzyme glutathione peroxidase is a well-known example of this oxidation-reductive function (Neve, 1989; Burk, 1991). Glutathione peroxidase levels in liver or in plasma are a good indicator for selenium concentrations in fish (Watanabe et al., 1997). Selenoproteins have important biological functions as Se homeostasis in brain is maintained primarily by the selenoprotein P, a Se-dependent protein (Nakayama et

al., 2007). Three main mechanisms of Se toxicity have been proposed. The first one is the replacement of sulphur with Se in proteins due to their chemical similarity preventing the chemical bonds necessary for the disulfide structure of the protein. The second and third one is the inhibition of methylation (a method of detoxification) and the generation of reactive oxygen species (ROS) respectively (Spallholz and Hoffman, 2002). SeMet is metabolized into methylselenol, either via the trans-sulphuration pathway or directly by the enzyme methioninase, and the subsequent redox cycling of methylselenol leads to the generation of ROS as reported for rainbow trout (Palace et al., 2004; Misra et al., 2010). Misra et al. (2010) detected methioninase activity in fish hepatocytes, and also demonstrated a rapid dose-dependent increase in intracellular ROS generation following an exposure to SeMet. Selenocysteine is also able to produce free radicals, while selenate causes toxic effects only after being reduced to selenite or selenole (Spallholz et al., 1993). The imbalance between prooxidant and antioxidants might lead to an oxidative stress condition which can be critical for organisms survival (Pacini et al., 2012, 2013). The antioxidant defense system includes enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione transferase (GST) and reduced glutathione. Acipenser baeri has a high growth rate, is tolerant to high stocking densities and is resistant to pathogens. Moreover, the Siberian sturgeon is the most commonly reared Acipenseridae in aquaculture throughout Europe (Gisbert and Williot, 2002) and not only for the valuable flesh, but especially for the esteemed caviar. Previously (Elia et al., 2007; Dörr et al., 2008; Elia et al., 2011; Pacini et al., 2012; Dörr et al., 2013) we indicated that the selenium content in commercial fish pellets can be higher than the concentration allowed by the EC law (0.5 mg kg1 ; 70/524/EEC). Therefore, in this study, we created pellets with Se-Cys to attain a Se concentration of 1.25 mg kg1 which can be easily found in fish feed, and other two diets (5 and 20 mg Se kg1) with higher Se concentrations. The aim of the study was to evaluate the effects of selenium on the antioxidant response of A. baeri. Siberian sturgeon was fed daily selenium diets (1.25, 5, 20 mg kg1) for 30 and 60 d under controlled conditions. Se concentration of the control feed was 0.32 mg kg1 . A further goal was to assess the dose – response relationship. Total Se concentration was measured in liver, kidney and muscle of each specimen. The levels of superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione S-transferase, and total glutathione were measured in liver and kidney of control and treated fish. In addition the index of lipid peroxidation (malondialdehyde) and the glyoxalase I and II enzymes were investigated in the same tissues. Since works on the effects of Se-cysteine on fish are scanty (Huang et al., 2012), the results reported in this study stand for the first information of the pro-oxidant effects of Se–Cys on the antioxidant response in the Siberian sturgeon.

2. Materials and methods

2.1. Experimental design

Selenium diets were manufactured in the laboratory at the Experimental Station of the Department of Agricultural, Forestry and Food Sciences of the University of Turin. Diets were prepared starting from a commercial fish diet for fingerlings (crude protein 55%, crude fats 16%, crude fiber 1%, crude ash 9.5%) having a Se concentration of 0.32 mg kg1 also used as control. The commercial control diet was finely grinded and then added with different amounts of Se-cysteine in order to attain the concentrations of 1.25 (Se1), 5 (Se2) and 20 (Se3) mg kg1 . Before and after the pelleting process each diet was tested for their Se content in order to confirm analytically its concentration using the method reported in the section of chemical analysis of selenium. Pellets were formed using a 3.5 mm diameter mold and stored in a controlled dark room at 6 C to avoid the auto-oxidation of lipids. Male Siberian sturgeon (n = 80; 1795.7 ± 447.6 g body weight, 74.7 ± 6.5 cm total length; mean ± SD) were fed once a day with a daily feeding rate of 1.5% body weight (bw d1) for 60 d. The experiment was conducted in an indoor flow-through system with two replicates for each experimental group. All administered feed was consumed and at no time feed was rejected during the trial. Mortality was recorded daily. After 30, and 60 d of exposure five fish of each Se-treated and control tank (4

2 replicates groups) were euthanized with an overdose of tricaine methanesulfonate (MS 222) (250 mg L1) (Sigma–Aldrich, St. Louis, MO, USA). Morphometric parameters were recorded and the Fulton condition factor (CF) = 100 * body weight (g)/[body length (cm)]3 was calculated for each specimen. Liver, posterior kidney and dorsal muscle were collected, placed in dry ice and subsequently stored at 80 C for chemical and biochemical analysis. The experiment was conducted in accordance with the European and national guidelines for the protection of animals applied for experimental and other scientific purposes (European Commission, Directive 86/609/EC and Italian Directive 116/1992, respectively).

2.2. Chemical analysis of selenium

Selenium concentration in tissues and feed was determined by means of an atomic absorption spectrometer (Perkin–Elmer AAnalyst 800) equipped with transversely heated graphite atomizer (THGA) system and with Zeeman-effect for background correction (Abete et al., 2007). An amount of 0.5 g of feed and 1 g of tissues previously homogenized was accurately weighed on the microanalytical balance and processed with a mixture of 8 mL of pure nitric acid 65% and 1.5 mL of hydrogen peroxide in a microwave digestion system (Ethos 1 – Milestone). The resulting was then filtered through a paper filter into a 25 mL volumetric flask and made up to volume with ultrapure water. Selenium detection was determined in the digested samples. Measurements were performed using a bandwidth of 0.7 nm at 196 nm using an electrodeless discharge lamp operated at 300 mA. Argon of 99.998% purity was used as inert gas, the flow rate being 250 mL min1 during all the stages except for atomization, when the flow was stopped. A mixture of Pd (NO3)2 and Mg(NO3)2 in nitric acid (0.2%) as matrix modifier was used. Selenium determination was carried out by the standard additions method, the concentrations were 25–50 lg L1 . The limit of quantification (LOQ) of the method was 0.22 mg kg1 in feed and 0.01 mg kg1 in tissues (Dörr et al., 2013). To assess the reliability of the procedure, all samples were analyzed in batches with blanks and certified reference materials with known selenium content: NIST 8436Durum wheat flour (Se concentration = 1.23 mg kg1 and NIST 1566b Oyster Tissue (Se concentration = 2.06 mg kg1). Recovered concentrations of the certified reference materials were within 10% of the certified values.

2.3. Biochemical analysis

The biochemical analyses were performed spectrophotometrically on the cytosolic fractions of liver and kidney as previously reported (Elia et al., 2003, 2008, Pacini et al., 2012, 2013). The concentration of total glutathione (GSH + 2GSSG) was measured at 412 nm and the oxidized glutathione was used as the standard. In detail, liver and kidney (0.5 g) were homogenized (1:5 w/v) in 5% sulphosalicilic acid with 4 mM ethylene diamine tetraacetic acid (EDTA), and centrifuged at 30 000g for 30 min. Total glutathione content was assayed in potassium phosphate (KP) buffer 100 mM, pH 7, EDTA 1 mM, glutathione reductase (GR) 1U, NADPH 4 mg mL1 and 5,50 -dithiobis (2-nitrobenzoic acid) (DTNB) 1.5 mg mL1 , both dissolved in NaHCO3 0.5%. Levels of lipid peroxidation products (Malondialdehyde; MDA) were analyzed according to the method of Shaw et al. (2004) with slight modification (Pacini et al., 2013). Liver and kidney (0.1– 0.3 g) were homogenized (1:10 w/v) in 20 mM 2-amino-2- hydroxymethyl-propane-1,3-diol (TRIS/HCl) pH 7.4 and 0.5 M butylated hydroxytoluene (BHT), centrifuged at 3000

g for 20 min at 4 C. The supernatant was derivatized in a 1 mL mixture containing 1-methyl-2-phenylindole (10.32 mM in acetonitrile/ methanol 3:1), 150 lL of HCl, and 200 lL of buffer (TRIS + HCl pH 7.4), sample, or MDA standard (0–4 lM of 1,1,3,3-tetramethoxypropane). The samples were incubated at 45 C for 60 min, centrifuged at 15 000g for 10 min and then read at 586 nm. Liver or kidney (0.2 g) were homogenized (1:10 w/v) in 100 mM TRIS/HCl buffer, pH 7.8, 100 lM phenylmethylsulphonyl fluoride (PMSF), aprotinin 0.008 TIU mL1 and bacitracin 0.1 mg mL1 , and centrifuged at 100 000g for 1 h at 4 C. The SOD Assay kit-WST (Sigma) was used to determine superoxide dismutase (SOD) in both tissues following the manufacturer's instructions. SOD was determined using a highly watersoluble tetrazolium salt WST-1 [2-(4-Iodophenyl)-3-

(4-nitrophenyl)-5-(2,4-disulfophenyl)- 2H-tetrazolium monosodium salt]. The rate of reduction with oxygen is linearly related to the xanthine oxidase (XO) activity and is inhibited by SOD. The activity of 50% inhibition of SOD was determined by the colorimetric method at 450 nm (Pacini et al., 2013). Catalase (CAT) activity was evaluated in Na-phosphate buffer pH 7 and 12 mM H_2O_2. The decrease in absorbance due to H_2O_2 consumption was read at 240 nm. Glutathione peroxidase (GPx) activity was determined at 340 nm following the oxidation of NADPH in 100 mM Na-phosphate buffer with pH 7.5, 1 mM EDTA, 0.12 mM NADPH, 2 mM GSH, 1 mM NaN_3, 1 U GR and 0.6 mM H_2O_2. Glutathione reductase (GR) activity was measured at 340 nm following the oxidation of NADPH in 100 mM Na-phosphate buffer with pH 7, 1 mM GSSG and 60 lM NADPH. Glutathione S-transferase (GST) activity was measured at 340 nm with 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and 1 mM GSH in 100 mM Na-phosphate buffer pH 6.5. Glyoxalase I activity was assayed in sodium phosphate (100 mM pH 6.8) and the formation of S-d-lactoylglutathione, from the hemimercaptal adduct of methylglyoxal (MG, 1.5 mM) and reduced glutathione (GSH, 1.5 mM), was assessed at 240 nm (Pacini et al., 2012). Glyoxalase II (GII) activity was measured at 412 nm following the GSH formation with 0.2 mM DTNB and 0.5 mM S-D-lactoylglutathione (LSG) in 100 mM MOPS buffer, pH 7 (Pacini et al., 2012). Enzyme activity was reported as specific activity and the protein concentration was determined according to Lowry et al. (1951). Biochemical analyses were performed in triplicate on each sample with a Varian spectrophotometer (Cary 50 Thermostat Cell Holder) at a constant temperature of 25 C. 2.4. S

2.4. Statistical analysis

Homogeneity of variance was checked by Levene test, $p < 0.05$, and if necessary a logarithmic data transformation was performed. Data are reported as mean and standard deviation and geometric mean and interval of confidence only when the logarithmic data transformation was applied. Two-way ANOVA was used (with time, concentration, and ‘‘time versus concentration’’ interaction as variables). To highlight the statistically significant differences among fish fed diets with different Se concentrations and controls, we used the Tukey’s Multiple Comparison Test ($p < 0.05$). The relationships between chemical and biochemical data were also evaluated by Linear Pearson correlation analysis ($p < 0.05$).

3. Results

Selenium (Se1 = 1.25 mg kg1 ; Se2 = 5 mg kg1 , Se3 = 20 - mg kg1) fed continuously for 60 d to sturgeon, did not cause mortality. After 30 d, the overall mean body weight and total length were 1872.6 ± 367.5 g and 74.6 ± 7.9 cm and after 60 d were 2049 ± 444.3 g and 76.1 ± 8.2 cm with no changes ($p > 0.05$) in weight and length among treatments at the end of the trial. The Fulton’s condition factor (0.45 ± 0.1) did not indicate differences between treated and control groups. All tissues accumulated selenium throughout the 60 d dietary Se exposure showing a dose-response relationship, and the highest levels were found in liver (Fig. 1A). In detail, Se levels in liver at T30 was 2 times higher in Se1, 4 times higher in Se2 and 14 times higher in Se3 specimens, when compared to own controls. From 30 d onwards, all Se groups showed higher concentrations than own controls. In addition, for the Se3 group we registered an increase of Se accumulation in liver and a decrease in kidney (Fig. 1B) while the exposure time progressed. In muscle, differently to liver and kidney, only the Se3 group showed higher accumulation in muscle (Fig. 1C), while Se accumulation in Se1 and Se2 groups were comparable to own controls. The selenium concentration measured in the 20 mg kg1 group reached a plateau from 30 d onwards. Malondialdehyde (MDA) concentration in Se-treated groups was different only in liver of the Se1T60 group, which was about 60% higher ($P < 0.05$), when compared to the control groups (Table 1). The hepatic total glutathione was almost similar in treated and controls groups (Table 1), while in kidney a higher level was found in the Se3T30 group, when compared to own control. The SOD activity was markedly higher in liver (Fig. 2A) of all Se treated groups at T30 and in kidney (Fig. 2B) of the Se3 group at T60, when compared to own controls. The

enzyme activity in both tissues was positively correlated with Se level in liver ($r = 0.48$, $p < 0.05$) and in kidney ($r = 0.53$, $p < 0.05$). A lowered CAT activity was measured in liver of all treated groups (Table 2), but was statistically significant different only for the Se3 group at T30. The activity in all Se-treated kidney was similar to that of control and was positively correlated ($r = 0.46$, $p < 0.05$) with the Se level in the corresponding tissue. Hepatic GPx activity (Fig. 3A) increased markedly for the Se3 group at T30, and in Se2 and Se3 groups at T60, while kidney showed higher activity (Fig. 3B) mainly for all three groups at T30, when compared to own controls. The activity in liver and kidney was positively correlated with Se accumulation in both tissues ($r = 0.50$, liver; $r = 0.47$, kidney; $p < 0.05$). GR activity was markedly higher in liver (Fig. 4A) of the Se2 and Se3 groups at T60, while in kidney it was nearly similar to the controls (Fig. 4B). Hepatic GST activity (Table 2) did not show statistically signifi- cant changes between treated and control groups. This activity was positively correlated with Se level in liver ($r = 0.53$, $p < 0.05$). The activity (Table 2) in kidney was statistical different only in the Se1 group (70%) at T30. No marked changes were observed in liver and kidney for GI activity between all Se exposed groups and controls at both endpoints (Table 2). Hepatic GII activity was depleted in the Se2 and Se3 groups at T30 and increased in the Se2 group at T60 (Table 2).

4. Discussion and conclusions

In the present study Se-Cys fed continuously for 60 d to sturgeon did not cause mortality and had no effects on growth in length and weight in Se-treated fish. A wide range of levels for Se requirement/toxicity for fish has been reported (Hamilton, 2004), which can be related to the different selenocompounds assimilated by fish species, time, concentration and mode of Se exposure. Particularly, at elevated concentrations the uptake, mobility, and toxicity of Se are regulated by its chemical form. For instance, reduced growth rate, poor feed efficiency and high mortality occurred at 13 and 15 mg Se kg1 diet in rainbow trout and channel catfish, respectively, when sodium selenite was used as the dietary selenium source for long time (Hilton et al., 1980). Reduction in weight, but no mortality were recorded in white sturgeon fed diets containing high Se levels (41.7–191.1 lg g1) for eight weeks (Tashjian et al., 2006). On the other hand, Arshad et al. (2011) indicated that growth of juvenile sturgeon Huso huso was improved by the administration of diets containing 11.56 and 20.26 lg g1 of selenomethionine for eight weeks. Low Se concentrations in liver. The distribution order of selenium levels in sturgeon tissues was the following: liver > kidney > muscle. Moreover, our results indicate that sturgeon accumulates Se in tissues with a clear dose-response relationship and Se levels in liver and muscle may have not reached the equilibrium concentrations after the 60 exposure days. High Se accumulation in kidney was also reported in white sturgeon exposed chronically with SeMet (Tashjian et al., 2006). Several factors like the fish species, selenium compounds and exposure time may have contributed to the different results obtained in our current investigation and in other studies previously reported (Tashjian et al., 2006; Huang et al., 2012). While selenium levels were measured in liver, kidney and muscle of sturgeon treated with the three Se concentrations, the oxidative stress indicators were measured only in liver and kidney, generally showing higher antioxidant enzymes levels than in fish muscle. The administration of Se enriched diets induced oxidative stress in both target tissues, mainly at the highest Se concentration. Aerobic tissues like kidney and primarily liver have a high potential to produce ROS, and consequently the enhancement of the antioxidant enzyme activities may be considered as a protective mechanism to offset the increase of cellular ROS concentration (Trenzado et al., 2006; Elia et al., 2010; Pacini et al., 2012). As reported by Miller and Hontela (2011) the SOD activity plays an important role in the defense against selenite toxicity in fish cells. In the present study, SOD activity in both tissues of sturgeon fed with the three Se enriched diets was generally induced, while CAT activity was lower in liver or unchanged in kidney. These finding might indicate that the detoxification of hydrogen peroxide, produced as a result of the catalytic activity of SOD, was not performed by catalase. In fact, glutathione peroxidase activity being complementary to catalase for the detoxification of hydroperoxides, showed a remarkable increase in liver and kidney of Se-treated specimens. The reduction of catalase activity would likely increase the oxidative stress in liver, which can be counterbalanced by the increased

activity of GPx in Se dosed sturgeon. This outcome might suggest that Se concentrations in liver were able to induce the enzyme activity as a result of enhanced detoxification ability versus the higher H2O2 concentration, being a metabolite of the Se reduction pathway. Generally, optimal GPx activity requires the best dietary supply of Se, since lower or higher Se levels than the adequate internal concentration lead to increased lipid peroxides levels and consequently to degenerative processes in tissues. The results of several studies indicated that selenium can affect differently the lipid peroxidation in fish either in vivo or in cell culture studies. An example are trout hepatocytes exposed to an increasing range of selenomethionine (0–1000 lM) for 24 h evidencing higher activity of SOD, CAT and GPx at the highest concentration; as a result the membrane lipid peroxidation increased with the increasing SeMet dose (Misra et al., 2012). However, it is also reported that Se may reduce lipid peroxidation. In rainbow trout treated with increasing concentrations of sodium selenite the malondialdehyde (MDA) level, an index of lipid peroxidation, was lower than that of control (Orun et al., 2005). Moreover, diets supplemented with 0.15–0.30 mg kg1 of sodium selenite or selenomethionine, reduced the MDA levels in serum and muscle of trout (Küçükbay et al., 2009). Contrary to the findings reported by those authors, the administration of the three Se concentrations did not cause important changes in levels of MDA in sturgeon. In fact, although an increasing trend for MDA values was observed during the trial, the statistical significant difference in MDA concentration was reached only in Se1 liver after 60 d. Thus Se enriched diets were not able to induce a marked peroxidation process in liver or kidney of sturgeon at both endpoints. This result may be due to the high GPx activity in Se treated sturgeon, that might have prevented the lipid peroxidation in liver. Furthermore, according to Watanabe et al. (1997), the glutathione peroxidase/ selenium ratio indicated that selenium supplied as selenocystine was a better source for plasma glutathione peroxidase than was selenium from selenomethionine. Previous studies have indicated that selenium decreased (Elia et al., 2007; Dörr et al., 2008; Miller et al., 2009; Misra et al., 2012) or increased (Elia et al., 2011; Pacini et al., 2012) the glutathione concentration in several aquatic species. In our experimental conditions, the concentration of GST, GR and total glutathione had a different response in both tissues. It seems that despite the very high Se accumulation in both tissues and mainly in liver, GST activity and glutathione content in liver were not affected by selenium and only an early increase of both concentrations was recorded in kidney. Moreover, an increased GR activity was recorded in liver after 60 d of Se treatment. The GR activity catalyzes the regeneration of GSH from GSSG thus providing the necessary GSH concentration for the GPx functioning, and for the GST to detoxify tissues from electrophilic xenobiotics. Since fish have an adequate concentration of cysteine and biosynthetic enzymes (Thomas and Wofford, 1984) the lack of the hepatic glutathione response might be a consequence of the balance process between regeneration and thiol consumption (Elia et al., 2003). The latter may be due to the marked increase of GPx activity, which exerted the main protective role in both tissues. Only few data are available for glyoxalases in selenium-exposed fish. Previously, we indicated that selenium (1 mg kg1 diet administered for several weeks) induced-peroxidative processes in fish caused an indirect increase of 2-ketoaldehydes formation in tench kidney (Pacini et al., 2012) and carp liver (Elia et al., 2011), thus enhancing the detoxification ability of both glyoxalases in the two cyprinid species. In our current study, while no changes of GI activity were noted for both Se treated tissues, the antioxidant GII enzyme in sturgeon showed a dose-related pattern: no effect in the Se1, reversible effect in the Se2 (the counteractive response decreased and then increased) and irreversible effect in the Se3 group. In conclusion, Siberian sturgeon accumulates Se in tissues with a dose-response relationship, particularly for liver and kidney. The distribution order of selenium in tissues was liver > kidney > muscle. This result is lined up with the findings achieved on the antioxidant markers evaluated in both tissues, in which a dose-response relationship for biomarkers was clearly recorded in liver. Among all biochemical indicators herein considered catalase and glyoxalase II were both overwhelmed by the highest selenium concentrations, while the other markers and mainly glutathione peroxidase activity in Siberian sturgeon provided an active defense role against the oxidative stress induced by selenium exposure. Our current results suggest that the Se-Cys dietary dose for which can be excluded toxic effects in Siberian sturgeon after a 60 d exposure period should be lower than 20 mg Se kg^{-1} .

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Table 1
MDA and total glutathione in liver and kidney of sturgeon.

	MDA	GSH + 2GSSG
Liver		
C T_{30}	73.22 ± 20.57	316.50 ± 56.59
Se_1 T_{30}	69.08 ± 10.78	259.50 ± 87.22
Se_2 T_{30}	79.73 ± 14.88	345.90 ± 73.82
Se_3 T_{30}	95.66 ± 17.73	296.50 ± 83.77
C T_{60}	80.48 ± 15.62*	341.40 ± 84.17
Se_1 T_{60}	127.80 ± 31.40*	363.80 ± 87.60
Se_2 T_{60}	116.50 ± 25.42	317.40 ± 85.98
Se_3 T_{60}	102.40 ± 28.92	281.10 ± 74.77
Kidney		
C T_{30}	29.42 ± 12.40	411.90 ± 37.70*
Se_1 T_{30}	29.03 ± 14.46	401.70 ± 99.76
Se_2 T_{30}	32.21 ± 10.63	395.10 ± 98.57
Se_3 T_{30}	46.31 ± 15.36	630.30 ± 88.80*
C T_{60}	24.66 ± 8.18	467.30 ± 38.40
Se_1 T_{60}	21.75 ± 11.61	547.50 ± 97.01
Se_2 T_{60}	34.67 ± 6.79	498.10 ± 67.11
Se_3 T_{60}	28.02 ± 5.69	566.10 ± 82.72

$C = 0.32$ mg kg^{-1} (control), $Se_1 = 1.25$ mg kg^{-1}, $Se_2 = 5$ mg kg^{-1}, $Se_3 = 20$ mg kg^{-1}. The results are reported as mean and standard deviation. Number of specimens for each experimental group = 10. The symbol (*) indicates significant statistical differences between treated samples (Se_1, Se_2, Se_3) and owns controls at different endpoints (30, 60 d) ($P < 0.05$).

Table 2
Antioxidant enzymes activity in liver and kidney of sturgeon.

	CAT	GST	GI	GII
Liver				
C T_{30}	148.30 ± 13.79*	176.80 ± 28.80	426.40 ± 104.0	84.12 ± 17.74*
Se_1 T_{30}	116.70 ± 23.27	221.10 ± 63.23	346.50 ± 61.68	61.12 ± 17.36
Se_2 T_{30}	106.50 ± 34.49	225.40 ± 58.64	352.60 ± 64.65	47.71 ± 15.92*
Se_3 T_{30}	85.90 ± 26.13*	237.80 ± 66.86	420.70 ± 98.28	42.59 ± 7.29*
C T_{60}	139.70 ± 32.84	187.10 ± 32.63	335.70 ± 96.48	87.12 ± 16.47*
Se_1 T_{60}	112.60 ± 24.15	209.3 ± 59.64	456.20 ± 77.67	106.80 ± 24.53
Se_2 T_{60}	104.60 ± 24.06	194.20 ± 42.55	425.40 ± 77.73	127.40 ± 21.60*
Se_3 T_{60}	100.10 ± 20.41	204.20 ± 61.56	430.60 ± 79.94	101.90 ± 23.97
Kidney				
C T_{30}	34.11 ± 6.65	88.37 ± 20.67*	175.10 ± 24.47	30.29 ± 4.97
Se_1 T_{30}	34.18 ± 6.01	150.20 ± 41.97*	192.00 ± 28.80	33.20 ± 4.25
Se_2 T_{30}	30.59 ± 12.14	147.10 ± 39.89	186.90 ± 33.92	23.36 ± 5.65
Se_3 T_{30}	35.06 ± 7.79	130.30 ± 49.58	209.50 ± 39.79	25.71 ± 2.23
C T_{60}	28.82 ± 10.72	74.30 ± 16.10	187.30 ± 25.85	29.21 ± 9.32
Se_1 T_{60}	26.57 ± 8.88	74.83 ± 25.65	247.90 ± 39.81	28.25 ± 5.89
Se_2 T_{60}	29.81 ± 9.83	75.88 ± 22.09	239.10 ± 44.78	26.74 ± 6.23
Se_3 T_{60}	35.67 ± 8.71	78.78 ± 26.29	254.90 ± 49.36	35.48 ± 11.04

$C = 0.32$ mg kg^{-1} (control), $Se_1 = 1.25$ mg kg^{-1}, $Se_2 = 5$ mg kg^{-1}, $Se_3 = 20$ mg kg^{-1}. The results are reported as mean and standard deviation. Number of specimens for each experimental group = 10. The symbol (*) indicates significant statistical differences between treated samples (Se_1, Se_2, Se_3) and owns controls at different endpoints (30, 60 d) ($P < 0.05$).

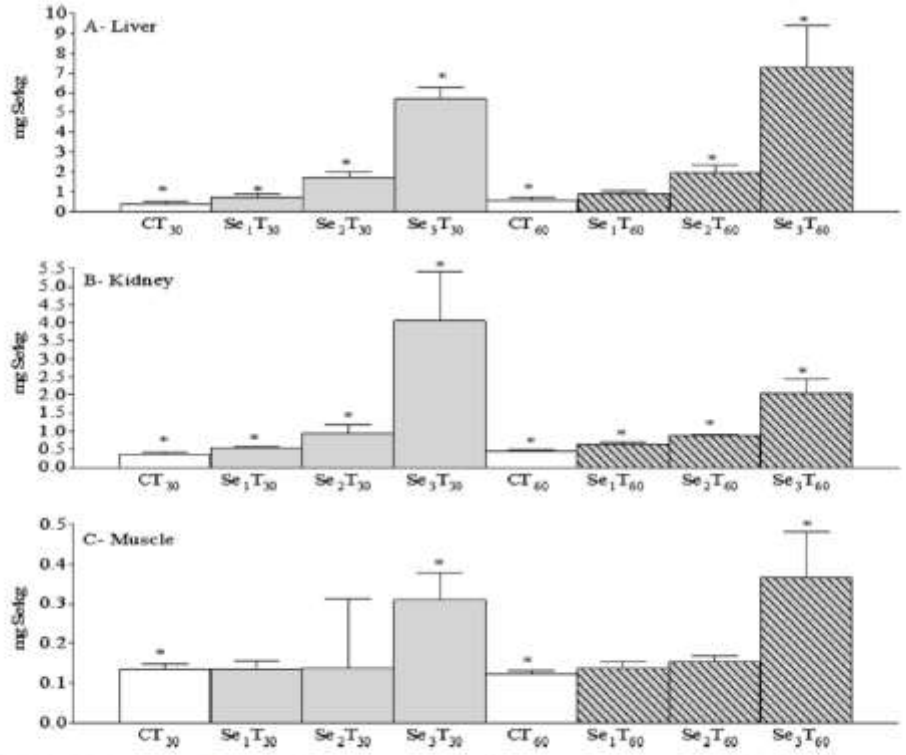

Fig. 1. Selenium levels in liver (A), kidney (B) and muscle (C) of sturgeon. C = 0.32 mg kg^{-1}; Se_1 = 1.25, Se_2 = 5, Se_3 = 20 mg kg^{-1}. The results are reported as geometric mean and 95% confidence intervals. The symbol (*) indicates significant statistical differences between treated samples (Se_1, Se_2, Se_3) and owns controls at 30 and 60 d ($P < 0.05$).

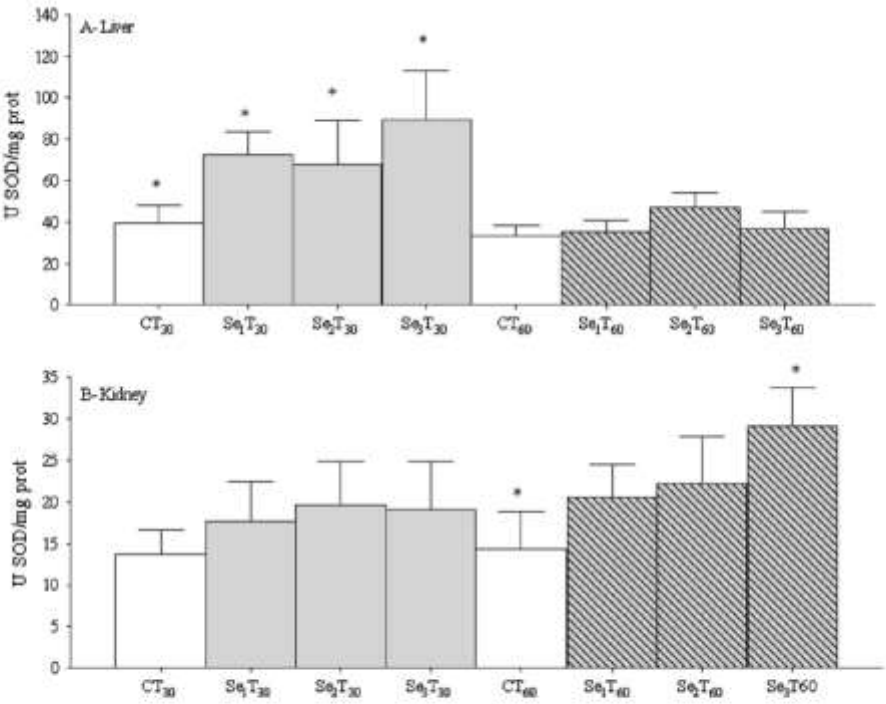

Fig. 2. Superoxide dismutase activity in liver (A) and kidney (B) of sturgeon. C = 0.32 mg kg^{-1}; Se_1 = 1.25, Se_2 = 5, Se_3 = 20 mg kg^{-1}. The results are reported as geometric mean and 95% confidence intervals for liver and as mean and standard deviation for kidney. The symbol (*) indicates significant statistical differences between treated samples (Se_1, Se_2, Se_3) and owns controls at different endpoints (30, 60 d) ($P < 0.05$).

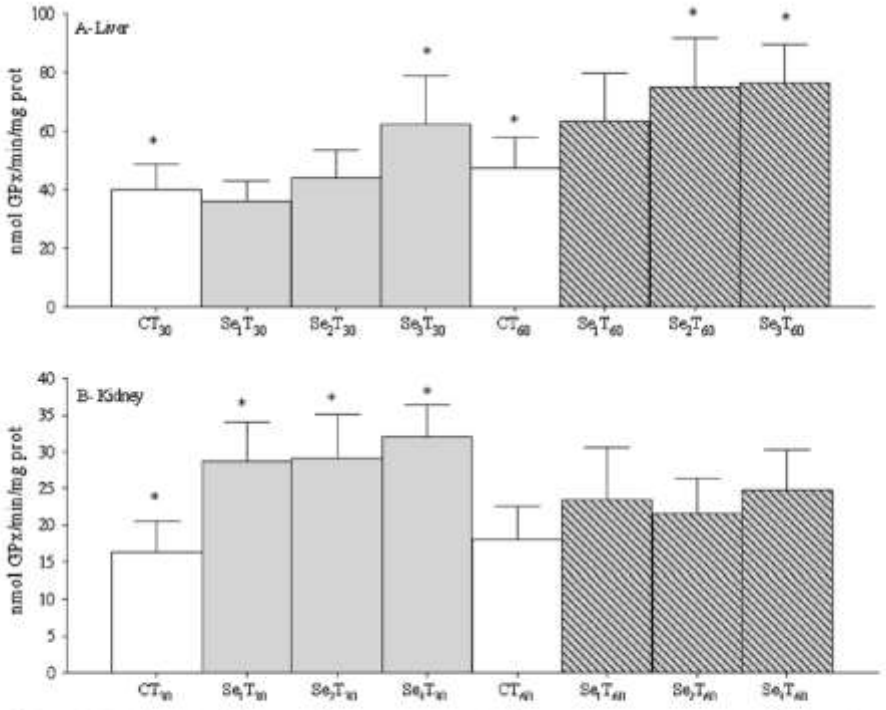

Fig. 3. Glutathione peroxidase activity in liver (A) and kidney (B) of sturgeon. $C = 0.32$ mg kg^{-1}; $Se_1 = 1.25$, $Se_2 = 5$, $Se_3 = 20$ mg kg^{-1}. The results are reported as geometric mean and 95% confidence intervals for liver and as mean and standard deviation for kidney. The symbol (*) indicates significant statistical differences between treated samples (Se_1, Se_2, Se_3) and own controls at different endpoints (30, 60 d) ($P < 0.05$).

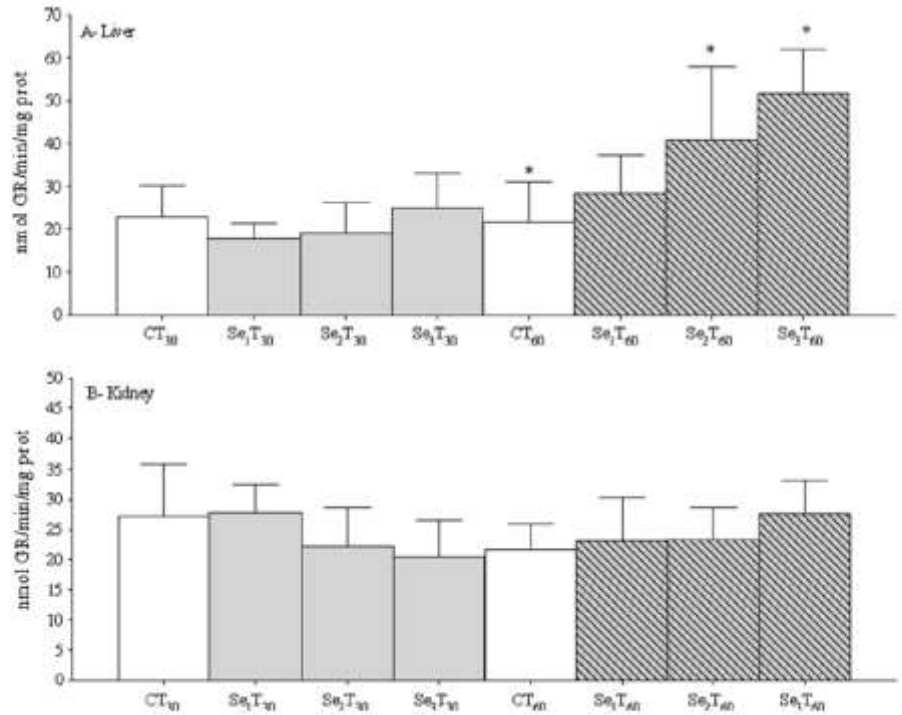

Fig. 4. Glutathione reductase activity in liver (A) and kidney (B) of sturgeon. $C = 0.32$ mg kg^{-1}; $Se_1 = 1.25$, $Se_2 = 5$, $Se_3 = 20$ mg kg^{-1}. The results are reported as geometric mean and 95% confidence intervals for liver and as mean and standard deviation for kidney. The symbol (*) indicates significant statistical differences between treated samples (Se_1, Se_2, Se_3) and owns controls at different endpoints (30, 60 d) ($P < 0.05$).