



Contents lists available at ScienceDirect

Animal Nutrition

journal homepage: <http://www.keaipublishing.com/en/journals/aninu/>

KeAi
ADVANCING RESEARCH
EVOLVING SCIENCE

Original research article

Responses of heat shock protein 70 and caspase-3/7 to dietary selenomethionine in juvenile white sturgeon

Weifang Wang^a, Seunghyung Lee^b, Silas S.O. Hung^c, Dong-Fang Deng^{b,*}

^a Qingdao Key Laboratory of Marine Fish Breeding and Biotechnology, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, China

^b School of Freshwater Sciences, University of Wisconsin-Milwaukee, Milwaukee 53204, USA

^c Department of Animal Science, University of California, Davis 95616-8521, USA

ARTICLE INFO

Article history:

Received 11 January 2016

Received in revised form

19 February 2016

Accepted 23 February 2016

Available online 15 March 2016

Keywords:

Apoptosis

Selenium toxicity

Stress protein

White sturgeon

ABSTRACT

An 8-week feeding trial was conducted to investigate the responses of juvenile white sturgeon (*Acipenser transmontanus*) to elevated dietary selenium (Se) based on the determination of the RNA/DNA ratio in muscle, heat shock protein 70 (Hsp70), and caspase-3/7 in muscle and/or liver tissues. Four semi-purified test diets were prepared by adding different levels of L-selenomethionine (0, 50, 100, and 200 mg/kg diet). The analytical determinations of total Se were 2.2, 19.7, 40.1, and 77.7 mg/kg diet. The sturgeon (initial body weight: 30 ± 2 g; mean ± SEM) were raised in indoor tanks provided with flow through freshwater (18–19 °C). There were three replicates for each dietary treatment with 25 fish per replicate. The liver and muscle tissues were collected at 4 and 8 weeks after feeding the test diets. A significant interaction between duration and levels of dietary Se exposures on RNA/DNA ratio in the muscle tissue was detected ($P < 0.05$). Although there was no significant main effect due to the duration of dietary Se exposures (i.e., 4 weeks versus 8 weeks) on muscle RNA/DNA ratio ($P \geq 0.05$), the ratio was significantly decreased with increasing dietary Se levels. Significant main effects were caused by the duration and levels of dietary Se exposures on Hsp70 in both the muscle and liver tissues, with significant increases in Hsp70 due to a longer exposure (8 weeks) and higher levels (40.1 and 77.7 mg Se/kg diet) of dietary Se. The caspase-3/7 activity in the liver were significantly higher in fish fed the diets containing 40.1 and 77.7 mg Se/kg diet than those fed the other diets. The toxic thresholds of Se in the muscle were estimated to be 32.2 and 26.6 mg Se/kg for the depressed specific growth rate and the induced Hsp70 response in muscle, respectively. This result indicated that the Hsp70 response in muscle is a more sensitive biomarker than the SGR of sturgeon for evaluating Se toxicity in white sturgeon. Results of the current study suggest that a mechanism involved with the activation of stress protein production and apoptosis protects white sturgeon from the lethal effect of Se.

© 2016, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Selenium (Se) is an essential nutrient and also a toxic trace element to all vertebrates, including fish, with a narrow margin between dietary essentiality and toxicity (Maier and Knight, 1994;

Hamilton, 2004). At normal dietary levels, Se is a component of glutathione peroxidase, which protects membranes and tissues against oxidative stress (NRC, 2011). At higher exposure levels, Se can induce oxidative damage or substitute sulfur during protein synthesis to form Se-contained protein analogs that change the structures or functions of normal proteins (Lemly, 1997; Spallholz and Hoffman, 2002).

Heat shock protein 70 (Hsp70) is the most conserved protein in the stress protein family and it is proposed as a specific indicator or biomarker of cellular stress (Stregeman et al., 1992; Sanders, 1993; Basu et al., 2002). Functioning as a molecular chaperon, Hsp70 plays a very important role in stabilizing cellular proteins to maintain cell integrity under stressful conditions, including the fluctuation of temperature, exposure to heavy metals, handling

* Corresponding author.

E-mail address: dengd@uwm.edu (D.-F. Deng).

Peer review under responsibility of Chinese Association of Animal Science and Veterinary Medicine.



Production and Hosting by Elsevier on behalf of KeAi

<http://dx.doi.org/10.1016/j.aninu.2016.02.001>

2405-6545/© 2016, Chinese Association of Animal Science and Veterinary Medicine. Production and hosting by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

stress, and alternation of feeding (Cara et al., 2005; Yengkokpam et al., 2008; Deng et al., 2009; Han et al., 2012; Wang et al., 2013; Zheng et al., 2015).

Apoptosis, a biological process of programmed cell death, is a tightly regulated mechanism to remove unwanted cells or damaged cells of an organism. It is mediated by a series of caspases, which are traditionally categorized into initiator caspases and effector caspases (Hengartner, 2000). Activation of caspase-3/7, which is primary effectors of apoptosis, will lead to irreversible commitment of a cell to apoptosis. Therefore, caspase-3/7 is considered a reliable marker for cells undergoing apoptosis. Although there is still very limited information available for understanding the protection mechanism for fish from Se toxicity, the influence of dietary Se on stress protein induction and apoptosis activation have been demonstrated in different research models, including myelodysplasia cell line (Gonçalves et al., 2013), isolated hepatocyte of rainbow trout (*Oncorhynchus mykiss*, Misra and Niyogi, 2009; Misra et al., 2012), and whole animals (Kaushal and Bansal, 2007, 2009; Hu et al., 2016).

White sturgeon is an aquaculture species in California with exceptional biological, commercial, and ecological value (Moyle, 2002). Listed as a species of concern, this ancient ray-finned fish is indigenous to the Pacific West Coast of North America (Moyle, 2002; NMFS, 2006). High concentrations of Se have been detected in their tissues with 30 and 15 mg Se/kg dry weight in liver and muscle tissues, respectively (Linville et al., 2002). These Se concentrations are well above the liver (12 mg Se/kg dry weight) and muscle (8 mg Se/kg dry weight) thresholds shown to cause toxic effects in freshwater and anadromous fish (Lemly, 2002). Recent studies also suggest that juvenile white sturgeon seem to be less sensitive to Se toxicity than other species of fish. For example, a toxic threshold that inhibited the growth of juvenile white sturgeon was 10–20 mg Se/kg diet, and whole body concentration was up to 34.4 mg Se/kg without any mortality (Tashjian et al., 2006). These threshold concentrations are much higher than most proposed dietary threshold levels (3–5 mg Se/kg diet; Lemly, 2002; Hamilton, 2004), and the whole body concentration threshold for mortality of other species of fishes (4.3–5.4 mg Se/kg, Hamilton et al., 1990; Cleveland et al., 1993). Furthermore, no mortality was observed in juvenile white sturgeon with a whole body concentration up to 47 mg Se/kg, but a lower concentration (27.8 mg Se/kg whole body) has caused a significant mortality in juvenile green sturgeon (*Acipenser medirostris*) (De Riu et al., 2014). A lower effective concentration has been determined for other species of fish, such as 5 mg Se/kg for rainbow trout (*Salmo gairdneri*, Hilton et al., 1980), 7.9 mg Se/kg for bluegill (*Lepomis macrochirus*, Lemly, 1993) and 5.4–6.1 mg Se/kg for razorback sucker (*Xyrauchen texanus*; Hamilton, 2001a, 2001b).

With the highly toxic thresholds of Se observed in white sturgeon, we hypothesize that this fish may have a better capacity to combat against Se toxicity. However, the mechanism for this to occur is yet to be understood. In the current study, we assume that the high tolerance of juvenile white sturgeon to Se toxicity may be partially related to their active protective mechanisms involved with stress protein production and apoptosis activity. Therefore, the objective of the current study was to determine the chronic effects of excess levels of dietary Se on the responses of Hsp70 levels and caspases-3/7 activities in juvenile white sturgeon.

2. Materials and methods

2.1. Experimental diets

Four semi-purified test diets (Table 1) were formulated by adding different concentrations of L-selenomethionine (SeMet,

Table 1
Formulation of the semi-purified test diets.

Ingredient ¹	Content, g/kg
Vitamin-free casein	310
Wheat gluten	150
Egg albumin	40
Dextrin	295
Corn oil	60
Cod liver oil	60
Cellufil hydrolyzed	40
Vitamin premix ²	10
BTM mineral premix ³	30
Choline chloride	5
Santoquin	0.2

¹ All of the ingredients were purchased from the USB Biochemical Corp. (Cleveland, OH, USA) except for the vitamin premix, which was purchased from ICN Nutritional Biochemicals (Aurora, OH, USA).

² The vitamin premix contained the following ingredients (g/kg premix): thiamine mononitrate (1.5), riboflavin (2.5), niacin (20), calcium pantothenate (5), pyridoxin HCl (2), vitamin B₁₂ (0.02), folic acid (1), biotin (0.15), inositol (50), ascorbic acid (50), para-amino benzoic acid (30), butylated hydroxyanisole (9), menadione sodium bisulfite (0.125), vitamin A acetate (500,000 units/g) (0.8), vitamin D (400,000 units/g) (0.6), vitamin E acetate (250 units/g) (48).

³ Bernhart and Tomarelli modified (BTM) mineral premix composition was prepared as described (Bernhart and Tomarelli, 1966).

Fisher Scientific, Pittsburgh, PA, USA; >99% purity). Dietary SeMet (0, 50, 100, and 200 mg/kg diet) were dissolved in distilled water before they were added to the semi-purified test diets. The total Se (molecular mass, 78.96) in SeMet is 40.26% of SeMet (molecular mass, 196.1). The final Se concentration in each diet was analyzed using the method described by Huang et al. (2012) and they were 2.2 ± 0.2 , 19.7 ± 0.6 , 40.1 ± 1.5 , and 77.7 ± 3.6 mg Se/kg for the 0, 50, 100, and 200 mg/kg diets, respectively. The analyzed Se concentrations in the diets were expressed as total Se levels, and the recovery rates of total Se in all the diets were greater than 88%. Proximate composition of the diets analyzed following the methods of AOAC (2000) was (% as fed): moisture, 8.0; protein, 42.8; lipid, 12.6; ash, 3.3, and gross energy 501.2 kcal/100 g diet.

2.2. Fish maintenance

Juvenile white sturgeon (initial body weight of 30 ± 2 g; mean \pm SEM) obtained from a local sturgeon farm (Sacramento, CA, USA) were randomly distributed into twelve 90-L circular fiberglass tanks, resulting in 25 fish per tank and 3 replicate tanks per treatment. The fish were conditioned to the semi-purified test diet without supplementation of SeMet for one week before they were fed the test diets. The protocols regarding fish maintenance and sample collection had been described by De Riu et al. (2014). Fish were batch weighed weekly, and feed rations were adjusted to 3.0% body weight per day (BW/d) for the first four weeks and 2.0% BW/d for the second four weeks. Feeding was managed using automatic feeders (Cui et al., 1997), and each tank was supplied with flow-through aerated well water (3 L/min). Water temperature, pH, and dissolved oxygen were measured daily and pH was monitored once a week during the feeding trial and they were 18–19 °C, 7–8, and 7–9 mg/L, respectively. The feeding trial followed a protocol approved by the Campus Animal Care and Use Committee at the University of California, Davis, USA.

2.3. Sample collection and analysis

White sturgeon juveniles were deprived of feed for 24 h prior to sampling. At the end of 4 and 8 weeks of exposure, three fish were randomly removed from each tank and euthanized in a tricaine

methanesulfonate solution (0.5 g/L, Argent Chemical Laboratories, Redmount, WA, USA). The liver and white muscle tissues (a cubical section 2 cm in length at the midpoint of the body) of each fish were dissected, and the resultant samples were pooled for each tank. The tissue samples were rinsed in double-distilled water before they were frozen in liquid nitrogen and stored at -80°C until ready for use. The frozen tissue was ground via a mortar and pestle, which was maintained in a cold condition with liquid nitrogen, and the homogeneous powder was subsampled for the subsequent analyses.

2.3.1. Determination of the RNA/DNA ratio in the muscle

Nucleic acids in the white muscle tissue were measured following the method described by Caldarone et al. (2001). In brief, muscle powder was vortexed with 1% sarcosyl Tris–EDTA buffer, followed by dilution with Tris–EDTA buffer to separate dissociated proteins from the nucleic acids. Fluorophore ethidium bromide was used to measure the total nucleic acids and RNase was then added to differentiate RNA from DNA. Standard curves for DNA and RNA were established from using ultrapure calf thymus DNA and 18S-rRNA. All of the chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA), except for the ethidium bromide, which was obtained from Fisher Scientific (Pittsburgh, PA, USA). The fluorescence activity was read at an excitation wavelength of 525 nm and an emission wavelength of 600 nm in a Spectra Max M2 micro plate reader (Molecular Devices Corporation, Sunnyvale, CA, USA).

2.3.2. Measurement of Hsp70 in muscle and liver tissues

Responses of Hsp70 in the muscle and liver tissues were measured by Western blotting following the method described by Deng et al. (2009). Briefly, 25 μg of protein of the tissue samples and Hsp70 standard (4 μg) (SPP-758; Assay Designs Inc., Ann Arbor, MI, USA) were loaded into a gel and run by one-dimensional SDS-PAGE. The separated proteins were then electroblotted onto a membrane (Millipore, Bedford, MA, USA). The membrane was incubated with the primary polyclonal Hsp70 antibody (Assay Designs Inc.) and then with the secondary antibody of peroxidase labeled anti-rabbit IgG (Amersham Biosciences, Piscataway, Pittsburgh, PA, USA). The antibody was detected using enhanced chemiluminescence reagents (Amersham Biosciences) and the membrane was exposed to hyperfilm (Amersham Biosciences). The band density on each film was quantified using a GS-710 calibrated imaging densitometer (Bio-Rad, Hercules, CA, USA). The relative band density was calculated by comparing the band density of each sample with the Hsp70 standard on the same film.

2.3.3. Determination of caspase-3/7 in the liver tissue

Caspase-3/7 activities in the liver tissues were determined using an Apo-ONE homogeneous Caspase-3/7 Assay kit (Promega Corporation, Madison, WI, USA) following the description by Deng et al. (2010). Frozen liver powder was homogenized in ice-cold T-PER tissue protein extraction reagent (Pierce, Rockford, IL, USA) containing a complete protease inhibitor cocktail (Roche Applied Science, Indianapolis, IN, USA) and phenylmethylsulfonyl fluoride (1.0 mmol/L, Sigma–Aldrich Corporate, St. Louis, MO, USA). The homogenate was centrifuged ($14,000 \times g$ at 4°C) for 10 min, and the separated supernatant was used to determine the protein concentration by an improved Lowry method (Bio-Rad, DC Protein Assay kit, Hercules, CA, USA). Fluorescence activity was determined at an excitation wavelength of 485 nm and an emission wavelength of 530 nm on a Spectra Max M2 micro plate reader (Molecular Devices Corporation, Sunnyvale, CA, USA). The amount of fluorescent product generated was proportional to the amount of caspase-3/7 cleavage activity presented in the test sample. The caspase

activity was expressed as a fluorescent reading per mg tissue protein per min.

2.4. Statistical analysis

All data were presented as the mean \pm SEM ($n = 3$). The data were square-root or log transformed and checked for violations of the assumptions for the analysis of variance (ANOVA). The data were subjected to a two-way ANOVA to determine if there were significant effects by feeding duration (weeks) and dietary Se concentration or their interactions. When the ANOVA results were significant ($P < 0.05$), differences among the means were determined by the Fisher LSD test. The data analyses were conducted using Statistica software (Version 9, StatSoft, Tulsa, OK, USA).

The samples collected for the current study were generated from the same feeding trial, which was published by De Riu et al. (2014). We used the data of specific growth rate (SGR; %/d) and muscle Se concentration published by De Riu et al. (2014) to run regression analyses with the parameters measured in the current study. Datasets were fitted to a model using a nonlinear least-squares (NLS) function from the standard library of R (R Core Team, 2015). The model describes a single breakpoint, which is the intersection of a straight line and another straight line with a positive or negative slope. The breakpoint represents a threshold level of Se in the muscle tissue. The model is written as

$$y = \beta_0 - \beta_1(\beta_2 - x) + \beta_3(x - \beta_2),$$

where $(x - \beta_2)$ is defined as zero at values of $x < \beta_2$, and $(\beta_2 - x)$ is defined as zero when $x \geq \beta_2$. The β_0 , β_1 , β_2 and β_3 were defined as the asymptote of the first segment, slope of the first segment, breakpoint (= a threshold level), and the slope of the second segment, respectively. More detailed information about the model can be found in Lee et al. (2014).

3. Results

A significant interaction between duration and levels of dietary Se exposures on RNA/DNA ratio in muscle tissue was detected ($P < 0.05$; Table 2). Although there was no significant main effect ($P \geq 0.05$) on the muscle RNA/DNA ratio due to Se exposure duration (4 weeks versus 8 weeks), the levels of dietary Se significantly decreased the ratio in fish fed the diets containing 40.1 and 77.7 mg Se/kg diet.

Significant main effects were detected on the Hsp70 response in both the muscle and liver tissues due to the exposure duration and dietary Se levels (Table 2). An 8 weeks of exposure induced significantly higher Hsp70 than did a 4 weeks of exposure. The responses of Hsp70 in both tissues were significantly induced in fish fed the diets containing ≥ 40.1 mg Se/kg compared with those fed 2.2 mg Se/kg diet, but were similar in the fish fed diets containing 19.7 and 40.1 mg Se/kg.

There was a significant main effect of dietary Se levels on caspase-3/7 activity in the liver (Table 2). The activities were significantly higher in sturgeon fed the diets with ≥ 40.1 mg Se/kg than those fed the diets with ≤ 19.7 mg Se/kg. The overall activity of caspase-3/7 tended to be higher at 4 than 8 weeks, but the difference was not statistically significant.

Based on a nonlinear least-squares analysis on the datasets of SGR and muscle Hsp70 at the end of 4-week's feeding, the responses of SGR and Hsp70 in response to the muscle Se concentrations were fitted to a two-slope straight broken-line regression model and a linear regression model, respectively [Fig. 1, SGR

Table 2
The RNA/DNA ratio, heat shock protein 70 (Hsp70) and caspase-3/7 in muscle and/or liver of juvenile white sturgeon, measured at the end of 4- or 8-week feeding with different levels of dietary selenium (Se) in the form of L-selenomethionine.

Feeding duration	Dietary Se, mg/kg	Muscle RNA/DNA ratio	Muscle Hsp70, % relative	Liver Hsp70, % relative	Liver caspase-3/7 activity, mg protein/min
Individual treatment means¹					
4 weeks	2.2	3.1 ^a	3.9	13.5	166.0
4 weeks	19.7	2.8 ^a	5.3	9.4	173.7
4 weeks	40.1	1.9 ^c	4.6	15.4	300.1
4 weeks	77.7	1.5 ^c	7.5	16.5	384.3
8 weeks	2.2	2.6 ^{ab}	11.4	15.6	139.8
8 weeks	19.7	2.7 ^a	10.7	15.6	191.7
8 weeks	40.1	2.3 ^b	16.9	33.3	253.1
8 weeks	77.7	1.8 ^c	21.7	62.2	269.0
Pooled SE		0.14	0.94	6.33	43.35
Means of main effect					
4 weeks		2.3	5.3 ^B	13.7 ^B	256.0
8 weeks		2.3	15.2 ^A	31.7 ^A	213.4
	2.2	2.8 ^a	7.7 ^c	14.5 ^{bc}	152.9 ^b
	19.7	2.8 ^a	8.0 ^{bc}	12.5 ^c	182.7 ^b
	40.1	1.9 ^b	10.7 ^b	24.4 ^{ab}	276.6 ^a
	77.7	1.7 ^b	14.6 ^a	39.4 ^a	326.6 ^a
Two-way ANOVA, P-value					
Duration		0.223	0.000	0.000	0.202
Dietary Se levels		0.000	0.000	0.006	0.001
Interaction		0.005	0.081	0.122	0.568

¹ The treatment means represent the average values of three tanks per treatment. No mean separation test was conducted for Hsp70 and caspase-3/7 because there was no significant interaction ($P > 0.05$) between duration and levels of dietary Se exposure.

^{A,B,a-c} Upper and lower case letters indicate significant differences within duration and levels of dietary Se exposure, respectively (Fisher's LSD procedure).

(%/d) = 2.7102 - 0.0190(22.2372 - x) - 0.1016(x - 22.2372), $R^2 = 0.8599$; $P > 0.05$; Hsp70 = 2.6482 + 0.1018x, $R^2 = 0.5072$; $P < 0.01$]. A two-slope straight broken-line regression model (Fig. 2)

was found to be the best model to describe the correlation between the muscle Se concentrations and the responses of SGR and muscle Hsp70 at the end of the 8-week feeding:

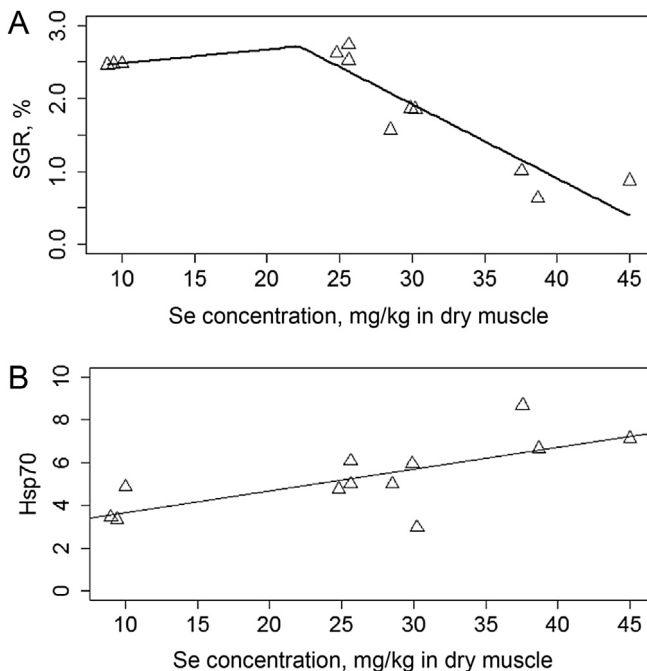


Fig. 1. A two-slope straight broken-line regression model was fitted to the dataset of specific growth rate (SGR; A), and a linear regression model was fitted to the dataset of heat shock protein 70 (Hsp70; B) in response to the Se concentrations in the dry muscle tissue of white sturgeon fed the test diets for 4 weeks. The equation for the SGR dataset (A) is $SGR (\%/d) = 2.7102 - 0.0190(22.2372 - x) - 0.1016(x - 22.2372)$ ($R^2 = 0.8599$; $P > 0.05$), where $(x - 22.2372)$ is defined as zero at values of $(x < 22.2372)$, and $(22.2372 - x)$ is defined as zero when $x \geq 22.2372$. The equation for the Hsp70 dataset (B) is $Hsp70 = 2.6482 + 0.1018x$ ($R^2 = 0.5072$; $P < 0.01$). The x represents Se concentration (mg/kg) in dry muscle tissue of white sturgeon.

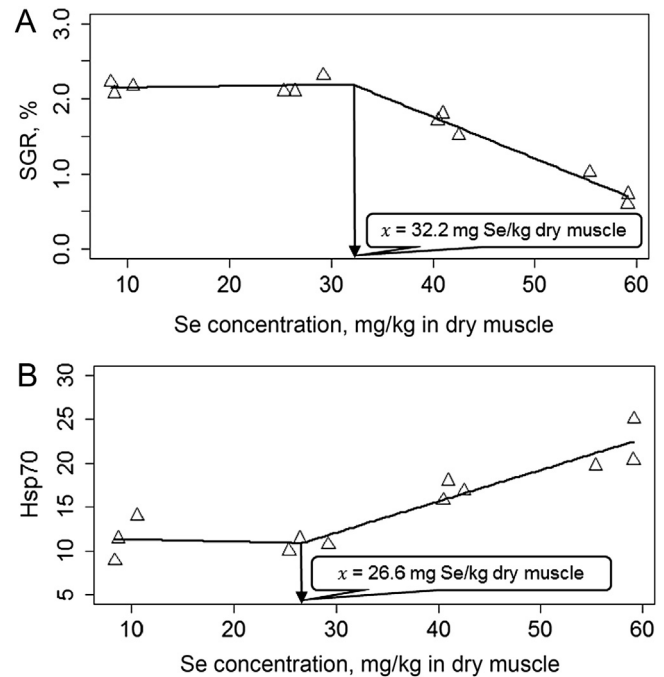


Fig. 2. A two-slope straight broken-line regression model was fitted to datasets of specific growth rate (SGR; A) and heat shock protein 70 (Hsp70; B) in response to the Se concentrations in the dry muscle tissue of white sturgeon fed the test diets for 8 weeks. The equation for the SGR (A) is $SGR (\%/d) = 2.1881 - 0.0019(32.1504 - x) - 0.0550(x - 32.1504)$ ($R^2 = 0.9785$; $P < 0.001$), where $(x - 32.1504)$ is defined as zero at the values of $(x < 32.1504)$ and $(32.1504 - x)$ is defined as zero when $x \geq 32.1504$. The equation for the Hsp70 dataset (B) is $Hsp70 = 10.8685 + 0.025(26.5728 - x) + 0.3561(x - 26.5728)$ ($R^2 = 0.8796$; $P < 0.001$), where $(x - 26.5728)$ is defined as zero at the values of $(x < 26.5728)$ and $(26.5728 - x)$ is defined as zero when $x \geq 26.5728$. The x represents the Se concentration (mg/kg) in dry muscle tissue.

$$\begin{aligned} \text{SGR (\%/d)} &= 2.1881 - 0.0019(32.1504 - x) \\ &\quad - 0.0550(x - 32.1504), \\ R^2 &= 0.9785; P < 0.001; \end{aligned}$$

$$\begin{aligned} \text{Hsp70} &= 10.8685 + 0.025(26.5728 - x) \\ &\quad + 0.3561(x - 26.5728), \\ R^2 &= 0.8796; P < 0.001. \end{aligned}$$

A toxic threshold level based on the 8-week feeding was determined to be 32.2 and 26.6 mg Se/kg dry muscle for the depressed SGR and the induction of Hsp70, respectively (Fig. 2).

4. Discussion

The results of the current study suggest that part of the reasons for the depressed growth of sturgeon presented by De Riu et al. (2014) fed the high doses of dietary Se is due to the adverse effect of Se on the protein synthesis measured by the RNA/DNA ratio in muscle tissue. It is also probably that extra energy might be required for the protection of sturgeon from Se toxicity by activation of stress protein production and apoptosis activities (i.e., physiological trade-offs) and thus less energy were used for their growth.

The ratio of RNA/DNA has been used to estimate the recent growth or nutritional condition of larval or juvenile fishes (Tanaka et al., 2008; Deng et al., 2010; Han et al., 2012). The application of this biomarker is based on the rationale that the quantity of DNA in a cell is normally stable but the quantity of RNA related to protein synthesis varies under different conditions. Thus, a decrease in the RNA/DNA ratio in the current study indicates that protein synthesis was decreased in the muscle of sturgeon fed a diet with excessive Se. However, there was no significant difference in the protein contents of whole fish collected from the same dietary Se-exposure trial (De Riu et al., 2014). This may be due to the different types of tissue (whole body versus muscle) and different measurements (crude protein and RNA/DNA ratio) used between the two studies. First, the crude protein content was measured based on the total nitrogen level, which is multiplied by a factor of 6.25. Thus, the result of the crude protein levels may not necessarily represent a true protein content of the fish if the sample contains other nitrogenous compounds, such as ammonia, urea, and nucleic acids. Second, muscle is a tissue primarily containing protein besides moisture and lipid, a major reservoir for protein storage in fish. Selenomethionine, which can substitute methionine during protein synthesis, will mainly end up in muscle tissue. Therefore, protein synthesis in muscles would be more responsive to dietary Se. Instead of measuring whole-fish protein content, an index for protein synthesis or protein content per se in the muscle tissue would be more reliable as a biomarker for evaluating the effect of dietary Se toxicity.

Selenomethionine could cause protein malformation during protein synthesis or induce the production of reactive oxygen species, which can result in cell damage as found in fish or isolated hepatocytes of rainbow trout (Hoffman, 2002; Misra et al., 2012). Thus, the expression of Hsp70 may play critical roles in repairing denatured protein or maintaining the integrity of cells stressed by an overdose of Se. This is supported by the up-regulation of Hsp70 in response to increasing dietary Se in the current study. The regulation was shown to be correlated differently with exposure duration and dietary Se levels. The Hsp70 response in the muscle of sturgeon was shown to be very sensitive to the different Se concentrations during the earlier exposure (4 weeks) because the response was linearly correlated with the Se concentration in the

muscle tissue of sturgeon. With the Se exposure being extended to the end of 8-week feeding, the Hsp70 inductions became less responsive to the muscle Se concentrations when the muscle Se concentrations were <26.6 mg/kg. This result indicated that there might be an adaptive mechanism developed in the sturgeon to protect them from Se toxicity when the Se accumulation in muscle is relatively low. When Se accumulations in the muscle reached the levels of ≥ 26.6 mg/kg, Hsp70 was significantly stimulated, suggesting an active protection by Hsp70 was involved and Se burden in the muscle tissues did not overwhelm the capacity of Hsp70 protection. However, the production and functioning of stress proteins are energy dependent processes (Mallouk et al., 1999) and probably impose costs on sturgeon exposed to the overdose of Se by influencing their energy distribution among the different physiological functions. This can partially explain why depressed growth, low energy storage, and poor swimming activity were recorded in juvenile white sturgeon fed the high Se diet (Tashjian et al., 2006; De Riu et al., 2014).

Induction of Hsp70 to protect fish from Se toxicity was recently reported in yellow catfish (*Pelteobagrus fulvidraco*) fed an overdose of Se in the form of Na_2SeO_3 (Hu et al., 2016). However, there is still a paucity of studies investigating the dose response of Hsp70 to dietary Se and the exact mechanism of how Hsp70 functions in the Se-stressed fish. It is possible that Hsp70 protects the stressed organism by stabilizing denatured proteins or by shifting the response from necrosis to apoptosis to avoid mortality. Our current preliminary results are limited in providing a conclusive explanation on the protective mechanism of Hsp70 in juvenile white sturgeon. This is an issue that will warrant future investigations.

The mechanisms of Se-induced apoptosis are associated with the chemical forms of Se and have been extensively studied in human cancer therapy (Kerr et al., 1972; Sanmartín et al., 2012). Selenium has been found to be involved with apoptosis through activation of caspase, modulation of mitochondrial functions of glutathione, and increasing reactive oxygen species levels (Misra et al., 2012; Sanmartín et al., 2012). Although very limited information is available on Se-induced apoptosis in fish, different mechanisms have been proposed. Previous studies on isolated hepatocytes of rainbow trout suggest that both organic and inorganic forms of Se can induce apoptosis through the pathway of caspase activation (Misra and Niyogi, 2009; Misra et al., 2012). A different mechanism in yellow catfish was proposed by Hu et al. (2016) such that an overdose of dietary Se increased cytoplasmic free- Ca^{2+} , which triggered cell apoptosis in the fish. In the current study, an increase of caspase-3/7 activity in juvenile white sturgeon in response to elevated dietary Se levels demonstrates that apoptosis is one of the pathways protecting the sturgeon from Se toxicity.

A tissue-based Se criterion has been recommended for the evaluation of Se toxicity for aquatic life (Hamilton, 2002). The rationale for the application of this biomarker is that a tissue-based Se integrates the effects due to route, duration, and dose of exposure, Se form, metabolic transformations, and modifying biotic and abiotic factors (Hamilton, 2002). Based on the previous finding by Tashjian et al. (2006), the observed effective dietary Se for depressed growth of white sturgeon is 41.7 mg Se/kg diet, which resulted in Se accumulation in muscle up to 36.8 mg/kg after 8 weeks of feeding. This observed muscle Se level is close to the Se threshold (32.2 mg/kg) for depressed SGR estimated by the broken line model in the current study. Furthermore, with a lower Se threshold (26.6 mg Se/kg muscle) for stimulating Hsp70 production, we suggest that Hsp70 is a more sensitive biomarker than SGR for the evaluation of Se toxicity in sturgeon.

5. Conclusions

An overdose of dietary SeMet decreased the RNA/DNA ratio, indicating depressed protein synthesis in the juvenile white sturgeon. Furthermore, white sturgeon seems to possess some active mechanisms, including activation of apoptosis and heat shock proteins to protect them from Se toxicity. Activation of these pathways protects the fish from mortality but compromises with depressed growth rate because extra energy may be required for the protection pathways. Based on the Se concentration in the muscle tissue, the toxic threshold was estimated to be 26.6 and 32.2 mg Se/kg for the induction of stress protein production and the depressed growth rate, respectively. The response of Hsp70 in muscle is dependent on the duration of dietary Se exposure and Se accumulation levels in muscle tissue. Our findings suggest that Hsp70 response in muscle is a more sensitive biomarker than the SGR of sturgeon for evaluating Se toxicity in white sturgeon.

Acknowledgment

This study was funded by CalFed Project number SP2006-035 and University of Wisconsin, Milwaukee (#150-25-3150-PRJ93WQ). We thank the Center of Aquatic Biology and Aquaculture, University of California-Davis for the use of the culture facilities. The infrastructure support of the Department of Animal Science and the College of Agriculture and Environmental Sciences of the University of California-Davis are also acknowledged.

References

- AOAC (Association of Official Analytical Chemists). Official methods of analysis. 17th ed. Arlington, Washington D.C.: Association of Official Analytical Chemists; 2000.
- Basu N, Todgham AE, Ackerman PA, Pibeau MR, Nakano K, Schulte PM, et al. Heat shock protein genes and their functional significance in fish. *Gene* 2002;295:173–83.
- Bernhart FW, Tomarelli RM. A salt mixture supplying the National Research Council estimates of the mineral requirements of the rat. *J Nutr* 1966;89:495–500.
- Caldarone EM, Wagner M, St Onge-Burns J, Buckley LJ. Protocol and guide for estimating nucleic acids in larval fish using a fluorescence microplate reader. *Northeast Fish. Sci. Cent. Ref. Doc.* 01-11. 166 Water Street, Woods Hole, MA: National Marine Fisheries Service; 2001. p. 2. 02543–1026.
- Cara JB, Aluru N, Moyano FJ, Vijayan MM. Food-deprivation induces HSP70 and HSP90 protein expression in larval gilthead sea bream and rainbow trout. *Comp Biochem Physiol* 2005;142B:426–31.
- Cleveland L, Little EE, Buckler DR, Wiedmeyer RH. Toxicity and bioaccumulation of waterborne and dietary selenium in juvenile bluegill (*Lepomis macrochirus*). *Aquat Toxicol* 1993;27:265–80.
- Cui YB, Hung SSO, Deng DF, Yang Y. Growth of white sturgeon as affected by feeding regimen. *Prog Fish-Cult* 1997;59:31–5.
- De Riu N, Lee JW, Huang SSY, Moniello G, Hung SSO. Effect of dietary selenomethionine on growth performance, tissue concentration, and histopathology in green and white sturgeon. *Aquat Toxicol* 2014;148:65–73.
- Deng DF, Wang CF, Lee SH, Bai SC, Hung SSO. Feeding rates affect heat shock protein levels in liver of larval white sturgeon (*Acipenser transmontanus*). *Aquaculture* 2009;287:223–6.
- Deng DF, Zheng KK, Teh FC, Lehman PW, Teh SJ. Toxic threshold of dietary microcystin (-LR) for quart medaka. *Toxicol* 2010;55:787–94.
- Gonçalves AC, Barbosa-Ribeiro A, Alves V, Silva T, Sarmento-Ribeiro AB. Selenium compounds induced ROS-dependent apoptosis in myelodysplasia cells. *Biol Trace Elem Res* 2013;154:440–7.
- Hamilton SJ. Rationale for a tissue-based selenium criterion for aquatic life. *Aquat Toxicol* 2002;57:85–100.
- Hamilton SJ. Review of selenium toxicity in aquatic food chains. *Sci Total Environ* 2004;326:1–31.
- Hamilton SJ, Buhl KJ, Faerber NL, Wiedmeyer RH, Bullard FA. Toxicity of organic selenium in the diet of Chinook salmon. *Environ Toxicol Chem* 1990;9:347–58.
- Hamilton SJ, Holley KM, Buhl KJ, Bullard FA, Weston LK, McDonald SF. The evaluation of contaminant impacts on razorback sucker held in flooded bottomland sites near Grand Junction, Colorado—1996. Final Report. Yankton, SD: US Geological Survey; 2001a.
- Hamilton SJ, Holley KM, Buhl KJ, Bullard FA, Weston LK, McDonald SF. reportThe evaluation of contaminant impacts on razorback sucker held in flooded bottomland sites near Grand Junction, Colorado—1997. Final Report. Yankton, SD: US Geological Survey; 2001b.
- Han D, Huang SSY, Wang W, Deng DF, Hung SSO. Starvation reduced heat shock protein responses in white sturgeon larvae. *Environ Biol Fish* 2012;93:333–42.
- Hengartner MO. The biochemistry of apoptosis. *Nature* 2000;407:770–6.
- Hilton JW, Hodson PV, Slinger SJ. The requirement and toxicity of selenium in rainbow trout (*Salmo gairdneri*). *J Nutr* 1980;110:2527–35.
- Hoffman DJ. Role of selenium toxicity and oxidative stress in aquatic birds. *Aquat Toxicol* 2002;57:11–26.
- Hu JR, Huang YH, Wang GX, Wu YX, Xian JA, Wang AL, Cao JM. Deficient and excess dietary selenium levels affect growth performance, blood cells apoptosis and liver HSP70 expression in juvenile yellow catfish *Pelteobagrus fulvidraco*. *Fish Physiol Biochem* 2016;42:249–61.
- Huang SS, Strathe AB, Wang WF, Deng DF, Fadel JG, Hung SSO. Selenocompounds in juvenile white sturgeon: evaluating blood, tissue, and urine selenium concentrations after a single oral dose. *Aquat Toxicol* 2012;109:158–65.
- Kaushal N, Bansal MP. Dietary selenium variation-induced oxidative stress modulates CDC2/cyclin B1 expression and apoptosis of germ cells in mice testis. *J Nutr Biochem* 2007;18(8):553–64.
- Kaushal N, Bansal MP. Diminished reproductive potential of male mice in response to selenium-induced oxidative stress: involvement of HSP70, HSP70-2, and MSJ-1. *Biochem Mol Toxicol* 2009;23(2):125–36.
- Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 1972;26:239–57.
- Lee S, Wang Y, Hung SSO, Strathe AB, Fangue NA, Fadel JG. Development of optimum feeding rate model for white sturgeon (*Acipenser transmontanus*). *Aquaculture* 2014;433:411–20.
- Lemly AD. Metabolic stress during winter increases the toxicity of selenium to fish. *Aquat Toxicol* 1993;27:133–58.
- Lemly AD. A teratogenic deformity index for evaluating impacts of selenium on fish populations. *Ecotoxicol Environ Saf* 1997;37:259–66.
- Lemly AD. Selenium assessment in aquatic ecosystem: a guide for hazard evaluation on water quality criteria. New York: Springer-Verlag; 2002. p. 161.
- Linville RG, Luoma SN, Cutter L, Cutter GA. Increase selenium threat as a result of exotic bivalve *Patamocorbula amurensis* into the San Francisco Bay Delta. *Aquat Toxicol* 2002;57:51–64.
- Maier KJ, Knight AW. Ecotoxicology of selenium in freshwater system. In: Ware GW, editor. Reviews of environmental contamination and toxicology. New York: Springer-Verlag; 1994. p. 31–48.
- Mallouk Y, Vayssier-Taussat M, Bonventre JV, Polla BS. Heat shock protein 70 and ATP as partners in cell homeostasis. *Int J Mol Med* 1999;4:463–74.
- Misra S, Niyogi S. Selenite causes cytotoxicity in rainbow trout (*Oncorhynchus mykiss*) hepatocytes by inducing oxidative stress. *Toxicol Vitro* 2009;23:1249–58.
- Misra S, Hamilton CH, Niyogi S. Induction of oxidative stress by Selenomethionine in isolated hepatocytes of rainbow trout (*Oncorhynchus mykiss*). *Toxicol Vitro* 2012;26:621–9.
- Moyle PB. Inland fishes of California. Berkeley: University of California Press; 2002. p. 106–13.
- NMFS (National Marine Fisheries Service). Endangered and threatened wildlife and plants: proposed threatened status for southern distinct population segment of North American green sturgeon. Federal Register; 2006. p. 17757–66.
- NRC (National Research Council). Nutrient requirements of fish and shrimp. Washington, D.C.: The National Academies Press; 2011. p. 175–6.
- RCore Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2015. <http://www.R-project.org/>.
- Sanders BM. Stress proteins in aquatic organisms: an environmental perspective. *Crit Rev Toxicol* 1993;23:49–75.
- Sanmartin C, Plano D, Sharma AK, Palop JA. Selenium compounds, apoptosis and other types of cell death: an overview for cancer therapy. *Int J Mol Sci* 2012;13(8):9649–72.
- Spallholz JE, Hoffman DJ. Selenium toxicity: cause and effects in aquatic birds. *Aquat Toxicol* 2002;57:27–37.
- Stegeman JJ, Brouwer M, Di Giulio RT, Forlin L, Fowler BA, Sanders BM, et al. Molecular responses to environmental contamination: enzyme and protein systems as indicators of chemical exposure and effect. In: Huggett RB, Kimerle RA, Mehrle PM, Bergman HL, editors. Biomarkers: biochemical, physiological and histological markers of anthropogenic stress. Boca Raton: Lewis Publishers; 1992. p. 235–347.
- Tanaka Y, Satoh K, Yamada H, Takebe T, Nikaido H, Shiozawa S. Assessment of the nutritional status of field caught larval pacific bluefin tuna by RNA/DNA ratio based on a starvation experiment of hatchery-reared fish. *J Exp Mar Biol Ecol* 2008;354:56–64.
- Tashjian DH, Teh SJ, Sogomonnyan A, Hung SSO. Bioaccumulation and chronic toxicity of dietary l-selenomethionine in the juvenile white sturgeon (*Acipenser transmontanus*). *Aquat Toxicol* 2006;79:401–9.
- Wang WF, Deng DF, De Riu N, Moniello G, Hung SSO. Heat shock protein 70 responses in tissues of white sturgeon and green sturgeon exposed to different stressors. *N Am J Aquacult* 2013;75:164–9.
- Yengkokpam S, Pal AK, Sahu NP, Jain KK, Dalvi R, Misra S, et al. Metabolic modulation in *Labeo rohita* fingerlings during starvation: hsp70 expression and oxygen consumption. *Aquaculture* 2008;285:234–7.
- Zheng KK, Wang WF, Hung SSO, Deng DF. Feeding rates affect expression of heat-shock protein 70 in green sturgeon fry. *N Am J Aquacult* 2015;77:206–10.