Comparative Study on Diet Added with Organic and Inorganic Selenium Forms Provided to Carps Exposed to Fipronil Insecticide

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Abstract Carps belonging to species *Cyprinus carpio* (carp) were fed on organic and inorganic selenium forms for 60 days to enable evaluating the biochemical profile of tissues exposed to fipronil (FPN) insecticide. Diphenyl diselenide [(PhSe)$_2$] (3.0 mg/kg) and sodium selenite (Na$_2$SeO$_3$) (0.75 mg/kg) were used as organic and inorganic selenium forms, respectively. Overall, the adopted organic and inorganic selenium forms were similarly capable of reestablishing oxidant and antioxidant stress parameters close to control levels. Fish exposed to fipronil have shown decreased acetylcholinesterase (AChE) activity in brain and muscle tissues. Brain tissues of fish supplemented with Na$_2$SeO$_3$ or (PhSe)$_2$ diets presented reestablished AChE levels in comparison to those of fish fed on standard diet. Liver tissues of fish fed on standard diet presented decreased δ-ALA-D activity after their exposure to FPN, whereas diets added with two selenium forms were efficient in reestablishing the levels of standard diets. Therefore, (PhSe)$_2$ and Na$_2$SeO$_3$ have potential to be used as supplementation factors in diets to feed *C. carpio*.

Keywords Diets · Diphenyl diselenide · Sodium selenide · Carp · Biochemical changes · Oxidative stress

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1 Introduction

The uncontrolled use of pesticides is a global issue that negatively affects the environment and human health. One of its negative effects lies on the contamination of aquatic ecosystems such as fish farming environments, a fact that leads to fish intoxication, since these animals can bioaccumulate the substances and transfer them to humans through the food chain in a biomagnification process (Clasen et al. 2018). Fipronil (FPN) is highly toxic to a wide range of non-target aquatic invertebrates used in fish diet (Pisa et al. 2015); its toxicity in fish changes from species to species. Similar to what happens with other aquatic organisms, insecticides often penetrate fish's body in a rapid and direct manner, either through ingestion or direct contact between contaminants and animals' gills, skin, scales, or mucus. Thus, it is necessary developing strategies focused on reversing or minimizing intoxication levels in organisms exposed to pesticides. FPN is a pesticide used at global scale. It was made available in the market in 1993 in order to help reduce pesticides' toxicity in the environment (Clasen et al. 2012). FPN is a highly active chiral

molecule that belongs to family phenyl pyrazole; it is widely used as broad-spectrum insecticide to treat seeds in different crops, mainly in rice crops, as well as to control pests in urban areas (Mize et al. 2008; Beggel et al. 2012). The bioactivity of FPN results from its ability to act at the γ-aminobutyric acid (GABA) receptor as noncompetitive blocker of the GABA-gated chloride channels of neurons in individuals' central nervous system (Romero et al. 2016). FPN forms four metabolites, namely fipronil sulfide, fipronil amide, fipronil-desulfinyl, and fipronil sulfone. These metabolites are more toxic to aquatic organisms than the parent compound. Furthermore, FPN and its metabolites have been detected in aquatic environments; therefore, it is a concerning factor since they are highly toxic to fish (Mize et al. 2008; Thuyet et al. 2013). Many studies have reported the toxic effects of fipronil on carps (Clasen et al. 2012; Qureshi et al. 2016; Ghazanfar et al. 2018). Although fipronil is widely used in countries such as Brazil, it was already banned in Europe due to its high toxicity level, as evidenced by severe issues observed in organisms exposed to it (Stevens et al. 1998).

In addition, studies have pointed out that exposure to fipronil changes the antioxidant ability and/or leads to increased reactive oxygen species (ROS) in fish tissues. *Cyprinus carpio* specimens exposed to fipronil presented increased superoxide dismutase (SOD) activity, decreased catalase (CAT) activity, as well as increased thiobarbituric acid reactive substances (TBARS) and protein carbonyl (PC) in different tissues (Clasen et al. 2012; Menezes et al. 2016). Moreover, pesticides can inhibit enzymes such as acetylcholinesterase (AChE) and δ-aminolevulinate dehydratase (δ-ALA-D) (Menezes et al. 2016). AChE inhibition leads to decreased breakdown and to subsequent accumulation of acetylcholine, a fact that can affect neuromuscular functions such as locomotion, as well as the behavior of organisms exposed to it (Serafini et al. 2019).

The use of organic or inorganic selenium compounds as preventive antioxidant substance is an alternative to minimize the toxic effects of pesticides. Selenium (Se) is an essential micronutrient to different organisms; it is found in proteins and enzymes—named selenoproteins—in the form of the amino acid known as selenocysteine (Barbosa et al. 2008). Selenium compounds have their own dose effect: low doses have beneficial effects on *C. carpio*, whereas high doses of it can be toxic to the species (Menezes et al. 2014). The authors of the current study have observed that 3.0 mg/Kg of diphenyl diselenide ((PhSe)$_2$) boosted carps' growth and improved their antioxidant defenses; however, disadvantages were observed at 5.0 mg/Kg of (PhSe)$_2$. The dose limit for toxic or beneficial effects depends on the exposed organisms, on the affected tissues and, mainly, on the selenium form such organisms were exposed to (Mézes and Balogh 2009; Misra et al. 2012). Molecular mechanisms associated with selenium toxicity remain unclear; they are supposedly related to the oxidation of thiols of biological importance (Barbosa et al. 2008; Menezes et al. 2016). Thus, it is essential conducting studies, such as the one by Betancor et al. (2015), to help elucidate this mechanism in fish. Numerous studies have already shown the antioxidant effects of selenium on fish (Elia et al. 2011; Siscar et al. 2015; Mansour et al. 2017); however, studies focused on investigating these effects on fish exposed to pesticides remain scarce in the literature. Accordingly, Menezes et al. (2012) have evidenced that diphenyl diselenide can be used as a powerful antioxidant against toxic effects of quinclorac herbicide on *C. carpio*.

Sodium selenite (Na$_2$SeO3) is an inorganic Se form; diets supplemented with sodium selenite have been poorly investigated in fish species. Menezes et al. (data not published) have shown that diets supplemented with Na$_2$SeO$_3$ at lower levels were more toxic than diets supplemented with diphenyl diselenide (PhSe)$_2$.

Fish species *Cyprinus carpio* (common carp) is distributed and grown worldwide, since it is highly valued as protein source in many countries (Elia et al. 2011). This species may be exposed to a wide range of pesticides, since it is found in Brazilian lakes and rivers throughout the year. Therefore, studies have been carried out to evaluate strategies focused on minimizing the effects of pesticides on fish after, or during, their exposure to these toxic substances, as well as on reducing human contamination associated with the food chain.

Therefore, the aim of the current study was to evaluate the assumed protective action of organic and inorganic selenium forms as potential antioxidants against biochemical changes in tissues of *C. carpio* individuals exposed to insecticides such as fipronil.

2 Materials and Methods

2.1 Diet Preparation and Experimental Design

The fish were divided in three groups: (1) control group (fish fed with a diet without (Se) $n = 24$), (2) organic Se group (fish fed with a diet supplemented with 3.0 mg/Kg (PhSe)$_2$, $n = 24$), and (3) inorganic Se group (fish fed with a diet supplemented with 0.75 mg/Kg Na$_2$SeO$_3$ $n = 24$). They were fed with these diets for 60 days. The experimental diet composition was based on previous studies from our research group (Menezes et al. 2012). Briefly, to obtain the diets containing (PhSe)$_2$ and Na$_2$SeO$_3$, these compounds were added to control diet and all the ingredients were completely mixed by adding distilled water before further homogenization. The diet pellets were stored at 4 °C until they were used and the fish were fed 3% biomass per day. The daily ratio was divided into two equal meals fed at 09:00 and 16:00 h. The concentration of (PhSe)$_2$ and Na$_2$SeO$_3$ chosen for treating fish was based on previous studies, which demonstrated that these did not cause overt signals of toxicity to carp (Menezes et al. 2016; data not published, respectively). After the feeding period of 60 days, groups were subdivided and allocated in 45 L boxes of fiberglass into six experimental groups of five or seven fish each group (duplicate): (1) control group (fish fed with a diet without Se); (2) (PhSe)$_2$ group (fish fed with a diet supplemented with 3.0 mg/kg of (PhSe)$_2$); (3) FPN group (fish fed with a diet without Se, and exposed to 0.65 μg/L fipronil for 192 h); (4) (PhSe)$_2$ + FPN group (fish fed with a diet supplemented with 3.0 mg/kg of (PhSe)$_2$ and exposed to 0.65 μg/L fipronil for 192 h); (5) Na$_2$SeO$_3$ group (fish fed with a diet supplemented with 0.75 mg/kg of (Na$_2$SeO$_3$); and (6) Na$_2$SeO$_3$ + FPN group (fish fed with a diet supplemented with 0.75 mg/kg of (Na$_2$SeO$_3$) and exposed to 0.65 μg/L fipronil for 192 h). The concentration of insecticide was chosen according to other experimental procedures carried out by our research group using this chemical compound (Clasen et al. 2012; Menezes et al. 2016). The concentration of insecticide in water was measured at the beginning, middle, and end of experimental, period by gas chromatography with electron capture detection (GC-ECD), according to Sabin et al. (2009). Water quality parameters were monitored daily and were maintained equal to those registered during the acclimation period.

2.2 Chemicals

The FPN insecticide (CAS 120068-37-3) used was an available commercial formulation (Standak®-BASF), containing 25% fipronil [(7)-5-amino-1-(2,6-dichloro-α-α-α−trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile].

Malondialdehyde (MDA), 2-thiobarbituric acid (TBA), sodium dodecyl sulfate (SDS), 2,4-dinitrophenylhydrazine (DNPH), bovine serum albumin, hydrogen peroxide (H$_2$O$_2$), and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of analytical reagent grade and purchased from Merck (Rio de Janeiro, Brazil). Diphenyl diselenide [(PhSe)$_2$] and sodium selenite (Na$_2$SeO$_3$) were synthesized according to literature methods (Paulmier 1986). Analysis of the ^{1}HNMR and ^{13}CNMR spectra showed analytical and spectroscopic data fully in line with its assigned structure.

2.3 Fish

Juvenile carp (weight, 18.28 ± 1.65 g; length, 10.20 ± 1.0 cm) were obtained from a fish farm (RS, Brazil). This fish farm is specially designed for the purpose of raising fish. The nurseries have a controlled supply and disposal system. The stocking rate is programmed as a commercial creation. Fish water comes from reservoirs with good sanitary conditions, free from sewage, and from products such as herbicides, insecticides, fungicides, and chlorine and the pH ranges from 7 to 8. Fish were acclimated to laboratory conditions for 15 days, in 250 L boxes of fiberglass prior to experiments. They were kept in continuously aerated tap water with a static system and with a natural photoperiod (12 h light/12 h dark). Water conditions were temperature 24.0 ± 1.0 °C, pH 7.4 ± 0.1 units, dissolved oxygen 7.1 ± 1.0 mg/L, non-ionized ammonia 0.3 ± 0.01 μg/L, and nitrite 0.05 ± 0.01 mg/L.

2.4 Sample Preparation

At the end of the exposure period, the fish were anesthetized with 50 mg/L clove oil for 3 min. Fish were then euthanized by spinal section and liver, gills, brain, and muscle were removed. The tissues were washed

with 150 mM saline solution, packed in teflon tubes, and kept at − 80 °C for future assays. The investigation was authorized by the board on experimentation on Animals of the Federal University of Santa Maria, reference number: 84/2009.

2.5 Chemical Analysis

2.5.1 Homogenate Preparation

For protein carbonyl and lipid peroxidation assay (TBARS), tissues were homogenized with 10 mM Tris-HCl buffer, pH 7.4, and the homogenate were centrifuged for 10 min at $3000 \times g$ at 5 °C, then the supernatant was used. For SOD, CAT, glutathione peroxidase (GPx), and glutathione-S-transferase (GST) assay, tissues were homogenized with 20 mM potassium phosphate buffer, pH 7.5 (1:20 dilution), centrifuged at $10,000g$ for 10 min at 4 °C. For non-protein thiols (NPSH) and ascorbic acid (AsA) levels assay, tissues were homogenized with 1.5 mL Tris-HCl 50 mM, pH 7.5, centrifuged at $3000g$ for 10 min. For δ-ALA-D assay, tissues were homogenized with 150 mM NaCl at the dilution of 1:7 (liver) or 1:3 (gills), centrifuged at $3000g$ for 10 min at 4 °C. For AChE assay, tissues were homogenized with 150 mM NaCl (1:10 dilution), centrifuged at $3000g$ for 15 min at 4 °C and supernatant was used as the enzyme source.

All biochemical analyses were made as described in Menezes et al. (2014, 2016).

2.5.2 Protein Determination

Protein was determined by the Comassie blue method using bovine serum albumin as standard. Absorbance of samples was measured at 595 nm (Bradford 1976).

2.6 Statistical Analysis

The values in all determinations are presented as means ± standard error of mean (S.E.M). Statistical analysis was performed using two-way analysis of variance (ANOVA) followed by Newman-Keuls post hoc comparison. Analyses were performed using Graph Prism (Version 6.0) and the minimum significance level was set at $p < 0.05$.

3 Results

3.1 Oxidative Damage Parameters

3.1.1 TBARS and Protein Carbonyl

This experiment was focused on evaluating the assumed pro-oxidant effects of diets supplemented with diphenyl diselenide $(PhSe)_2$ and sodium selenite (Na_2SeO_3) on carps. Fish fed on standard diet and exposed to fipronil have shown increased TBARS levels in the analyzed tissues, except for the brain tissue, which did not show TBARS levels different from the ones observed in fish fed on standard diet, who were not exposed to the aforementioned insecticide. Na_2SeO_3 was effective in reducing TBARS levels in the liver and gills of carps, when compared with the control group who had experienced significantly increased levels of it after their exposure to fipronil; however, it was not effective in TBARS levels in carps' muscle tissues (Figs. 1A, 2A, and 3A, respectively). $(PhSe)_2$ per se decreased TBARS levels in carps' liver, gills, and brain (Figs. 1A, 2A, and 4A) compared with the control group, who had experienced increased levels of it after their exposure to this insecticide in the control group; however, a similar result was not observed in carp' muscle tissues (Figs. 1A, 2A, and 3A, respectively).

There was no difference between protein carbonyl levels observed in the liver and gills of fish fed on standard diet alone and levels observed in fish exposed to FPN, although these fish presented increased protein carbonyl levels in muscle and brain tissues. Individuals in the Se groups, who were exposed to fipronil, did not show changes in hepatic protein carbonyl levels in comparison to the control group (individuals who were not exposed to $(PhSe)_2$ or Na_2SeO_3, as shown in Fig. 1B. Organic and inorganic Se forms provided to the group exposed to fipronil were effective in decreasing carbonyl protein levels in the gill, muscle, and brain tissues of carps compared with control group, who had experienced increased levels of it after their exposure to this insecticide (Figs. 2B, 3B, and 4B, respectively).

3.2 Antioxidant Parameters

3.2.1 SOD Activity

SOD activity in the liver of fish fed on standard or $(PhSe)_2$ diet and exposed to fipronil did not change
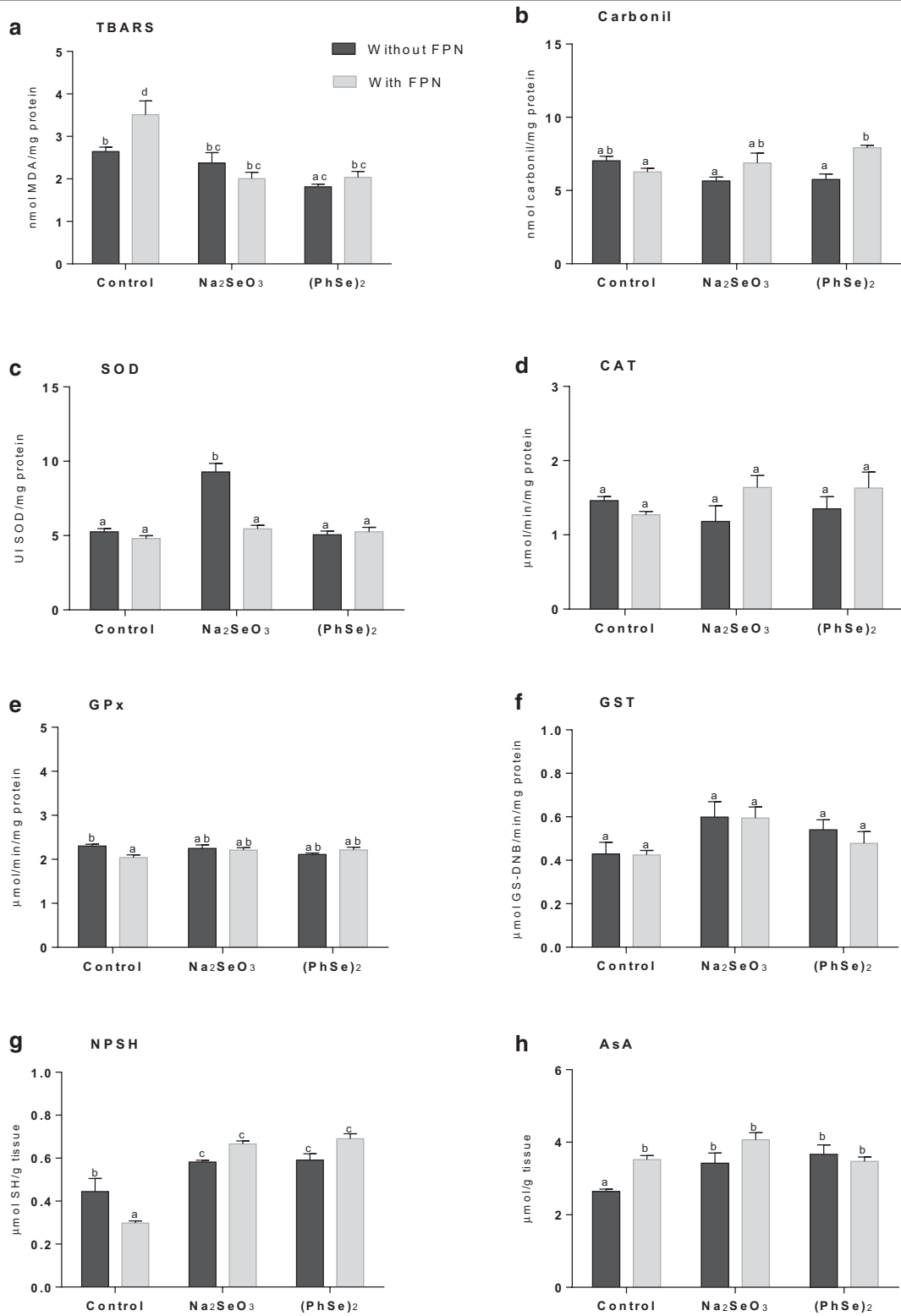
Fig. 1 TBARS (A), protein carbonyl (B), SOD (C), CAT (D), GPx (E), GST (F), NPSH (G), and AsA (H) in liver of carp fed with diets containing Na_2SeO_3 and/or $(PhSe)_2$ (mg/kg) for 60 days and exposed to FNP insecticide. Data are reported as mean ± SEM ($n = 8$). Different letters indicate differences between groups ($p < 0.05$)/(two-way ANOVA/ Newman Keuls)
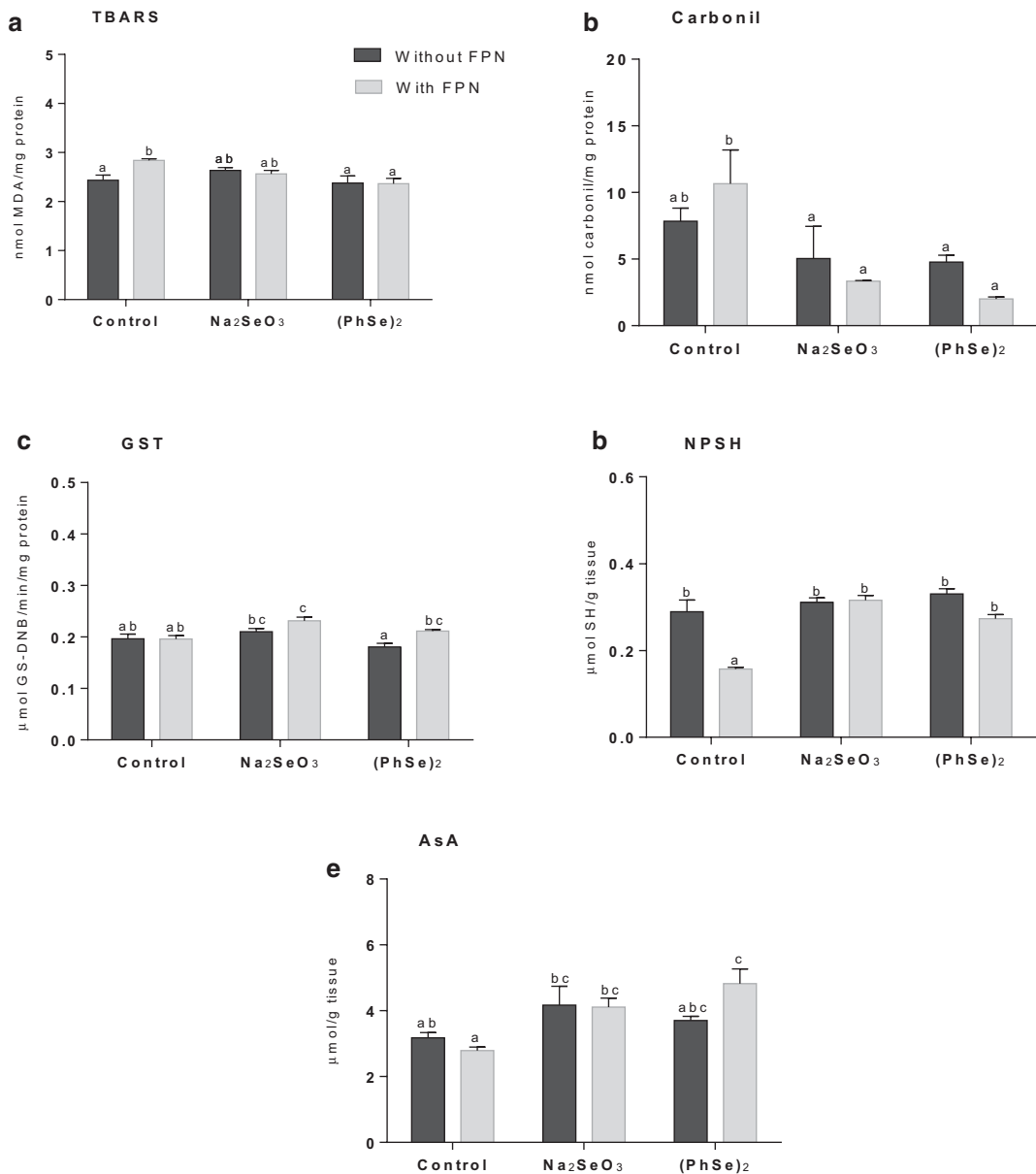
Fig. 2 TBARS (A), protein carbonyl (B), GST (C), NPSH (D), and AsA (E) in gills of carp fed with diets containing Na_2SeO_3 and/or $(PhSe)_2$ (mg/kg) for 60 days and exposed to FNP insecticide. Data are reported as mean ± SEM ($n = 8$). Different letters indicate differences between groups ($p < 0.05$)/(two-way ANOVA/Newman Keuls)

(Fig. 1C). Na_2SeO_3 per se led to increased SOD activity in carps' liver (Fig. 1C).

3.2.2 CAT Activity

The liver of fish fed on standard diet and on diets supplemented with Na_2SeO_3 or $(PhSe)_2$ who were exposed to fipronil did not show any change in CAT activity (Fig. 1D).

3.2.3 GPx Activity

Fish exposure to FPN has led to decreased GPx activity in the control group (standard diet). GPx activity in carps' liver did not change in individuals fed standard on diet and on diets supplemented with Na_2SeO_3 or $(PhSe)_2$ who were exposed to fipronil (Fig. 1E).
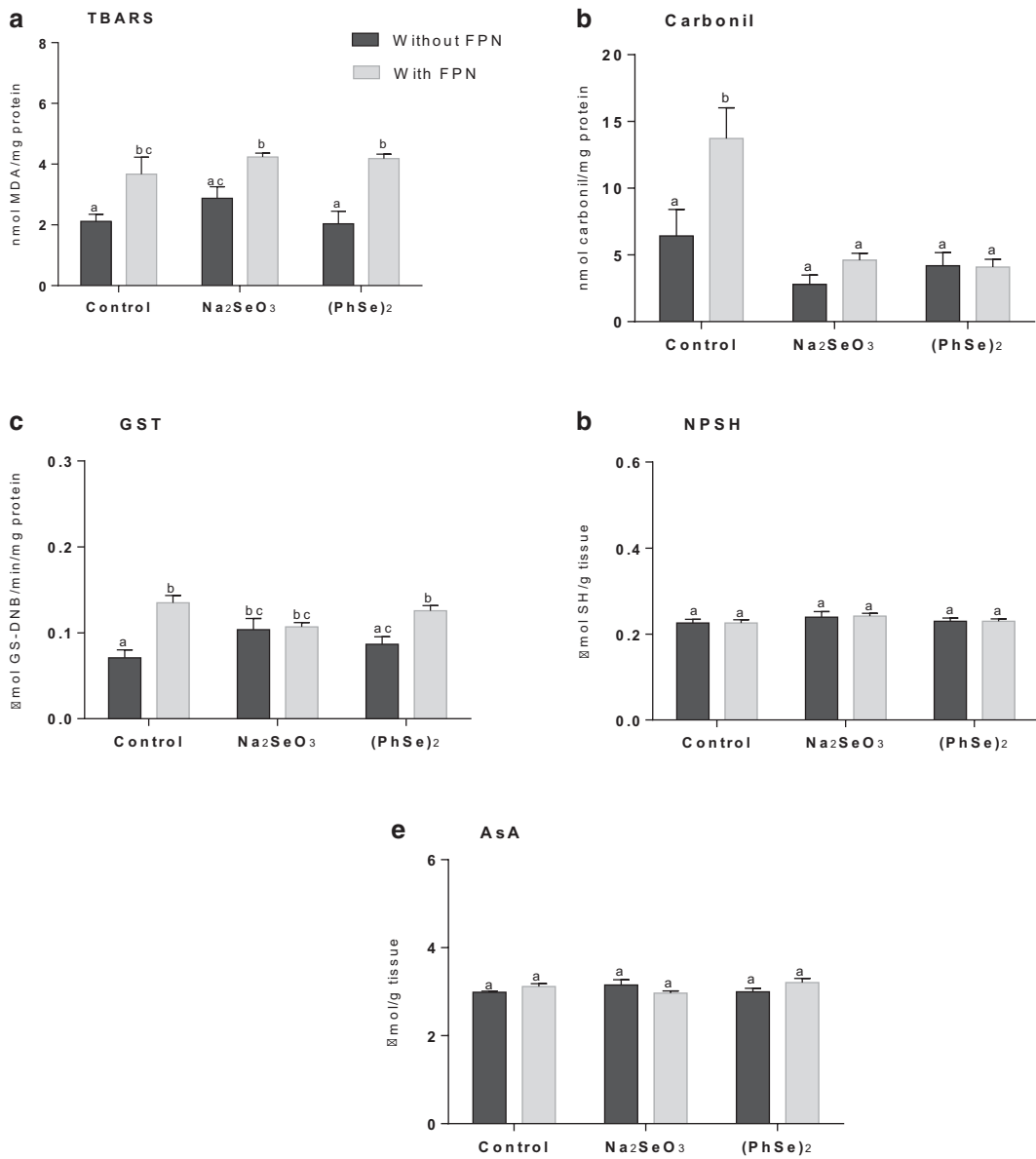
Fig. 3 TBARS (A), protein carbonyl (B), GST (C), NPSH (D), and AsA (E) in muscle of carp fed with diets containing Na_2SeO_3 and/or $(PhSe)_2$ (mg/kg) for 60 days and exposed to FNP insecticide. Data are reported as mean $\pm$ SEM ($n = 8$). Different letters indicate differences between groups ($p < 0.05$)/(two-way ANOVA/Newman Keuls)

3.2.4 GST Activity

GST activity in the liver of fish fed on standard diet, or on diets supplemented with Se, who were exposed to fipronil (Fig. 1F) did not change. FPN has increased GST activity in the gills of fish fed on the $(PhSe)_2$ diet (Fig. 2C). Fish exposure to fipronil has also increased GST activity in the muscle tissues of individuals fed on standard and $(PhSe)_2$ diets (Fig. 3C). $(PhSe)_2$ per se has increased GST activity in carps' brain tissue (Fig. 4C). GST activity in the gill and muscle tissues of fish fed on diet supplemented with Na_2SeO_3 was not different from that of fish fed on the diet supplemented with Na_2SeO_3 and exposed to fipronil (Figs. 2C and 3C).
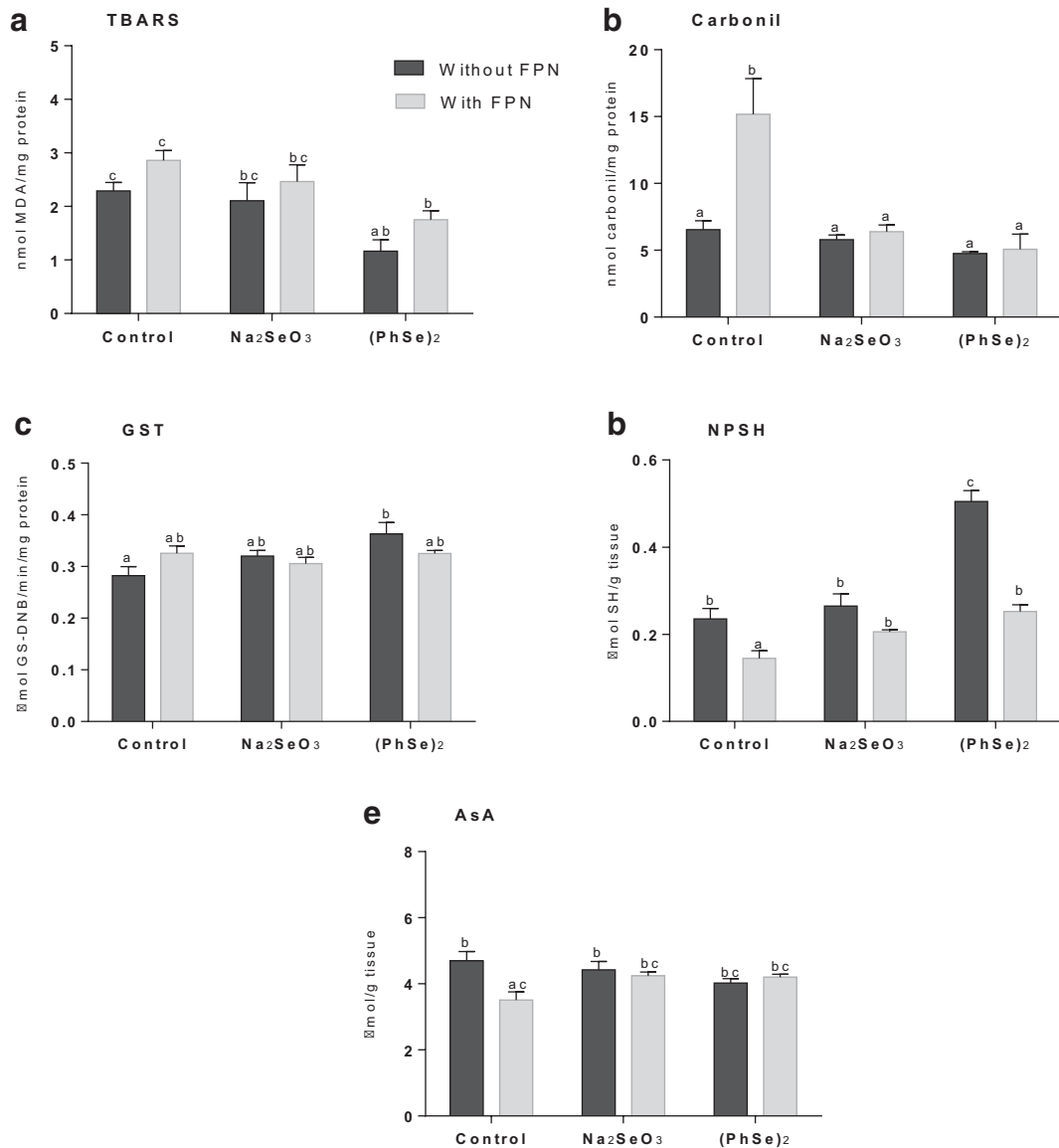
Fig. 4 TBARS (A), protein carbonyl (B), GST (C), NPSH (D), and AsA (E) in brain of carp fed with diets containing Na_2SeO_3 and/or $(PhSe)_2$ (mg/kg) for 60 days and exposed to FNP insecticide. Data are reported as mean $\pm$ SEM ($n = 8$). Different letters indicate differences between groups ($p < 0.05$)/(two-way ANOVA/Newman Keuls)

3.2.5 NPSH and Ascorbic Acid Levels

Fish exposed to fipronil and fed on standard diet have shown decreased NPSH levels in liver, gill, and brain tissues; however, did not show any changes in muscle tissues (Figs. 1G, 2D, 4D, and 3D, respectively). Fish fed on diets supplemented with Na_2SeO_3 or $(PhSe)_2$ and exposed to fipronil have shown increased NPSH levels in their liver (Fig. 1G). $(PhSe)_2$ per se has increased NPSH levels in carps' brain tissues (Fig. 4D).

The liver of fish fed on standard diet and on diets supplemented with Na_2SeO_3 or $(PhSe)_2$ who were exposed to fipronil have shown increased ascorbic acid levels (Fig. 1H). The gills of fish fed on the diet supplemented with Na_2SeO_3 or $(PhSe)_2$ who were exposed to fipronil have shown increased ascorbic acid levels in comparison to the gills of fish fed on standard diet and exposed to fipronil (Fig. 2E). The brain tissue of fish fed on standard diet and exposed to fipronil has shown decreased ascorbic acid levels (Fig. 4E). The brain tissue
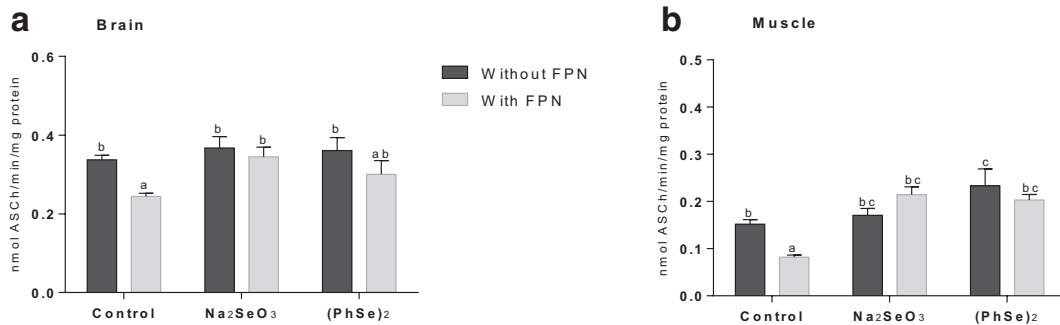
Fig. 5 AChE activity in brain (A) and muscle (B) of carp fed with diets containing Na_2SeO_3 and/or $(PhSe)_2$ (mg/kg) for 60 days and exposed to FNP insecticide. Data are reported as mean ± SEM ($n = 8$). Different letters indicate differences between groups ($p < 0.05$)/(two-way ANOVA/Newman Keuls)

of fish fed on the Na_2SeO_3 or $(PhSe)_2$ diets did not show any change in ascorbic acid levels, although the supplementation of these diets has enabled the reestablishment of AsA levels (Fig. 4E).

3.2.6 AChE Activity

Brain and muscle tissues of fish fed on standard diet and exposed to fipronil have shown decreased AChE activity (Fig. 5A, B). The brain tissues of fish fed on the diets supplemented with Na_2SeO_3 or $(PhSe)_2$ have shown reestablished AChE levels in comparison to the brain tissues of fish fed on standard diet (Fig. 5A). The muscle tissues of fish fed on the diets supplemented with Na_2SeO_3 diet have shown reestablished AChE levels in comparison to the muscle tissues of fish fed on standard diet and exposed to fipronil (Fig. 5B). Furthermore, $(PhSe)_2$ per se has increased AChE levels in carps' muscle tissues (Fig. 5B) in comparison to fish fed on standard diet. Fish fed on the diet supplemented with $(PhSe)_2$ and exposed to fipronil have shown reestablished AChE levels in comparison to fish fed on standard diet.

3.2.7 δ-ALA-D Activity

Fish exposure to fipronil has led to decreased δ-ALA-D activity in the liver of individuals fed on standard diet (Fig. 6A). The liver of fish fed on the diet supplemented with Na_2SeO_3 and exposed to fipronil has shown increased δ-ALA-D activity (Fig. 6A). No change in δ-ALA-D activity was observed in carps' gills (Fig. 6B).

4 Discussion

Climate changes and anthropogenic activities associated with urbanization and agricultural practices are factors contributing to increase the amount of toxic substances in water bodies; consequently, these substances affect aquatic communities such as fish. Fipronil is largely used in Southern Brazil; however, several studies have shown that this insecticide is
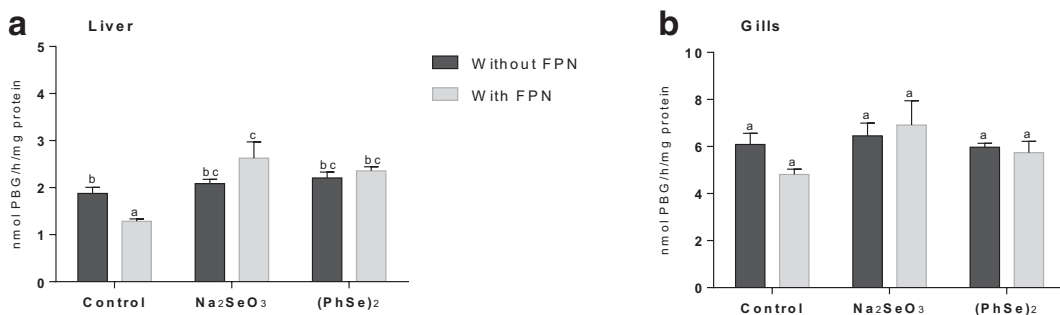

Fig. 6 ALA-D activity in liver (A) and gills (B) of carp fed with diets containing Na_2SeO_3 and/or $(PhSe)_2$ (mg/kg) for 60 days and exposed to FNP insecticide. Data are reported as mean ± SEM ($n = 8$). Different letters indicate differences between groups ($p < 0.05$)/(two-way ANOVA/Newman Keuls)

toxic to fish. Furthermore, fipronil toxicity in fish changes from species to species (Clasen et al. 2012; Qureshi et al. 2016). Selenium plays a key role in many biological processes; besides, it protects individuals from several diseases. Assumingly, selenium can improve antioxidant capacity by increasing the activity of antioxidant enzymes, as well as the content of different antioxidants. Many studies have shown the antioxidant role played by different selenium forms in fish (Zhou et al. 2009; Elia et al. 2011; Ashouri et al. 2015). However, there are only few studies in the literature focused on evaluating the antioxidant role played by selenium in fish exposed to pesticides (Monteiro et al. 2009), a fact that evidences the need of conducting further studies in this topic. According to Menezes et al. (2016), (PhSe)$_2$ was capable of reversing changes resulting from fish exposure to fipronil in two different species. Studies focused on assessing the use of Na$_2$SeO$_3$ as a compound to reverse changes observed in fish exposed to pesticides remain scarce in the literature.

Overall, the enzymatic antioxidant activity and non-enzymatic antioxidant levels observed in fish fed on Na$_2$SeO$_3$ or (PhSe)$_2$ were significantly better, similar or superior to those of the control and of fish subjected to other treatments with FPN. Results have shown that FPN led to oxidative stress, as well as to increased TBARS (liver, gills, and muscles), and PC levels in carps' gill, muscle, and brain tissues. In addition, Na$_2$SeO$_3$ and (PhSe)$_2$ were capable of protecting the animals' liver and gill tissues from these disorders. The current results corroborate Menezes et al. (2012, 2016), who observed similar outcomes in carps exposed to a different pesticide. Both (PhSe)$_2$ and Na$_2$SeO$_3$ compounds used in the current study have shown similar ability to restore TBARS and protein carbonyl levels after the increased levels caused by fish exposure to the insecticide. Marins et al. (2018) have shown that Se-supplemented diet was capable of preventing TBARS level increase in liver and white muscle in *C. carpio* exposed to 2 μg L^{-1} of atrazine. Therefore, this outcome has indicated the antioxidant potential of these selenium compounds.

Fipronil-induced lipid peroxidation was associated with disturbances observed in other oxidative stress parameters such as thiol or ascorbic acid levels. Na$_2$SeO$_3$ and (PhSe)$_2$ have increased the levels of non-enzymatic antioxidants, ascorbic acid,

and non-protein thiols in all evaluated tissues; besides, they likely acted in the restoration of TBARS and protein carbonyl levels in fish subjected to treatments with fipronil + Na$_2$SeO$_3$ and fipronil + (PhSe)$_2$. Similarly, they increased GST levels in muscle and brain tissues. These changes have clearly shown the beneficial role played by diets supplemented with selenium in oxidants ant antioxidants' stability in fish exposed to fipronil.

Acetylcholinesterase enzyme activity in the brain and muscle tissues of fish has shown the same positive response. *C. carpio* individuals exposed to FPN in the current study have shown AChE activity inhibition. Many studies have reported AChE activity inhibition in aquatic animals exposed to pesticides (Menezes et al. 2016; Clasen et al. 2018; Singh et al. 2018). However, increased AChE activity was observed in fish treated with Na$_2$SeO$_3$ or (PhSe)$_2$, a fact that evidenced the protective effect of Se on fish.

Fish exposed to FPN presented decreased δ-ALA-D levels in the liver. δ-ALA-D inhibition can lead to 5-aminolevulinate substrate accumulation, which, in its turn, can intensify oxidative stress and generate reactive species (Altinok et al. 2012). Thus, δ-ALA-D inhibition can lead to ALA accumulation and contribute to oxidative stress caused by FPN. Thus, fish fed on the diet supplemented with Na$_2$SeO$_3$ who were exposed to FPN have shown increased δ-ALA-D activity in the liver, thus indicating the potential to reduce oxidative stress due to the presence of this compound in the diet.

Results in the current study suggest that Se—in the forms of Na$_2$SeO$_3$ and (PhSe)$_2$—is potentially useful to help prevent oxidative damage induced by FPN, mainly due to its ability to adjust cellular antioxidant capacity and to prevent oxidative stress.

5 Conclusions

The current study has shown that (PhSe)$_2$ and Na$_2$SeO$_3$ treatments have significantly acted in reducing the oxidative damage caused by PNF observed by the reduction of TBARS and carbonyl protein levels in gill liver and brain, mainly. Improvement in antioxidant activity mainly due to increased GST in gills, NPSH in liver, gills, and brain and AsA in gills. These results suggest that (PhSe)$_2$ and Na$_2$SeO$_3$ may be used to feed fish farmed in fish and aquaculture systems.

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