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# Effects of diphenyl diselenide on growth, oxidative damage, and antioxidant response in silver catfish

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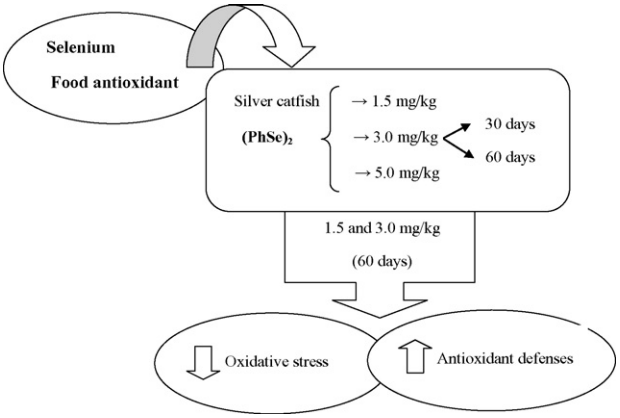
## HIGHLIGHTS

- Selenium is element participates of various metabolic routes.
- $(PhSe)_2$ in different concentrations in diet were investigated on silver catfish.
- 1.5 and 3.0 mg/kg of $(PhSe)_2$ increased antioxidant defenses of fish.
- The best results were obtained after 60 days of feeding with $(PhSe)_2$.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The aim of this study was to evaluate the effects of dietary diphenyl diselenide [$(PhSe)_2$] at different concentrations (1.5, 3.0, and 5.0 mg/kg) on growth, oxidative damage and antioxidant parameters in silver catfish after 30 and 60 days. Fish fed with 5.0 mg/kg of $(PhSe)_2$ experienced a significant decrease in weight, length, and condition factor after 30 days and these parameters increased after 60 days. Thiobarbituric acid reactive substances (TBARS) and protein carbonyl (PC) decreased in the liver of silver catfish supplemented with $(PhSe)_2$ after 30 days at all concentrations, while after 60 days these parameters decreased in liver, gills, brain, and muscle. Supplementation with $(PhSe)_2$ induced a decrease in catalase (CAT) activity from liver only after 60 days of feeding. Superoxide dismutase (SOD) decreased at 5.0 mg/kg after 30 and 60 days and glutathione peroxidase (GPx) was enhanced at 1.5 and 3.0 mg/kg after 30 and 60 days. Silver catfish supplemented for 30 days showed a significant increase in liver glutathione S-transferase (GST) at 3.0 mg/kg, while after 60 days GST activity increased in liver at 1.5, 3.0, and 5.0 mg/kg and in gills at 3.0 and 5.0 mg/kg of $(PhSe)_2$. After 30 days, non-protein thiols (NPSH) did not change, while after 60 days NPSH increased in liver, gills, brain, and muscle. In addition, ascorbic acid (AA) levels after 30 days increased in liver at three concentrations and in gills and muscle at 1.5 mg/kg, while after 60 days, AA increased at all concentrations in all and tissues tested. Thus, diet supplemented with $(PhSe)_2$

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for 60 days could be more effective for silver catfish. Although the concentration of 5.0 mg/kg showed decreased growth parameters, concentrations of 1.5 and 3.0 mg/kg, in general, decreased oxidative damage and increased antioxidant defenses.



## 1. Introduction

Selenium (Se) is an essential nutrient in the diet of a variety of organisms, because it is important for growth, development, and physiological function (Hamilton, 2004; Kim and Kang, 2014). Se is an antioxidant mineral present in various selenoproteins that contain one or more selenocysteine residues in their active sites. This element participates in various metabolic routes, especially those involved in the antioxidant system, as its presence in active sites of glutathione peroxidase (GPx) (Nogueira et al., 2004; Li et al., 2008; Monteiro et al., 2009). However, nutritional requirements and toxic levels of Se exhibit a fine partition, and this element may be either essential or toxic, depending on its concentration. In fact, at high cellular concentrations, Se may be utilized in place of sulfur, causing errors in protein synthesis and damage to other molecules (e.g. lipids and DNA) as well as reproductive impairment, larval deformities, and mortality (Muscatello et al., 2006).

Diphenyl diselenide [$(PhSe)_2$] is a synthetic organoselenium compound that has been considered a potential pharmacological and antioxidant agent in various experimental models, such as fish, rats, and mice (Menezes et al., 2012; Costa et al., 2013; Fiuza et al., 2015). The exposure of fish to xenobiotics is a main cause of oxidative damage due to increased production of reactive species oxygen (ROS) in exposed organisms (Murussi et al., 2014; Pretto et al., 2014). Thus, the use of antioxidants such as Se in the diet may permit animals to overcome, healthy and without damage, adverse conditions that may occur (Monteiro et al., 2007). Menezes et al. (2012) evaluated the effects of $(PhSe)_2$ on oxidative damage and antioxidant profile in different tissues of *Cyprinus carpio*. The authors observed that fish fed a diet without $(PhSe)_2$ and exposed to Facet® herbicide showed oxidative damage in different organs, while $(PhSe)_2$ treatment reversed oxidative damage by increasing some antioxidant defenses. Thus, $(PhSe)_2$ could be a powerful antioxidant. However, for other species of fish, the requirement in terms of concentration and form as well as the threshold dose for its opposing toxic properties has not yet been established.

The silver catfish (*Rhamdia quelen*), is a fish species with an extensive geographic distribution, occurring from southern Mexico to central Argentina. Its features, such as tolerance to management, omnivorous feeding behavior, ability to grow throughout the winter, and high yield, place it in a prominent position among the native species of interest to aquaculture in the region of southern Brazil (Fracalossi et al., 2004; Baldisserotto, 2009).

Studies have evidenced the possibility that dietary supplementation with certain vitamins and minerals can increase disease resistance, prevent negative effects of stress, and minimize the toxicity of contaminants in fish (Borba et al., 2007; Monteiro et al., 2009; Menezes et al., 2012, 2014a). The objective of this study was to evaluate the efficacy of dietary supplementation with different concentrations of $(PhSe)_2$ for 30 and 60 days in silver catfish, in order to establish the concentration that warrants further exploration as a potential supplement in silver catfish nutrition.

## 2. Materials and methods

### 2.1. Chemicals

The reagents chemicals used in this study were obtained from Sigma Chemical Co. (St. Louis, MO, USA) and Merck (Rio de Janeiro, Brazil). Diphenyl diselenide [$(PhSe)_2$] was synthesized according with Paulmier (1986). Analysis of the $^1$HNMR and $^{13}$CNMR spectra showed analytical and spectroscopic data in agreement with its assigned structure.

### 2.2. Fish

Silver catfish (body length, 7.0 ± 1.0 cm; mean weight, 18.0 ± 1.0 g) of both sexes were acquired from a local fish farm (RS, Brazil). Before the experiment, the fish were acclimated to the laboratory conditions for 15 days, in 250 L fiberglass boxes. They were kept in continuously aerated dechlorinated tap water with a static system and with a natural photoperiod (12 h light/12 h dark). During the acclimation, water temperature was maintained at 22.5 ± 1.0 °C, the dissolved oxygen content was 7.21 ± 1.0 mg/L, pH between 7.3 and 7.7, non-ionized ammonia 0.3 ± 0.01 μg/L and nitrite 0.05 ± 0.01 mg/L. In this period of acclimation, fish were fed once a day with commercial fish pellets (Supra, Brazil). Feces and food residues were removed by syphon and filter system was used to keep the quality of water.

### 2.3. Diet preparation and experimental design

During the experiment, silver catfish were divided in four groups (n = 20 per group): (1) control, (2) $(PhSe)_2$ 1.5 mg/kg, (3) $(PhSe)_2$ 3.0 mg/kg and (4) $(PhSe)_2$ 5.0 mg/kg. The fish of control group were supplemented with diet without $(PhSe)_2$. All fish were fed during 30 and 60 days and $(PhSe)_2$ was added to the control diet. Diet formulation was performed based in previous studies from our group (Menezes et al., 2012, 2014b). Diets were made into pellets (5 mm, diameter) and stored at 4 °C until fed. During the experiment, silver catfish were fed with 3% biomass per day. The daily ration was divided into two equal meals fed at 09:00 and 16:00 h. The feces and food residues were removed by syphoning. Temperature, dissolved oxygen, pH, ammonia and nitrite were evaluated daily and were maintained similar values to those recorded during the acclimation period.

At end of the each experimental period (30 and 60 days), ten fish of each group (n = 10) were weighed and measured. Total length (TL) and body weight (W) for each fish were recorded to evaluate growth and condition factor K (K = (weight / length$^3$) × 100). Afterwards, the fish were anesthetized with 50 mg/L clove oil and euthanized by punching the spinal cord behind the opercula. Liver, gills, brain, and muscle were quickly collected and stored at −80 °C for posteriors analysis. The study was approved by the Committee on Ethics and Animal Welfare of Federal University of Santa Maria, under the number: 84/2009.

### 2.4. Biochemical analysis

At end of the each experimental period (30 and 60 days), ten fish of each group (n = 10) were used to biochemical analysis. In liver, gills, brain, and muscle the lipid peroxidation (LPO) levels was estimated using the thiobarbituric acid reactive substances (TBARS) assay of according to Buege and Aust (1978) and the protein carbonyl (PC) determination was measured by the method described by Yan et al. (1995). In liver, the superoxide dismutase (SOD) activity was determined as described by Misra and Fridovich (1972) based on inhibition of the radical superoxide reaction with adrenalin. Catalase (CAT) activity in liver was determined as reported by Nelson and Kiesow (1972). Glutathione peroxidase (GPx) activity in liver was measured by following the rate of NADPH oxidation at 340 nm by the coupled reaction with glutathione reductase as described by Paglia and Valentine (1987). The glutathione