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Serum and hepatic oxidative damage induced by a diet contaminated with fungal mycotoxin in freshwater silver catfish *Rhamdia quelen:* Involvement on disease pathogenesis

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ABSTRACT

It has been recognized that oxidative stress is implicated in the initiation and progression of diseases due to the excessive formation of free radicals and impairment of the antioxidant defense system, contributing to the mortality of affected animals. The occurrence of a disequilibrium between the antioxidant/oxidant status in serum and liver of freshwater fish fed with aflatoxin B_1 (AFB_1) remains poorly understood and limited to only a few oxidant variables. Thus, the aim of this study was to evaluate whether an AFB_1-contaminated diet causes disturbance on the antioxidant and oxidant status in silver catfish (*Rhamdia quelen*) of freshwater. Serum and hepatic reactive oxygen species (ROS), metabolites of nitric oxide (NOx), and lipid hydroperoxide increased on days 14 and 21 post-feeding in animals that received AFB_1 contaminated diet compared to the control group (basal diet), while protein carbonylation levels increased on day 21 post-feeding. On the other hand, serum and hepatic antioxidant capacity against peroxyl radical and vitamin C levels, as well as glutathione peroxidase and catalase activities were lower on days 14 and 21 post-feeding in animals that received AFB_1 contaminated diet compared to the control group. No difference was observed between groups regarding the superoxide dismutase activity and glutathione levels. Based on these evidences, an AFB_1-contaminated diet causes a disturbance on serum and hepatic antioxidant/oxidant system due to lipid and protein damage elicited by excessive ROS and NOx production. Also, the antioxidant defense system was unable to avoid or minimize ROS and NOx deleterious effects, and consequently, the oxidative damage. In summary, this disturbance can contribute to understand the pathophysiology and mortality of fish after the consumption of AFB_1-contaminated diets.

1. Introduction

Feed is considered vital to fish production and factors that affect its quality and safety are significant impediment to aquaculture, as contamination with aflatoxin B_1 (AFB_1), which is the most occurring aflatoxin found in fish feed [1,2]. The occurrence of AFB_1 in fish feed has significantly increased due to the extensive use of plant-based ingredients to minimize production costs, since a high percentage of worldwide crops are contaminated by aflatoxins before or post-harvest [3].

AFB_1 is a secondary toxic metabolite produced by certain fungi belonging to the genus *Aspergillus flavus* and *Aspergillus parasiticus* that occur as natural contaminants of fish food [4], being associated with significant negative impact in fish health, such as decreased growth and weight gain in Nile tilapia (*Oreochromis niloticus*) [5], hepatic damage in sea bass (*Dicentrarchus labrax*) [6], behavioral abnormalities in silver catfish (*Rhamdia quelen*) [7], and some alterations linked to antioxidant/oxidant status in serum of *O. niloticus* [8], but the occurrence of disturbances in the antioxidant/oxidant status of *R. quelen* fed with AFB_1-contaminated diet remains unknown. Oxidative stress is defined as a disturbance of antioxidant/oxidant status in favor of the later, which happens when the production of free radicals is faster than they are scavenged by antioxidant mechanisms [9]. This imbalance contributes to lesions of macromolecules and impairment of physiological metabolism [10]. In order to avoid or reduce the production of free radicals, such as reactive oxygen species (ROS), fish may activate

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several antioxidant mechanisms as a primary antioxidant defense system composed by the enzymes catalase (CAT) and superoxide dismutase (SOD). A secondary antioxidant defense system formed by glutathione peroxidase (GPx) and glutathione reductase may also be activated. Both mechanisms are needed to limit the prooxidant activity of ROS [11]. Although some alterations of antioxidant/oxidant status have already been reported in fish fed with AFB_1, a complete and expressive evaluation of antioxidant/oxidant system remains unknown, since so far only few parameters such as lipid peroxidation and CAT activity in serum and liver of Nile tilapia [8] and common carp (*Cyprinus carpio*) [12] were determined. Also, it is important to emphasize that these studies did not evaluate the direct effect of AFB_1 because a mixture of aflatoxins (AFB_1 and AFB_2) was provided in fish diet. Thus, our hypothesis is that AFB_1 can cause an increase of free radicals and activation and/or inhibition of antioxidant defenses of silver catfish fed with a contaminated diet. In this context, the aim of this study was to evaluate whether an AFB_1-contaminated diet could cause disturbances in the antioxidant/oxidant status of freshwater silver catfish.

2. Material and methods

2.1. AFB_1 and fish maintenance

AFB_1 (molecular weight: 312.27 g/mol; from *Aspergillus flavus*) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and used to contaminate fish feed. Healthy fish for experimental purposes were collected from a fish farm located in Southern Brazil, and water quality parameters (dissolved oxygen, temperature, total ammonia, and non-ionized ammonia levels) were recently published in details by Baldissera et al. [7].

2.2. Diet and the experimental study

A basal diet was formulated as established in details by Baldissera et al. [7], and was experimentally contaminated with AFB_1 (1177 ppb kg/feed), as preconized by Lopes et al. [13].

A total of thirty-six juvenile silver catfish (90.32 ± 7.54 g; 25 ± 3.5 cm) were used as the experimental model to evaluate parameters linked to the oxidative stress. The animals were divided into two groups named: control (C) and aflatoxin (A) with 18 animals each, and subdivided into six subgroups with 6 animals each (C1, C2, and C3; A1, A2, and A3). The control subgroups (C1, C2, and C3) received a basal diet, while the aflatoxin groups (A1, A2, and A3) were fed with an AFB_1 contaminated diet. Fish received the experimental diet twice a day (9 a.m. and 5 p.m.) at a proportion of 5% of total biomass for 7 days (subgroups C1 and A1), 14 days (subgroups C2 and A2), and 21 days (subgroups C3 and A3).

2.3. Sample collection and tissue preparation

Total blood samples were collected in tubes without anticoagulant on days 7 (subgroups C1 and A1), 14 (subgroups C2 and A2), and 21 (subgroups C3 and A3) after the use of a natural anesthetic (eugenol 30 mg/L), followed by spinal cord section according to the Ethics Committee recommendations. Thereafter, liver tissue was removed and dissected in a glass dish over ice to evaluate the oxidant/antioxidant status. Blood samples were centrifuged at 1000 × *g* for 15 min at 4 °C to obtain serum that was stored at −20 °C until measurement of oxidant/antioxidant parameters. Liver was homogenized (1:10 w/v) in a glass Potter tube with Tris-HCl buffer (10 mM, pH 7.4) and centrifuged at 2000 × *g* for 10 min, and the supernatant used to evaluate the oxidative parameters.

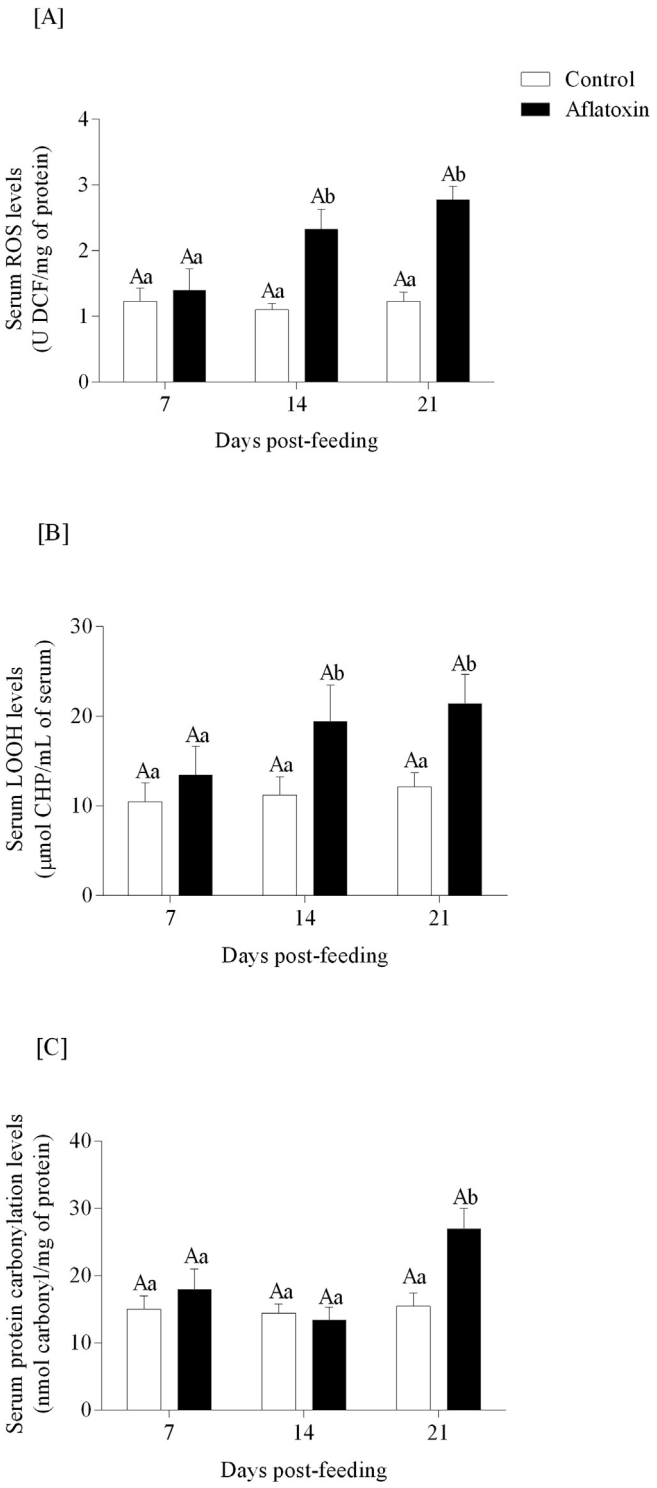

Fig. 1. Serum reactive oxygen species (ROS) [A], lipid peroxidation (LOOH) [B] and protein carbonylation levels [C] in silver catfish fed with a diet contaminated by aflatoxin B_1 (AFB_1) compared to the control group (basal diet). Lowercase letters indicate significant difference between treatment groups at the same time. Capital letters indicate significant difference between times for the same treatment. Bilateral two-way analysis of variance (ANOVA) for independent samples followed by the Tukey post hoc test ($p < 0.05$; $n = 6$ per group).

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