Original Article

Effects of trichlorfon on ecotoxicological biomarkers in farmed *Colossoma macropomum* (tambaqui)

Efeitos do triclorfon sobre biomarcadores ecotoxicológicos em tambaqui (*Colossoma macropomum*) de cativeiro

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Abstract

Producers of fish have been looking for viable alternatives for the management of *Colossoma macropomum* (tambaqui) in confinement systems in order to avoid the harm and subsequent losses caused by parasitic diseases. One alternative used by farmers is pesticides, such as trichlorfon, which has a genotoxic effect. Thus, this study aimed to evaluate the changes in gene expression due to the side effects of trichlorfon in tambaqui. Two treatments were used based on LC_{50}-96h of 0.870 mg/L using 30% and 50% trichlorfon with exposure periods of 48, 72 and 96 h. For differential expression of the genes in the liver, real-time PCR was performed for the *AChE*, *GST*, *CYP2J6*, *CYP2C8*, *18S* and *GAPDH* genes. After 96 h of exposure to trichlorfon, an alteration in the gene expression profile of the antioxidant defense system (GST) of the tambaqui was observed. It was also observed that this organophosphate did not affect the expression of genes related to the isoenzymes that are responsible for the biotransformation of xenobiotics in phase I (2J6 and 2C8) and cholinesterase *AChE*. It was concluded that the reduction in gene expression of *GST* suggests a decrease in metabolization capacity in phase II.

Keywords: pesticide, organophosphate, xenobiotic, Amazonian fish, gene expression.

Resumo

Os produtores de peixes têm procurado alternativas viáveis para o manejo do *Colossoma macropomum* (tambaqui) em sistemas de confinamento, para evitar prejuízos causados por doenças parasitárias. Uma alternativa utilizada pelos piscicultores são os pesticidas, como por exemplo o triclorfon, que apresenta efeito genotóxico. Dessa forma, este estudo teve como objetivo avaliar as alterações na expressão gênica sob os efeitos colaterais do triclorfon em peixes. Foram utilizados dois tratamentos baseados na CL_{50}-96h de 0,870 mg/L usando 30% e 50% de triclorfon com períodos de exposição de 48, 72 e 96 horas. Para expressão diferencial dos genes no fígado, foi realizada a PCR em tempo real para os genes *AChE*, *GST*, *CYP2J6*, *CYP2C8*, *18S* e *GAPDH*. Após 96 h de exposição ao triclorfon, observou-se uma alteração no perfil de expressão gênica do sistema de defesa antioxidante (GST) do tambaqui. Observou-se também que este organofosforado não afetou a expressão dos genes relacionados às isoenzimas responsáveis pela biotransformação de xenobióticos na fase I (2J6 e 2C8) e da colinesterase *AChE*. Concluiu-se que a redução na expressão gênica da *GST* sugere uma diminuição na capacidade de metabolização na fase II.

Palavras-chave: pesticida, organofosforado, xenobiótico, peixe Amazônico, expressão gênica.

1. Introduction

Brazilian aquaculture has consistently intensified due to the favorable conditions that the country presents for its development, and a large part of Brazilian aquaculture production is represented by fish farming (Barçante and Sousa, 2015; Schulter and Vieira-Filho, 2017). Fisheries in the Amazon are an extractive activity and are determined by the level of the water in the rivers, with great production in the dry season, and scarcity during the flood season, which influences the final price of the product (Brasil, 2003). One alternative for minimizing the effects of this seasonality is the breeding of fish in captivity, which, in addition to providing a balance between supply and demand in the regional market and stabilizing prices throughout the year (Brasil, 2003).

Brazil has several native species that are produced in captivity. Among them is the *Colossoma macropomum* (tambaqui), which is produced in diverse fish farming in several regions of the country and on the South American continent due to the growth and development of fish farming (Goulding and Carvalho, 1982; Izel et al., 2014). The tambaqui is the most produced native fish in aquaculture in Brazil according to 2023 data. (Peixe BR, 2021; IBGE, 2021).

Colossoma macropomum belongs to the class Osteichthyes, order Characiformes and family Serrasalmidae. It is the second-largest freshwater scaled fish in South America and is known as the largest characiform of the Amazon (Dairiki and Silva, 2011; Morais et al., 2017). In confined environments, it presents good potential for growth, high resistance and great commercial value, in addition to the ease of obtaining juveniles and wide appreciation by consumers (Gomes et al., 2010; Pedroza-Filho et al., 2016). These aspects have stimulated the commercial rearing of the species as result of the decline of its natural populations and have boosted studies focused on the species regarding its physiology, genetics, preservation, ecology and production technologies, among other aspects (Morais et al., 2017). Fish farming is a profitable activity (Barros et al., 2016) and, currently, most of the commercialized tambaqui are reared in captivity (Peixe BR, 2021). With the evolution of the aquaculture sector, fish farms have been looking for viable and technological alternatives for the management of tambaqui in confinement systems in order to avoid losses caused by mortality and problems in production, such as the control of parasitic diseases (Silva et al., 2013; Abdel-Moneim et al., 2012; Queiroz and Rotta, 2016).

One solution that is used by fish farmers are the pesticides; however, their indiscriminate use in agriculture and animal husbandry is responsible for environmental contamination and causes risks to human health (Abdel-Moneim et al., 2012). Fish are highly vulnerable to aquatic pollutants and can bioaccumulate toxic substances from the water, mainly substances with an apolar physicochemical nature. (Abdel-Moneim et al., 2012; Kerambrun et al., 2011; Cazenave et al., 2014). In addition, these contaminants can generate great economic losses in fishery production due to fish mortality (Ullah, 2015).

The most widely used pesticides in the world today belong to the class of organophosphates (Santana and Cavalcante, 2016). Trichlorfon (dimethyl, 2,2,2-trichloro-1-hydroxyethyl phosphonate), an organophosphate compound with genotoxic effect (Timoroğlu et al., 2014; Costa, 2019), is one of the most widely used products for controlling a variety of ectoparasites in fish farming (Venturini et al., 2015). Thus, the use of biomarkers is essential for the evaluation of aquatic ecosystems (Dalzochio and Gehlen, 2016) that suffer from anthropological actions such as the use of pesticides. Fish need to cope with all the stressors by altering their physiological processes to the limit of adaptation that, in turn, are controlled by their genes (Sinha et al., 2012).

Genotoxic damage has been used as an important biomarker in toxicological studies because it detects DNA damage in different cell types, such as double helix and chromosome breakage in animals exposed to xenobiotics (Guilherme et al., 2010; Santos and Martinez, 2012; Souza-Filho et al., 2013). Biomarkers of stress can serve as important tools for biomonitoring and in the development of risk assessment protocols (Costa-Silva et al., 2015). The most commonly used biomarkers are the enzymes that are involved in the detoxification process of xenobiotics and their metabolites, such as biotransformation enzymes and antioxidant enzymes (Van der Oost et al., 2003).

Despite these variations in the response pattern of the detoxification processes indicated by various studies, in general, the metabolism of xenobiotics, in which organophosphate-based pesticides are included, occur in two phases known as Phase I and Phase II. Phase I is responsible for introducing a polar group into the molecule and involves reactions of oxidations, reductions and hydrolysis of the molecule. Phase II is a conjugation phase, whereby the products of Phase I are bound to other molecules that make them capable of being eliminated from the body. The main enzymes involved in Phase I of metabolism are enzymes of the CYP (cytochrome oxidase) family, which are composed of several isoenzymes and participate in the oxidation of the compounds (Santana et al., 2022). The main enzyme of Phase II is glutathione S-tranferase (GST), which is the first enzyme to be bound to the metabolite of Phase I and its activity is most recognized in the liver, the main organ involved in the metabolism of xenobiotics (Hodgson, 2010).

For most drugs and contaminants, phase I reactions are catalyzed by enzymes of the cytochrome P450 (CYP) superfamily, which transport and biotransform these xenobiotics (Van der Oost et al., 2003). CYP450 is a family of hemoproteins that catalyze monooxygenation reactions, as result of which, P450 accelerates the elimination of many toxic substances and compounds (Rojas-Garcia et al., 2011).

Glutathione-S-transferase (GST) is a family of enzymes with a key role in the general biotransformation of xenobiotics and endogenous substances (Van der Oost et al., 2003). It catalyzes the conjugation of glutathione with xenobiotics in the phase II biotransformation system and has been widely recognized for playing a significant role in the process in freshwater fish (Braz-Mota et al., 2015). Changes in enzyme levels may be efficient biomarkers for monitoring organophosphate pesticides in aquatic environments (Abhijith et al., 2016). The GST enzyme is used as a biomarker of exposure of fish to aquatic pollutants;

and both its induction and inhibition have been reported in fish (Coelho et al., 2011; Santana and Cavalcante, 2016).

The neurological, physiological and behavioral responses of animals are also extremely sensitive to environmental contamination (Menezes et al., 2011; Hued et al., 2012; Shiogiri et al., 2012; Harayashiki et al., 2013; Cazenave et al., 2014; Braz-Mota et al., 2015). Analyses of acetylcholinesterase (AChE) activities using muscle and brain (Lopes et al., 2014) have been used to evaluate the occurrence of aquatic pollution by contaminants such as organophosphorus pesticides (Barbieri and Ferreira, 2011; Glusczak et al., 2011), including trichlorfon (Sinha et al., 2010; Mataqueiro et al., 2014), in addition to other toxic agents (Sinha et al., 2012; Mela et al., 2013; Bonifacio et al., 2016).

A number of studies have confirmed that cholinesterases are suitable for monitoring the occurrence of this class of pesticides in fish (Assis et al., 2010), and several methodologies have been developed using these enzymes to monitor the presence of pesticides in the aquatic environment (Maheswari et al., 2014; Bonifacio et al., 2016).

In general, the responses of the biomarkers to exposure to pesticide residues have been used to indicate stress in aquatic organisms (Kerambrun et al., 2011), since they suggest responses that affect the biological condition of fish, provide insight into the health of these organisms (Vidal-Dorsch et al., 2012) and allow an early indication of the ecotoxicological impact (Venturini et al., 2015). Thus, the aim of this study was to evaluate gene expression for *AChE*, *GST* and *CYP450* with a view to monitoring the potential effects of trichlorfon in tambaqui.

2. Materials and Methods

2.1. Acquisition and acclimation of animals

Juvenile specimens of *C. macropomum* (tambaqui) were obtained at the Experimental Farm of the Federal University of Amazonas (UFAM), located on state highway BR 174, km 38, Ramal de Presidente Figueiredo, in the state of Amazonas, Brazil. Before the experiment, the animals were acclimated during the period of 90 days in 300 L polyethylene tanks in the Wet Laboratory of Morphophysiology, Parasitology and Genetics of Aquatic Animals. The fish were fed three times a day with commercial feed enriched for growth with 28% crude protein (Nutripiscis®), until they were at the ideal size for the beginning of the experiment. The physic-chemical conditions of the water, such as dissolved oxygen, pH and temperature were monitored daily.

This study was submitted to the Ethics Committee of the Federal University of Amazonas (UFAM), under the registration CEUA/UFAM 030/2018.

2.2. Experimental design

For the experiment, 8 tambaqui specimens were distributed among 15 tanks with a controlled volume of 60 L each. The juvenile animals weighed approximately 24.86 ± 7.9 g and had a fork length of about 10.5 ± 1.2 cm. Based on the LC_{50-96h} described by Silva et al. (2019) (0.870 mg/L), two

nominal concentrations of triclorfon were determined for the animals' exposure: 30% of the LC_{50-96h}, corresponding to 0.261 mg/L, and 50% of the LC_{50-96h}, corresponding to 0.435 mg/L. The drug used in this experiment containing triclorfon [dimetil (2,2,2 – tricloro – 1 hidroxietil) fosfonato] is Masoten® (Bayer S.A.), which contains, in 100 g of product, 80 g of triclorfon and 20 g of vehicle. The triclorfon solution was prepared in advance with the chosen nominal concentrations, and the weighed drug was mixed with distilled water for complete dissolution. Then, the solutions corresponding to each concentration were added to their respective tanks, homogenized using a spatula until it is no longer possible to observe lumps with the naked eye, which corresponded to: control condition (C0), without the addition of triclorfon; condition 1 (C1), with the addition of 0.261 mg/L; and condition 2 (C2), with the addition of 0.435 mg/L. The tanks were randomly assigned to receive the treatments.

The animals were collected at exposure times of 48, 72 and 96 h , with each collection time group having its respective control group collected as well. Furthermore, the animals were not fed during the 96 h experiment, water circulation remained closed during this period, and air circulation remained open. Water parameters such as dissolved oxygen (6.1 ± 0.5 mg/L), pH (6.3 ± 0.4), and temperature (27.1 ± 0.1 ºC) were measured throughout the experiment. Figure 1 presents the schematic design of the experimental setup.

The animals were slaughtered using the medullary section procedure (Pedrazzani et al., 2007) after the proposed period and liver tissue was collected. The samples were placed in 1.5 mL microtubes containing 800 µL of Trizol® reagent, then macerated and stored in a freezer at -80 ºC.

2.3. RNA extraction and cDNA synthesis

Extraction of total RNA was performed using the Trizol® reagent extraction protocol (Invitrogen, Applied Biosystems) following the manufacturer's guidelines. To verify the integrity of the extracted RNA, electrophoresis in 1% agarose denaturing gel was used. Total RNA samples were treated with DNAse I-Ambion RNAse-free (Applied Biosystems) (manufacturer's protocol). After the treatment of the samples with DNAse I, the synthesis of complementary DNA (cDNA) was performed. For this, the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems) was used, following the manufacturer's guidelines. In order to verify the effectiveness of the reaction, the synthesized cDNA was quantified in a chemiluminescence imager (FluorQuant™, Loccus Biotecnologia), for subsequent dilution of the samples for real-time PCR.

2.4. Analysis of gene expression

The specific primers *AChE*, *GST*, *Cyp450 2j6* and *Cyp 2c8* (Table 1) were designed from sequences obtained from the genome of *C. macropomum* available on the GenBank, NCBI platform, BioProject PRJEB 40318, accession number GCA_904425465. The sequences obtained were saved in the ".fasta" format. The design of the primers was carried out
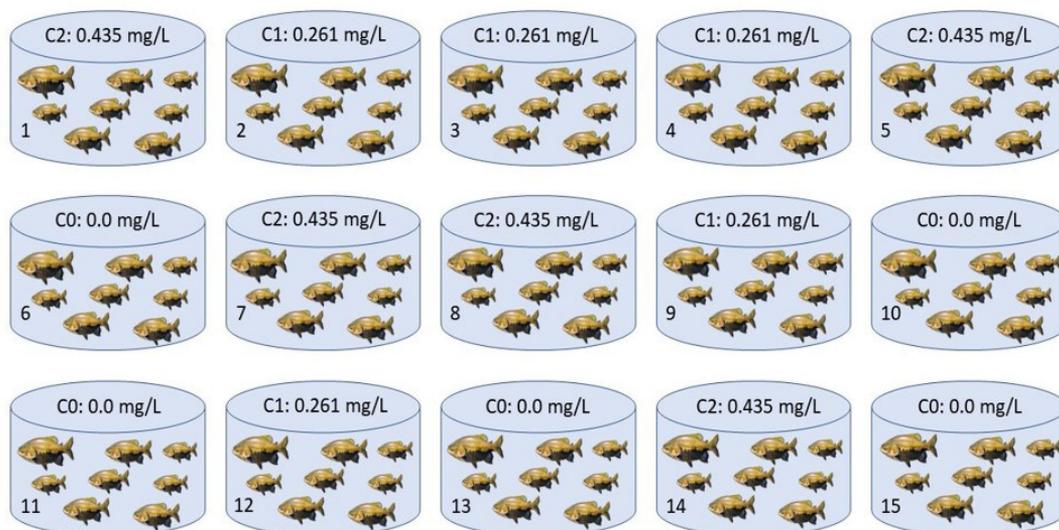
Figure 1. Experimental design with the specification of the tanks, conditions used and quantity of fish per tank. The experiment was completely randomized to ensure statistical results. C0 – control condition; C1 – concentration 1. 30% of LC_{50-96h}; and C2 – concentration 2. 50% of LC_{50-96h}.

Table 1. Sequences of qPCR primers for *AChE*, *GST*, *CYP2J6*, *CYP2C8*, *18S* and *GAPDH* in *Colossoma macropomum*.

Gene	Primer sequences	Amplicons
GST	Forward: GCTCCCTACACCATCACTTAC	100
	Reverse: TTACCACAATCTCCTTCCACTC	
	Forward: AGTGGAAGGAGATTGTGGTAAA	102
	Reverse: CCAGGTCACCATCCTGAAAT	
AChE	Forward: GAAATGACACCGAGCTGATTAAC	119
	Reverse: CACAACAGGCACAAAGGAATAG	
	Forward: AACAGCAGGTCAAGGGAATC	101
	Reverse: CTCGATGGTCAAACAGGTAGAG	
CYP2J6	Forward: CGGCAGATTTGTGAAGAGAGA	113
	Reverse: GCATCAGAGAGGTGAAGAAGAG	
CYP2C8	Forward: GATGAGGTTCTGGAGGGTAAAG	116
	Reverse: AACGCAGTTGGGACAGTAAT	

on the Integrated DNA Technologies (IDT DNA) platform, using the Primer Quest Tool.

For the RT-qPCR assay, the qPCR thermal cycler (Amplio 96®, Loccus Biotecnologia) was used and the SYBR Green Master Mix PCR reagent (Applied Biosystems) was used in), following the manufacturer's guidelines. Differences in gene expression were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). The reference genes for tambaqui *18S* and *gapdh* were selected as normalizers in the reaction (Nascimento et al., 2016).

The reactions were analyzed in triplicate (technical replicates) for the detection of errors, and negative controls were also analyzed for the elimination of contamination. The efficiency of each gene was calculated by means of a

serial dilution curve of the PCR products obtained from the experimental and control samples and, from the data obtained, a graph of the cycle of initiation of detection of the amplified product (Ct) versus the log10 of the relative number of copies per serial dilution was prepared. A linear regression was performed to determine the angular coefficient of the line (S) used to determine the amplification efficiency using the formula developed by Pfaffl (2001).

For the detection of the difference in the levels of the evaluated genes, the relative quantification method was used (Pfaffl, 2001). This method is a modification of the comparative Ct method ($\Delta\Delta CT$) based on the quantification of the gene of interest relative to the reference gene and the efficiency in reverse transcription. The relative expression ratio is based on amplification efficiency and CT variation of the control or calibrator group and the other groups of interest relative to the reference gene. All results were analyzed and presented as mean +/- standard error of mean (SEM).

2.5. Statistical analysis

Data were presented as the mean ± standard error of mean (SEM; n=3). The Sigma Plot 11.0 statistical package (Systat Inc., USA) and GraphPad 5.0 (USA) were used for statistical analysis and graph preparation, respectively. Before the analyses, normality and homoscedasticity were tested to evaluate the parametric presupposition of the data. When deemed to be normal and homoscedastic, the data were tested using two-way analysis of variance (two-way ANOVA) considering trichlorfon concentration x exposure period as factors. Tukey's post-hoc test was used to contrast between means when differences were detected in the two-way ANOVA. Differences were significant when $p<0.05$.

3. Results

Exposure to the organophosphate trichlorfon promoted a reduction in the genetic expression of the GST gene in the liver of the tambaqui. The change was influenced by the interaction between the concentration of trichlorfon x exposure period (F=4.291; p=0.014), (Table 2). The fish exposed to trichlorfon at a concentration of 30% for 96 h showed reduced gene expression when compared to animals subjected to the same treatment at 48 and 72 h (p<0.05). In addition, the expression was also lower in relation to the control group and the 50% trichlorfon group at 96 h (p<0.05; Figure 2).

Gene expression levels of acetylcholinesterase (AChE) in the liver (F=0.314; p=0.734) were not altered by exposure to trichlorfon. The gene expression remained stable, with no effects in relation to the exposure period. There were no interactions between organophosphate concentration and trichlorfon exposure period (Figure 3, and Table 2).

CYP2J6 (F=0.414; p=0.668) and CYP2C8 (F=0.189; p=0.830) expression levels were not significantly influenced by organophosphate concentrations, exposure periods (F=0.0578; p=0.994/F=1.310; p=0.297) or by the interaction between the treatments (F=0.609; p=0.662/F=0.310). F=1.402; p=0.278), respectively (Figure 4).

4. Discussion

Some recent studies have highlighted the effects caused by organophosphates, directly or indirectly, in vertebrate or invertebrate aquatic animals; in fish, they compromise metabolism, leading to death (Das, 2013; Kayhan et al., 2013). Silva et al. (2019) observed swim bladder problems and tissue necrosis in fish exposed to trichlorfon.

In Brazil, the drug Masoten® (Bayer), which is based on the organophosphate trichlorfon, is used commonly in fish farming for the treatment of ectoparasitosis. However, around the world, the side effects that can be caused by the use of this compound have been noted, not only in fish, but also in several other animals. The toxicity, not only of trichlorfon, but of all organophosphate compounds, begins during the process of metabolism of the compound. Depending on the compound to be metabolized, metabolites or reactive oxygen species (ROS) are generated that can be more toxic to the animals (Latif et al., 2022). Several enzymes are directly responsible for the metabolism of trichlorfon in the body (Santana et al., 2022). In this study, the gene expression levels of *GST* gene, indicative of oxidative stress, *Cyp2j6* and *Cyp2C8* genes, which are considered participants in the metabolization of the compound, and *AChE*, the enzyme that is directly blocked by the compound, were evaluated in tambaqui.

Regarding the enzyme acetylcholinesterase (AChE), several studies have pointed out that both its enzymatic
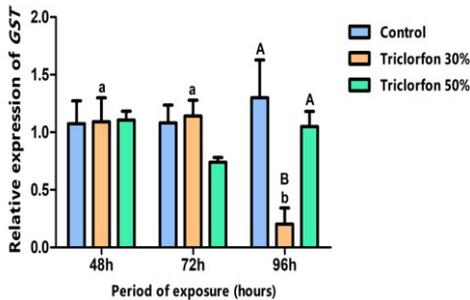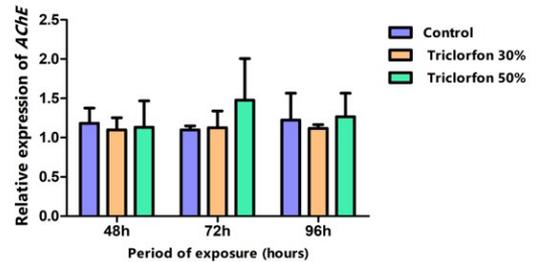

Figure 2. Relative gene expression of *GST* in juvenile *Colossoma macropomum* exposed to trichlorfon for 48 and 96 h (n=3). Uppercase letters indicate differences between the control group and trichlorfon concentrations for the same exposure period, and lowercase letters indicate differences between the same trichlorfon concentration and the evaluated exposure periods (p<0.05).

Figure 3. Relative gene expression of acetylcholinesterase (*AChE*) in the liver of *Colossoma macropomum* exposed to trichlorfon for 48 and 96 h (n=3). The significance level adopted in the Tukey test was p<0.05.

Table 2. Source of variation of the two-way ANOVA for Colossoma macropomum exposed to trichlorfon for 48 and 96 h. The F-test indicates whether the variances are different and p indicates which means-treatments are different.

Gene	Source of variation	F	p	Gene	Source of variation	F	p
AChE	Concentration	0.314	0.734	CYP2C8	Concentration	0.189	0.830
	Period	0.088	0.915		Period	1.310	0.297
	Conc. x Period	0.171	0.951		Conc. x Period	1.402	0.278
CYP2J6	Concentration	0.414	0.668	GST	Concentration	2.674	0.098
	Period	0.0578	0.994		Period	1.330	0.291
	Conc. x Period	0.609	0.662		Conc. X Period	4.291	0.014*

*Asterisk indicates statistical difference between treatments.
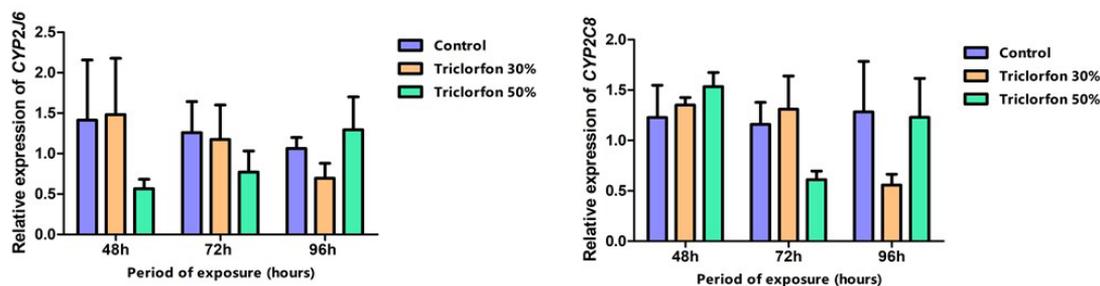
Figure 4. Relative expression of *CYP2J6* (A) and *CYP2C8* (B) in juvenile *Colossoma macropomum* livers exposed to trichlorfon for 48 and 96 h (n=3). The significance level adopted in the Tukey test was $p<0.05$.

activity and its expression levels vary according to the tissue analyzed, as well as the organophosphate to which the animal was subjected and that the organs most affected in terms of their activity are the muscle and the brain. In *Oreochromis niloticus* (Nile tilapia), trichlorfon exposure inhibited 85% of *AChE* enzymatic activity in muscle after 96 h of exposure (Guimarães et al., 2007). One study carried out with *Rhamdia quelen* (silver catfish) observed that there was a reduction in the enzymatic activity of AChE in the brain of the animal when subjected to exposure to trichlorfon (Baldissera et al., 2019). In *Cyprinus carpio* (common carp), a similar result was found in the brain (Wang et al., 2022). In tambaqui, Duncan et al. (2020) describes that there was inhibition of more than 90% of the enzyme activity of AChE in the brain, muscle and intestine.

The results found in our study show that AChE gene expression in the liver did not show a significant difference regarding the dose of trichlorfon and the exposure time. Similar results were found by Sinha et al. (2010), who observed no significant difference in the levels of AChE gene expression in liver of catfish (*Pangasiadon hypophthalmus*). However, a decrease in AChE gene expression was observed in the gills of the same animals. Similar results were also found in the analysis of the tambaqui transcriptome, in which AChE was not observed as a differentially expressed gene, therefore, without changes in gene expression levels when compared to the control group (Silva, 2023).

Our results showed that the AChE response in aquatic organisms depends on the organophosphate used. In tambaqui, the commercial product Malathion®, which has malathion as its active ingredient, also did not alter the enzymatic activity of AChE in different tissues (Souza et al., 2020, 2021), which similar to what was observed by Silva (2023). Thus, despite AChE being considered in the current literature to be a good biomarker for organophosphate pollution, our review and our studies indicate that its usefulness as a biomarker of aquatic contamination is totally dependent on the tissue analyzed, considering both its enzymatic activity and its levels of gene expression.

Regarding the *CYP450* variant enzymes, it was observed that the concentration of trichlorfon and period of exposure to the compound did not alter the gene expression of the cytochrome in the two isoenzymes *Cyp 2j6* and *Cyp 2c8* analyzed in the present study. A change in the induction of *CYP* enzymes for biotransformation of xenobiotics can interfere with the dynamics of the pathways of degradation and synthesis of hormones and vitamins (Soares et al., 2016). These factors have zootechnical relevance for captive specimens that need to present healthy and satisfactory growth for the consumer market. In studies similar to ours, in analysis of the liver transcriptome of tambaqui, alterations were observed in the expression profiles of the CYP450 variants *Cyp2J6* and *Cyp2C8* (Silva, 2023; Carvalho et al., 2023). However, the analyses of expression carried out in the present study provided evidence that this altered expression was not statistically significant in relation to the control group. Despite this, the appearance of CYP450 transcripts may indicate that these enzymes are good ecotoxicological biomarkers for the contaminant trichlorfon in the genome of this native species. In addition, other factors such as genetic polymorphisms, regulation by cytokines, hormones, epigenetic factors influence the expression of CYPs (Zanger and Schwab, 2013). MicroRNAs may be the main regulatory mechanism responsible for altering Cyp450 activity after the inflammatory process (De Jong et al., 2020) and inhibit gene expression largely post-transcriptionally (Guo et al., 2010). Gene expression of cytochromes belonging to subfamilies 2C and 2J during the inflammatory process can occur through transcriptional dysregulation and inhibition of gene expression in a post-transcriptional manner and interact with microRNAs (Vizzini et al., 2021).

One of the first and main signs that there is toxicity from xenobiotics in the cell is the imbalance caused and, consequently, the reduction in antioxidant defense and oxidative stress (Khatib et al., 2022). GST is a superfamily of enzymes responsible for the conjugation of GSH to xenobiotics and they are the most studied enzymes because they are considered bio-markers of biotransformation and oxidative stress (Santana et al., 2022). A study of *C. carpio* (common carp) evaluated the activity of enzymes involved in oxidative stress, neurotoxicity and cortisol levels in gills and liver during one and two weeks of exposure to trichlorfon, subjecting the animals to different temperatures. As one of the markers of oxidative stress, the activity of GST was evaluated, which showed that, at a temperature of 25 °C, it has a higher activity and that two weeks of exposure is when it presents a higher activity of the enzyme at the highest concentration used (4.0 mg/L^{-1}) (Woo and Chung, 2020).

On the other hand, Duncan et al. (2020), who evaluated the enzymatic activity of GST in tambaqui during 96 h of exposure to trichlorfon, observed that GST did not have its activity significantly altered in several tissues, including the liver, even with greater exposure to the compound. On the other hand, our results indicate a decrease in the expression of this enzyme in the liver after 96 h of exposure to a 30% concentration of trichlorfon. Our results also suggest that *GST* is not involved in detoxification of trichlorfon. Therefore, the lack of catalytic activation as well as the reduction in *GST* gene expression suggests an inability of phase II to conjugate GSH directly to trichlorfon and/or its metabolites. This can prolong and potentiate the effects of intoxication.

Thus, based on the results of the present study, we observed a significant decrease in GST gene expression in the group exposed to the organophosphate trichlorfon, mainly at a concentration of 30% of LC_{50-96h} and for the exposure period of 96 h. On the other hand, the expression levels of *AChE*, *Cyp450 2J6* and *Cyp 2c8* were not altered in relation to their relative expression in the liver. New studies are being developed to investigate the relationship of these enzymes with the stress caused by trichlorfon in other tissues**.**

5. Conclusions

After 96 h of exposure, there was a change in the gene expression of the species antioxidant defense system (GST). The reduction in GST gene expression suggests a decrease in the rate of metabolism in Phase II. This effect can prolong the action of the contaminant on organisms.

Acknowledgements

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