



Physiological parameters of *Piaractus mesopotamicus* (Osteichthyes: Characidae) exposed to a biopesticide based on *Bacillus thuringiensis*

WAGNER S. MARIANO^{1,2}, SAULO B. AZEVEDO³, FRANCISCO L. GOMES², LIANA B.D. LIMA², SANDRO E. MORON² and MARCOS TAVARES-DIAS^{1,4}

¹Programa de Pós-Graduação em Biodiversidade e Biotecnologia/BIONORTE, Universidade Federal do Amapá/UNIFAP, Rodovia Juscelino Kubitschek, Km 2, 68903-419 Macapá, AP, Brazil

²Universidade Federal do Tocantins/UFT, Campus Universitário de Araguaína, Avenida Paraguai, s/n, Setor Cimba, 77824-838 Araguaína, TO, Brazil

³Programa de Pós-Graduação em Zoologia, Museu Paraense Emílio Goeldi, Avenida Governador Magalhães Barata, 376, 66040-170 Belém, PA, Brazil

⁴Embrapa Amapá, Rodovia Juscelino Kubitschek, Km 6, 68903-419 Macapá, AP, Brazil

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Abstract: This study investigated the physiological effects of a pesticide based on *Bacillus thuringiensis* (Dipel-WP[®]) added in the water and diet of *Piaractus mesopotamicus* during 24 and 48 h. It was added 0.13 g of de *B. thuringiensis* per kg of commercial feed; and for the fish subjected to the biopesticide in the water of the tanks, it was added 0.13 g/L of the biopesticide. Plasma levels of sodium, chloride, potassium, cholesterol, glucose, triglycerides, cortisol, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), hematocrit, hemoglobin, erythrocytes number, mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), number of thrombocytes and leukocytes were determined. Cortisol, lactate, glucose, total protein, cholesterol, triglycerides, ALT, AST, sodium, potassium, hematocrit, hemoglobin, MCV, number of erythrocytes, leukocytes, lymphocytes, eosinophils and PAS-positive granular leukocytes suffered alterations derived from the addition of *B. thuringiensis* in water and diet of the fish. The toxicity of the concentrations of biopesticide in short-term exposure in water and diet of the fish led to blood alterations (increase or decrease). Therefore, care must be taken to avoid a possible prolonged contamination in the tanks of fish farming by agricultural pollution based on *B. thuringiensis*.

Key words: Biochemistry, Blood, Farm fish, Freshwater fish.

INTRODUCTION

Agricultural production systems in the contemporary world use high levels of pesticides, a growing practice in proportion to the increase in

food production (Angelo et al. 2010, Gusmão 2013). Biopesticides have been used as an alternative to chemical pesticides and are considered more advantageous because they are less toxic and cause damage only to target insects. In addition, biopesticides are used in small concentrations and have a rapid decomposition (Angelo et al. 2010, Mariano et al. 2017, Lobo et al. 2018). Bacterial-

Correspondence to: Marcos Tavares-Dias
E-mail: marcos.tavares@embrapa.br
ORCID: <https://orcid.org/0000-0002-8376-1846>

based biopesticides have been used to control invertebrates, mainly pest insects that invade the various crops of soybean, corn and cotton (Mariano et al. 2015).

Currently, biopesticides based on *Bacillus thuringiensis* (Bt) have been widely used in biological control of crop pests in several countries since they are not considered to be environmentally harmful. *Bacillus thuringiensis* is a gram-positive bacterium of the family Bacillaceae with growth temperature range between 10 and 45°C. The main characteristic that distinguishes this bacteria species from others of the same genus is the intracellular presence of protein crystals with entomopathogenic activities for several species of pest insects. Such protein crystals are the agents that act directly in the digestive tract of the target insects, leading them to death (Angelo et al. 2010, Lobo et al. 2018). Spores of Bt have advantages over vegetative cells because they remain stable for long periods, have the ability to grow rapidly, tolerate a wide range of physiological conditions, involve simple production processes, and are inexpensive. They can be used in commercial products because they are naturally suitable for ingestion by animals (Mendoza-Estrada et al. 2016).

Studies reported that Bt has no toxicity to non-target species, including fish (Jackson et al. 2002, Meher et al. 2002, Ahmad et al. 2011), but it produces toxic and highly specific proteins against protozoans, insects, helminths and mites of agricultural and veterinary importance (Mendoza-Estrada et al. 2016). Thus, Bt has been recommended for control of trematodes *Centrocestus formosanus* in ornamental fish (Mendoza-Estrada et al. 2016). However, for *Oreochromis niloticus*, exposure to Bt increased erythrocyte apoptosis (Grisolia et al. 2009) and in *Colossoma macropomum*, it decreased the number of total erythrocytes (Mariano et al. 2017). In *Clarias gariepinus*, intraperitoneal inoculation with Bt increased the leukocytes number (Omoya and Akharaiyi 2015). Therefore,

little is known about the physiological effects of Bt in fish. Hematological parameters can provide information on physiological response of fish due to the close association of the circulatory system with the external environment. Blood analysis allows the assessment of health and potential pathological disorders that can affect the homeostasis of fish (Tavares-Dias and Mataqueiro 2004, Ranzani-Paiva et al. 2013, Qadir et al. 2014, Omoya and Akharaiyi 2015, Fazio 2019), for example, when biopesticides are used. The aim of study was to investigate the effects of a pesticide based on Bt in blood parameters of *Piaractus mesopotamicus*, when administered in the tank water or diet.

MATERIALS AND METHODS

FISH AND ACCLIMATION

A total of 90 fingerlings of *P. mesopotamicus* (121.5 ± 28.9 g) obtained from a commercial fish farming in Araguaína, Tocantins State (Brazil) was transported to the Laboratory of Zoophysiology and Biochemistry of the Federal University of Tocantins (UFT), in Araguaína (TO). These fishes were acclimated for 30 days in 500 L water tanks and were given fish feed containing 32% crude protein. A system of constant water was maintained in the tanks and the temperature was kept at 25°C and pH 7.0. The organic matter that accumulated at the bottom of the tanks was removed once a day, and dissolved oxygen level was of 6.2 ± 2.3 mg/L. This study was developed in accordance with the principles adopted by the Brazilian College of Animal Experimentation (COBEA) and were strictly according to the protocols and rules of the Committee on Ethics of Animal Use of the Embrapa Amapá (N° 002- CEUA).

PREPARATION OF THE DIETS WITH BIOPESTICIDE BASED ON *Bacillus thuringiensis*

Dipel-WP® biopesticide (Sumitomo Chemical, Brazil) containing spores of *B. thuringiensis* var.

kurstaki was used in the diet for *P. mesopotamicus*. It was added 0.13 g of this biopesticide per kg of commercial feed (Mariano et al. 2017) to omnivorous fish containing 32% crude protein. The rations were then allowed to dry in a microbiological oven at 37°C during 12 h to activate the Bt spores. Later, a bacterial growth test was performed to confirm the presence of Bt in the prepared diets. A sterile swab was used in the prepared rations and the material was seeded in Petri dishes with Mueller-Hintom agar medium. After 24 hours in a microbiological oven at 37°C, the Gram test was performed to identify Bt. The rations were then used to feed *P. mesopotamicus* for 24 or 48 h. These rations were prepared daily to avoid storage.

EXPERIMENTAL DESIGN WITH BIOPESTICIDE BASED ON *Bacillus thuringiensis*

Two trials using Bt-based biopesticide were performed: one with addition in the feed and another with addition of the biopesticide in the culture tanks of *P. mesopotamicus*. For the trials with fish exposed to the 0.13 g/L of biopesticide (Mariano et al. 2017) directly in the tank water (150 L) were used. Fish were divided in three groups: (1) control group, (2) group exposed to water with Bt during 24 h and (3) group exposed to water with Bt during 48 h. For each group a total of 15 fishes were used, with 5 fishes per replicate and 3 replicates per treatment. The fish were fed *ad libitum* two time every day, and no waste of ration was observed.

Fishes fed with ration containing the biopesticide were kept in tanks of 150 L and divided into three groups: (1) control group, (2) group fed with Bt during 24 and (3) group fed with Bt during 48 h. For each group a total of 15 fishes were used, with 5 fishes per replicate and 3 replicates per treatment. The fishes were fed *ad libitum* two time every day and no wastes of ration was observed. The fishes of the control group were fed with commercial ration without the

biopesticide, which was only moistened with water and dried in a microbiological oven at 37°C, during 12 h. This control group was used for comparison in both trials, with addition of the biopesticide in the feed and in water of cultivation.

During all trials the behavior (erratic swimming, lethargy, loss of balance and loss appetite) of the fish were analyzed.

A system of constant water was maintained in the tanks and the temperature was kept at $25.0 \pm 1.1^\circ\text{C}$ and $\text{pH } 7.0 \pm 1.0$. The organic matter that accumulated at the bottom of the tanks was removed once a day and dissolved oxygen was $6.0 \pm 2.1 \text{ mg/L}$.

BLOOD COLLECTION AND ANALYSIS

Samples of blood were collected by puncture of the caudal vessel with heparinized syringes (5000 IU) and were divided in two aliquots for determination of blood parameters. One aliquot was used for determination of hematocrit by the microhematocrit method, hemoglobin concentration by the cyanometahemoglobin method and count of the total erythrocytes in Neubauer chamber. From these data, the hematimetric indices were determined: Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin Concentration (MCHC). Blood smears were confectioned and stained with a panoptic staining (Laborclin®) and used to determine the number of leukocytes and total thrombocytes (Ranzani-Paiva et al. 2013). The nomenclature of the leukocytes followed the recommendations of Tavares-Dias and Mataqueiro (2004).

Another blood aliquot was centrifuged to obtain plasma that was frozen at -20°C and then used for biochemical analysis. Cortisol was determined using the DBC Kit (CAN-E-270®), which allowed direct quantitative determination of this enzyme-immunoassay and reading at 450 nm in a microplate reader. Concentrations of potassium

(K⁺), sodium (Na⁺), chloride (Cl⁻), glucose, lactate, total proteins, cholesterol, triglycerides, Aspartate Amino Transferase Enzyme (AST) and Enzyme Alanine Amino Transferase (ALT) were determined using kits from Labtest (MG, Brazil) for each purpose.

STATISTICAL ANALYSIS

All the data were initially assessed with regard to the assumptions of normal distribution and homoscedasticity, using the Shapiro–Wilk and Bartlett tests, respectively. Data with not a normal distribution were analyzed using Kruskal–Wallis followed by the Dunn test to compare the medians. Data with a normal distribution were analyzed using ANOVA-One way followed by the Tukey's test, to make comparisons between the means (Zar 2010).

RESULTS

During the trials of 24 and 48 h with *P. mesopotamicus*, there was no mortality or behavioral changes in the fish, except the loss of appetite. In fish exposed to tank water with Bt-based biopesticide, cortisol levels after 24 h were similar ($p>0.05$) to the controls but decreased ($p<0.05$) after 48 h in relation to control fish. Plasma concentration of lactate decreased ($p<0.05$) after 24 h in relation to the control. Glucose levels decreased ($p<0.05$) after exposure of fish for 24 and 48 h to water containing Bt when compared to controls (Figure 1).

In fish fed diets containing Bt, cortisol levels were not affected ($p>0.05$) after 24 and 48 h, while lactate levels decreased ($p<0.05$) after 48 h when compared to control fish. However, glucose levels decreased ($p<0.05$) after 24 h of feeding when compared to control but were similar ($p>0.05$) after 48 h (Figure 1).

In fish exposed to water containing biopesticide based on Bt, plasma levels of total protein and

MCHC were not different ($p<0.05$) between the treatments. Cholesterol levels increased ($p<0.05$) after 48 h of exposure but were similar to control after 24 h while plasma levels of triglycerides decreased and AST. Plasma levels of ALT, sodium, hematocrit, hemoglobin and MCV increased ($p<0.05$) after 24 and 48 hours of exposure when compared to control. The total number of erythrocytes decreased after 48 h of exposure when compared to controls. Potassium concentration decreased ($p<0.05$) after 48 h of fish exposure to water containing Bt but was similar ($p>0.05$) in fish after 24 h exposure and control. Chloride concentrations increased ($p<0.05$) in fish after 24 h of exposure to Bt but were similar to control after 48 h of exposure (Table I).

In *P. mesopotamicus* fed a diet containing Bt, levels of total protein, cholesterol, potassium and erythrocyte decreased ($p<0.05$) after 24 and 48 h compared to control, while hematocrit, hemoglobin and MCV increased ($p<0.05$). The MCHC and levels of sodium and chloride were similar ($p>0.05$) between all treatments and controls. The levels of AST and ALT increased after 48 h of diet with Bt, while triglycerides levels increased after 48 h and decreased after 24 h, compared to controls (Table I).

In fish exposed to water containing Bt, the total number of thrombocytes did not presented alteration ($p>0.05$) between treatments. Number of total leukocytes, lymphocytes and PAS-GL increased ($p<0.05$) after 24 and 48 h when compared to controls, but there were no differences between these evaluated periods. Number of monocytes and eosinophils increased ($p<0.05$) after 24h of exposure to water containing Bt, but there were no differences ($p>0.05$) after 48 h when compared to the control. In fish fed with diets containing Bt-based biopesticide, the number of total thrombocytes, lymphocytes, monocytes, eosinophils and PAS-GL was similar ($p>0.05$) between treatments. However, the total number of leukocytes and neutrophils was similar ($p>0.05$)

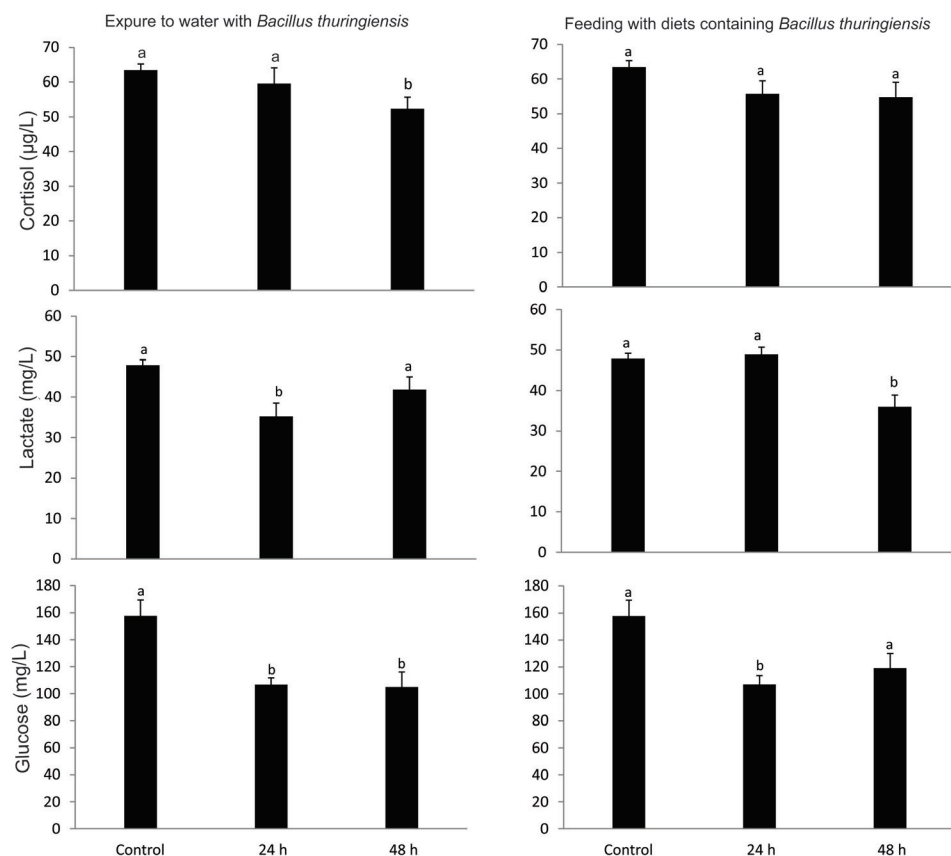


Figure 1 - Plasma levels of cortisol, lactate and glucose of *Piaractus mesopotamicus* exposed to water or diets containing biopesticide based on *Bacillus thuringiensis*. Values express mean and standard deviation. Different letters indicate differences by the Tukey test ($p < 0.05$).

after 24 and 48 h feeding on Bt, but both were higher ($p > 0.05$) than the controls (Table II).

DISCUSSION

Glucose, lactate and blood cortisol levels in fish have been often used as physiological indicator of stress (Wendelaar Bonga 1997, Barton 1997, 2002, Tavares-Dias and Mataqueiro 2004, Urbinati et al. 2015, Khansari et al. 2017). Ionic alterations can be caused by stress, and cortisol exerts both effects: mineralocorticoid and glucocorticoid (Barton 1997, 2002, Mommsen et al. 1999, Khansari et al. 2017). Thus, the secondary effects of stress can lead to metabolic adjustments in plasma levels of glucose, lactate and glycogen, as well as in levels of chloride, sodium, potassium, protein and

osmolarity (Urbinati et al. 2015, Khansari et al. 2017). However, in *P. mesopotamicus*, the increase or decrease in Na^+ , K^+ and Cl^- concentrations were not caused by cortisol, since this hormone presented reduction only after 48 h in fish exposure to water with biopesticide based on Bt. The lactate levels also decreased only after 48 h of feeding with diet based on Bt. Possibly, these ionic alterations were caused by the reduction in feed intake and/or to mobilization of ions to vital organs (i.e. liver, kidney, gills and muscles) due to toxic effects of biopesticide based on Bt. Sarma et al. (2011) reported accumulation of ions in the vital organs of *Channa punctatus* exposed to pesticide, once the gills and kidneys represent the main organs responsible for osmotic regulation in

TABLE I
Blood parameters of *Piaractus mesopotamicus* exposed to biopesticide based on *Bacillus thuringiensis*.

Exposure to water with <i>Bacillus thuringiensis</i>			
Parameters	Control	24 h	48 h
Total protein (g/dL)	7.2 ± 0.4 ^a	5.8 ± 0.8 ^a	7.5 ± 0.3 ^a
Cholesterol (mg/dL)	122.3 ± 15.1 ^a	132.2 ± 29.7 ^{ab}	174.2 ± 29.8 ^c
Triglycerides (mg/dL)	154.2 ± 22.8 ^a	77.1 ± 32.0 ^b	86.9 ± 8.38 ^b
Sodium (mmol/L)	105.3 ± 52.7 ^a	382.1 ± 49.4 ^b	318.7±28.4 ^b
Potassium (mmol/L)	15.5 ± 0.9 ^a	13.3 ± 2.6 ^{ab}	11.3±0.9 ^b
Chloride (mmol/L)	64.4 ± 10.1 ^a	82.4 ± 6.5 ^b	65.8 ± 19.6 ^{ab}
AST (UL)	11.7 ± 7.32 ^a	25.4 ± 7.5 ^b	19.0 ± 6.34 ^{ab}
ALT (UL)	9.7 ± 7.6 ^a	21.1 ± 9.0 ^b	24.9 ± 13.7 ^b
Hematocrit (%)	21.3 ± 3.5 ^a	31.23 ± 3.2 ^b	32.8 ± 3.3 ^b
Hemoglobin (g/dL)	4.9 ± 0.7 ^a	5.9±0.2 ^b	5.9±0.6 ^b
Erythrocytes (x 10 ⁶ /μL)	2.039 ± 0.150 ^a	1.870 ± 0.100 ^{ab}	1.690 ± 0.310 ^b
MCV (fL)	105.4 ± 20.4 ^a	155.6 ± 29.1 ^b	185.9 ± 46.6 ^b
MCHC (g/dL)	22.0 ± 3.0 ^a	21.6 ± 4.0 ^a	18.5 ± 3.3 ^a
Feeding with diets containing <i>Bacillus thuringiensis</i>			
Parameters	Control	24 h	48 h
Total protein (g/dL)	7.2 ± 0.4 ^a	5.7 ± 1.5 ^b	4.8 ± 0.8 ^b
Cholesterol (mg/dL)	122.3 ± 15.1 ^a	88.1 ± 18.1 ^b	79.7 ± 18.1 ^b
Triglycerides (mg/dL)	154.2 ± 22.8 ^a	63.7 ± 5.1 ^b	181.8 ± 27.2 ^c
Sodium (mmol/L)	105.3 ± 52.7 ^a	110.4 ± 39.2 ^a	78.4 ± 12.9 ^a
Potassium (mmol/L)	15.5 ± 0.9 ^a	11.7 ± 1.9 ^b	11.6 ± 1.8 ^b
Chloride (mmol/L)	64.4 ± 10.1 ^a	53.8 ± 10.7 ^a	45.6 ± 6.4 ^a
AST (U/L)	11.7 ± 7.32 ^a	24.0 ± 6.31 ^a	77.1 ± 21.0 ^b
ALT (U/L)	9.7 ± 7.6 ^a	16.1 ± 6.3 ^{ab}	26.7 ± 10.2 ^b
Hematocrit (%)	21.3 ± 3.5 ^a	28.8 ± 3.6 ^b	32.3 ± 2.6 ^b
Hemoglobin (g/dL)	4.9 ± 0.7 ^a	5.4 ± 0.7 ^{ab}	6.0 ± 0.7 ^b
Erythrocytes (x 10 ⁶ /μL)	2.039 ± 0.150 ^a	1.700 ± 0.210 ^b	1.660 ± 0.220 ^b
MCV (fL)	105.4 ± 20.4 ^a	173.1 ± 44.6 ^b	196.5 ± 20.6 ^b
MCHC (g/dL)	22.0 ± 3.0 ^a	19.8 ± 3.8 ^a	18.7 ± 2.0 ^a

Values express means ± standard deviation. AST: Aspartate Amino Transferase, ALT: Alanine Amino Transferase, MCV: Mean corpuscular volume, MCHC: Concentration of mean corpuscular hemoglobin. Different letters, in the same line, indicate differences by the Tukey test (p<0.05).

freshwater fish. In addition, the feeding also plays an important role in ionoregulation and in other blood parameters of the fish (Liew et al. 2015).

In general, the plasma levels of total protein, triglycerides, glucose and cholesterol in *P. mesopotamicus* fed Bt-containing ration were reduced, probably due to reduction in feed intake, which affects the energy demand of fish.

Analyses of the activity of specific enzymes has been used to verify how the metabolism of animals reacts to different conditions, being possible to diagnose adverse metabolic conditions (Sampath et al. 2002, Kori-Siakpere et al. 2010, Qadir et al. 2014, Liew et al. 2015). The enzymes AST and ALT are important in the process of catabolism of amino acids and are available in the blood when

TABLE II
Thrombocytes and leukocytes of *Piaractus mesopotamicus* exposed to biopesticide based on *Bacillus thuringiensis*.

Exposure to water with <i>Bacillus thuringiensis</i>			
Parameters	Control	24 h	48 h
Thrombocytes (μL)	118,363 \pm 269,630 ^a	141,457 \pm 39,475 ^a	140,107 \pm 31,273 ^a
Leukocytes (μL)	82,268 \pm 8033 ^a	196,099 \pm 51,700 ^b	191,208 \pm 58,213 ^b
Lymphocytes (μL)	31,999 \pm 4816 ^a	75,122 \pm 24,671 ^b	101,390 \pm 28,526 ^b
Neutrophils (μL)	37,973 \pm 7349 ^a	83,781 \pm 25,467 ^b	68,680 \pm 24,644 ^b
Monocytes (μL)	3464 \pm 1250 ^a	12,736 \pm 4142 ^b	5889 \pm 2236 ^a
Eosinophils (μL)	5284 \pm 2067 ^a	13,604 \pm 3236 ^b	3393 \pm 1675 ^a
PAS-GL (μL)	3548 \pm 1450 ^a	10,865 \pm 6492 ^b	11,856 \pm 4255 ^b
Feeding with diets containing <i>Bacillus thuringiensis</i>			
Parameters	Control	24 h	48 h
Thrombocytes (μL)	118,363 \pm 269,630 ^a	146,611 \pm 31,540 ^a	132,170 \pm 23,646 ^a
Leukocytes (μL)	82,268 \pm 8033 ^a	113,903 \pm 42,173 ^{ab}	158,166 \pm 53,891 ^b
Lymphocytes (μL)	31,999 \pm 4816 ^a	48,527 \pm 16,314 ^a	59,663 \pm 15,894 ^a
Neutrophils (μL)	37,973 \pm 7349 ^a	48,276 \pm 2481 ^b	75,199 \pm 31,408 ^b
Monocytes (μL)	3464 \pm 1250 ^a	4164 \pm 941 ^a	5836 \pm 2321 ^a
Eosinophils (μL)	5284 \pm 2067 ^a	4918 \pm 1750 ^a	5617 \pm 2460 ^a
PAS-GL (μL)	3548 \pm 1345 ^a	8018 \pm 1690 ^a	11,851 \pm 4345 ^a

Values express means \pm standard deviation. PAS-GL: PAS-positive granular leukocytes. Different letters, in the same line, indicate differences by the Dunn test ($p < 0.05$).

there are tissue damages, mainly in hepatocytes (Sampath et al. 2002, Kori-Siakpere et al. 2010, Bawa et al. 2017). In *P. mesopotamicus* exposed to biopesticide based on Bt, the increase in plasma levels of ALT suggests possible hepatic damages, since this enzyme is found predominantly in the liver (Kori-Siakpere et al. 2010, Bawa et al. 2017, Uncumusaoğlu 2018). In addition, changes in AST plasma levels suggests that the heart and skeletal muscles can had be affected by the action of Bt. However, it has been reported that biopesticides based on Bt are not toxic to fish (Jackson et al. 2002, Meher et al. 2002, Ahmad et al. 2011).

The hematocrit is a good indicator of the adverse effects of environmental factors to fish (Tavares-Dias and Mataqueiro 2004, Ranzani-Paiva et al. 2013). In *P. mesopotamicus* exposed to biopesticide based on Bt, it was found a decrease in the number of total erythrocytes, but with increased hematocrit, hemoglobin concentration and MCV as

compensatory effect. This increase in hematocrit was caused by an increase in the number of immature erythrocytes (erythroblasts), as indicated by the increase in MCV, but no change in amount of hemoglobin by erythrocytes occurred, as indicated by no change in MCHC levels. Similarly, in *Colossoma macropomum* fed diet containing 20 mg/L biopesticide based on Bt per kg ration, a decline in the number of total erythrocytes but no change in hematocrit and hemoglobin concentration was reported (Mariano et al. 2017). In *O. niloticus*, exposure to Bt increased erythrocytes apoptosis (Grisolia et al. 2009), which may reduce the number of erythrocytes in fish exposed to biopesticide based on Bt. These data indicated that biopesticide based on *B. thuringiensis* have adverse effects on the physiology of fish. The disparity in the toxic potential of biopesticides based on Bt may perhaps be related to the differences in susceptibility and tolerance related to fish species. In addition, the

magnitude of toxic effects of biopesticides also may be related to size of fish. However, these issues need still be investigated.

Piscine thrombocytes are cells responsible for hemostasis and are involved secondarily in immune defense (Tavares-Dias and Mataqueiro 2004, Ranzani-Paiva et al. 2013, Fazio 2019). In *P. mesopotamicus* exposed to biopesticide based on Bt, the number of total thrombocytes was not affected, indicating that hemostasis was not impaired. The pesticide can change the functions of vital organs such as liver and kidney, disrupting the hemostatic function in fish exposed (Qadir et al. 2014); however, studies on biopesticides based on Bt have not carried out until the present moment.

In fish, stress response is related to a decreased function of the immune system, but it is dependent on the intensity and duration of the stressor. Consequently, an activation of the immune cells may also occur (Barton 2002, Tort 2011, Urbinati et al. 2015). Leukocytes are particularly useful in studies of physiology because they are altered by stress and can be directly related to stress hormone levels (Davis et al. 2008, Khansari et al. 2017, Fazio 2019). However, in *P. mesopotamicus* there were alterations in the number of leukocytes without increase in cortisol levels. Fish exposed to water with biopesticide based on Bt had leukocytosis caused by the increase in numbers of lymphocytes, neutrophils, monocytes, eosinophils and PAS-GL. In contrast, subcutaneous inoculation with 100 µL of 10⁶ CFU/mL Bt caused leukopenia due to a decrease in the percentage of monocytes, neutrophils, and eosinophils in blood of *Clarias gariepinus* (Omoya and Akharaiyi 2015). Therefore, such differences in these results may be related to the pathway of Bt exposure and fish species.

CONCLUSIONS

Concentrations of the biopesticide based on Bt used in water and diet, in short exposure time, led

to blood alterations due to toxicity, which affected the homeostasis of *P. mesopotamicus*. Therefore, as biopesticide based on Bt was toxic for fish exposed, due to the close relationship of fish with the aquatic environment and the dispersion of them in agriculture, care must be taken to avoid a possible contamination by these agricultural pollutant in fish farming tanks.

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AUTHOR CONTRIBUTIONS

WSM contributed with the data collection and text elaboration. **SBA, FLG, LBDL** and **SEM** contributed with execution of trials. **MTD** was the coordinator of this work.

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