

## CLINICAL SAFETY OF IVERMECTIN TREATMENT IN NILE TILAPIA, *Oreochromis niloticus*

### SEGURANÇA CLÍNICA DO TRATAMENTO COM IVERMECTINA EM TILÁPIAS DO NILO, *Oreochromis niloticus*

H. C. P. SILVA<sup>1</sup>; S. L. OLIVEIRA<sup>2</sup>; C. C. COSTA<sup>2</sup>; B. C. C. IBELLI<sup>1</sup>; B. J. CAVALLI<sup>1</sup>;  
V. L. SCARABEL<sup>1</sup>; M. F. ARACAT<sup>2</sup>; L. F. RODRIGUES<sup>2</sup>; M. A. A. BELO<sup>1\*</sup>

#### SUMMARY

Ivermectin belongs to the group of avermectins, a group of macrocyclic lactones produced by *Streptomyces avermitilis*, this drug is widely used in the treatment of endoparasitoses and ectoparasitosis in different species of mammals around the world. Based on this need to develop an adequate sanitary management of fish farms and the therapeutic importance of macrocyclic lactones, this study aimed to evaluate the clinical safety of ivermectin administered orally as part of the feed for Nile tilapia (*O. niloticus*). For this, 56 tilapias (*O. niloticus*),  $\pm 100\text{g}$ , were placed in 7 aquariums (n=8) with a capacity of 100 L of water, supplied with running water devoid of chlorine, with the following treatments being preserved: T0 (Control, untreated), T1 and T2 (treated with 125 and  $625\mu\text{g}/\text{kg}^{-1}$  of ivermectin, respectively). Blood samples were collected for hemogram, leukogram and evaluation of serum biochemical parameters, in addition to organs such as spleen, liver and kidneys (cranial and caudal) for somatic evaluation. The results admired an increase in serum levels of ALT, AST and glycemia, a reduction in cholesterol and triglyceride values at the highest dose, in addition to a decrease in total protein levels and liver somatic analysis. There was no change in hematological parameters, while the leukocyte profile indicated a significant increase in total leukocytes in treated tilapia with lymphocytosis and neutrophilia. Therefore, treatment with high doses of ivermectin can harm the health of Nile tilapia, requiring additional studies to establish safe therapeutic protocols for these animals.

**KEY-WORDS:** Avermectins, macrocyclic lactones, endoparasitosis, ectoparasitosis, teleost fish

#### RESUMO

A ivermectina pertence ao grupo das avermectinas, um grupo de lactonas macrocíclicas produzidas pela *Streptomyces avermitilis*, esse medicamento é amplamente utilizado no tratamento de endoparasitoses e ectoparasitoses em diferentes espécies de mamíferos ao redor do mundo. Partindo-se dessa necessidade de desenvolver um manejo sanitário adequado de pisciculturas e da importância terapêutica das lactonas macrocíclicas, esse estudo teve por objetivo avaliar a segurança clínica da ivermectina administrada via oral incorporada à ração em tilápias do Nilo (*O. niloticus*). Para isso foram utilizadas 56 tilápias (*O. niloticus*),  $\pm 100\text{g}$ , acondicionadas em 7 aquários (n=8) com capacidade de 100 L de água, abastecidos com água corrente desprovida de cloro, sendo constituído os seguintes tratamentos: T0 (Controle, não tratado), T1 e T2 (tratados com 125 e  $625\mu\text{g}/\text{kg}$  de ivermectina, respectivamente). Foram coletadas amostras de sangue para determinação do hemograma, leucograma e avaliação de parâmetros bioquímicos séricos, além de órgãos como baço, fígado e rins (cranial e caudal) para avaliação somática. Os resultados mostraram aumento nos níveis séricos de ALT, AST e glicemia, redução nos valores de colesterol e triglicérides na dose mais alta, além da diminuição nos níveis de proteína total e análise somática hepática. Não houve alteração nos parâmetros hematológicos, enquanto o perfil leucocitário indicou aumento significativo nos leucócitos totais em tilápias tratadas, com marcada linfocitose e neutrofilia. Portanto, o tratamento com altas doses de ivermectina pode prejudicar a saúde das tilápias do Nilo, sendo necessário estudos adicionais para se estabelecer protocolos terapêuticos seguros aos animais.

**PALAVRAS-CHAVE:** Avermectinas, lactonas macrocíclicas, endoparasitose, ectoparasitose, peixes teleósteos.

<sup>1</sup> Laboratory of Animal Pharmacology and Toxicology, Brazil University, Descalvado/SP, Brazil.

<sup>2</sup> Department of Pathology, Reproduction, and One Health - Faculdade de Ciências Agrárias e Veterinárias (FCAV) - UNESP Jaboticabal, SP.

\* Corresponding author: Marco Antonio de Andrade Belo, 950 Hilario da Silva Passos Avenue, Pq. Universitário, Descalvado/SP, ZipCode: 13690-000, Brazil.

## INTRODUCTION

Aquaculture has been the fastest-expanding food production sector worldwide over the past 50 years, growing at an average of 5.3% per year since the turn of the century, with Nile tilapia (*Oreochromis niloticus*) responsible for more than 50% of Brazilian production (FAO, 2022).

In the management of intensive fish farming, controlling helminth infections is a challenge, as the availability of licensed anthelmintic medications is limited (Morales-Serna et al., 2018), and medications must be effective and clinically safe for fish (Alves et al., 2019). Furthermore, there are predisposing factors that can intensify parasitism in these species, such as poor water quality, temperature changes, poor nutrition, a lot of decomposition of organic matter, inadequate transport, and high stocking density (Garcia et al., 2003; Piazza et al., 2006).

In addition to reducing animal productivity, parasites can lead to increased susceptibility to other diseases, causing immense losses to production (Varó et al., 2010). Therefore, they can also generate public health problems, as zoonotic diseases may be carried together (Silva et al., 2011). In this context, there is a great demand for products with antiparasitic action (Varó et al., 2010).

Ivermectin belongs to the avermectin group (Scarpelli et al., 1999), being widely used to combat endoparasites and ectoparasites in several animal and human species (Silva et al., 2015). Even though its use in other species is a success, in fish it is still controversial, despite its proven effectiveness, it may present possible toxicity to the species (Varó et al., 2010; Cardoso, 2013). Innocuousness is a relevant factor when studying the administration of pharmacological compounds. According to Klaassen (2013), every substance is potentially toxic, and the correct dose is what differentiates the medicine from the poison. On the other hand, the analysis of biochemical and hematological parameters can point out important information for diagnosis and prognosis of the morbid conditions of an individual or their population (Oliveira et al., 2022; Costa et al., 2022). In this context, little is known about the clinical safety of ivermectin in bony

fish. Therefore, this investigation has experimentally studied and identified the harmlessness of treatment with this avermectin in Nile tilapia (*Oreochromis niloticus*), through biochemical and hematological analyzes.

## MATERIAL AND METHODS

### 2.1. Fish

Fifty-six tilapia (*O. niloticus*) weighing  $\pm 100$ g, belonging to the same spawning from Aquabel fish farm (Porto Ferreira, SP, Brazil), were randomly distributed in 7 tanks (n=8), with a capacity of 100 L of water each, supplied with running water devoid of chlorine, coming from an artesian well with a flow rate of 1 L/min. After being transported to the tanks, fish were acclimatized for 15 days, the time necessary for the plasma cortisol concentration and osmolarity to return to their baseline levels. In the first three days of acclimatization, the animals were exposed to NaCl solution at a concentration of 6.0g/L (Carneiro & Urbinati, 2001). Water quality parameters were determined twice daily throughout the experimental period using a YSI-63 pH meter and a YSI-55 oximeter, and their values remained within the range appropriate for the welfare of tropical fish (Boyd, 1990) (dissolved oxygen =  $4.07 \pm 0.89$  mg L<sup>-1</sup>; temperature =  $27.64 \pm 2.05^\circ\text{C}$ ; pH =  $7.64 \pm 0.54$ ; and conductivity =  $208.29 \pm 97.57\mu\text{S/cm}$ ). All experimental procedures were approved by the Animal Use Ethics Committee (CEUA), at Universidade Brasil (UB) under protocol n° 1900036/19.

### 2.2. Experimental design

Tilapia were randomly distributed in the tanks to establish the following treatments: T0 (control group, not treated with Ivermectin); T1 and T2 (treated with 125 and 625 $\mu\text{g}$  of ivermectin/kg of body weight, respectively). Eight animals were sampled per treatment in 3 periods, that is, 5, 7, and 9 days post-treatment (DPT) (Table 1), to collect blood samples for hemogram and serum biochemical determination, as well as organs such as spleen, liver and kidneys (cranial and caudal) for somatic evaluation.

**Table 1.** Distribution of tilapia in different treatments with Ivermectin.

Groups	Treatments	Days post-treatment with ivermectin		
		5	7	9
T0	Control (without Ivermectin treatment)	N=8	N=8	N=8
T1	Treated with 125 $\mu\text{g}$ of ivermectin/kg of b.w.	N=8	N=8	N=8
T2	Treatment with 625 $\mu\text{g}$ of ivermectin/kg of b.w.	N=8	N=8	N=8

### 2.3. Experimental diet

The commercial extruded feed, containing 36% crude protein (Nutripiscis® - Presence Company), was chosen to formulate the experimental diets for tilapia. Feeding was conducted twice a day (at 8 AM and 5 PM), with the administration of 2% of the biomass in the tanks. To prepare the diets, the feed was weighed daily in proportion to the average weight of tilapia in each tank. Subsequently, the antiparasitic Ivermectin was added at doses of 125 and 625 µg/kg of body weight and homogenized in 2% vegetable oil, composing the diets for T1 and T2, respectively. For diet standardization and nutritional balance, 2% vegetable oil was added to the control group's diet (T0).

### 2.4. Fish Anesthesia

Tilapia were anesthetized by immersion in an aqueous solution of benzocaine at a ratio of 1:10,000 for blood collection and 1:500 at the time of euthanasia. Benzocaine was diluted in 98% alcohol (0.1 g/mL), making up the volume to 1L (Wedemeyer, 1970). Initially, pre-anesthesia was performed, during which the water level in the tanks was reduced to a volume of 10L, and 0.1g of benzocaine already diluted in 98% alcohol was added. Subsequently, each fish was transferred to a container with 1L of water containing 0.1g of benzocaine. Both procedures were carried out under aeration to minimize the stress caused by handling. Once the operculum ceased movement, the fish was removed, and blood was collected. Finally, the animal was transferred to another container with 0.5g of benzocaine diluted in 1L of water for euthanasia.

### 2.5. Hematological Analysis

Blood samples of 3mL were collected from the caudal vessel of each animal, aliquoted into two sets: one heparinized syringe (5,000 IU) and another without heparin, for obtaining plasma and serum, respectively. During the syringe exchange (with and without heparin), the needle was not withdrawn from the vessel, so there was no blood loss. The hemogram was performed using a hemocytometer (Neubauer chamber) and Natt and Herrick solution (1952) at a ratio of 1:100 (v:v). Hematocrit was determined by the microhematocrit centrifugation technique. The concentration of circulating hemoglobin was assayed using the LabQuest biochemical reagent for reading at a wavelength of 540nm, and the mean corpuscular volume (MCV) values were obtained by calculating  $MCV = (HT/HE) \times 100$  and the mean corpuscular hemoglobin concentration (MCHC) was calculated as  $CHCM = (HG / HT) \times 100$ . The differential leukocyte count was performed on blood smears with a count of 200 cells, establishing the percentage of each type of cell of interest after prior smears staining with May-Grünwald Giemsa Wright (Belo et al., 2013).

### 2.6. Serum Biochemistry Analysis

Following protocol described by Aracati et al. (2021), blood samples from fish without anticoagulant were centrifuged at 10,000 rpm for 10 minutes at 4°C to obtain serum. The determination of alkaline phosphatase (ALP), triglycerides, cholesterol, total proteins (TP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) was carried out using a semi-automatic biochemical analyzer (Model LabQuest® - Bioplus Company), and the fish's blood glucose level was determined using the Accu-Chek Performa device.

### 2.7. Assessment of the Somatic Organ Index

After euthanasia, as described in section 2.4, the fish were weighed and dissected via three longitudinal cuts: one from the anus to the operculum, another from the anus to the head following the lateral line, and a third passing through the pectoral fin. This dissection provided a comprehensive view of all organs. For morphometric evaluation according to Weibel et al. (1969), the livers, caudal kidneys, cranial kidneys, and spleens of tilapia were collected and subsequently weighed to express the hepatic, renal, and splenic somatic indices, calculated using the formula: Somatic Index = organ weight.

### 2.8. Statistical Analysis

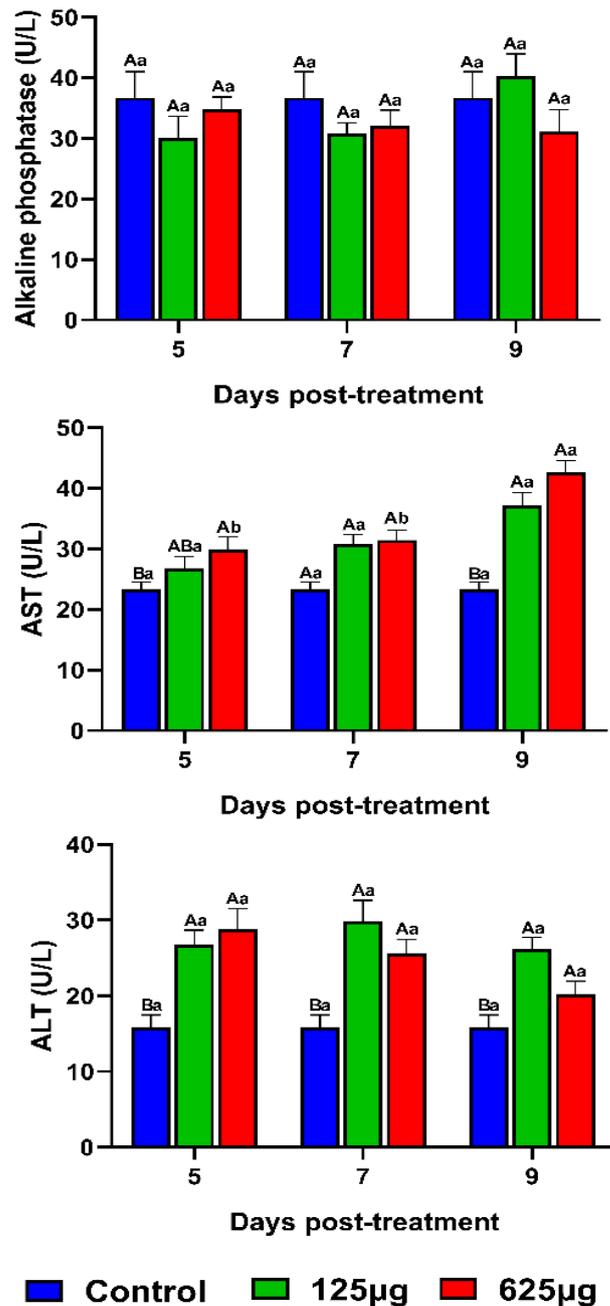
The experimental design for clinical safety assessment was completely randomized in a 3 x 3 factorial scheme (three treatments: 125µg, 625µg, and control X three evaluation periods: 5, 7, and 9 DPT). Analysis of variance to compare different experimental groups was performed using the non-parametric Kruskal-Wallis test with GraphPad Prism software version 9.0. Differences were considered significant when  $p < 0.05$ .

## RESULTS

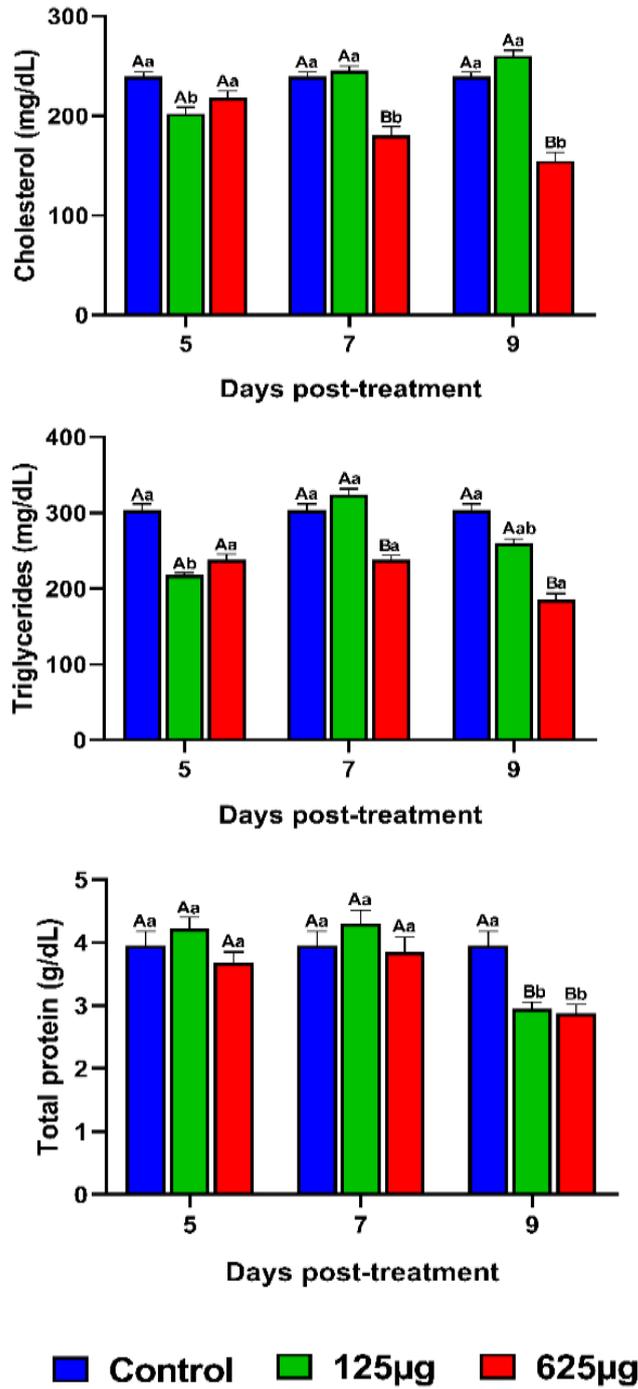
### 3.1. Serum Biochemistry

In the evaluation of liver cytotoxicity, tilapia treated with the antiparasitic ivermectin did not show significant changes in alkaline phosphatase (Figure 1A). However, serum levels of ALT and AST were higher in animals treated with both doses (125 and 625µg) 5 DPT, with 9 DPT being intensified, mainly in relation to AST (Figure 1B and 1C).

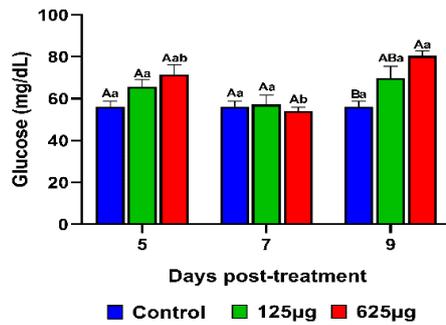
In the assessment of liver functionality (Figure 2), there was a decrease in serum cholesterol and triglyceride values in tilapia treated with the highest dose (625 µg) 7 and 9 DPT compared to fish from the control group and the lowest dose used (125 µg) (Figures 2A and 2B). The results revealed that tilapia treated with ivermectin showed a decrease in serum total protein levels of both doses 9 DPT, when compared to the control group (Figures 2C). Glucose assessment showed a significant difference in both doses 9 DPT, with a more significant increase in the highest dose when compared to the control group (Figure 3).



**Figure1** - Analysis of alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of tilapia treated with ivermectin. The means (n=8) followed by the same letter do not differ according to the Kruskal-Wallis test ( $P < 0.05$ ). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between different experimental days.



**Figure 2** - Analysis of cholesterol, triglycerides and total protein in tilapia treated with ivermectin. The means (n=8) followed by the same letter do not differ according to the Kruskal-Wallis test (P<0.05). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between different experimental days.

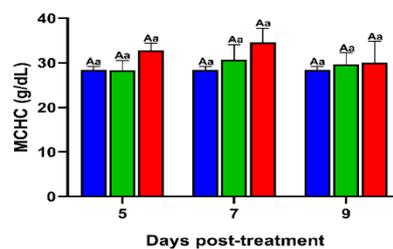
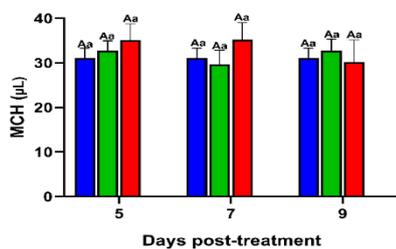
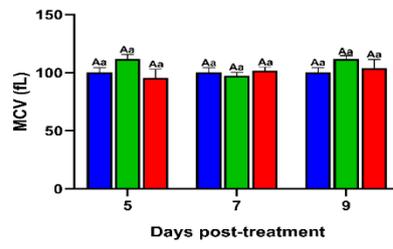
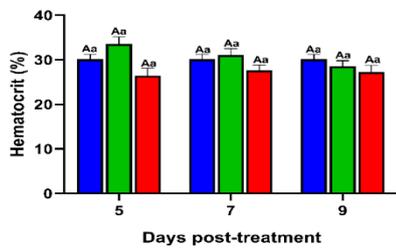
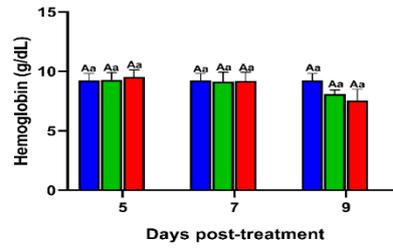
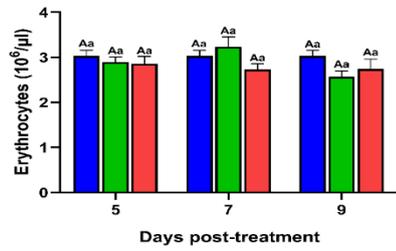


**Figure 3.** Analysis of blood glucose levels in tilapia treated with ivermectin. The means (n=8) followed by the same letter do not differ according to the Kruskal-Wallis test ( $P < 0.05$ ). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between different experimental days.

### 3.2. Hematological analysis

Tilapia treated with ivermectin did not show significant difference ( $P \geq 0.05$ ) in the number of erythrocytes,

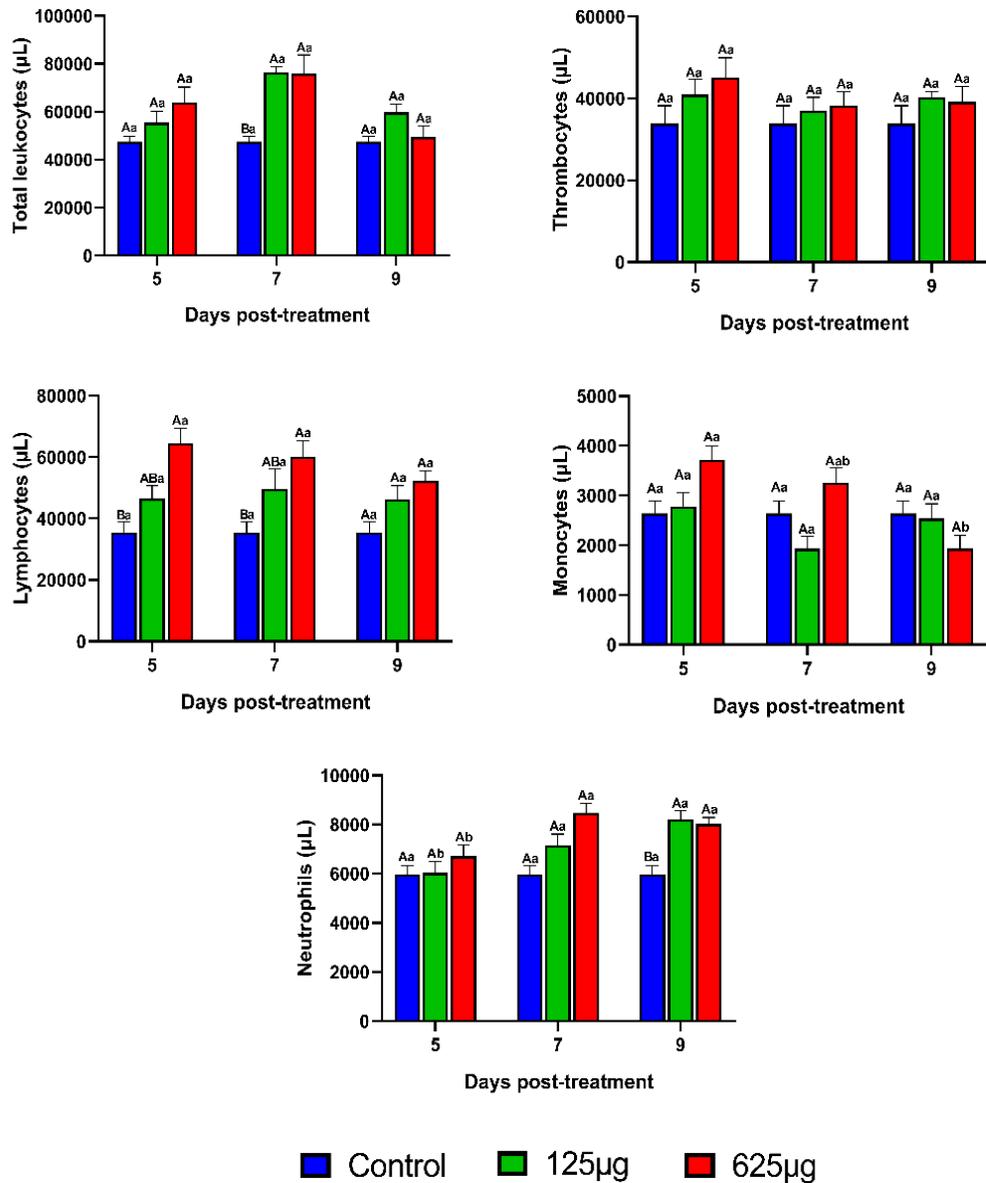
hemoglobin concentration, percentage of hematocrit, MCV, MCH and MCHC when compared to fish from control group (Figures 4A, 4B, 4C, 4D, 4E, and 4F, respectively).



■ Control ■ 125µg ■ 625µg

Leukocyte profile evaluation demonstrated that ivermectin treated-tilapia showed a significant increase ( $P < 0.05$ ) in total leukocytes at 7 DPT when compared to fish in the control group (Figure 5A). This result was also observed in the neutrophil counts at 9 DPT (Figure 5E). Furthermore, lymphocytes showed similar

responses on all evaluation days, with an increase in treated animals compared to control animals, and this response was only significant on 5 and 7 DPT (Figure 5C). There was no significant difference ( $P > 0.05$ ) in the differential count of monocytes and thrombocytes (Figures 5B and 5D).

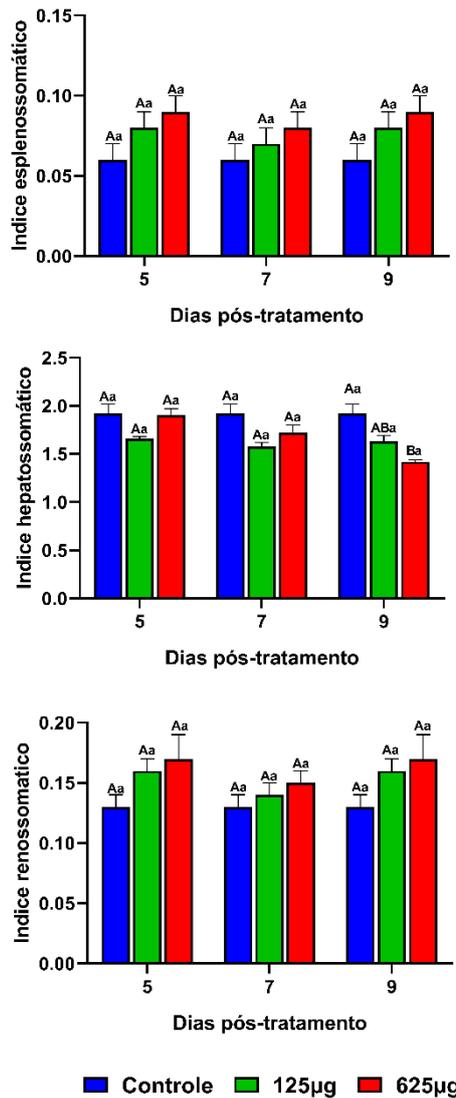


**Figure 5** - Leukogram analysis of tilapia treated with ivermectin. The means (n=8) followed by the same letter do not differ according to the Kruskal-Wallis test (P<0.05). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between different experimental days

### 3.3. Morphometric Analysis

Despite an increase in spleen and kidney somatic values of tilapia treated with ivermectin, these findings did not show a significant difference ( $p \geq 0.05$ ) when compared to control animals (Figures 6A and 6C,

respectively). On the other hand, a decrease was observed in liver somatic index of ivermectin treated-tilapia, and these findings were significant ( $p < 0.05$ ) in fish treated with 625mg compared to control fish 9DPI (Figure 6B).



**Figure 6** - Somatic evaluation of spleen, liver and kidney of tilapia treated with ivermectin. The means (n=8) followed by the same letter do not differ according to the Kruskal-Wallis test ( $P < 0.05$ ). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between different experimental days.

## DISCUSSION

Hematological analyzes can be used as indicators of the health status of fish, allowing the detection of morbid conditions (Oliveira et al., 2022). Treatment with ivermectin did not result in harmful changes in red blood cells profile of tilapia, corroborating the findings of Lozano et al. (2021) who studied ivermectin in *Prochilodus lineatus* (Curimatá) exposed to environmental concentrations at doses of 0.5 and 1.5  $\mu\text{g L}^{-1}$ . Madrid et al. (2021) observed a decrease in red blood cell counts in Amazonian fish (*Corydoras schwartzi*) 7 days post-treatment with a dose of 860  $\mu\text{g mg}$  of ivermectin/kg, although the averages observed were around half the value found for the control fish, these findings were not statistically significant when compared to control fish. For these authors high doses of 220 and 860  $\mu\text{g}$  of ivermectin/kg did not affect the blood cells counting in general. On the other hand, Katharios et al. (2002) report changes in

erythrograms, such as a decrease in hematocrit and an increase in hemoglobin levels in sea bream (*Sparus aurata*) treated with 100-800  $\mu\text{g}$  of ivermectin through intraperitoneal route. According to Varó et al. (2010), oral doses between 50 and 200  $\mu\text{g}$  of ivermectin/kg body weight have been commonly used in farmed fish, however side effects for fish have not been reported (Varó et al., 2010). The results observed in tilapia treated with 125 and 625  $\mu\text{g}$  of ivermectin/kg of b.w. for nine consecutive days corroborate the findings of these authors.

there were higher counts of leukocytes, lymphocytes and neutrophils in animals subjected to treatment with ivermectin. These findings suggest that this macrocyclic lactone may have irritated the intestinal mucosa and resulted in inflammatory changes. The increase in circulating leukocytes is a typical response during the course of both chronic and acute inflammatory reactions (Charlie-Silva et al., 2020; Aracati et al. 2022; Conde et al., 2022). Similar results

with the increase in circulating leukocytes were observed by other authors, such as Ogueji et al. (2019) who studied the exposure of African catfish (*Clarias gariepinus*) to ivermectin at concentrations of 9 to 25 µg/L-1. The use of abamectin also resulted in an increase in white blood cells in tilapia (Mansour et al., 2022).

When xenobiotics bind to the cell membrane of hepatocytes, they can cause cellular damage and result in the leakage of liver enzymes into the bloodstream (Belo et al., 2012). This means that the interaction of these substances with the liver cell membrane can compromise its integrity, leading to the release of enzymes normally found inside liver cells into the bloodstream. This leakage of liver function enzymes may be indicative of liver damage or dysfunction caused by exposure to xenobiotics. Tilapia treated with ivermectin showed an increase in serum levels of ALT and AST, as observed by Ogueji et al. (2020) in which acute administration of ivermectin to fish resulted in significant and duration-dependent increases in ALT and AST concentrations, which may indicate damage to liver cells. Similar findings were also reported following acute concentration exposure of the drug ibuprofen to juvenile African Catfish (*Clarias gariepinus*) (Saravanan et al. 2012; Ogueji et al. 2017). These enzymes are specific indicators of liver disorders (Harper, 1979), their high levels in the bloodstream suggest that high doses of ivermectins lead to hepatocellular damage, however, histopathological studies are required to elucidate pathological changes resulting from this toxic effect.

Tilapia treated with a higher dose of ivermectin showed a decrease in serum cholesterol, triglycerides and total protein values associated with an increase in glucose. These results suggest that the high dose of ivermectin resulted in gluconeogenesis, by mobilizing triglyceride reserves, cholesterol and alter protein metabolism to the production of glucose, probably due to the endogenous glucocorticoid effect due to the increased energy demand caused by stress caused by ivermectin exposure (Mommensen et al. 1999; Belo et al. al. 2005; El-Sayed et al. 2007). Our results corroborate the findings of Firat and Tutus (2020) who reported a significant increase in plasma glucose level after 96h in Nile tilapia exposed to 10 ppb abamectin considering. Higher glucose levels are demanded during the stress response (Mansour et al. 2020). On the other hand, Kushwaha, et al. (2020) reported a significant decrease in serum proteins in Mozambique tilapia (*Oreochromis mossambicus*) exposed to 45 and 55 ppb abamectin for 48 h.

In the evaluation of liver somatic analysis, a significant decrease was observed in animals from different treatments, possibly due to prolonged exposure to higher concentrations of ivermectin. Liver somatic index can indicate the harmful effects of chemical stressors on fish growth and physiology (Ucán-Marín et al., 2012; Corsi et al., 2003). In mammals, De Marco et al. (2002) had already observed a rapid reduction in the hepatic somatic index during brief exposure to ivermectin.

Therefore, it is crucial that future studies delve into issues for a more comprehensive understanding of the risks associated with the use of ivermectin in tilapia, aiming to protect the health, well-being and balance of aquatic ecosystems for appropriate therapeutic use in fish farms.

## CONCLUSION

Treatment with high doses of ivermectin may have negative effects on the health of Nile tilapia, affecting leukocyte parameters, liver function, lipid and protein metabolism, as well as somatic characteristics of the liver. These effects as a whole are important to assess the potential risks of tilapia exposure to ivermectin to assist in health management for fish farms and minimize the negative impacts of its administration in aquatic environments.

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