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SAFETY OF ORAL DOXYCYCLINE TREATMENT IN NILE TILAPIA, *Oreochromis niloticus*

SEGURANÇA DO TRATAMENTO ORAL COM DOXICICLINA EM TILÁPIA DO NILO, *Oreochromis niloticus*

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SUMMARY

This study aimed to evaluate the clinical safety of doxycycline treatment (Sandoz Pharmacetical Industry from Brazil Ltd.), administered orally incorporated into the feed of Nile tilapia, *Oreochromis niloticus*. For this purpose, 75 Nile tilapia (± 300g) from the same spawning were randomly distributed in 15 tanks (n=5), containing 100 L of water each, supplied with running water free from chlorine, to establish the following treatments: T0 (Control - not treated with doxycycline); T1, T2, T3 and T4 (treated with 10, 20, 40 and 80mg of doxycycline/kg of body weight, respectively), in which 5 animals per treatment were sampled in 3 periods: 2, 4 and 8 days post-treatment (DPT). Blood samples were collected for hemogram determination and serum biochemical evaluation, as well as spleen, liver and kidneys (cranial and caudal) for somatic and histopathological evaluation. The results showed no significant difference among treatments in the serum values of creatinine, total protein, cholesterol, triglycerides, globulin, glycemia and alkaline phosphatase enzyme activity. However, serum levels of AST (aspartate aminotransferase) were significantly higher ($P<0.05$) in animals treated with 80 mg of doxycycline in the longest period of treatment (8 days). In the hematological evaluation of tilapia treated with doxycycline, no significant changes ($P>0.05$) were observed in erythrocyte counts, hematocrit, MCV, HCM and CHCM values. Doxycycline-treated tilapia did not present significant difference in the somatic analysis of spleen, liver and kidney when compared to control group. Therefore, the results demonstrated the clinical safety of oral treatment with doxycycline at doses of 10, 20, 40 and 80 mg/kg of b.w., although transient changes in liver functionality were observed after eight days of treatment with the dose of 80mg/kg.

KEY-WORDS: Tetracyclines. Innocuity. Cichlids. Aquaculture. Teleost fish. Sanitary management.

RESUMO

Este estudo teve por objetivo avaliar a segurança clínica do tratamento com doxiciclina (Sandoz do Brasil Indústria Farmacêutica Ltda.), administrada via oral incorporada à ração em tilápias do Nilo, *Oreochromis niloticus*. Para tal, foram utilizadas 75 tilápias no Nilo (±300g) oriundas da mesma desova foram distribuídas aleatoriamente em 15 tanques (n=5), contendo 100 L de água cada, abastecidos com água corrente desprovida de cloro, para constituir os seguintes tratamentos: T0 (Controle - não tratado com doxiciclina); T1, T2, T3 e T4 (tratados com 10, 20, 40 e 80mg de doxiciclina/kg de p.v., respectivamente. 5 animais por tratamento foram amostrados em 3 períodos: 2, 4 e 8 dias pós-tratamento (DPT). Foram coletadas amostras de sangue para determinação do hemograma e avaliação do bioquímico sérico, além de órgãos como baço, fígado e rins (cranial e caudal) para avaliação somática e histopatológica. Os resultados não mostraram diferença significativa entre os tratamentos nos valores séricos de creatinina, proteína total, colesterol, triglicerídeos, globulina, glicemia e atividade da enzima fosfatase alcalina. No entanto, os níveis séricos de AST (aspartato aminotransferase) foram significativamente maiores ($P<0,05$) nos animais tratados com 80 mg de doxiciclina no período mais longo de tratamento (8 dias). Na avaliação hematológica de tilápias tratadas com doxiciclina, não foram observadas alterações significativas ($P>0,05$) nas contagens de eritrócitos, valores de hematócrito, VCM, HCM e CHCM. Tilápias tratadas com doxiciclina não apresentaram diferença significativa na análise somática do baço, fígado e rim quando comparadas aos animais do grupo controle. Portanto, os resultados

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demonstraram a segurança clínica do tratamento oral com doxiciclina nas doses de 10, 20, 40 e 80 mg/kg de peso corporal, embora alterações transitórias na funcionalidade hepática tenham sido observadas após oito dias de tratamento com a dose de 80mg/kg.

PALAVRAS-CHAVE: Tetraciclinas. Inocuidade. Ciclídeos. Aquicultura. Peixe teleósteo. Manejo Sanitário
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INTRODUCTION

The development of synthetic antimicrobials from tetracycline class, initiated with doxycycline in 1967, which presents better oral absorption and prolonged serum half-life (18-22 hours) (Holmes et al., 2009). Besides, compared to the original tetracycline, the synthetics have better dosage plan and are more easily absorbed when ingested with food (Smith and Leyden, 2005).

Doxycycline has activity against a wide range of Gram-positive and Gram-negative, as well as some protozoa such as malaria (Holmes et al., 2005), considered a bacteriostatic drug, since its action mechanism occurs by the inhibition of bacterial proteins synthesis, reversibly binding to the 30S ribosomal subunit, and preventing the association of aminoacyl-tRNA with the bacterial ribosome (Holmes et al., 2009).

The intensification of fish production results in numerous challenges, among which stands out the sanitary control of animals. In general, the increase in specimens per area, added to management practices, results in the occurrence of stress, a physiological condition responsible for immunosuppressing fish defense systems, predisposing them to the most diverse diseases (Belo et al, 2005; 2012a). Thus, infectious agents that apparently cause little damages to fish populations in their natural habitat can become precursors of diseases with great economic importance when subjected to rearing conditions (Eto et al., 2020; Farias et al., 2020).

Few antimicrobials are approved for use in aquaculture, according to the FDA - Food and Drug Administration, the regulatory agency for the use of drugs in the United States, only oxytetracycline, florfenicol and sulfadimethoxine associated with ormethopine can be added to feed and used for bacterial control in aquaculture (FDA, 2022). For Moraes et al. (2022), in addition to being effective in controlling bacterial diseases in fish, these drugs must be clinically safe for animals and the aquatic environment, making the approval process for new drugs extremely complex.

According to Resolution XXVIII, an OIE (World Organization for Animal Health) list of Antimicrobial Agents of Veterinary Importance, established at the 75th International Committee during the General Session, highlighted among others the potential use of doxycycline in fish production. However, it is necessary to design effective therapeutic protocols for its use in aquatic organisms (OIE, 2015). This synthetic tetracycline has a broad spectrum of antimicrobial action, and it has been successfully used in animal production through oral route in poultry (Gutiérrez et al., 2017) and swine (Morés et al., 2015).

Nakamura (1982) proved the therapeutic efficacy of 20mg doxycycline/kg of b.w., administered orally in the feed, for the treatment of streptococcosis in Yellow-tail, *Seriola quinquiradiata*. For this author, doxycycline demonstrated stability in the diet, very low toxicity for animals, confirming its therapeutic safety, in addition to presenting rapid absorption and systemic distribution. In another study, the clinical safety of doxycycline was observed after eight months of treatment in Australian lungfish (*Neoceratodus fosteri*), submitted to doxycycline therapy for the treatment of mycobacteriosis caused by *Mycobacterium marinum, M. fortuitum, M. chelonae* and *M. peregrinum*, resulting in the healing and no side effects for the chronic treatment (Strike et al., 2017).

Three hundred isolates of *Aeromonas hydrophila* and *Edwardsiella tarda* were tested for 16 antimicrobials. This study revealed among the tested drugs that 70% of the isolates were sensitive to doxycycline (Lee and Wend, 2017). In fish farming, the use of other tetracyclines such as oxytetracycline is indicated for the treatment of infectious diseases such as: septicemia caused by *Edwardsiella tarda* and *Edwardsiella ictaluri*, septicemia caused by *Aeromonas* spp. and *Pseudomonas fluorescens*, among others (Austin and Newaj-Fyzul, 2017).

Therefore, it is extremely important to study the effect of antimicrobial treatment on fish health, which generally are performed through hematological, histopathological and biochemical exams, characterized as essential parameters of fish biological conditions. In this context, we evaluated the clinical safety of 10, 20, 40 and 80 mg of doxycycline /kg of body weight, orally administered in tilapia, *Oreochromis niloticus*.

MATERIAL AND METHODS

Fish and experimental conditions

For the clinical study of Doxycycline safety were used 75 Nile tilapia (approximately 300g) from the same spawning (Aquabel farm, Porto Ferreira/SP, Brazil), conditioned in 15 tanks (100 liters of water each, n=5) filled with chlorine free running water from an artesian well with a recirculation system with a flow rate of 1 liter per minute. After being transported to the appropriate tanks, the fish were acclimatized for 15 days, the time necessary for the plasma cortisol concentration and osmolarity return to baseline levels. In the first three days of acclimatization, the animals were bathed with a NaCl solution at a concentration of 6.0 g/L (Carneiro and Urbinati, 2001).

The animals received extruded commercial feed with 36% crude protein (Nutripiscis® - Presence Company), constituting the basal diet. The fish were fed twice a day, at 8:00 am and 5:00 pm, corresponding to 2% of the biomass in the tanks. Water quality parameters were determined twice a day throughout the experimental period using pHmeter YSI-63 and oximeter Y-55, and their values remained within the range suitable for the well-being of tropical fish (Boyd, 1990) (dissolved oxygen = 4.07 ± 0.89 mg L^{-1}; temperature = 27.64 ± 2.05°C; pH = 7.64 ± 0.54; and conductivity = 208.29 ± 97.57µS/cm).

The Ethics Committee for the Use of Animals (CEUA), UNESP/FCAV approved the experimental procedures, under protocol nº 5315/20.

Experimental design

Fish were randomly distributed in 15 tanks (100L of water, n=5) to compose the following treatments: T0 (control group, not treated with Doxycycline); T1, T2, T3 and T4 (treated with 10, 20, 40 and 80mg of doxycycline /kg of b.w., respectively. Five animals were sampled per treatment in 3 periods: 2, 4 and 8 days (Table 1), to collect blood samples for hemogram and biochemical determination, in addition to organs such as spleen, liver and kidneys (cranial and caudal) for somatic and histopathological evaluation.

Table 1 - Distribution of tilapia in different treatments with Doxycycline.

Groups	Treatments	Days after starting Doxycycline treatment		
		2	4	8
T0	Control (not treated with Doxycycline)	N=5	N=5	N=5
T1	Treated com 10 mg/kg de p.v. de Doxycycline	N=5	N=5	N=5
T2	Treated com 20 mg/kg de p.v. de Doxycycline	N=5	N=5	N=5
T3	Treated com 40 mg/kg de p.v. de Doxycycline	N=5	N=5	N=5
T4	Treated com 80 mg/kg de p.v. de Doxycycline	N=5	N=5	N=5

Experimental diet

The commercial extruded food, containing 36% crude protein, 12% moisture, 70 g/kg ethereal extract, 140 g/kg mineral matter, 50 g/kg crude fiber, 25 g/kg calcium, 8 g/kg phosphorus and vitamin C 350 mg/kg. (Nutripiscis® - Presence Company) was the option to compose the experimental diets of tilapia. Feeding was performed twice a day (8:00 am and 5:00 pm), with administration of 2% of the biomass in the tanks. To prepare the diets, the ration was weighed daily in proportion to the fish average weight in each tank. Then, Doxycycline (Doxycycline: Sandoz Pharmacetical Industry from Brazil Ltd.) was added at doses of 10, 20, 40 and 80mg/kg b.w. and homogenized in 2% of vegetable oil, composing the diets of T1, T2, T3 and T4, respectively. For the standardization of diets and nutritional balance, 2% of vegetable oil was added to the diet of the control group (T0).

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, in which the water level of the tanks was lowered to a volume of 10L and was added 0.1g of benzocaine already diluted in 98º alcohol. Soon after, each fish was transferred to a container with 1L of water with 0.1g of benzocaine, both procedures were performed under aeration to minimize the stress caused by handling. As soon as the operculum stopped moving, 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.

Blood sampling and hematological analysis

Five fish per treatment (one tank for each treatment), after being anesthetized, approximately three mL of blood samples were collected from the caudal vessel of each animal at 2, 4 and 8 days post-treatment (DPT), which were aliquoted into two sets: one heparin-coated syringe (5000 IU) and one without anticoagulant, for obtaining plasma and serum, respectively. During the exchange of syringes (with and without heparin), the needle was not removed from the vessel, so no blood was lost. The hemogram was performed using a hemocytometer (Neubauer chamber) and Natt and Herrick (1952) solution in the proportion 1:100 v:v). Hematocrit was determined by the microhematocrit centrifugation technique. And circulating hemoglobin, using Rabkin's reagent for reading at a wavelength of 540nm and the mean corpuscular volume (MCV) values were obtained by calculating MCV = (HT/HE)*100 and mean corpuscular hemoglobin concentration (CHCM) by calculating CHCM = (HG/HT)*100. Differential leukocyte counts were performed in blood extensions with a count of 200 cells, establishing the percentage of each cell type of interest, after previous staining of the extensions with May-Grünwald Giensa Wright (Farias et al., 2016).

Serum biochemical assessment

Blood samples from fish without anticoagulant were centrifuged at 10,000 rpm during 10 minutes at 4°C to obtain serum and determine alkaline phosphatase (AF), aspartate aminotransferase (AST), creatinine, albumin, total protein, cholesterol, triglycerides and globulin, using a semiautomatic biochemical analyzer (Model LabQuest® - Bioplus Company) and the glycemia of the fish was determined using the Accu-Chek Performa instrument.

Morphometric and histopathological evaluation of organs

After 2, 4 and 8 days of treatment, the tilapia were euthanized by immersion in an aqueous solution of benzocaine (1:500) until the anesthetic plane was deepened and the opercular movements were completely lost. Then, they were weighed and dissected by a ventral longitudinal section, 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 allowed a wide view of all organs. For morphometric evaluation, according to Weibel et al. (1969), liver, caudal kidney and spleen of tilapia were collected, which were weighed to express the hepatic, renal and splenic somatic index, calculated by the formula: Somatic index = organ weight X 100/body weight. For histopathological examinations, were evaluated liver, caudal kidney and spleen. Immediately after collecting a fragment of each organ, they were fixed in 10% formalin and after 24 hours they were transferred to 70% alcohol, being sent for preparation of the pieces in a veterinary pathology laboratory, embedded in paraffin and cuts of 5µm will be stained in hematoxylin and eosin. The reading was done in an optical microscope to determine possible pathological changes.

Statistical analysis

The experimental design for clinical safety assessment was entirely randomized in a 5 x 3 factorial scheme (five treatments: 10, 20, 40, 80 and control X three evaluation periods: 2, 4 and 8 DPT). Analyzes of variance to compare the different experimental groups were performed using the GLM (General Linear Model) procedure of the SAS program, version 9.3 (Statistical Analysis Software, 2012). Significant differences ($P<0.05$) were estimated based on Tukey's test at 95% confidence level.

RESULTS

Serum biochemical analysis

In the analysis of hepatic cytotoxicity of tilapia treated with doxycycline (Figure 1), no significant changes were observed in serum alkaline phosphatase enzyme activity. However, serum levels of AST were significantly higher ($P<0.05$) in animals treated with 80 mg of doxycycline in the longest period of treatment (8 days), compared to animals treated with the lowest dose (10mg) and control group. This result was also observed over time, in which the treatment with 80 mg increased its values in the later period of treatment.

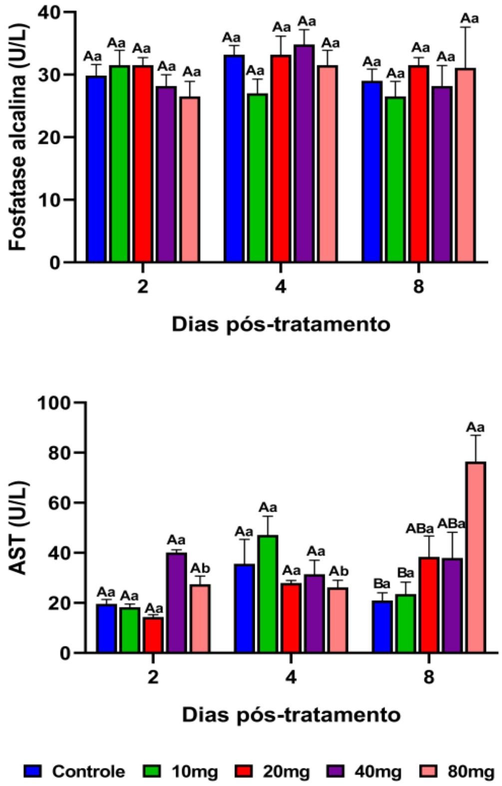

Figure 1 - Alkaline phosphatase and aspartate aminotransferase (AST) analysis of tilapia treated with doxycycline. Means (n=5) followed by the same letter do not differ by Tukey's test ($P<0.05$). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

In the evaluation of the hepatic functionality of the treatment with Doxycycline (Figure 2), no significant changes were observed in the serum values of creatinine, total protein, cholesterol, triglycerides and globulin. However, after 4 days of treatment with 80 mg of doxycycline an albumin peak was observed and its values returned to their baseline levels on the eighth day.

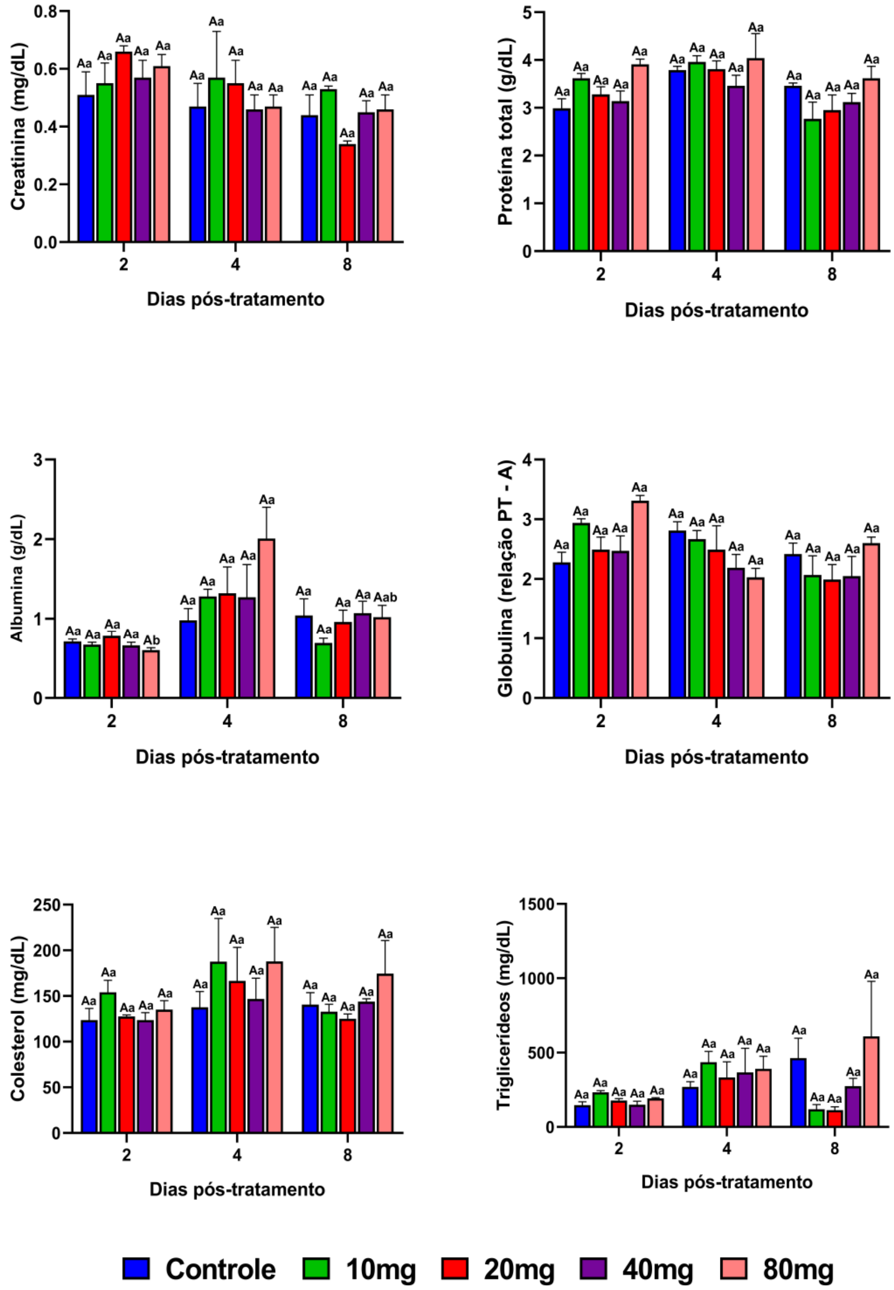

Figure 2 - Analysis of creatinine, total protein, albumin, globulin, cholesterol and triglycerides of tilapia treated with doxycycline. Means (n=5) followed by the same letter do not differ by Tukey's test (P<0.05). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

Glycemia assessment (Figure 3) revealed a decrease after 4 days of treatment in the control group and treated with 10 mg of doxycycline, after 8 days of treatment, this decrease was observed in all groups, except for the one treated with 80 mg of doxycycline that no decrease was observed, remaining constantly high throughout the evaluation period.

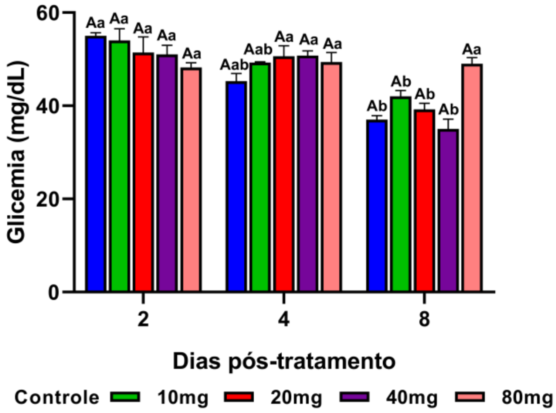

Figure 3 - Analysis of blood glucose in tilapia treated with doxycycline. Means (n=5) followed by the same letter do not differ by Tukey's test (P<0.05). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

Hematological analysis

In the hematological evaluation of tilapia treated with doxycycline (Figure 4) no significant changes (P>0.05) were observed in hematocrit, MCV, HCM and CHCM values. However, it was observed that after 8 days of treatment there was a significant decrease (P<0.05) in serum hemoglobin values in animals treated with 40 and 80 mg of doxycycline compared to animals in the control group. Over time, it was observed that animals treated with 40 mg had a significant decrease in hemoglobin levels and animals treated with 80 mg had an increase in circulating erythrocytes.

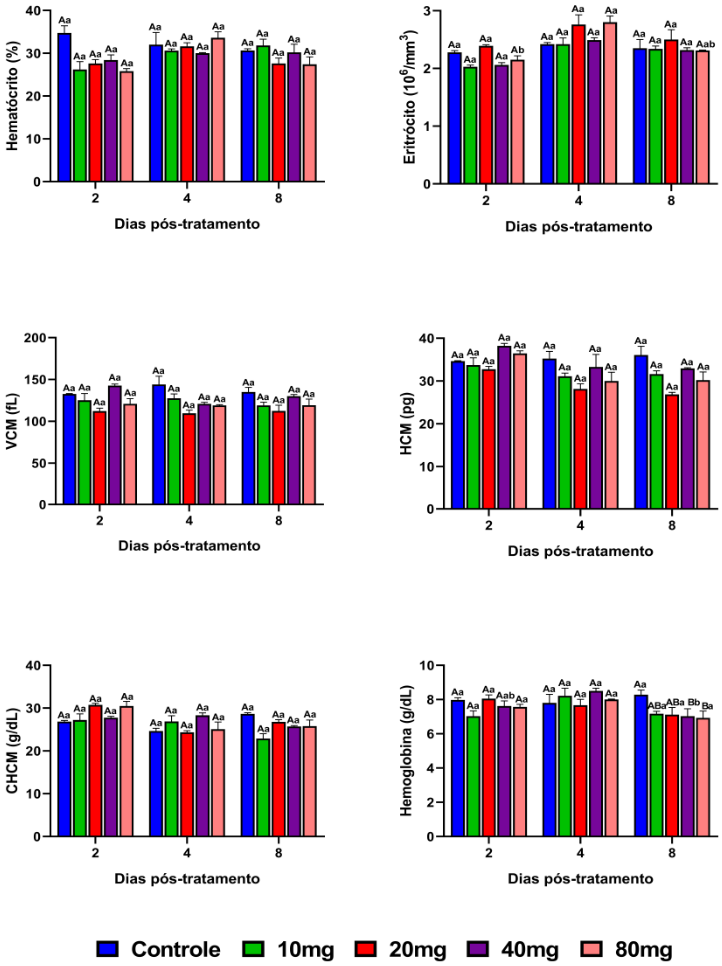

Figure 4 - Hematological analysis of tilapia treated with Doxycycline. Means (n=5) followed by the same letter do not differ by Tukey's test (P<0.05). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

In the leukocyte evaluation (Figure 5), there were no significant changes ($P \geq 0.05$) in the global leukocyte count between the different treatments. It was observed a significant increase of lymphocytes ($P<0.05$) on the second day after the start of treatment in animals treated with 80 mg when compared to fish treated with 20 mg/kg, but both did not show significant variations when compared to control tilapia. The evaluation of treatments over time revealed a decrease in monocytes and neutrophils 4 days after treatment with 80 mg doxycycline, which returned to normal levels after 8 days.

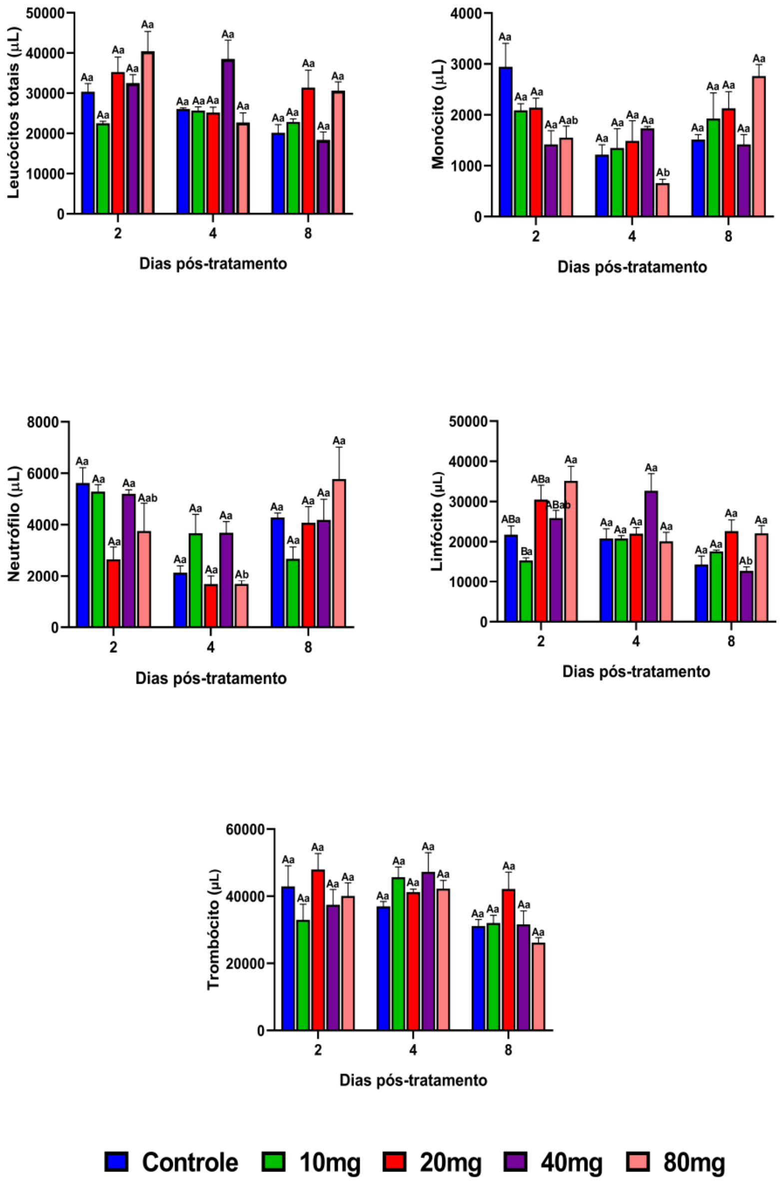

Figure 5 - Leukocyte analysis of tilapia treated with Doxycycline. Means (n=5) followed by the same letter do not differ by Tukey's test ($P<0.05$). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

Morphometric and Histopathological analysis

In the somatic analysis of spleen, liver and kidney, no significant changes were observed in the animals of the different treatments and the control group (Figure 6).

Histopathological changes

The histopathological study of tilapia treated with doxycycline showed changes in the liver with loss of hepatic cord architecture, presence of pyknotic nucleus, hydropic degeneration with necrosis of hepatocytes and hepatic sinusoid dilatation (Figure 7). Such histopathological findings were observed more frequently in animals treated with 80 mg of doxycycline. Histological sections of splenic and renal tissues did not show significant changes when compared to animals in the control group. However, it is worth mentioning that studies that aim to quantify and comparatively analyze the formation of melanomacrophages centers in splenic tissues can help to understand their participation during the body's detoxification process.

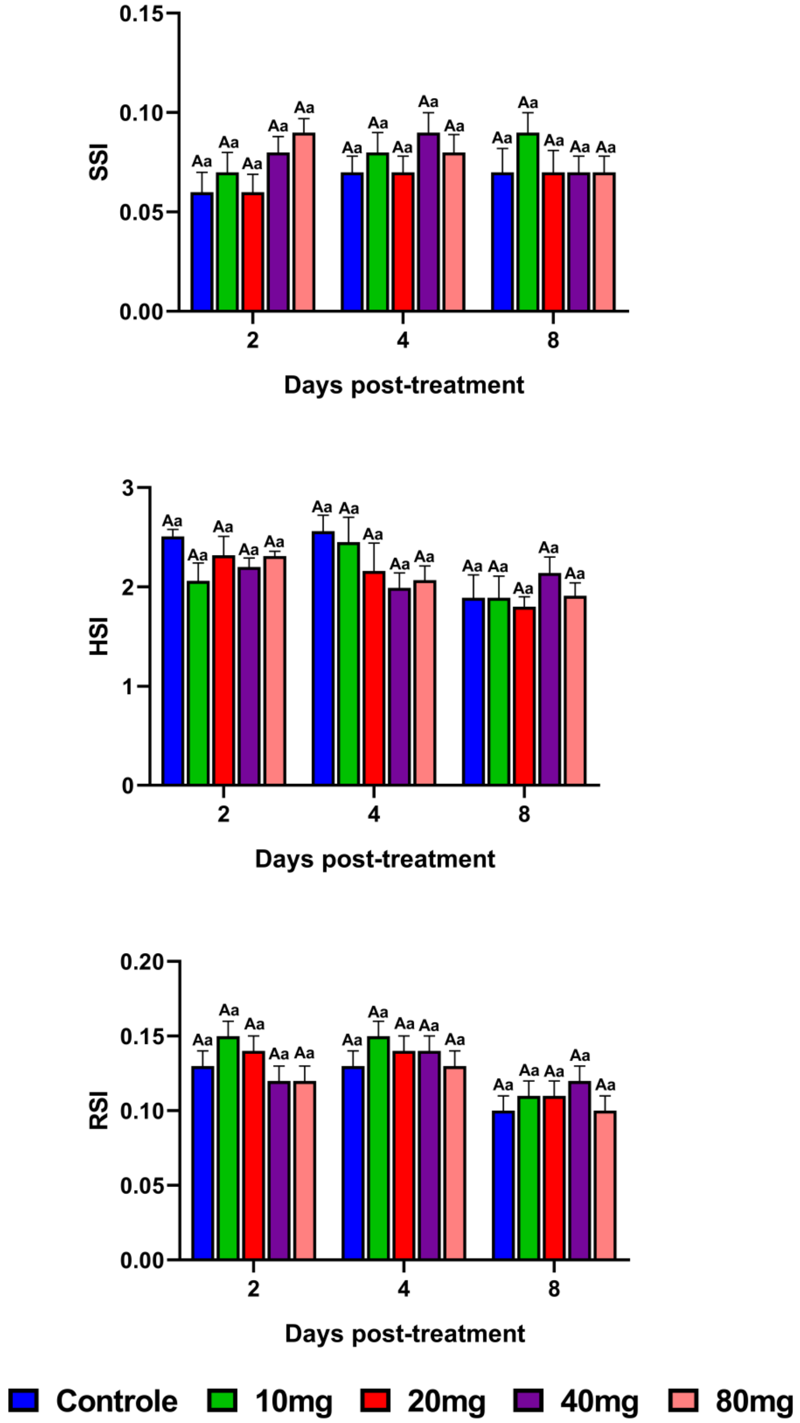

Figure 6 - Somatic evaluation of spleen, liver and kidney of tilapia treated with Doxycycline. Hepatic somatic index (HSI), splenic somatic index (SSI) and renal somatic index (RSI). Means (n=5) followed by the same letter do not differ by Tukey's test (P<0.05). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

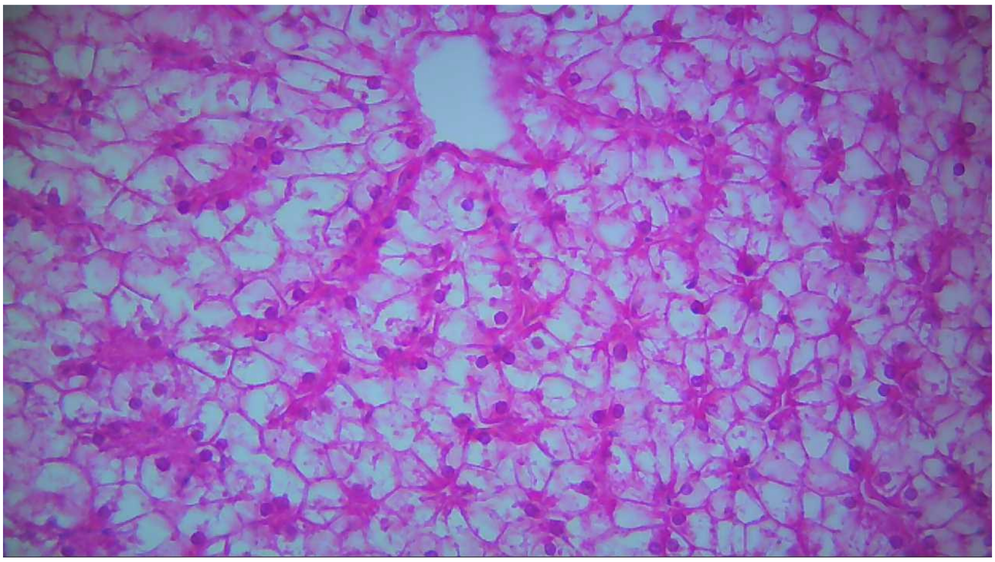

Figure 7 - Photomicrograph of histological section of the liver of tilapia treated with doxycycline. Loss of architecture of the hepatic cords, presence of pyknotic nuclei, hydropic degeneration with necrosis of hepatocytes and hepatic sinusoid dilatation are observed. H.E stain, 40x magnification.

DISCUSSION

Under outbreak conditions, the use of drugs in aquaculture is indispensable, however, there are few drugs registered for use in fish, which leads to the indiscriminate use of various chemical substances (Carraschi et al., 2017; Charlie-Silva et al., 2020). The study of clinical safety in fish becomes relevant, and this happens through hematological and biochemical tests, in which they are essential parameters to assess the physiological condition of fish (Mahmoud et al. 2018) considering the use of pharmaceutical molecules in aquaculture.

In the hematological evaluation of the tilapia treated with doxycycline, no changes were observed in the hematocrit, MCV, HCM and CHCM values, corroborating the findings of Costa et al. (2022), in which the tilapia treated with zileuton did not show any change between the different concentrations of the drug administered during an acute inflammatory reaction caused by *Aeromonas hydrophila*.

Hemoglobin can indicate the oxygen content in the blood and under stress conditions anoxia can occur, ceasing the oxidative phosphorylation mechanism, being the alternative pathway of energy synthesis in the body (Ramesh et al., 2018). In the study, higher doses of doxycycline (40 and 80 mg/kg) for eight consecutive days decreased hemoglobin concentration. However, no evidence of anemia was observed, as there was no decrease in the circulating values of red blood cells, as well as in the percentage of hematocrit, discarding the hypothesis that treatment with this tetracycline would have resulted in hemolytic changes. Feng et al. (2017) demonstrated that doxycycline binds to the hydrophobic cavity of bovine hemoglobin through multiple interactions in a mechanism similar to drugs with albumin. Engebretson and Hey-Hadavi (2011) observed a decrease in hemoglobin A1c during chronic treatment with doxycycline for three months in patients with type-2 diabetes. For these authors, tetracyclines can inhibit the glycation of non-enzymatic proteins.

Similar effects were observed in tilapia, in which the decrease in circulating hemoglobin values in fish treated with 80 mg of doxycycline after eight days of treatment were significantly related to the increase in blood glucose. The metabolic breakdown of glucose (glycolysis) is an important pathway for ATP production, and its levels may change in the blood when the animal is in high-energy demand, being strongly influenced by catecholamines, glucocorticoids and thyroid hormones (Ramesh et al., 2018). Tilapia treated with 80 mg of doxycycline showed a transient increase in circulating albumin values on the fourth day of treatment, returning to baseline values on the eighth day. Hyperalbuminemia usually occurs when there are changes in fluid-electrolyte balance, as the loss of fluid volume from the plasma extracellular compartment results in hemoconcentration, reflecting an increase in serum albumin, this mechanism is clearly described in mammals (Belo et al., 2012b).

There are few data on the influence of antimicrobials on the biochemical indices of fish serum and those that have been published so far are mainly focused on the effects of oxytetracycline (Bojarski et al., 2020) with little information on the use of doxycycline in fish. In the liver evaluation, tilapia treated with 80 mg of doxycycline for eight consecutive days had increased serum AST levels, as observed by Oyeniran et al. (2021) in which fish treated with different antibiotics had higher AST activities compared to the control, suggesting changes in liver cytotoxicity. Bojarski et al. (2020) observed an increase in AST in fish exposed to oxytetracycline, possibly resulting from a transient cytotoxic effect during treatment with this tetracycline.

The increase in the metabolic degradation of the drug in hepatocytes can result in the production of free radicals that favor the increase of lipid peroxidation and consequently changes in the permeability of the hepatocyte plasma membrane, which can result in extravasation of this enzyme, as well as in the most severe cases cause cytotoxicity (Moraes et al., 2018; Aracati et al., 2021; Oliveira et al., 2021).

The data on the hematological and immunological response in fish after antibiotic treatment are complex, as the results obtained by several authors are likely to depend on the antibiotic dosage, infectious processes and the sensitivity of various fish species (Kondera et al., 2020). Leukocytes play a vital role in the immune system of fish, comprising the cellular element of innate immunity and releasing humoral substances such as cationic antimicrobial peptides, components of the complement system, lectins and cytokines (Do Huu et al., 2016). According to Popal et al. (2017) the change in the level of leukocytes is an indication of activation of the immune response. In the present study, no changes were observed in the number of circulating leukocytes during treatment with this tetracycline, suggesting the low toxicity of doxycycline treatment not resulting in inflammatory changes. The decrease in the number of monocytes and neutrophils on the fourth day after treatment with 80 mg of doxycycline corroborates the findings of Maklakova et al., (2011) in rainbow trout (*Oncorhynchus mykiss*) injected with five doses of 20 mg/kg of oxytetracycline.

In the somatic analysis of spleen, liver and kidney, no significant changes were observed in the animals of the different treatments and the control group, these results are in agreement with Moraes (2017), in which there was no difference in relation to the weight of the organ and animal weight in study of the clinical safety of amoxicillin for the treatment of streptococcosis in Nile tilapia. The same result was also observed by Dobšíková et al. (2013) who evaluated oxytetracycline in biometric indices in common carp (*Cyprinus carpio L.*). Such results corroborate the results of erythrocyte, leukocyte and biochemical studies of tilapia, suggesting that the drug has not compromised liver and kidney functions, resulting from inflammatory processes in these tissues.

All fish in this study, regardless of the treatment received, did not show any behavioral and/or clinical changes such as erratic swimming, lethargy, increased opercular beating, skin damage or loss of appetite during treatment for eight days with doxycycline.

Therefore, the results observed as a whole demonstrate the clinical safety of treatment with doxycycline administered orally at doses of 10, 20, 40 and 80 mg/kg (bodyweight), although transient changes in liver functionality were observed after eight days of treatment with the dose of 80mg/kg.

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