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CLINICAL SAFETY OF SULFAMETHOXAZOLE-TRIMETHOPRIM TREATMENT IN NILE TILAPIA (*Oreochromis niloticus*)

SEGURANÇA CLÍNICA DO TRATAMENTO COM SULFAMETHOXAZOL-TRIMETOPRIMA EM TILAPIAS DO NILO (OREOCHROMIS NILOTICUS)

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SUMMARY

The safety and tolerability of pharmaceuticals, due to the complexity of assessing adverse effects, require the conduction of multiple studies. This necessitates extensive research and clinical trials to confirm the safety and efficacy of medications. This study evaluated the clinical safety of oral Sulfamethoxazole-trimethoprim (SMZ-TMP) treatment in Nile tilapia (*Oreochromis niloticus*) through hematological and biochemical assessments. A total of 63 animals (±100g) were reared in 9 tanks (n=7). Following acclimation, the treatments administered were: T0 (control, not treated with SMZ-TMP); T1 and T2 (treated with 30-6 and 120-24 mg of SMZ-TMP/kg b.w., respectively). Seven animals per treatment were sampled at 4, 8, and 12 days post-treatment, with an additional recovery period where animals were fed only commercial feed until day 16 for clinical recovery evaluation. Blood samples were collected for hematological and serum biochemical analysis, as well as somatic organ analysis. The results showed that SMZ-TMP, especially at high doses, caused hematological changes such as increased erythrocyte counts and hemoglobin levels, as well as microcytosis. Treatment with SMZ-TMP/kg modulated the response of fish white blood cells by altering circulating leukocyte levels associated with lymphocyte counts. In addition, it resulted in disorders with reductions in protein and creatinine levels, associated with anorexia and mortality in fish treated with 120-24 mg of SMZ-TMP/kg of bw, demonstrating the toxic effect of overdose treatment. However, further studies should be conducted to determine the clinical safety of different therapeutic protocols involving the use of SMZ-TMP in tilapia.

KEY-WORDS: Teleost fish. Cichlids. Hematological parameter. Antimicrobial, bacterial diseases, health management

RESUMO

A segurança e a tolerabilidade de fármacos, devido à complexidade na avaliação de efeitos adversos, exigem a realização de múltiplos estudos. Isso torna indispensável a realização de diversos estudos e ensaios clínicos para confirmar a segurança e a eficácia dos medicamentos. Este estudo avaliou a segurança clínica do tratamento com Sulfametoxazol-trimetoprima (SMZ-TMP) em tilápias do Nilo (*Oreochromis niloticus*) administrado via oral, por meio de avaliações hematológicas e bioquímicas. Para tal, foram utilizados 63 animais (±100g) acondicionados em 9 tanques (n=7). Após aclimatação, foram ministrados os seguintes tratamentos: T0 (controle, não tratado com SMZ-TMP); T1 e T2 (tratados com a proporção 30-6 e 120-24 mg de SMZ-TMP/kg de p.v., respectivamente). Sete animais foram amostrados por tratamento em 4 períodos: 4, 8, 12 dias pós-tratamento com SMZ-TMP além de um período adicional que, após 12 dias, os animais foram alimentados apenas com ração comercial até o 16º dia para avaliação da recuperação clínica. Foram coletadas amostras de sangue para análise hematológica e bioquímico sérico, além da análise somática dos órgãos. Os resultados mostraram que o SMZ-TMP, especialmente em doses altas, causou alterações hematológicas como aumento do número de eritrócitos e nos valores de hemoglobina, além de microcitose. O tratamento com SMZ-TMP/kg modulou a resposta de células brancas dos peixes por alterar os valores circulantes de leucócitos associados às contagens de linfócitos. Além de resultar em distúrbios com reduções nos níveis de proteína e creatinina, associados ao quadro de anorexia e mortalidade em peixes tratados com 120-24 mg de SMZ-TMP/kg de p.v., demonstrando o efeito tóxico do tratamento com sobre dose. Contudo, outros estudos devem ser realizados para determinar a segurança clínica de diferentes protocolos terapêuticos envolvendo o uso de SMZ-TMP em tilápias.

PALAVRAS-CHAVE: Peixe teleósteos. Ciclídeos. Parâmetro hematológico. Antimicrobiano, doenças bacterianas, manejo sanitário

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INTRODUCTION

With the increase in fish farming, the sector has faced a higher incidence of outbreaks of opportunistic infectious diseases, mainly caused by bacteria, which generates significant economic losses (Farias et al., 2016). In response to these outbreaks, the use of antimicrobials during sanitary management has become a common practice (Oliveira et al., 2022). However, the lack of drugs officially registered by the World Organization for Animal Health (OIE) for use in fish leads to the indiscriminate use of several substances (Aracati et al., 2021; Costa et al., 2022a; Oliveira et al., 2024). Therefore, it is essential to carry out studies that evaluate the safety of new molecules in fish, using hematological and biochemical parameters to monitor the health and physiological condition of the animals (Mahmoud et al., 2018; Oliveira et al., 2021; Silva et al., 2023).

The combination of sulfamethoxazole and trimethoprim (SMZ-TMP) is widely used in the treatment of bacterial infections in humans and animals, including fish (Phu et al., 2015). This combination is usually administered orally in feed or directly in the water of breeding tanks. Evaluating hematological and biochemical parameters is essential to determine the safety and efficacy of SMZ-TMP use in fish (Liu et al., 2013). Sulfamethoxazole acts as a sulfonamide antimicrobial that inhibits the synthesis of folic acid in bacteria, while trimethoprim, an antibiotic from the diaminopyrimidine group, blocks the enzyme dihydrofolate reductase, enhancing the antibacterial effect (Lunden and Bylund, 2002). However, the indiscriminate use of this combination can lead to the development of bacterial resistance, compromising the efficacy of treatments. Therefore, it is essential that clinical safety studies be conducted before using SMZ-TMP in aquaculture, in order to avoid negative impacts on fish health and the aquatic environment (Liu et al., 2013). This concern is especially relevant considering the significant growth of aquaculture at a global level. In 2022, global aquaculture production reached a record 223.2 million tons, with an increase of 4.4% compared to 2020, according to FAO (2024). The organization highlighted the importance of the achievements, but emphasized the need for transformative actions to increase the sustainability of aquatic food systems. Brazilian fish production in 2023 was 887,029 tons, an increase of 53.25% since 2014, consolidating Brazil as the fourth largest producer of tilapia in the world, a species that represents 65% of national production (PeixeBR, 2024).

Considering the need to develop new antimicrobials for fish farming and the socioeconomic relevance of tilapia farming, this study aims to evaluate the clinical safety of SMZ-TMP, as well as its possible effects on the physiological responses of teleost fish.

MATERIAL AND MÉTHODS

Fish and packaging

For the clinical safety study of SMZ-TMP, 63 Nile tilapia (±100 g) from the same spawning of the Piscicultura Projeto Peixes (Sales de Oliveira, SP, Brazil) were used, housed in 9 tanks (100 L of water each, n=7) supplied with dechlorinated water (Figure 1). After being transported to the appropriate tanks, the fish were acclimated for 15 days, the time necessary for the plasma cortisol concentration and osmolarity to return to baseline levels. In the first three days of acclimation, the animals were bathed in a NaCl solution at a concentration of 6.0 g/L (Carneiro and Urbinati, 2001). The animals received commercial extruded feed with 35% crude protein (Nutripiscis - Empesa ADM®), constituting the basal diet. The fish were fed twice a day, at 8:00 a.m. and 4:00 p.m., corresponding to 2% of the tank biomass. Water quality parameters were determined twice a day throughout the experimental period using a YSI-63 pH meter and a Y-55 oximeter, and values were recorded that remained within the range suitable for the well-being of tropical fish (Boyd, 1990). All experimental procedures were approved by the Ethics Committee on the Use of Animals (CEUA) of Universidade Brasil, under protocol number 230027.

Experimental design

Tilapia were randomly distributed into 9 tanks (100 L of water, n = 7) to constitute the following treatments: T0 (control group, not treated with SMZ-TMP); T1 and T2 (treated with 30-6 and 120-24 mg/kg-1 of bw of SMZ-TMP, respectively). To evaluate the possible physiological changes of the animals caused by SMZ-TMP, seven animals from both treatments (T1 and T2) were treated for 12 days with SMZ-TMP, after which they were administered only commercial feed for four more days, without the addition of vegetable oil and SMZ-TMP, called the recovery period, totaling 16 days of analysis. Seven animals per treatment (T1 and T2) were sampled in four periods: 4, 8, and 12 days post-treatment (DPT) and after the 16th day (recovery period). Blood samples were collected to determine the blood count, leukogram, serum biochemical parameters and organs such as spleen, liver and kidneys (cranial and caudal) for somatic and histopathological evaluation.

Experimental diet

The extruded commercial feed containing 35% crude protein, 13% moisture, 75 g/kg ether extract, 130 g/kg mineral matter, 45 g/kg fibrous matter, 30 g/kg calcium, and 10 g/kg phosphorus (Nutripiscis - ADM® Company) was used to compose the experimental diets of tilapia. Feeding was carried out twice a day (8:00 a.m. and 4:00 p.m.), with 2% of the biomass of the tanks administered. To prepare the diets, the feed was weighed daily in proportion to the average weight of the tilapia in each tank. Then, sulfamethoxazole-trimethoprim (Sulfamethoxazole + trimethoprim: Bravet® Laboratório Bravet LTDA.) was added at doses of 30-6 and 120-24 mg/kg-1 b.w. and homogenized in 2% vegetable oil, composing the T1 and T2 diets, respectively.

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), completing the volume to 1 L (Wedemeyer, 1970). Initially, pre-anesthesia was performed, in which the water level in the tanks was lowered to a volume of 10 L by adding 0.1 g of benzocaine already diluted in 98° alcohol. Soon after, each fish was transferred to a container containing 1 L of water with 0.1 g of benzocaine. As soon as the operculum stopped moving, the fish was removed and blood collected. Finally, the animal was transferred to another container containing 0.5 g of benzocaine diluted in 1 L of water for euthanasia.

Blood collection and hematological analysis

Seven fish per treatment (one tank for each treatment) were anesthetized. Approximately two mL of blood samples were collected from the caudal vessel (Figure 4) of each animal at 4, 8, 12 and 16 days after treatment (DPT). They were aliquoted into two sets: a syringe coated with heparin (5000 IU) and another without heparin, to obtain plasma and serum, respectively. During the exchange of syringes (with and without heparin), the needle was not removed from the vessel to avoid blood loss. The blood count was performed using a hemocytometer (Neubauer chamber) and Natt and Herrick's solution (1952) in a 1:100 (v:v) ratio. Hematocrit was determined by the microhematocrit centrifugation technique. As for circulating hemoglobin, Labtest hemoglobin reagent was used for reading at a wavelength of 540 nm and the mean corpuscular volume (MCV) values were obtained by calculating MCV = (HT/RBC)*10, mean corpuscular hemoglobin concentration (MCHC) by calculating MCHC = (HG/HT)*100, and mean corpuscular hemoglobin (MCH) by calculating MCH = (HB/RBC)*10). The differential leukocyte count was performed in blood smears with a count of 200 cells, establishing the percentage of each cell type of interest, after prior staining of the smears with May-Grünwald Giensa Wrigth (Farias et al., 2016).

Serum biochemical evaluation

Blood samples from fish without anticoagulant were centrifuged at 10,000 rpm for 5 minutes at 4°C to obtain serum and determine total protein, albumin, globulin (total protein – albumin ratio), creatinine, using a semi-automatic biochemical analyzer (Model LabQuest® - Bioplus Company) and the fish's blood glucose was determined using the Accu-Chek Performa device (Belo et al., 2012).

Assessment of hepatic, renal and splenic somatic index

After each determined experimental period (4, 8, 12 and 16 DPT), tilapia were euthanized by immersion in an aqueous solution of benzocaine (1:500) until the anesthetic plane was deepened and opercular movements were completely lost. They were then weighed and dissected by a longitudinal ventral cut, 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 (Figure 5). For morphometric evaluation according to Weibel et al. (1969), the liver, caudal kidney, cranial kidney and spleen of the tilapia were collected and weighed to express the hepatic, renal and splenic somatic indices, calculated by the formula: Somatic index = organ weight X 100/body weight.

Clinical and behavioral assessment

The aeration system was turned off daily and the animals in the tanks were examined for possible behavioral changes, clinical signs, and mortality that could result from treatment with SMZ-TMP. Therefore, anorexia, excitability, lethargy, altered respiratory rate (opercular beat), disorientation, skin hemorrhages, fin corrosion, eye ulcers, exophthalmos, abdominal distension, changes in tissue pigmentation, among others, were evaluated and recorded.

Water quality monitoring

Water quality monitoring was carried out daily at 9:00 am and 5:00 pm, evaluating the hydrogen potential, electrical conductivity, oxygen concentration, and water temperature using a portable pH meter and oximeter "YSI – 63" and "YSI- 55", respectively.

Statistical analysis

The experimental design for evaluating clinical safety was completely randomized in a 3 x 4 factorial scheme (three treatments: control, 30-6mg and 120-24mg X four evaluation periods: 4, 8, 12 and 16 days. Analyses 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 the Tukey test at the 95% confidence level.

RESULTS

Clinical and behavioral analysis

During the clinical and behavioral evaluation of tilapia treated with SMZ-TMP, it was observed that, at 3 DPT, the animals treated with the highest dose (T2) showed signs of anorexia. At 6 DPT, one fish in this group died, and other individuals in the same tank exhibited yapping behavior at the surface of the water. Additionally, one fish in the high-dose group jumped out of the tank, resulting in its death. At 7 DPT, another fish in this group also jumped out of the tank. Between 9 and 10 DPT, the death of two fish treated with the lowest dose (T1) was recorded, and the signs of anorexia persisted. During the recovery period, the fish resumed their normal food intake.

Hematological analysis

In the erythrocyte count (Figure 1A), it was observed that the animals treated with the highest dose (T2) showed a significant increase ($p<0.05$) in the number of erythrocytes and a decrease in MCV at 12 DPT compared to the control group (Figure 1D).

Tilapia treated with SMZ-TMP showed a significant increase ($P < 0.05$) in the hemoglobin analysis 4 DPT in the group that received the highest dose of the

drug (T2) when compared to fish in treatment T1, but both were statistically similar ($p \geq 0.05$) to the results observed in fish in the untreated control group (Figure 1B). No significant changes ($P \geq 0.05$) were observed between the different concentrations of the drug administered, as well as there were no changes over time in the hematocrit percentage (Figure 1C), as well as in the MCH and MCHC calculations (Figure 1E and 1F).

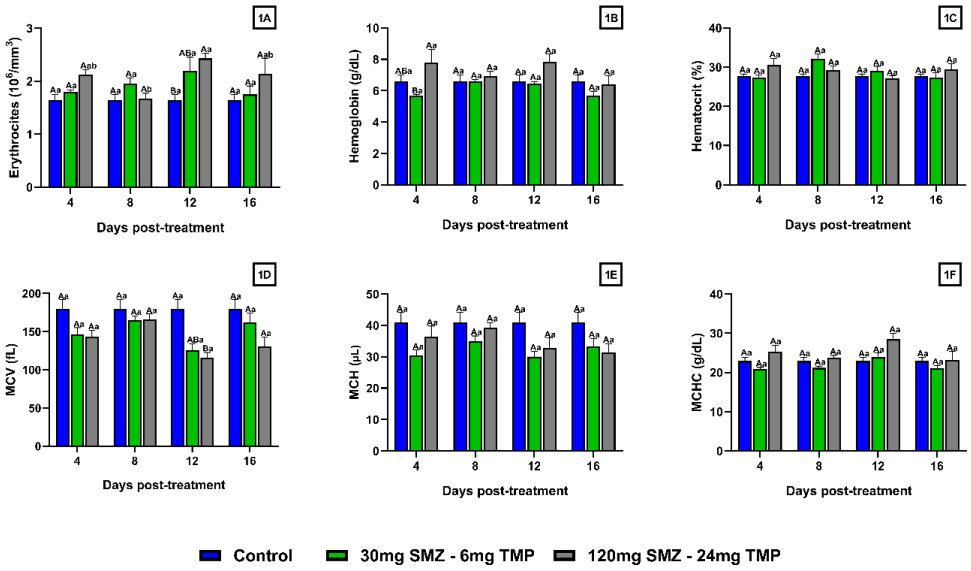

Figure 1 - Mean values (± standard error) observed in the hematological analysis of tilapia treated with trimethoprim-sulfamethoxazole. Means (n=7) followed by the same letter do not differ by Tukey's test (P<0.05). A: Erythrocytes; B: Hemoglobin; C: Hematocrit; D: Mean Corpuscular Volume (MCV); E: Mean Corpuscular Hemoglobin (MCH); F: Mean Corpuscular Hemoglobin Concentration (MCHC). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

Serum biochemical analysis

Serum creatinine biochemical evaluation in animals treated with both doses of SMZ-TMP revealed a significant decrease at 8 DPT for T1, and at 12 DPT for both treatments (T1 and T2). Throughout the treatment period, a significant reduction in serum creatinine levels was observed in both SMZ-TMP treatment groups at 16 DPT (Figure 2A).

In the evaluation of total protein (Figure 2B), a decrease in total protein was observed in T2 at 8 DPT compared to the control group. While throughout the treatment, tilapia treated with SMZ-TMP with both doses presented a peak in circulating serum values of total protein in the recovery period (16 DPT). The results revealed that tilapia treated with SMZ-TMP did not present significant differences ($p \geq 0.05$) in serum glucose levels between fish subjected to the different treatments, despite the decrease in mean glucose values observed in T2 animals at 12 and 16 DPT (Figure 2C).

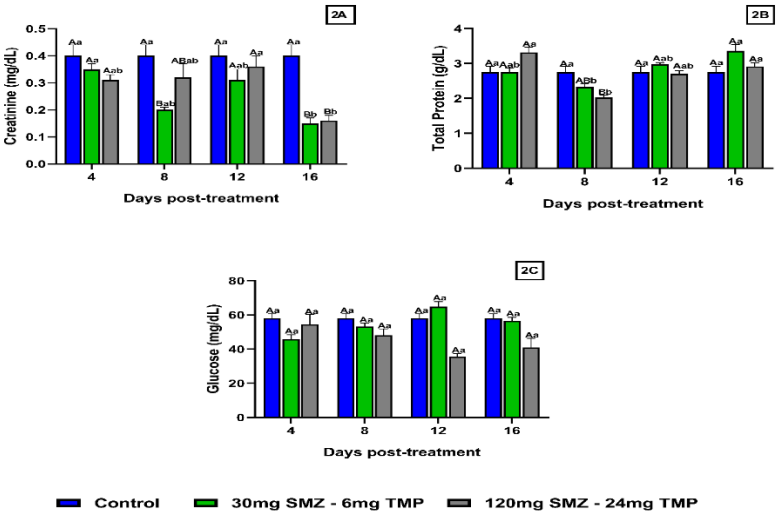

Figure 2 - Mean values (±Standard error) observed in the biochemical analysis. Means (n=7) followed by the same letter do not differ by Tukey's test (P<0.05). A: Creatinine; B: Total protein; C: Glycemia. Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

Leukogram

The evaluation of the leukocyte profile showed that, over time, the animals in group T1 presented a significant decrease ($p<0.05$) in the number of leukocytes, associated with a significant decrease in lymphocytes (Figure 3A and 3D). While in the differential count of granulocytes, monocytes and thrombocytes (Figures 3B, 3C and 3E), they did not present any significant change ($P \geq 0.05$).

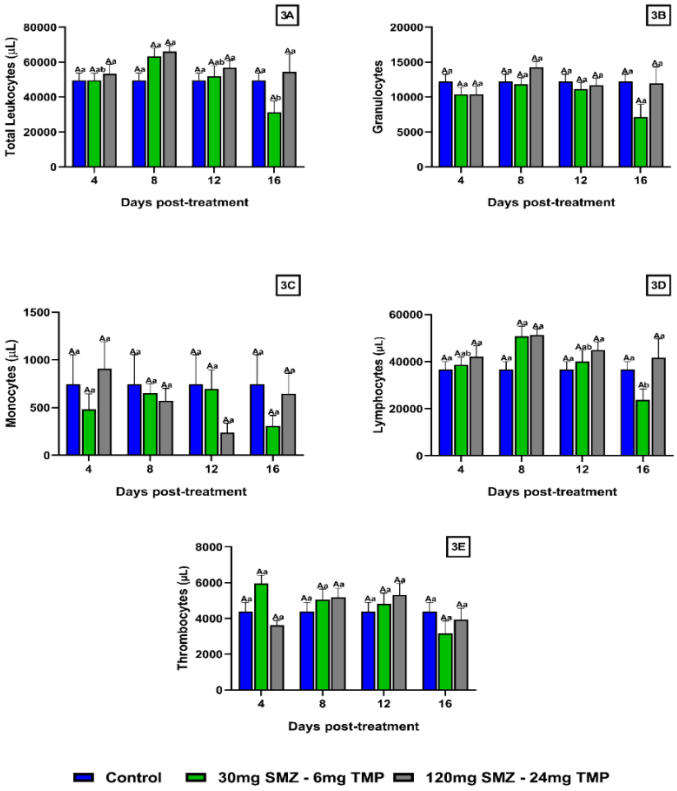

Figure 3 - Mean values (±Standard error) observed in the leukocyte analysis of tilapia treated with trimethoprim-sulfamethoxazole. Means (n=7) followed by the same letter do not differ by Tukey's test ($P<0.05$). A: Total Leukocytes; B: Granulocytes; C: Monocytes; D: Lymphocytes; F: Thrombocytes. Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

Analysis of the hepatic, renal and splenic somatic index

In the weight assessment, together with the somatic analysis of the spleen, liver and kidney, no significant changes were observed in the animals of the different treatments and in the control group (Figure 4).

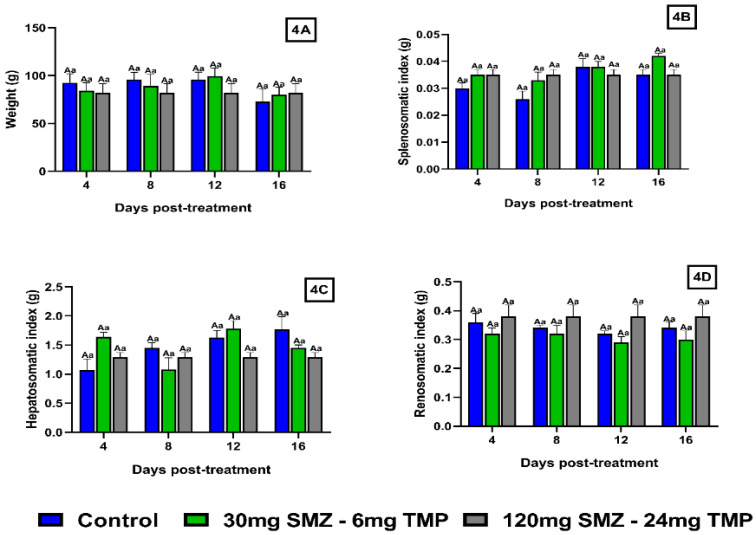

Figure 4 - Mean values (±Standard error) observed in the somatic evaluation of spleen, liver and kidney of treated tilapia. Means (n=7) followed by the same letter do not differ by Tukey's test ($P<0.05$). A: Weight; B: Splenosome Index; C: Hepatosomatic Index; D: Renosomatic Index. Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

DISCUSSION

Analysis of blood parameters is an essential tool for assessing the toxic effects of xenobiotics in fish (Bojarski & Witeska, 2020). However, most of the available data on the hematological effects of antibacterials in fish focus on oxytetracycline (Dobšíková et al., 2013; Hoseini et al., 2019), while information on other antibacterials is scarce (Costa et al., 2022b; Oliveira et al., 2022). These studies mostly involve the administration of relatively high doses of antibiotics, either via diet or parenterally. Exposure to antibiotics can induce several hematological alterations, such as an increase in red blood cell parameters (RBC, Ht, Hb) or an anemic response. Tilapia treated with the highest dose (120-24 mg/kg) of SMZ-TMP showed a significant increase in the number of erythrocytes associated with a decrease in mean corpuscular volume. Changes in microcytosis may result in a decrease in the capacity of red blood cells to transport oxygen, representing stress to the fish. According to Belo et al., (2014), this fact would result in changes in the hydro-electrolytic balance associated with the responses of catecholamines that would act on splenic contraction and consequently increase the number of circulating erythrocytes, justifying the findings of this study. In addition, the concentration of circulating hemoglobin was higher in this group. On the other hand, Saglam and Yonar (2009) reported a reduction in the erythrocyte count in rainbow trout fed 400 mg/kg of sulfamerazine for 7 and 14 days, compared to untreated fish.

Regarding SMZ-TMP, there are few data on its hematological effects in fish. However, Sampaio et al. (2016) observed that the addition of 400 mg/kg of sulfamethasone to the diet for 11 days did not significantly influence the erythrogram and leukogram parameters in Nile tilapia. This corroborates our findings, which demonstrate that, although SMZ-TMP can affect some hematological parameters, such as the number of erythrocytes and hemoglobin concentration, these effects appear to be dose-related and are less pronounced for other hematological indices.

On the other hand, exposure to antibiotics has been associated with a reduction in leukocyte levels in fish (Lunden and Bylund, 2002). In this study, tilapia treated with SMZ-TMP showed an increase in the number of leukocytes influenced by the increase in lymphocyte counts after 8 days of treatment, however, in the recovery period, fish treated with the therapeutic dose (30-6 mg/kg) presented leukopenia with lymphopenia, suggesting a modulation of the immune response induced by exposure to antimicrobial treatments. While in the differential count of granulocytes, monocytes, and thrombocytes, they did not show any significant changes in tilapia. Rutkoski et al. (2022) evaluated the effects of environmental concentrations of sulfamethoxazole (SMX) and oxytetracycline (OTC) in bullfrog tadpoles (Lithobates catesbeianus). The findings of these authors corroborate our results, as they demonstrated an absence of significant variations in the numbers of monocytes, eosinophils, basophils, and thrombocytes when compared to the control group. These observations are similar to the existing literature, which indicates that antibiotics can cause both increases and decreases in leukocyte counts (Bojarski et al., 2020). Caipang et al. (2009) observed that the immune response of fish to antibiotics depends on the type of drug, the procedure adopted, and the species of fish. In contrast, Lundén and Bylund (2002) did not detect significant effects on the immune response of rainbow trout after oral administration of 30 mg/kg of sulfadiazine associated with trimethoprim, concluding that these antimicrobials could be used to prevent diseases in fish without compromising their hematological health.

Protein plays an essential role in the maintenance and growth of fish, providing energy, especially under stressful conditions. Exposure to xenobiotics can interfere with biochemical reactions, resulting in changes in protein levels. In the present study, the decrease in protein levels observed at the dose of 120-24 mg/kg, 8DPT, which is four times the recommended therapeutic dose (Anyogu et al., 2018), suggests an inhibition of protein synthesis. This result may reflect the stress caused by the drug, similar to that observed in rainbow trout (*Oncorhynchus mykiss*) exposed to sulfamethasone, in which the decrease in protein was associated with a reduction in protein synthesis or degradation due to drug-induced stress (Saglam and Yonar, 2009). Another hypothesis that justifies the change in serum protein levels would be associated with the anorexia observed, mainly in tilapia treated with 120-24 mg/kg. During the recovery period at 16 DPT, when the fish were receiving only commercial feed, without the presence of the antibiotic, an increase in total protein levels was observed. This increase indicates that the effect of SMZ-TMP on protein metabolism was transient, reflecting an adaptive recovery of the fish after the removal of the drug-induced stress. The absence of the antibiotic allowed protein metabolism to return to normal, with increased protein synthesis necessary for the restoration of physiological homeostasis.

Creatinine concentration in animals treated with SMZ-TMP decreased significantly at 8 DPT for the 30-6 mg/kg dose, and at 12 DPT for both the 30-6 mg/kg and 120-24 mg/kg doses. This continuous reduction in creatinine levels at 16 DPT in both treated groups suggests a possible impact of the treatments on its production, which occurs relatively constantly and is generated from the degradation of phosphocreatine, a protein that is obtained from food and also synthesized by the liver, kidneys, and pancreas. The decrease in creatinine levels may be associated with malnutrition with loss of muscle mass or changes in liver function, similar to the effects observed with oxytetracycline and florfenicol in previous studies (Reda et al., 2013), which resulted in pathological changes in the liver of treated fish. The reduction in creatinine could be associated with the anorexia observed in tilapia treated with these antimicrobials or indicate a decrease in the liver's ability to participate in the biosynthesis of this substance, possibly due to hepatoxicity associated with treatment with SMZ-TMP, but additional analyses, such as histopathology, are necessary to confirm these findings.

Behavioral evaluation of tilapia suggests that the antibiotic SMZ-TMP may have reduced palatability or, alternatively, indicate possible toxicity at the highest dose (120-24 mg/kg), which is four times the recommended dose. The occurrence of anorexia, mortality, and

behavioral disturbances, such as yapping and jumping out of the tank, reinforces the hypothesis that the high dose may be associated with significant adverse effects. These findings highlight the need for further investigation to determine whether the observed effects are due to low drug acceptance or dose-related toxicity.

Organ somatic indices are relevant parameters in the analysis of the clinical safety assessment of the drug, because they may undergo changes that indicate disorders of increased size in acute pathological conditions due to circulatory changes associated with inflammatory processes with occurrences of congestion and edema, as well as organ shrinkage in chronic pathological processes as observed in liver cirrhosis (Khan et al., 2022). Tilapia treated with SMZ-TMP did not show changes in the somatic indices of the liver, kidney and spleen organs, similar to the results observed by Costa et al. (2022b) and Oliveira et al. (2022) in which tilapia treated with lincomycin and doxycycline respectively, did not show somatic changes after treatment.

The clinical safety study of SMZ-TMP treatment demonstrated that these antimicrobials can cause haematological changes with an increase in the number of circulating erythrocytes associated with a decrease in mean corpuscular volume, with a decrease in creatinine and protein levels, as well as affecting fish behaviour, especially at high doses. Although no changes in somatic organ indices were observed, additional analyses are crucial for a complete assessment of the clinical safety of SMZ-TMP. Careful monitoring and further studies are essential to confirm the safety of this drug at different dosages.

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