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CLINICAL SAFETY OF ORAL LICOMYCIN TREATMENT IN NILE TILAPIA (*Oreochromis niloticus*)

SEGURANÇA CLÍNICA DO TRATAMENTO VIA ORAL COM LINCOMICINA EM TILAPIAS (Oreochromis niloticus)

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SUMMARY

Lincomycin is a broad-spectrum antimicrobial acting against Gram-positive bacteria, widely used in veterinary medicine. In fish, there are only limited *in vitro* data, thus requiring the design of effective therapeutic protocols for their use in aquatic organisms. In this context, the objective was to evaluate the clinical safety of lincomycin treatment, administered orally in tilapia, through hematological, biochemical and somatic index evaluation. A total of 136 tilapia (±100g) were randomly distributed in 17 tanks (100L of water, n=8) to constitute the following treatments: T0 (control group, not treated with lincomycin); T1, T2, T3 (treated with 10, 20 and 40mg/kg^{-1} of lincomycin b.w., respectively) and T4 physiological standard (reference values). Eight animals were sampled per treatment in 4 periods: 2, 4 and 8 days post-treatment (DPT), and a group that was treated for 8 days with the drug and then treated only with commercial feed until the 12th day (recovery period). Tilapia treated with lincomycin had no difference in the hematological and leukocyte evaluation, in the hepatic, renal and splenic somatic index. However, they presented a transient increase in the values of ALT, AST, cholesterol, triglycerides and creatinine, which returned to normal levels after the period of recovery (12DPT). Furthermore, an increase in total protein, albumin and globulin levels was observed in treated animals. It is concluded that although there were some transient changes during the experiment, lincomycin has a good clinical safety margin at doses of 10, 20 and 40mg/Kg^{-1} b.w. for Nile tilapia.

KEY-WORDS: Antimicrobial drugs. Aquaculture. Cichlids. Safety. Teleost Fish.

RESUMO

A lincomicina é um antimicrobiano de amplo espectro atuando contra bactérias gram-positivas, amplamente utilizada na medicina veterinária. Em peixes existem apenas dados limitados *in-vitro*, necessitando assim, de delineamento de protocolos terapêuticos eficazes para seu uso em organismos aquáticos. Desse modo, objetivou-se avaliar a segurança clínica do tratamento com lincomicina, administrada por via oral em tilápias, por meio da avaliação hematológica, bioquímica e índice somático hepático, renal e esplênico. Foram utilizadas 136 tilápias (±100g), distribuídas aleatoriamente em 17 tanques (100L de água, n=8) para constituir as repetições dos diferentes tratamentos: T0 (grupo controle, não tratado com lincomicina); T1, T2, T3 (tratados com 10, 20 e 40mg/kg^{-1} de p.v. de lincomicina, respectivamente) e T4 padrão fisiológico (valores de referência). Oito animais foram amostrados por tratamento em 4 períodos: 2, 4 e 8 dias pós-tratamento (DPT) com lincomicina, e um grupo que foi tratado por 8 dias com o fármaco e após isso tratado apenas com ração comercial até o 12º dia (período de recuperação). Tilápias tratadas com lincomicina não tiveram diferença na avaliação hematológica e leucocitária, no índice somático hepático, renal e esplênico, entretanto, apresentaram um aumento transitório nos valores de ALT, AST, colesterol, triglicérides e creatinina, que retornaram aos níveis normais após o período de recuperação (12DPT). Ademais, foram observados um incremento nos níveis de proteína total, albumina e globulina nos animais tratados. Conclui-se que embora tenha ocorrido algumas alterações transitórias ao decorrer do experimento, a lincomicina apresenta boa margem de segurança clinica nas doses de 10, 20 e 40mg/Kg^{-1} p.v para tilápia do Nilo.

PALAVRAS-CHAVE: Antimicrobiano. Aquicultura. Ciclídeos. Inocuidade. Peixes Teleósteos.

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INTRODUCTION

The aquaculture sector is increasingly recognized for its essential contribution to global food security in the 21st century (FAO, 2022). Brazilian aquaculture has grown in recent years and is positioned as an important agribusiness sector (OLIVEIRA et al., 2022). However, intensive management practices with high population density and excessive fish handling result in stress, responsible for loss of performance, reproduction and resistance to pathogens (BELO et al., 2005; 2012). Bacterial infections cause significant mortality and enormous economic losses, estimated in billions of dollars annually. Particularly, opportunistic bacteria pose a serious threat to aquaculture (FARIAS et al., 2020).

According to the 86th General Session of the International Commission from OIE (World Organization for Animal Health) in 2018, the list of important antimicrobials in veterinary medicine was updated, and lincomycin has significant potential for use in fish. However, it requires design of effective therapeutic protocols for its use in aquatic organisms (OIE, 2021), associated with ecotoxicological (MORAES et al., 2022) and clinical safety criteria for the target species (OLIVEIRA et al., 2022). Veterinary clinical pathology studies involving serum biochemical analyzes of liver and kidney function, as well as hematological studies help in the diagnosis of morbid conditions (ARACATI et al., 2021a,b; OLIVEIRA et al., 2021).

Lincomycin is a broad-spectrum lincosamide antibiotic that is specifically active against Gram-positive bacteria. (SPÍŽEK & ŘEZANKA, 2017). Lincosamides inhibit bacterial protein synthesis by binding to the 50S ribosome subunit, which preferentially binds to the 23S rRNA from 50S subunit (PYÖRÄLÄ et al., 2014). The bactericidal or bacteriostatic effect depends on the drug concentration at the site of infection and the sensitivity of the infected organism (KHAN et al., 2022). In fish, only limited *in vitro* data are available (REVINA et al., 2017), and little is known about its action in Nile tilapia, requiring the design of effective therapeutic protocols for its use. Therefore, the objective of this study was to evaluate the clinical safety of lincomycin, administered orally in Nile tilapia (*Orechromes niloticus*).

MATERIAL AND METHODS

Fish

136 tilapias (± 100g) belonging to the same spawn and from the Aquabel fish farm (Porto Ferreira, SP) were placed in 17 experimental tanks (n=8, 100 L each) with running water without chlorine, from an artesian well with a flow of 1 L /min. After being transported to the tanks, the fish were acclimatized for 15 days, the time required for the plasma cortisol concentration and osmolarity to return to baseline levels. In the first three days of acclimatization, NaCl was added at a concentration of 6.0 g/L in each tank, favoring the fish hydroelectrolytic balance (CARNEIRO & URBINATI, 2001) . The water quality was determined twice a day (at the time of feeding), temperature and dissolved oxygen concentration, measured by the YSI equipment (model 55), and the pH and electrical conductivity by the YSI equipment (model 63). All experimental procedures were approved by the Animal Ethics Committee of UNESP/FCAV protocol nº 5639/22.

Experimental Design

Tilapia randomly distributed in 17 tanks (n=8), constituted the following treatments: T0 (control group, not treated with lincomycin); T1, T2, T3 (treated with 10, 20 and 40mg/kg^{-1} p.v. of lincomycin, respectively) and T4 physiological standard (reference values). Doses were recommended according to Lee et al. (2022), who studied lincomycin in *Paralichthys olivaceus*. To evaluate the possible animal physiological alterations caused by 8 days of treatment with lincomycin, after this period, it was administered for another 4 days only commercial feed, without the addition of lincomycin, called the recovery period, totaling 12 days of analysis. Eight animals were sampled per treatment (T0, T1, T2, and T3) in 4 periods: 2, 4 and 8 days post-treatment (DPT) and after in the 12th day (recovery period).

Composition of experimental diets

The commercial extruded feed containing 32% crude protein, 6% ether extract, 5.5% crude fiber and 12% mineral matter (Nutripiscis – Presence Company) was used to compose the experimental diets of tilapias. Feeding was carried out twice a day (9 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 fish average weight in each tank. Then, lincomycin (Lincomicin 300: Labyes do Brasil Ltda at doses of 10, 20 and 40 mg/kg^{-1} b.w. was added and homogenized in 2% vegetable oil, composing the diets of T1, T2 and T3 respectively. For standardization of diets and nutritional balance, 2% vegetable oil was added to the diet of the control group (T0).

Fish Anesthesia

Fish were anesthetized by immersion in an aqueous solution of benzocaine at a ratio of 1:10,000 for blood collection and 1:500 for euthanasia. Benzocaine was diluted in 98° alcohol (0.1 g/mL), completing the volume to 1L (WEDEMEYER, 1970). Initially, pre-anesthesia was performed, in which the water level in the tanks was lowered to a volume of 10L by adding 0.1g of benzocaine already diluted in 98º alcohol. Soon after, each fish was transferred to a container containing 1L of water with 0.1g of benzocaine. Both procedures were performed in aeration to minimize stress caused by handling. After paralyzing the operculum movements, fish were removed and blood were collected. Finally, fish were transferred to another container containing 0.5g of benzocaine diluted in 1L of water for euthanasia.

Hematological analysis

Eight fish per treatment (one tank for each treatment), after being anesthetized, 2 mL of blood samples were collected from the caudal vessel of each animal at 2, 4, 8 and 12 days after treatment (DPT), and aliquoted into two sets: one 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, so that there would be no blood loss. The hemogram was performed using a hemocytometer (Neubauer chamber) and Natt and Herrick's solution (1952) in the proportion 1:100 (v:v). Hematocrit was determined by the microhematocrit centrifugation technique. Hemoglobin was determined using Rabkin's reagent at a wavelength of 540nm. Differential leukocyte counts were performed in blood smears with a count of 200 cells, establishing the percentage of each cell type of interest, after previous staining 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), alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, triglycerides and cholesterol, using a semiautomatic biochemical analyzer (Model LabQuest® - Bioplus Company), and fish glycemia were determined using the Accu-Chek Performa device.

Somatic Index

After 2, 4, 8 and 12 days of treatment, the tilapias were euthanized by immersion in an aqueous solution of benzocaine (1:500) until the anesthetic plane deepened and complete loss of opercular movements. Then, fish were weighed and dissected by a ventral longitudinal 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 broad view of all organs. For morphometric evaluation according to Weibel et al. (1969), liver, caudal kidney, cranial 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.

Statistical analyzes

The experimental design was completely randomized in a 4 x 4 factorial scheme (four treatments: 10, 20, 40 and control by four evaluation periods: 2, 4, 8 and 12 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 the 95% confidence level, according to Snedecor and Cochran (1984).

RESULTS

Hematological analysis

Tilapia treated with lincomycin did not show any significant change ($P> 0.05$) between the different drug concentrations administered, as well as, they did not show change over time regarding the number of erythrocytes, hemoglobin concentration, percentage of hematocrit as well as the VCM, HCM and CHCM calculations (Figure 1).

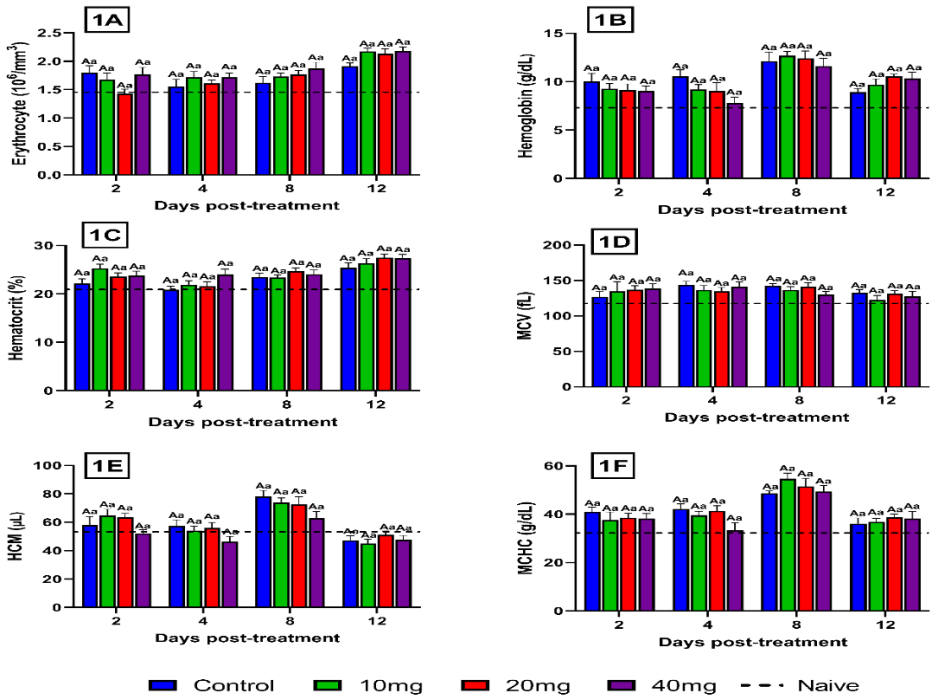

Figure 1 - Hematological analysis of tilapia treated with lincomycin. Means (n=8) followed by the same letter do not differ by Tukey's test ($P<0.05$). A: Erythrocyte; B: Hemoglobin; C: Hematocrit; D: Mean Corpuscular Volume (MCV); E: Mean Corpuscular Hemoglobin; F: Mean Corpuscular Hemoglobin Concentration (CHCM). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

Leukocyte profile evaluation (Figure 2) demonstrated that tilapia treated with lincomycin did not present any significant change (P> 0.05) in total leukocytes, as well as in the differential count of neutrophils, monocytes, lymphocytes and thrombocytes.

Serum biochemical evaluation

In the evaluation of hepatic cytotoxicity, tilapia treated with lincomycin did not have significant changes in alkaline phosphatase. However, serum levels of ALT and AST were higher in animals treated with 20 and 40 mg of lincomycin 4DPT, compared to animals treated with 10mg and control group (Figure 3). However, it has been observed that these serum values of ALT and AST have been regressing over time.

In the evaluation of liver and kidney functionality, there was a transient increase in serum values of triglycerides, cholesterol and creatinine in tilapia treated with 40 mg of lincomycin 8 DPT. However, after the recovery period, the values returned to normal levels (Figure 4) . The results revealed that tilapia treated with lincomycin showed an increase in serum levels of total protein, albumin and globulins 4DPT, when compared to the control group. In the evaluation of glycemia, no significant difference was observed between fish submitted to different treatments.

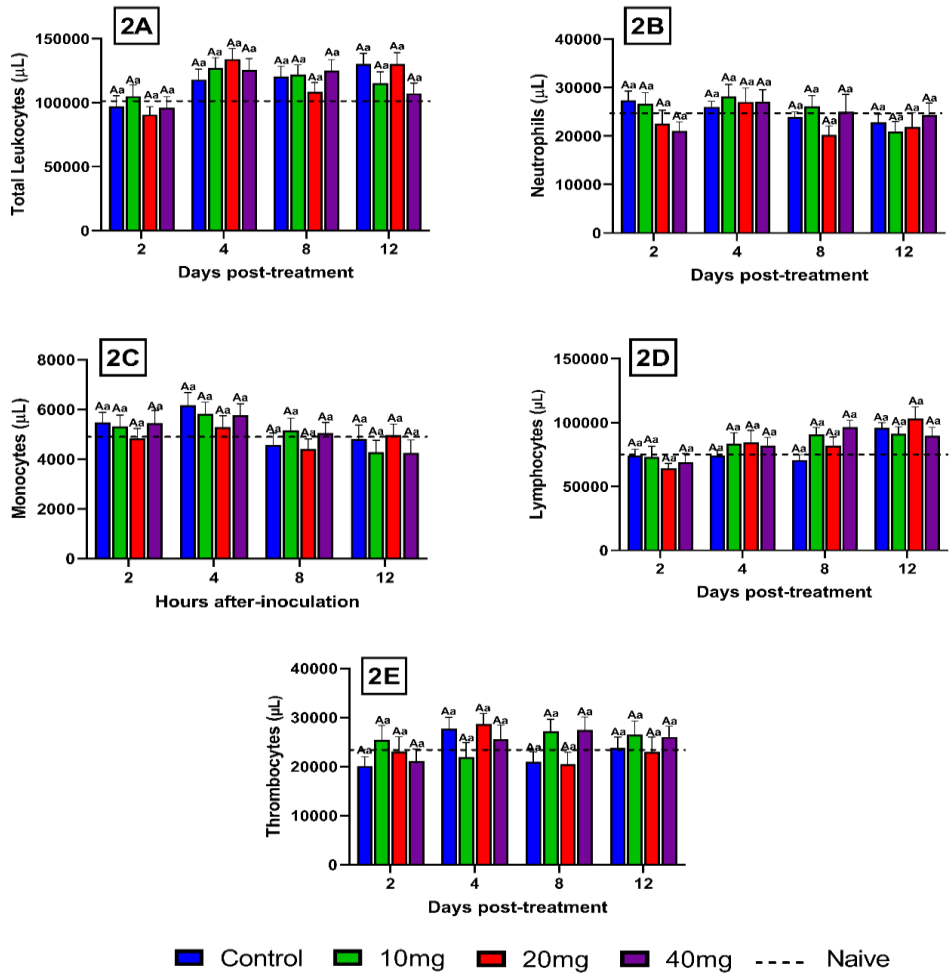

Figure 2 - Leukocyte analysis of tilapia treated with lincomycin. Means (n=08) followed by the same letter do not differ by Tukey's test (P<0.05). A: Total Leukocytes Erythrocyte; B: Neutrophils; C: Monocytes; D: Lymphocytes; E: Thrombocytes. Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

Somatic index

In the weight evaluation together with the somatic analysis of the spleen, liver and kidney, no significant alterations were observed in the animals of the different treatments and in the control group (Figure 5).

DISCUSSION

Tilapia treated with lincomycin did not show any alteration in the hematological and leukocyte evaluation. these results agree with those found in the treatment of growing pigs by Harvey et al., (1995), where the administration of lincomycin did not result in significant alteration in hematological parameters. Costa et al. (2022) and Oliveira et al. (2022) did not observe differences between hematological parameters in clinical safety studies in tilapia with zileuton and doxycycline, respectively. In contrast, Amer et al. (2017) studied oral administration of lincomycin in rats, and they found a significant decrease in the number of erythrocytes,

hemoglobin concentration and percentage of hematocrit at a daily dose of 500 mg/kg for 21 days. Possibly the difference observed between our results is due to the difference in drug concentration and days of treatment, where in the present study, the highest dose administered was 40mg/kg^{-1} for 8 days.

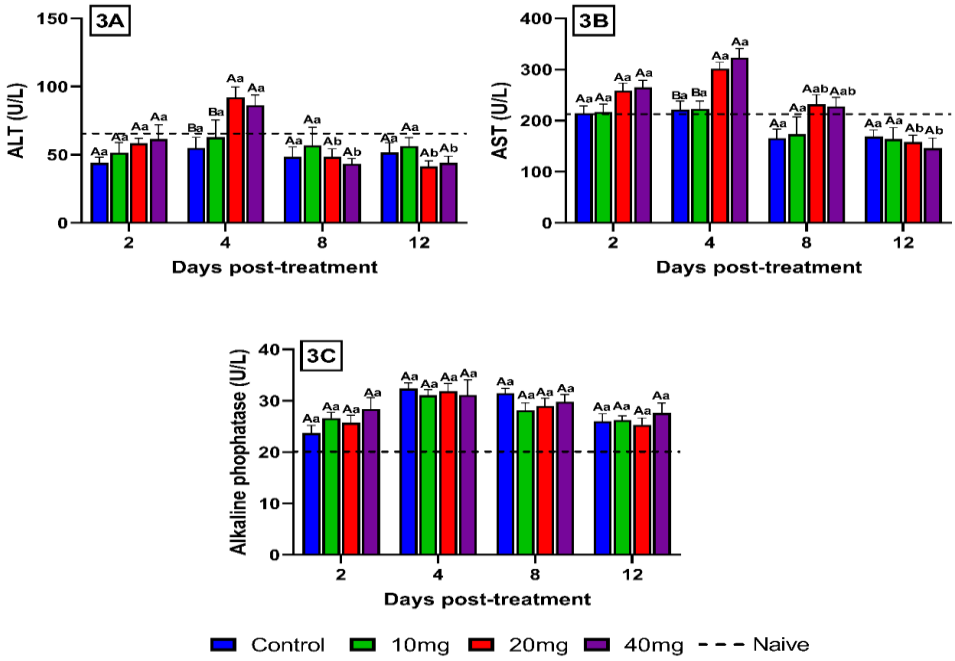

Figure 3 - Mean values (± Standard error) observed in the analysis of serum enzymatic activity of A: Alanine aminotransferase (ALT); B: Aspartate aminotransferase (AST); C: Alkaline phosphatase (FA) from tilapia treated with lincomycin. Means (n=8) followed by the same letter did not differ by Tukey's 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.

Studies evaluating oral administration of Lincomycin in rats (AMER et al., 2017) and in beagles (GRAY et al., 1964) observed a transient increase in ALT and AST, with their values returning to baseline levels, corroborating the findings of our study with tilapias. This increase in enzymatic activity can be attributed to changes in the permeability or cytotoxicity of hepatocytes in the drug metabolic degradation, leading to their release from the cytoplasm into the bloodstream after damage or rupture of the plasma membrane (OYENIRAN et al., 2021).

There are few data on the deleterious effects of antimicrobials on serum biochemical indices in fish and those published so far are mainly focused on the effects of oxytetracycline, amoxicillin and doxycycline (BOJARSKI et al., 2020; MORAES, 2018; OLIVEIRA et al., 2022) with little information on the use of lincomycin in tilapia.

According to Jwad et al. (2015), lincomycin may have harmful effects on hematology and serum biochemical variables, and may interfere with liver and kidney functions. In the study, there was a transient increase in triglycerides and cholesterol at dose of 40mg, possibly due to impaired liver function, and their values returned to normal levels within reference intervals previously recorded for tilapia under normal health conditions (MAUEL et al., 2007; HRUBEC et al., 2000). A study evaluating the oral administration of lincomycin in rats (AMER et al., 2017) also observed the same response profile.

The creatinine level is an indicator of renal function, therefore, the significant increase in its serum content after lincomycin administration can be attributed to the temporary impairment of the renal functional capacity, which can cause oxidative damage in the renal tubules, or probably due to the effect of this chemical antibiotic on the creatinine production rate, which leads to increase its biosynthesis (JWAD et al., 2015).

Furthermore, treatment with lincomycin resulted in increases in total protein, albumin, and globulins. Fortuoso et al. (2019) observed a similar variation, when evaluating glycerol monolaurate in the diet of broilers, which may suggest that lincomycin may have helped in the tilapia defense mechanisms, and an improvement in the immune response of this evaluated species could be observed. According to Belo et al. (2021) serum proteins, albumin and globulin play important roles in immune responses. Increases in serum protein, albumin, and globulin levels are thought to be associated with a stronger innate immune response in fish (BELO & CHARLIE-SILVA, 2022). On the other hand, the increase in serum levels of circulating proteins may result from changes in fluid-electrolyte balance with fluid loss in the extracellular compartment (Charlie-Silva et al. 2019; 2020). However, these effects are usually accompanied by an increase in the red blood cell counts and percentage values of hematocrit, justifying the hemoconcentration. This correlation between hematology and protein profile was not observed in tilapia treated with lincomycin.

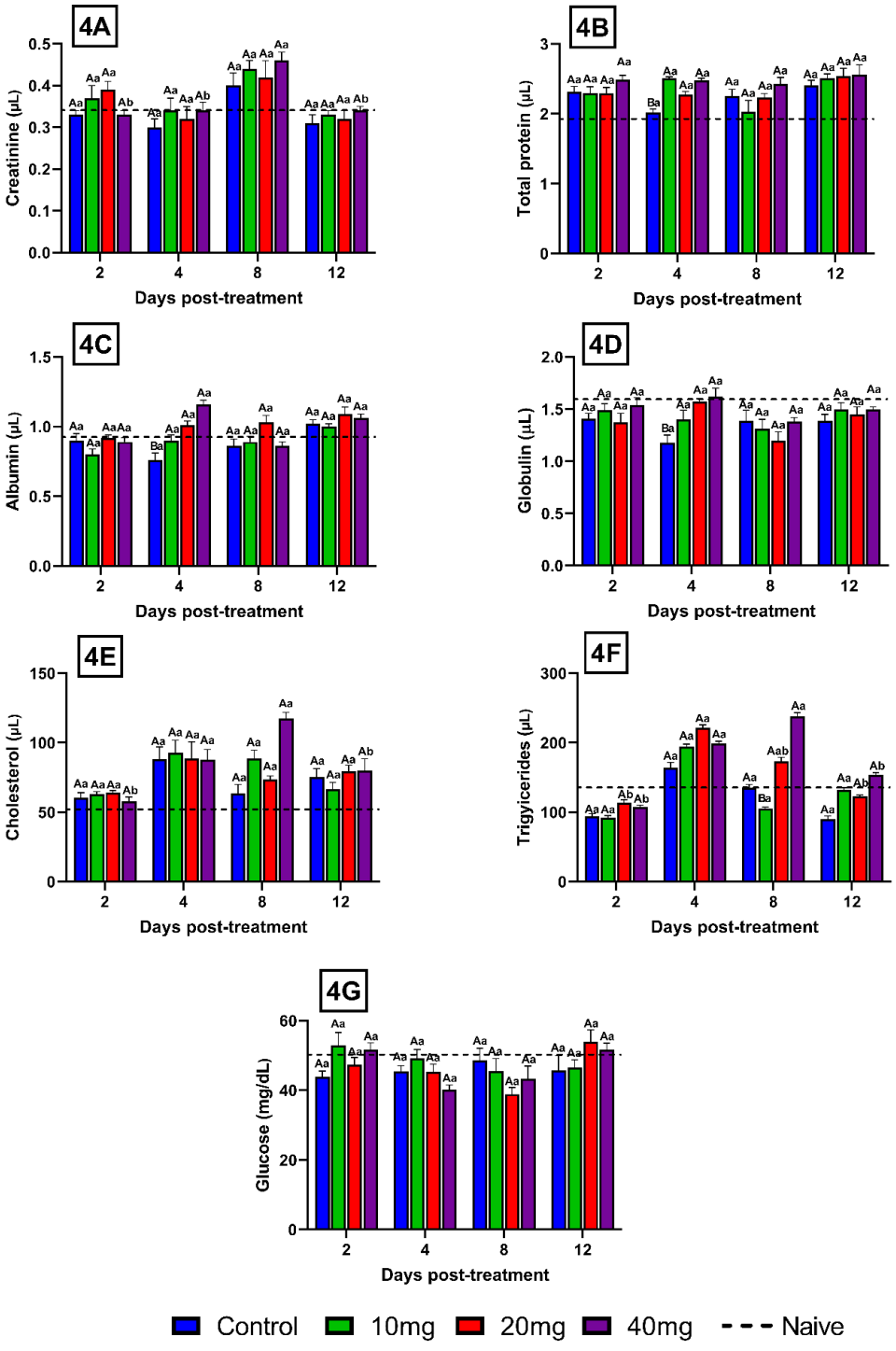

Figure 4 - Analysis of creatinine, total protein, albumin, globulin, cholesterol, triglycerides and glucose of tilapia treated with lincomycin. Means (n=8) followed by the same letter do not differ by Tukey's test (P<0.05). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

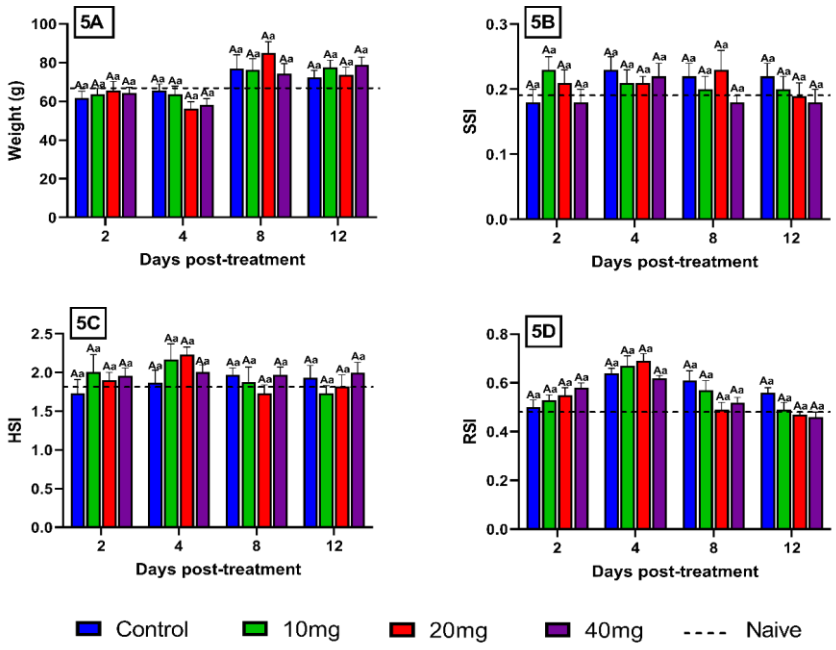

Figure 5 -. Somatic evaluation of spleen, liver and kidney of tilapia treated with lincomycin. Hepatic somatic index (HSI), splenic somatic index (SSI) and renal somatic index (RSI). Means (n=8) followed by the same letter do not differ by Tukey's test (P<0.05). Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

Organs somatic indices are relevant parameters in the safety evaluation analysis because they establish a proportionality between the organs and the animal size (KLAN et al., 2022). Tilapias treated with lincomycin did not show alterations in the somatic indices of liver, kidney and spleen, similar to the results observed by Oliveira et al. (2022) in which treated tilapia showed no difference in relation to the animal organ weight in a study of the clinical safety of doxycycline. A similar result was also observed by Dobšíková et al. (2013) who evaluated oxytetracycline in biometric indices in common carp (*Cyprinus carpio* L.).

Although transient changes in liver and kidney enzymes were observed at higher doses of lincomycin (40mg), treated tilapia did not show changes in hematological and somatic variables. It is worth noting that behavioral and clinical changes were not observed during the study period. Therefore, oral administration of lincomycin at doses of 10, 20 and 40mg/Kg^{-1} b.w. for eight days demonstrated clinical safety for tilapia.

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