ARS VETERINARIA, Jaboticabal, SP, v.40, n.3, 050-058, 2024. ISSN 2175-0106
DOI: http://dx.doi.org/10.15361/2175-0106.2024v40n3p50-58

EVALUATION OF THE CLINICAL SAFETY OF ORAL CURCUMIN TREATMENT IN NILE TILAPIA (*Oreochromis niloticus*)

AVALIAÇÃO DA SEGURANÇA CLÍNICA DO TRATAMENTO VIA ORAL DE CURCUMINA EM TILÁPIAS DO NILO (*Oreochromis niloticus*)

K. S. ARAUJO[1]; B. A. R. KLINGER[2]; M. E. PULTZ[1]; C. CARLINO-COSTA [3]; E. C. VIEIRA[1]; L. F. RODRIGUES[3]; M. F. ARACATI[3]; S. L. OLIVEIRA[3]; M. A. A. BELO[3]

SUMMARY

Curcumin is considered a multifunctional feed additive, with immunostimulant properties. Given this potential, it is crucial to deepen studies on the safety of substances like curcumin in fish, ensuring its safe and effective use for sustainable aquaculture. In this context, the present study aimed to evaluate the clinical safety of curcumin in tilapia, administered orally via feed, through hematological and biochemical analyses. A total of 63 fish (±100g) from a commercial fish farm, Sales de Oliveira/SP, were randomly distributed in 9 tanks of 100 L each, supplied with chlorine-free water. The treatments were: T0 (control, without curcumin), T1 and T2 (treated with 100 and 400 mg/kg of curcumin, respectively). Seven fish were sampled per treatment at four time points: 4, 8, and 12 days post-treatment (DPT), along with an additional recovery period up to day 16. Blood samples were collected for hematological and biochemical analysis, along with somatic evaluation of the organs. The results showed a significant increase in the number of erythrocytes and hematocrit in tilapia treated with curcumin, particularly at doses of 400 mg/kg at 12DPT and 100 mg/kg at 16DPT. There were no significant differences in the leukogram, total protein levels, or somatic index of the organs between the groups. Serum creatinine levels temporarily decreased, returning to baseline levels, and a significant peak in glucose levels was observed at 16DPT, especially at the 400 mg/kg dose. Further studies are needed to elucidate the mechanisms involved and confirm the clinical safety of curcumin over longer periods.

KEY-WORDS: Teleost fish. Cichlids. Turmeric. Hematological parameters. Nutraceuticals

RESUMO

A curcumina é considerada um aditivo alimentar multifuncional com potencial imunoestimulante, sendo crucial aprofundar os estudos sobre a inocuidade desta substância em peixes, visando garantir seu uso seguro e eficaz para a sustentabilidade da aquicultura. Neste contexto, o presente estudo teve como objetivo avaliar a segurança clínica da curcumina em tilápias, administrada oralmente incorporada à ração, por meio de análises hematológicas e bioquímicas. Foram utilizados 63 peixes (±100g) oriundos da piscicultura Projeto Peixes, Sales de Oliveira/SP, mantidos em 9 tanques de 100 L, abastecidos com água sem cloro. Os tratamentos foram: T0 (controle, sem curcumina), T1 e T2 (100 e 400 mg/kg de curcumina, respectivamente). Sete animais foram amostrados por tratamento em 4 períodos: 4, 8 e 12 dias pós-tratamento (DPT), além de um período adicional de recuperação clínica até o 16º dia. Coletaram-se amostras de sangue para análises hematológicas e bioquímicas, além da avaliação somática dos órgãos. Os resultados mostraram um aumento significativo no número de eritrócitos e no hematócrito nas tilápias tratadas com curcumina, principalmente nas doses de 400 mg/kg aos 12DPT e 100 mg/kg aos 16DPT. Não houve diferenças significativas no leucograma, nos níveis de proteína total ou no índice somático dos órgãos entre os grupos. Os níveis de creatinina sérica diminuíram temporariamente, com retorno aos valores basais, e observou-se um pico significativo nos níveis de glicemia aos 16DPT, principalmente na dose de 400 mg/kg. Mais estudos são necessários para elucidar os mecanismos envolvidos e confirmar a segurança clínica da curcumina em períodos mais longos.

PALAVRAS-CHAVE: Peixe teleósteos. Ciclídeos. Cúrcuma. Parâmetro hematológico. Nutracêuticos.

[1] Laboratory of Animal Pharmacology and Toxicology, Brasil University (UB), Descalvado/SP, Brasil.
[2] Department of Clinic and Surgery for Small Animals, UNICEP, São Carlos/SP, Brasil.
[3] Department of Pathology, Reproduction and One Health, UNESP/FCAV, Jaboticabal/SP, Brasil.

Submetido: 10/09/2024 Aprovado: 28/09/2024

INTRODUCTION

The use of natural nutraceuticals in aquafeed has proven to be an essential strategy to promote aquaculture sustainability (Dawood et al., 2021). Among these nutraceuticals, several medicinal herbs have been widely applied, demonstrating efficacy as growth promoters and immunostimulants (Zhu et al., 2020). Curcumin, a bioactive polyphenol present in turmeric (*Curcuma longa*), stands out for its multiple beneficial effects on growth performance, metabolic and physiological functions, immunity, antioxidant capacity, and disease resistance in several fish species (Alagawany et al., 2021).

Chemically identified as (1E6E)1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, curcumin has anti-inflammatory, antioxidant and antitumor properties, making it a promising therapeutic agent for both humans and animals, in addition to being widely recognized for its low toxicity and food safety (Alagawany et al., 2021; Zheng et al., 2018). Studies show that curcumin supplementation improves the growth of species such as crucian carp (*Carassius auratus*), Nile tilapia (*Oreochromis niloticus*), and large yellow croaker (*Larimichthys crocea*), in addition to positively affecting hematological, immunological, and disease resistance parameters in fish such as Nile tilapia, common carp (*Cyprinus carpio*), and rainbow trout (*Oncorhynchus mykiss*) (Jiang et al., 2016; Mahmoud et al., 2017; Ji et al., 2021; El-Barbary, 2018; Yonar, 2018; Yonar et al., 2019).

Fisheries and aquaculture are crucial pillars for global food security, especially in the 21st century (FAO, 2022). In Brazil, aquaculture has shown significant growth, consolidating itself as a strategic sector of agribusiness (Costa et al., 2022a,b). Tilapia production, in particular, offers great potential for expansion, providing a source of high-quality protein (Aracati et al., 2022). However, the intensification of production systems imposes health challenges, such as the increased spread of diseases in high-density population environments, generating economic losses and risks to public health (Oliveira et al., 2024). Faced with these challenges, curcumin supplementation emerges as a promising alternative. Its antimicrobial, anti-inflammatory and immunomodulatory properties can not only improve resistance against pathogens but also reduce the need for the use of synthetic antimicrobials, promoting sustainability and safety in aquaculture (Alagawany et al., 2021).

Therefore, the evaluation of the clinical safety of new compounds, such as curcumin, becomes essential. Hematological and biochemical tests are indispensable tools for monitoring the health and welfare of animals (Mahmoud et al., 2018). Thus, considering the development of fish farming and the socioeconomic importance of tilapia farming, the present study aims to investigate the clinical safety of curcumin and its potential effects on the physiological responses of teleost fish.

MATERIAL AND MÉTHODS

Fish and packaging

For the clinical safety study of curcumin, 63 Nile tilapia (±100 g) from the same spawning of the Projeto Peixes Fish Farm (Sales de Oliveira, SP, Brazil) were used, housed in 9 tanks (100 L of water each, n=7) filled with dechlorinated water. 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 basal 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, with values recorded that remained within the range appropriate 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 230030.

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 curcumin); T1 and T2 (treated with 100 and 400 mg/kg-1 of bw of curcumin, respectively). To evaluate the possible physiological changes of the animals caused by the drug, 7 animals from both treatments (T1 and T2) were treated for 12 days with curcumin, after which they were administered only commercial feed for 4 more days, without the addition of vegetable oil and curcumin, called the recovery period, totaling 16 days of analysis. Seven animals per treatment were sampled in groups T1 and T2 in four periods: 4, 8 and 12 days post-treatment (DPT) and on the 16th day, corresponding to the recovery period. In the control group (T0), the animals were sampled in a single period (n = 7). 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 am and 4 pm), with administration of 2% of the biomass of the tanks. To prepare the diets, the feed was weighed daily in proportion to the average weight of the tilapia in each tank. Then, curcumin was added at doses of 100 and 400 mg/kg1 bw 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 of 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 group (T1 and T2, one tank for each treatment) were anesthetized and approximately 2 mL of blood were collected from the caudal vessel of each animal at 4, 8, 12 and 16 DPT. In the T0 group, collection was performed in a single initial period. Samples were aliquoted into two sets: a syringe coated with heparin (5000 IU) to obtain plasma, and another without heparin, to obtain serum. 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. Circulating hemoglobin was measured using Labtest's hemoglobin reagent for reading at a wavelength of 540 nm, and 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 and 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 (Aracati et al., 2021).

Assessment of hepatic, renal and splenic somatic index

After 4, 8, 12 and 16 days of treatment, the 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. 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 any behavioral changes, clinical signs, and mortality that could result from curcumin treatment. 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 oxygen 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, 100mg and 400X 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 curcumin, it was observed that, at 8 DPT, the animals in group T2, exposed to the highest dose (400 mg/kg) showed intermittent episodes of anorexia, characterized by alternating periods of food intake and food refusal. At 10 DPT and 14 DPT, the mortality of two fish belonging to this group was recorded. However, during the recovery period, the fish resumed regular food consumption.

Hematological analysis

In the erythrocyte count (Figure 1A), it was found that the animals treated with curcumin showed a significant increase in the number of erythrocytes ($p<0.05$), especially at the dose of 400 mg/kg at 12 DPT and at the dose of 100 mg/kg at 16 DPT. The same pattern was observed in the hematocrit percentage (Figure 1B) compared to the control group. Regarding hemoglobin (Figure 1C), the response was similar, but without statistical significance. During the evolution of treatment with curcumin at the dose of 100 mg/kg (T1), a decrease in the mean corpuscular volume

(MCV) was observed at 16 DPT (Figure 1D) and an increase in the mean corpuscular hemoglobin concentration (MCHC) at 7 DPT compared to the values of 4 DPT (Figure 1F). No significant changes (P ≥ 0.05) were observed in mean corpuscular hemoglobin (MCH) calculations (Figure 1E).

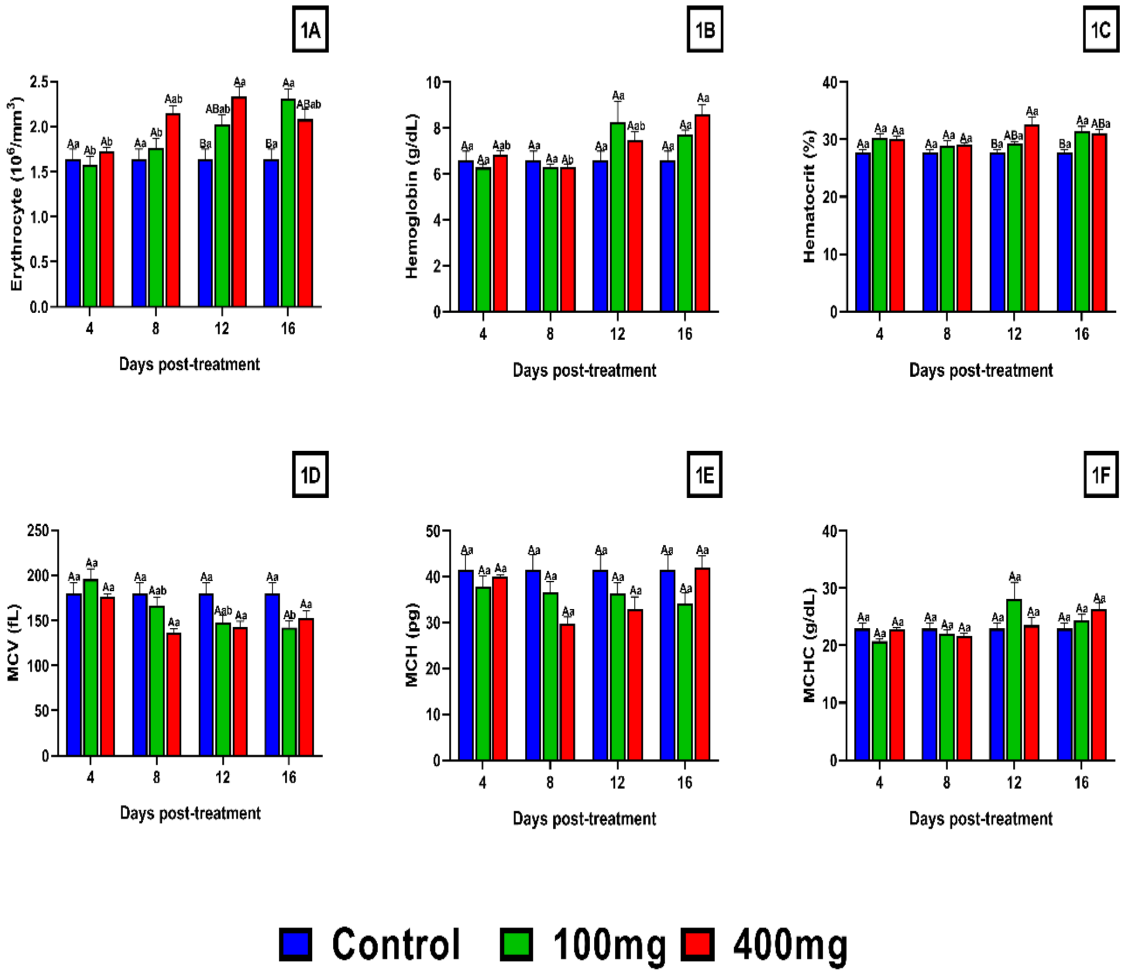

Figure 1 - Mean values (± standard error) observed in the hematological analysis of tilapia treated with curcumin. Means (n=7) followed by the same letter do not differ by Tukey's test (P<0.05). A: Erythrocytes; B: Hemoglobin; C: Hematocrit; D: Mean Corpuscular Volume (MCV); E: Mean Corpuscular Hemoglobin (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.

Leukogram

Analysis of the leukocyte profile did not show statistically significant differences in the total number of leukocytes (Figure 2A), as well as in the differential count of monocytes, granulocytes, lymphocytes and thrombocytes (Figures 2B to 2E)

Serum biochemical analysis

Serum creatinine biochemical evaluation (Figure 3A) in animals treated with both doses of curcumin revealed a decrease in levels at 4 and 8 days DPT, being significant only at 4 DPT at the highest dose T2 (400 mg/kg) compared to the control group. As treatment progressed, values returned to levels similar to those of the control group.

In the evaluation of total protein (Figure 3B), there was no significant difference between the groups. However, throughout the treatment, an increase was observed at 12 DPT in both doses, compared to the values at 8 DPT.

The results also indicated that tilapia in group T2 presented a peak in glycemia at 16 DPT compared to the control group and the 100 mg/kg dose. This glycemic increase was observed gradually throughout the treatment with 400 mg/kg of curcumin (Figure 3C).

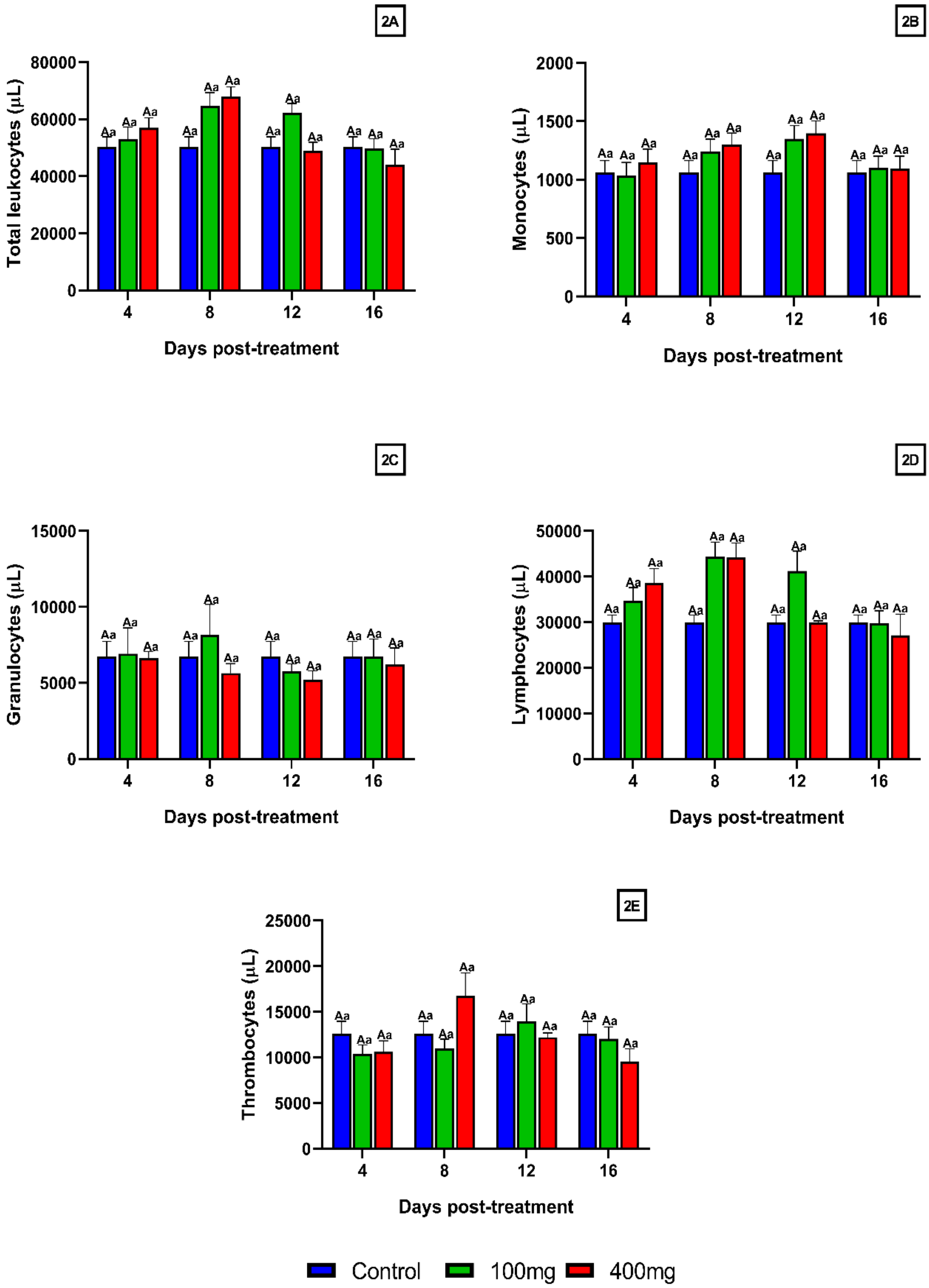

Figure 2 -- Mean values (±Standard error) observed in the leukocyte analysis of tilapia treated with curcumin. Means (n=7) followed by the same letter do not differ by Tukey's test (P<0.05). A: Total Leukocytes; B: Monocytes; C: Granulocytes; D: Lymphocytes; E: Thrombocytes. Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

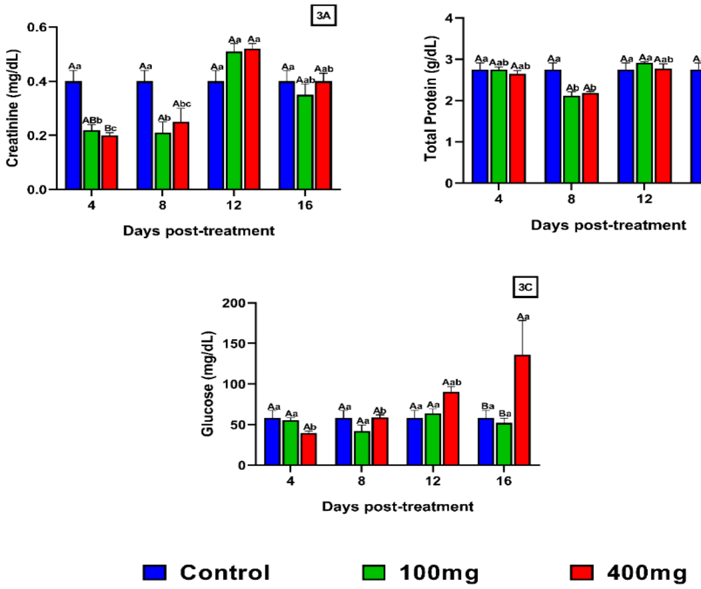

Figure 3 - Mean values (±Standard error) observed in the biochemical analysis. Means (n=7) followed by the same letter do not differ by Tukey's test (P<0.05). A: Creatinine; B: Total protein; C: Glycemia. Capital letters compare the different treatments within each experimental day, lowercase letters compare the evolution of each treatment between the different experimental days.

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).

.

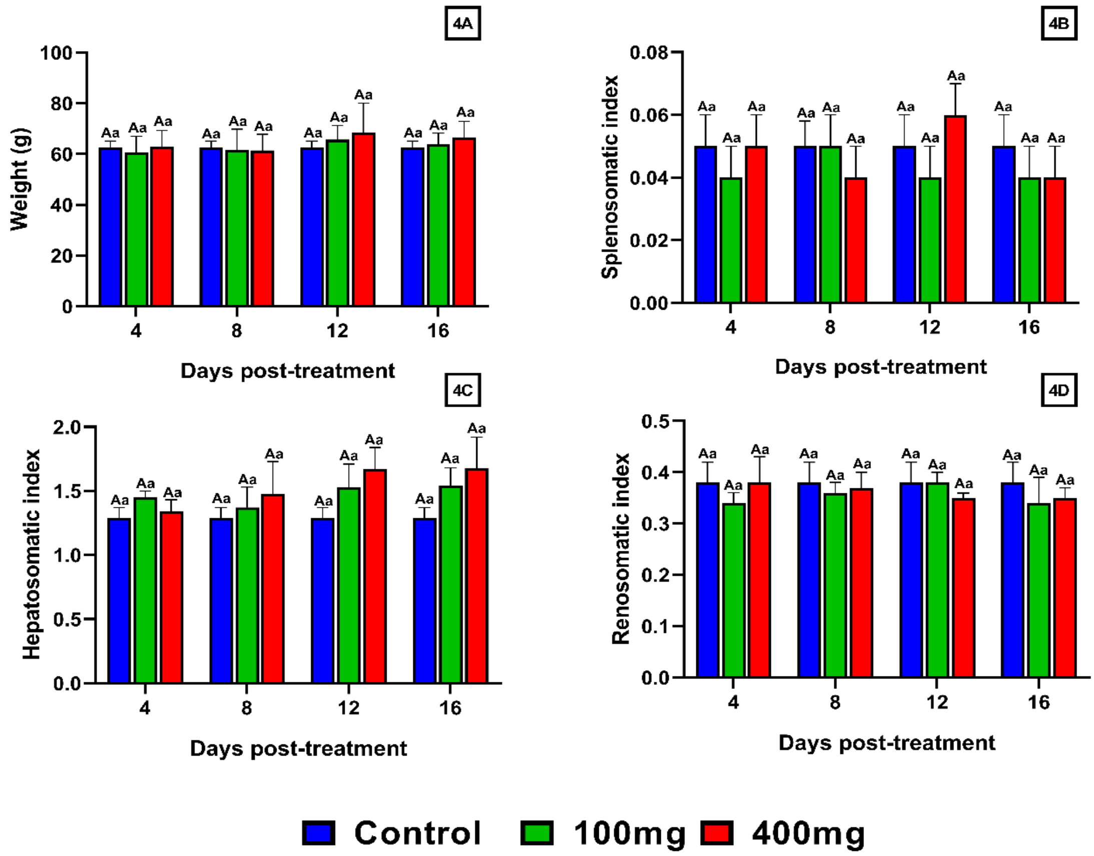

Figure 4 - Mean values (±Standard error) observed in the somatic evaluation of spleen, liver and kidney of treated tilapia. Means (n=7) followed by the same letter do not differ by Tukey's test (P<0.05). A: Weight; B: Splenosome Index; C: Hepatosomatic Index; D: Renosomatic Index. Capital letters compare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

DISCUSSION

In the present study, the results indicate that curcumin treatment in tilapia promoted a significant increase in the number of erythrocytes and hematocrit ($p<0.05$), especially at the dose of 400 mg/kg at 12 DPT and at the dose of 100 mg/kg at 16 DPT. Hemoglobin analysis showed a similar response, but without statistical significance. This increase in the number of erythrocytes may suggest a stimulating effect of curcumin on erythropoiesis, possibly due to its antioxidant and anti-inflammatory properties, which may improve the ability of fish to produce red blood cells (Aggarwal et al., 2007). Similar studies in other fish species (Sahu et al., 2008) also observed that curcumin can positively influence hematological parameters, corroborating our findings. The precise mechanisms by which curcumin exerts these effects are not yet fully elucidated. However, they are likely to involve modulation of oxidative stress in the bone marrow, promoting the proliferation of erythroid precursors and protecting these cells from oxidative damage (Bisht et al., 2010).

The absence of statistical difference in leukogram in our study may be explained by the relatively short duration of administration of the substance compared to previous studies. For example, Mohamed et al. (2020) observed a significant increase in leukogram values in fish after supplementation with curcumin (200 mg/kg) for 60 consecutive days. However, in our experiment, the administration period was a maximum of 12 days, which may have been insufficient to promote detectable changes in the leukocyte profile. This suggests that the immune response to bioactive compounds may be related to the duration of exposure, with a longer period being necessary to observe significant effects on the immune system, as reported by other studies (Mohamed et al., 2020; Yonar et al., 2019; Abd El-Hakim et al., 2020).

Curcumin treatment resulted in a decrease in serum creatinine values. This effect, followed by a return to baseline levels similar to the control group with the progression of treatment, suggests a temporary action of curcumin on renal function or creatinine metabolism. Creatinine is a common marker used to assess renal function, and elevated levels may indicate renal impairment or increased muscle breakdown (Levei and Coresh, 2012). The observed reduction in creatinine levels may suggest that curcumin may be associated with a beneficial effect on renal function, which is widely documented in the literature. Previous studies indicate that curcumin may exert protective effects on the kidneys, mainly through its anti-inflammatory and antioxidant properties, which reduce oxidative stress and inflammation in renal tissue (Katouah, 2019). In mammalian models, curcumin has been shown to protect against toxin-induced renal injury by reducing serum creatinine levels and other enzymes indicative of renal injury (Venkatesan et al., 2000). A study by Kumar et al. (2017) showed that curcumin improved cisplatin-induced nephrotoxicity and potentiated its anticancer activity in rats by decreasing creatinine levels and other markers of renal damage. These effects may be related to curcumin's ability to modulate the expression of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase, in addition to inhibiting the production of reactive oxygen species (ROS), which results in less oxidative damage to renal tissue (Aggarwal, 2007). Another hypothesis that justifies the change in serum creatinine levels would be associated with the anorexia observed, especially in tilapia treated with 400 mg of curcumin/kg. Decreased creatinine levels may be associated with malnutrition with loss of muscle mass (Bartholomae et al., 2022).

Curcumin treatment did not result in changes in serum protein levels, suggesting that curcumin, at the doses used, did not substantially affect protein synthesis or degradation in tilapia. Serum total protein is an indicator of hepatic protein synthesis and the nutritional status of the organism, reflecting the liver's ability to produce essential proteins such as albumin and globulins (Gabay and Kushner 1999; Belo et al., 2012). Although the increase was not significant between groups, the elevation at 12DPT suggests that curcumin may influence the synthesis or stability of circulating proteins. This increase may be related to the modulation of liver function, since curcumin is known for its hepatoprotective properties and for influencing the expression of genes involved in protein synthesis (Khan et al., 2019).

The results demonstrated that tilapia treated with 400 mg/kg curcumin showed a significant peak in blood glucose at 16 DPT, i.e., during the recovery period, which may be attributed to several factors related to the effect of curcumin on glucose metabolism. Although curcumin is known for its anti-inflammatory and antioxidant properties, it can also influence glucose metabolism in complex ways (Zhang et al., 2013). Studies in mammals indicate that curcumin can alter glucose homeostasis, possibly by modulating the expression of enzymes involved in gluconeogenesis and endocrine function (Zhong et al., 2022).

Somatic analysis of organs such as spleen, liver, and kidney revealed no significant alterations, which is consistent with the absence of toxicity observed in other clinical and biochemical parameters. These findings are promising, considering that curcumin can be used as a dietary supplement in fish, improving specific aspects of health without compromising the function of vital organs. Recent studies corroborate these findings, pointing to curcumin as a potential therapeutic and prophylactic agent in aquaculture, due to its antioxidant and anti-inflammatory properties. For example, Ahmadifar et al. (2021) highlight the role of curcumin in improving the immune response in fish, while Paulpandi et al. (2023) reported the efficacy of curcumin in promoting hematopoiesis under oxidative stress conditions. However, it is essential that further studies be conducted to elucidate the specific mechanisms by which curcumin influences hematological and biochemical parameters in tilapia, in addition to determining the optimal dosages to optimize its benefits without causing adverse effects.

REFERENCES

ABD EL-HAKIM, Y. M., EL-HOUSEINY, W., ABD ELHAKEEM, E. M., EBRAHEIM, L. L., MOUSTAFA, A. A., MOHAMED, A. A. R. Melamine and curcumin enriched diets modulate the haemato-immune response, growth performance, oxidative stress, disease resistance, and cytokine production in *Oreochromis niloticus*. **Aquatic Toxicology,** 220, 105406, 2020.

AGGARWAL, B.B.; SURH, Y-J; SHISHODIA, S (Ed.). The molecular targets and therapeutic uses of curcumin in health and disease. **Springer Science & Business Media**, 2007.

AHMADIFAR, E., YOUSEFI, M., KARIMI, M., FADAEI RAIENI, R., DADAR, M., YILMAZ, S., ABDEL-LATIF, H. M. Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. **Reviews in Fisheries Science & Aquaculture**, 29(4), 478-511, 2021.

ALAGAWANY, M.; FARAG, M. R.; ABDELNOUR, S. A.; DAWOOD, M. A.; ELNESR, S. S.; DHAMA, K. Curcumin and its different forms: A review on fish nutrition. **Aquaculture**, v. 532, p. 736030, 2021.

ARACATI, M. F., RODRIGUES, L. F., DE OLIVEIRA, S. L., RODRIGUES, R. A., CONDE, G., CAVALCANTI, E. N. F., ... & DE ANDRADE BELO, M. A. Astaxanthin improves the shelf-life of tilapia fillets stored under refrigeration. **Journal of the Science of Food and Agriculture**, 102(10), 4287-4295, 2022.

ARACATI, M. F.; RODRIGUES, L. F.; OLIVEIRA, S. L.; MORAES, A. C.; PRADO, E. J. R.; FERNANDES, D. C.; ETO, S. F.; CHARLIE-SILVA, I.; BELO, M. A. A. Clinical safety of zafirlukast treatment during acute inflammatory reaction in Nile tilapia (*Oreochromis niloticus*). ***Ars Veterinaria***, v. 37, p. 67-73, 2021.

BARTHOLOMAE, E.; KNURICK, J.; JOHNSTON, C.S. Serum creatinine as an indicator of lean body mass in vegetarians and omnivores. **Frontiers in Nutrition**, v. 9, p. 996541, 2022.

BELO, M. A. D. A., MORAES, J. R. E. D., SOARES, V. E., MARTINS, M. L., BRUM, C. D., MORAES, F. R. Vitamin C and endogenous cortisol in foreign-body inflammatory response in pacus. **Pesquisa Agropecuária Brasileira**, 47, 1015-1021, 2012.

BISHT, K.; WAGNER, K. H.; BULMER, A. C. Curcumin, resveratrol and flavonoids as anti-inflammatory, cyto-and DNA-protective dietary compounds. **Toxicology**, v. 278, n. 1, p. 88-100, 2010.

BOYD, C.E. Water Quality in Ponds for Aquaculture, Alabama Agricultural Experiment 416 Station, Auburn University, AL, USA, 1990.

CARNEIRO, P. C. F.; URBINATI, E. C. Salt as stress response mitigator of matrinxã Brycon cephalus during transport. **Aquaculture Research**, v. 32, p. 297-304, 2001.

COSTA, C. C., OLIVEIRA, S. L., ARACATI, M. F., RODRIGUES, L. F., COLTURATO, L., MONTASSIER, H. J., & BELO, M. A. A. Clinical safety of treatment with Zileuton, 5-Lox inhibitor, during acute inflammatory reaction in Nile Tilapia (*Oreochromis niloticus*). ***Ars Veterinaria***, 38(1), 23-30, 2202a.

COSTA, C. C., OLIVEIRA, S. L., ARACATI, M. F., RODRIGUES, L. F., MONTASSIER, H. J., & BELO, M. A. A. (2022b). Clinical safety of oral licomycin treatment in Nile tilapia (*Oreochromis niloticus*). ***Ars Veterinaria***, 38(4), 190-198, 2022b.

DAWOOD, M. A.; GEWAILY, M. S.; MONIER, M. N.; YOUNIS, E. M.; VAN DOAN, H.; SEWILAM, H. The regulatory roles of yucca extract on the growth rate, hepato-renal function, histopathological alterations, and immune related genes in common carp exposed with acute ammonia stress. **Aquaculture**, v. 534, p. 736287, 2021.

EL-BARBARY, M. I. Impact of garlic and curcumin on the hepatic histology and cytochrome P450 gene expression of aflatoxicosis Oreochromis niloticus using RT-PCR. **Turkish Journal of Fisheries & Aquatic Sciences**, v. 18, p. 405–415, 2018.

FAO (Food and Agriculture Organization of the United Nations). O Estado Mundial da Pesca e Aquicultura 2022 (SOFIA).http://www.fao.org/3/CA0191EN/CA0191EN.pdf. Acessado: Junho de 2024.

FARIAS, T. H. V.; LEVY-PEREIRA, N.; DE OLIVEIRA ALVES, L.; DE CARLA DIAS, D.; TACHIBANA, L.; PILARSKI, F.; ... RANZANI-PAIVA, M. J. T. Probiotic feeding improves the immunity of pacus, Piaractus mesopotamicus, during Aeromonas hydrophila infection. **Animal Feed Science and Technology**, v. 211, p. 137-144, 2016.

GABAY, C; KUSHNER, I. Acute-phase proteins and other systemic responses to inflammation. **New England journal of medicine**, v. 340, n. 6, p. 448-454, 1999.

JI, R. L.; XIANG, X. J.; LI, X. S.; MAI, K. S.; AI, Q. H. Effects of dietary curcumin on growth, antioxidant capacity, fatty acid composition and expression of lipid metabolism-related genes of large yellow croaker fed high-fat diet. **British Journal of Nutrition**, v. 126, n. 3, p. 1–10, 2021.

JIANG, J.; WU, X. Y.; ZHOU, X. Q.; FENG, L.; LIU, Y.; JIANG, W. D.; WU, P.; ZHAO, Y. Effects of dietary curcumin supplementation on growth performance, intestinal digestive enzyme activities and antioxidant capacity of crucian carp *Carassius auratus*. **Aquaculture**,

v. 463, p. 174–180, 2016. https://doi.org/10.1016/j.aquaculture.2016.05.040.

KATOUAH, H. A. Curcumin Clubbed Preventive for Renal Damage. **International Journal of Drug Development & Research** 11: 51, v. 54, p. 51-54, 2019.

KHAN, E. A., MA, J., XIAOBIN, M., JIE, Y., MENGYUE, L., HONG, L.; LIU, A. Safety evaluation study of lincomycin and spectinomycin hydrochloride intramuscular injection in chickens. **Toxicology Reports**, 9, 204-209, 2022.

KUMAR, P.; BARUA, C. C.; SULAKHIYA, K.; SHARMA, R. K. Curcumin ameliorates cisplatin-induced nephrotoxicity and potentiates its anticancer activity in SD rats: potential role of curcumin in breast cancer chemotherapy. **Frontiers in pharmacology**, v. 8, p. 132, 2017.

LEVEY, A.S.; CORESH, J. Chronic kidney disease. **The lancet**, v. 379, n. 9811, p. 165-180, 2012.

MAHMOUD, H. K.; AL-SAGHEER, A. A.; REDA, F. M.; MAHGOUB, S. A.; AYYAT, M. S. Dietary curcumin supplement influence on growth, immunity, antioxidant status, and resistance to Aeromonas hydrophila in Oreochromis niloticus. **Aquaculture,** v. 475, p. 16–23, 2017. https://doi.org/10.1016/j.aquaculture.2017.03.043.

MAHMOUD, S.; HASABELNABY, S.; HAMMAD, S.; SAKR, T. Antiviral nucleoside and nucleotide analogs: a review. **Journal of Advanced Pharmacy Research**, v. 2, n. 2, p. 73-88, 2018.

MOHAMED, A. A. R., EL-HOUSEINY, W., ABD ELHAKEEM, E. M., EBRAHEIM, L. L., AHMED, A. I. ABD EL-HAKIM, Y. M. Effect of hexavalent chromium exposure on the liver and kidney tissues related to the expression of CYP450 and GST genes of Oreochromis niloticus fish: Role of curcumin supplemented diet. **Ecotoxicology and Environmental Safety**, *188*, 109890, 2020.

NATT, M.P.; HERRICK, C.A. A new blood diluents for counting the erythrocytes and leucocytes of the chicken. **Poultry Science** v. 31, p. 735-738, 1952.

OLIVEIRA, S. L., DA COSTA, C. C., ARACATI, M. F., RODRIGUES, L. F., MONTASSIER, H. J., CONDE, G., ... & DE ANDRADE BELO, M. A. Doxycycline treatment modulates the immune response of tilapia and controls Aeromonas hydrophila infection. **Aquaculture**, 582, 740504, 2024.

PAULPANDI, M., RAJAGANESH, R., KAVITHAA, K., KALAIARASI, G., & ARUL, N. Curcumin-loaded a-lactalbumin nanoparticles neuroprotective effects against permethrine-induced neurotoxicity. *Inorganic Chemistry Communications*, *158*, 111586, 2023.

=SAHU, S., DAS, B. K.; MISHRA, B. K.; PRADHAN, J.; SAMAL, S. K.; SARANGI, N. Effect of dietary Curcuma longa on enzymatic and immunological profiles of rohu, Labeo rohita (Ham.), infected with Aeromonas hydrophila. **Aquaculture Research**, v. 39, n. 16, p. 1720-1730, 2008.

SAS Institute Inc. (2012) SAS/STAT software changes and enhancements through computer program. Release 9.3. Cary: SAS Institute.

VENKATESAN, N., PUNITHAVATHI, D., ARUMUGAM, V. Curcumin prevents adriamycin nephrotoxicity in rats. British journal of pharmacology, 129(2), 231-234, 2000.

WEDEMEYER, G. Stress of anesthesia with MS-222 and benzocaine in rainbow trout (Salmo gairdneri). **Journal of the Fisheries Board of Canada**, v. 22, n. 5, 1970.

WEIBEL, E. R.; STAUBLI, W.; GNAGI, H. R.; HESS, F. A. Correlated morphometric and biochemical studies on the liver cell. ***Journal of Cell Biology***, p. 68-91, 1969.

YONAR, M. E. Chlorpyrifos-induced biochemical changes in Cyprinus carpio: ameliorative effect of curcumin. **Ecotoxicology and Environmental Safety**, v. 151, p. 49–54, 2018. https://doi.org/10.1016/j.ecoenv.2017.12.065.

YONAR, M. E.; MIŞE YONAR, S.; IŞPINAR, Ü.; URAL, M. S. Effects of curcumin on haematological values, immunity, antioxidant status and resistance of rainbow trout (Oncorhynchus mykiss) against Aeromonas salmonicida subsp. achromogenes. **Fish & Shellfish Immunology**, v. 89, p. 83–90, 2019. https://doi.org/10.1016/j.fsi.2019.03.038.

ZHANG, D. W.; FU, M.; GAO, S. H.; LIU, J. L. Curcumin and diabetes: a systematic review. **Evidence-Based Complementary and Alternative Medicine**, v. 2013, n. 1, p. 636053, 2013.

ZHENG, B.; PENG, S., ZHANG, X.; MCCLEMENTS, D. J. Impact of delivery system type on curcumin bioaccessibility: Comparison of curcumin-loaded nanoemulsions with commercial curcumin supplements. **Journal of Agricultural and Food Chemistry**, v. 66, n. 41, p. 10816-10826, 2018.

ZHONG, Y.; XIAO, Y.; GAO, J.; ZHENG, Z.; ZHANG, Z.; YAO, L.; LI, D. Curcumin improves insulin sensitivity in high-fat diet-fed mice through gut microbiota. **Nutrition & Metabolism**, v. 19, n. 1, p. 76, 2022.

ZHU, Fei. A review on the application of herbal medicines in the disease control of aquatic animals. **Aquaculture**, v. 526, p. 735422, 2020.