ORIGINAL RESEARCH

Recirculating aquaculture systems affects hematological parameters and increases ectoparasite susceptibility in Nile tilapia *Oreochromis niloticus*

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ABSTRACT. We herein evaluated the hematology and parasitology of Nile tilapia previously raised in an earthen pond and transferred to a recirculating aquaculture system (RAS) to verify any possible influence of RAS on such health parameters. For this, 780 tilapias with an initial average weight and length of 91.05 ± 27.08 g and 17.45 ± 1.91 cm, respectively, were distributed in three tanks (2.0 m^3) attached to the RAS, where they remained for 54 d. Throughout the period, water quality parameters remained within the range suitable for the cultivation of the species. Tilapia growth performance was satisfactory in the RAS, reaching a final biomass of close to 300 kg and survival of 98%. Hematological and parasitological analyses at the end of 54 d showed possible stress in RAS, with a significant increase in neutrophils from 12.15 ± 6.66 (earthen pond) to 21.43 ± 11.68% (RAS) and erythrocytes from 1.81 ± 0.24 (earthen pond) to 2.13 ± 0.14 (RAS), and a significant decrease in lymphocytes from 22.4 ± 2.66 (earthen pond) to 13.67 ± 3.38 (RAS). Furthermore, parasitological analysis showed a significant increase in the number of parasitized fish (25% to 63%) and abundance (3.55 ± 6.44 to 9.37 ± 9.99) after 54 d. It was concluded that tilapia cultivation in RAS can cause hematological changes and increase parasitism.

Key words: Intensive fish farm, blood count, Monogenea.

Los sistemas de recirculación de acuicultura afectan los parámetros hematológicos y aumentan la susceptibilidad a los ectoparásitos en la tilapia del Nilo *Oreochromis niloticus*

RESUMEN. Evaluamos la hematología y parasitología de tilapias del Nilo previamente criadas en un estanque de tierra y transferidas a un sistema de acuicultura de recirculación (RAS), para verificar cualquier posible influencia del RAS en dichos parámetros de salud. Para ello, se distribuyeron 780 tilapias con un peso y longitud promedio inicial de 91.05 ± 27.08 g y 17.45 ± 1.91 cm en tres tanques (2.0 m^3) adjuntos al RAS, donde permanecieron durante 54 d. Durante todo el período los parámetros de calidad del agua se mantuvieron dentro del rango adecuado para el cultivo de la especie. El comportamiento del crecimiento de la tilapia fue satisfactorio en el RAS, alcanzando una biomasa final cercana a los 300 kg y una supervivencia del 98%. Los análisis hematológicos y parasitológicos al final de los 54 d mostraron un posible estrés en el RAS, con un aumento significativo de neutrófilos de 12,15 ± 6,66 (estanque de tierra) a 21,43 ± 11,68% (RAS) y de eritrocitos de 1,81 ± 0,24 (estanque de tierra) a 2,13 ± 0,14 (RAS), y una disminución significativa de linfocitos de 22,4 ± 2,66 (estanque de tierra) a 13,67 ± 3,38 (RAS). Además, el análisis parasitológico mostró un aumento significativo en el número de peces parasitados (25% a 63%) y abundancia (3,55 ± 6,44 a 9,37 ± 9,99) después de los 54 d. Se concluyó que el cultivo de tilapia en RAS puede provocar cambios hematológicos y aumentar el parasitismo.

Palabras clave: Piscifactoría intensiva, hemograma, Monogenea.

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INTRODUCTION

Recirculating aquaculture systems (RAS) emerge in the aquaculture sector as powerful sustainable tools. When compared to other cultivation technologies, RAS presents economic advantages as it requires a smaller area to obtain the same productivity, better sanitary control during production, allows ideal production growth throughout the year, reducing seasonality in the product supply and allows production close to the consumer market (Ebeling and Timmons 2012). In addition to reducing environmental impacts, RAS can optimize productivity in harmony with the pillars of sustainability, contributing to a healthy and prosperous future for the planet (Ebeling and Timmons 2012; Owatari et al. 2022a).

However, the implementation of RAS technology foresees challenges that exceed implementation costs and specialized labor. Perspectives related to environmentally friendly practices in aquaculture must also consider the welfare of farmed animals (Dara et al. 2023). In Brazil, RAS are intended for the cultivation of ornamental fish and for research purposes (Valenti et al. 2021). However, its use in commercial fish production, such as Nile tilapia *Oreochromis niloticus*, has not yet been established.

In the Brazilian aquaculture sector, Nile tilapia stands out as the most produced fish species, reaching the mark of 550,000 t in 2022, representing 63.9% of Brazilian production, where the most predominant cultivation models are those conducted in earthen ponds (PeixeBR 2023). Nile tilapia, as well as other fish species, can present good productive performance when stocked at high densities; however, a considerable increase in stocked biomass will result in a greater amount of waste, and consequently, rapid deterioration in water quality (Roveda et al. 2024), impacting the fish health and enabling infectious and parasitic outbreaks (Tavares-Dias and Martins 2017).

A quick and accurate way to access fish health is through monitoring hematological parameters (Fazio 2019). Hematological analyses are important tools for diagnosing possible infectious processes caused by both bacteria and parasites (Ishikawa et al. 2008). In homeostasis, fish blood is composed of cells that remain in different proportions; however, when a disturbance occurs that interferes with the animals' health, these proportions are altered, generating an indication of anomalies. Such changes can occur due to biotic and abiotic factors, such as parasites and changes in water quality (Ranzani-Paiva and Silva-Souza 2004).

The growth and intensification of tilapia production has faced obstacles that affect the productivity and quality of the fish, with health being one of the main obstacles detected (Tavares-Dias and Martins 2017; Owatari et al. 2020). Thus, the present study evaluated the health aspects of Nile tilapia cultivated in RAS in southern Brazil, evaluating water quality variables and fish health with hematological and parasitological analyses, before and after stocking in the RAS system.

MATERIALS AND METHODS

The Ethics Committee on the Use of Animals (CEUA/UFSC 9594280417) approved the study using eugenol (75 mg l^{-1}) as an anesthetic. The experiment was conducted using an indoor recirculating aquaculture system (RAS). Initially, the system underwent a sanitary vacuum, in which the RAS was completely cleaned and disinfected. After three days of sanitary vacuum, the system was filled with water and chlorinated at a concentration of 200 mg l^{-1}, in accordance with the recommendations of Timmons and Ebeling (2010), and was recirculated for four days until the chlorine was completely volatilized (Alcon® Labcon Cloro Test kit). Then, the system was cycled to mature the biological filter. During this phase, the average values of the water quality variables were dissolved oxygen (DO) 8.3 mg l^{-1}, temperature 37.4 °C, pH 6.8,

electrical conductivity 205.8 μS cm^{-1}, salinity 0.1, alkalinity 34 mg $CaCO_3$ l^{-1} and hardness 35 mg l^{-1}.

For bacterial growth, 100 g of the commercial fertilizer ammonium sulfate with a concentration of 21% N_2 (Adubel –salts, from Buschle and Lepper SA) were diluted in 14 l of water and added to the RAS experimental units to stimulate the growth of nitrifying bacteria. Bacteria of the genera *Nitrosomonas* and *Nitrobacter*, cultivated at the Laboratório de Camarões Marinhos of the Federal University of Santa Catarina (LCM/UFSC) were inoculated into the system, according to method 9245 (Eaton 2005). Daily dilutions of ammonium sulfate were added after monitoring the quality of the water, increasing its concentration by 50% for a week, and then maintaining it at 1.9 kg of ammonium sulfate (containing 399 g of ammonium), totaling an addition of 29.12 mg l^{-1} of ammonium per day. When the nitrite concentration reached safe levels, fish were introduced into the RAS.

For the experiment, 780 Nile tilapia with initial average weight and length of 91.05 ± 27.08 g and 17.45 ± 1.91 cm, respectively, from an earthen pond were used. Before stocking in the RAS, fifty tilapias were subjected to health assessment with parasitological and hematological analyses. All fish received an oxytetracycline bath (50 mg l^{-1}) for one hour during three days before being introduced into the RAS (Noga 2010). After this procedure, fish were measured, weighed, and stoked in the experimental units, reaching an initial total biomass of approximately 70 kg. Fish were fed commercial feed containing 36% crude protein, twice a day (10 am and 4 pm) until apparent satiety. The feed consumption was estimated as consumption × feed protein content × K ($K = 0.092$), in order to quantify the ammonia nitrogen introduced per meal into the RAS and the daily total (Timmons and Ebeling 2010).

The RAS was composed of a 5.7 m^3 biofilter, two mechanical filters (sand filter and rotary filter) and three 2.0 m^3 black fiberglass cylindrical stocking tanks as experimental units (cultivation units), composed of independent modules that could be connected or disconnected from the RAS at any time. The water inlet was located on the surface, while the water outlet was at the bottom through a central drain. The flow rate was 119 l min^{-1} and the residence time in the biofilter was 48 min. Oxygen was supplied by three air diffusers connected to a radial air compressor (ELAM, model CRE-05 of 7.5 CV, 8.2 m^3 air min^{-1}). Initially, daily water replacements were carried out, replacing only the water lost through evaporation and backwashing, totaling an approximate volume of 750 l day^{-1}. Starting on day 27th after fish stocking, two daily backwashes were necessary, totaling a replacement of 1,500 l day^{-1}.

Dissolved oxygen and temperature were measured with the YSI 550A probe. pH, salinity, and electrical conductivity were measured with the YSI 63 probe. Alkalinity and hardness were analysed in the laboratory according to Golterman et al. (1978) and by titration and with edetetic acid, respectively. For the quantification of nitrogenous compounds, the colorimetric method was used with a Micronal B582 spectrophotometer, following Bendschneider and Robinson (1952) and Koroleff (1976).

After inoculating the RAS with nitrifying bacteria and ammonium sulfate, water quality monitoring was conducted daily at 4 pm until the stocking of fish. The alkalinity and hardness were conducted every four days. After fish stocking, water parameters continued to be measured daily at 4 pm in the experimental units. For total ammonia, nitrite and nitrate analyses, water samples were collected from the cultivation units, after the rotary filter between points 1 and 2.1 (Figure 1) and after the sand filter between points 4 and 5 (Figure 1). Alkalinity and hardness were measured at intervals of 4 days and water was collected for analysis before entering the biofilter between points 1 and 2 (Figure 1). The experimental period lasted 54 days.

After being removed from the earthen pond and at the end of the cultivation phase in the RAS, hematological and parasitological analysis of fish were conducted. For hematological analysis, ten fish from each cultivation unit were sedated with eugenol (75 mg l^{-1}) and then the blood was col-

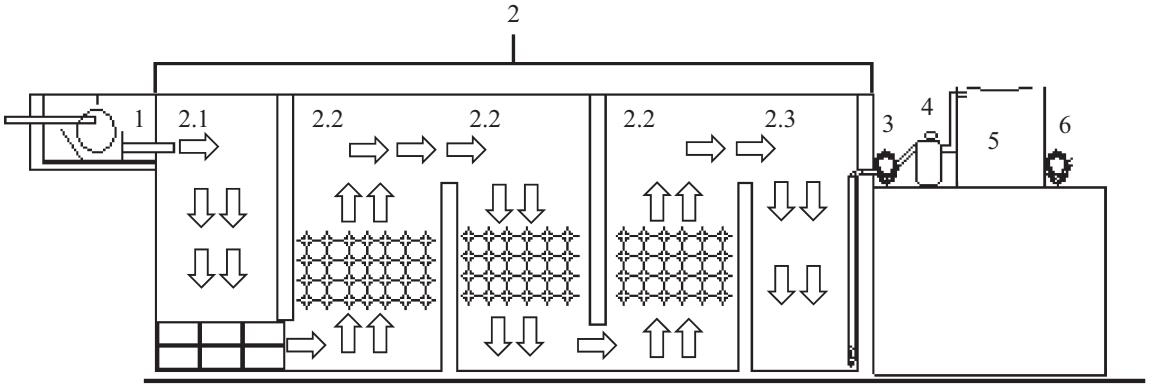

Figure 1. Schematic of the water treatment unit. 1) rotating filter, with 30 μ mesh, 2) biofilter, 2.1) water reception compartment from the cultivation units, containing 355 kg of oyster shell, 2.2) compartment with biological support for fixing nitrifying bacteria, 2.3) compartment for collecting biologically filtered water, 3) 1.5 HP motor pump (Jacuzzi, model 15 B-T), 4) sand filter (Jacuzzi, model 30 TP), 5) 5 m^3 equalizing box for water storage and temperature homogenization, 6) 1.0 HP motor pump (Jacuzzi, model 1 A-T) delivers water to the experimental units.

lected by puncturing the caudal vessel with the aid of insulin syringes emulsified with 10% Hemstab EDTA anticoagulant. Blood extensions were prepared and stained with May-Grunwald/Giensa (Rosenfeld 1947) for total and differential counts of organic defense blood cells (leukocytes and thrombocytes). The differential count of organic defense blood cells followed the methodology proposed by Ishikawa et al. (2008) and the erythrocyte count on a hemocytometer and hematocrit according to Goldenfarb et al. (1971). Parasitological analysis was performed after blood collection, according to Jerônimo et al. (2011). The prevalence rate, average intensity and average abundance of parasites were calculated according to Bush et al. (1997). The hematological and parasitological parameters of fish were compared between initial and final samples.

To verify the growth performance of fish, the following production indexes were calculated: survival (%) = (number of final fish / number of initial fish) × 100; weight gain = (final weight-initial weight); specific growth rate (SGR) = [(ln final weight - ln initial weight) / (time) × (100)]; feed conversion (FC) = (amount of feed / weight gain); and condition factor (K) = [(total weight) / ($length^3$) × (100)].

Data were subjected to the Kolmogorov-Smirnov test to assess whether the distribution was within the normality curve and the Levene test to verify homoscedasticity. Data that met the prerequisites of normality and homoscedasticity were subjected to the Student t test. All analyses had a significance of 5% (Zar 2009).

RESULTS AND DISCUSSION

To evaluate the performance of the RAS, water quality parameters were evaluated separately and divided into the biofilter maturation phase and after the stocking of the fish (Table 1). During the cultivation period, water quality variables remained within the comfort range for Nile tilapia (Mengistu et al. 2020). Nile tilapia contributes to the development of aquaculture around the world (FAO 2022) and is among the main farmable freshwater species (Geletu and Zhao 2023). This is due to its rusticity, including greater resistance to environmental variations and diseases, when compared to other species, as it survives and grows in varied environmental conditions (PeixeBR 2023), with great potential for growth at temperatures ranging between 27 °C and 32 °C and dissolved oxygen above 5 mg l^{-1} (Mengistu et al. 2020).

Table 1. Water quality variables during the maturation phase of the biological filter and after 54 days of cultivation of Nile tilapia in a recirculating aquaculture system (RAS). Mean values are presented as mean ± SD. BB = before the biofilter. AB = after the biofilter. TAN = total ammonia nitrogen. NH_3 = toxic ammonia. NO_2 = nitrite. NO_3 = nitrate. (*) Asterisk indicates significant difference.

Variables	Maturation phase	Stocking fish phase	p-value
Dissolved oxygen (mg l^{-1})*	7.61 ± 0.20	5.53 ± 0.24	0.0079
Temperature (°C)	27.24 ± 0.22	27.99 ± 0.19	0.3046
pH*	6.92 ± 0.59	7.48 ± 0.21	0.0486
Alkalinity (mg $CaCO_3$ l^{-1})*	28.99 ± 5.44	33.35 ± 2.45	< 0.0001
Hardness (mg $CaCO_3$ l^{-1})	36.73 ± 3.26	36.85 ± 2.57	0.5282
Salinity (‰)*	0.17 ± 0.08	0.37 ± 0.07	0.0373
Electric conductivity (μS cm^{-1})*	385.44 ± 163.76	732.57 ± 52.87	0.0331
TAN (BB) (mg l^{-1})*	3.12 ± 6.28	0.274 ± 0.15	0.0089
TAN (AB) (mg l^{-1})*	1.635 ± 4.26	0.02 ± 0.013	0.0002
NH_3 (BB) (mg l^{-1})*	0.285 ± 0.90	0.007 ± 0.01	0.0021
NH_3 (AB) (mg l^{-1})*	0.041 ± 0.14	0.001 ± 0.00	0.0161
NO_2 (BB) (mg l^{-1})*	3.263 ± 3.79	0.042 ± 0.03	0.0399
NO_2 (AB) (mg l^{-1})*	1.353 ± 2.16	0.022 ± 0.04	0.0321
NO_3 (BB) (mg l^{-1})*	42.56 ± 2.09	34.73 ± 2.47	0.0451
NO_3 (AB) (mg l^{-1})*	46.04 ± 3.73	35.83 ± 3.61	0.0071

The average amounts of feed offered in the morning period were significantly lower ($p < 0.05$) (1,755.22 ± 475.8 g) when compared to the afternoon period (1,927.64 ± 408.55 g), resulting in an average consumption of 3,682.86 ± 693.37 g of feed daily. According to Timmons and Ebeling (2010), 3% of feed supplied daily to fish will be converted into TAN, i.e. for every 1,000 g of feed, 30 g of TAN will be produced in the system; and for every 0.3 g of TAN generated in the system, 1.0 m² of specific surface area is needed for the adhesion of nitrifying bacteria to efficiently oxidize ammonia to nitrate. Therefore, the RAS must be equipped with a biofilter that allows the development of healthy colonies of nitrifying bacteria, capable of carrying out ammonia oxidation. In the present study, the inoculation of nitrifying bacteria such as those of the genera *Nitrosomonas* and *Nitrobacter*, which obtain their energy from the oxidation of ammonia or nitrite (Owatari et al. 2022a), probably accelerated the biofilter maturation process and maintained the concentrations of nitrogenous compounds in the stocking fish phase within acceptable limits in the RAS.

Water quality standards may vary depending on the target species (Arana 2010). However, the deterioration of such water quality standards is one of the main factors affecting fish health (Dara et al. 2023), causing changes in the blood count (Fazio 2019), as well as increasing the presence of parasites (Lacerda et al. 2018). The hematological analysis is a bioindicator of the health of fish and the environment in which they live, and this assessment elucidates changes and morphological disorders of blood cells caused by aggressive agents (Fazio 2019). Thus, in the erythrogram it is possible to identify potential anemic processes, while the leukogram provides information about infectious processes and other states of homeostatic imbalance (Tavares-Dias et al. 2009). Hematolog-

ical values found in the present study (Table 2) are within the expected range for the species (Tavares-Dias and Faustino 1998). Nevertheless, when compared with other results from RAS studies (Moraes et al. 2022; Owatari et al. 2022b), values are completely different, indicating that hematological responses can vary according to the condition faced by animals in environments subject to stress.

The numbers of lymphocytes showed a significant decrease ($p < 0.05$) between the beginning and end of the experiment, while the number of erythrocytes and neutrophils showed a significant increase ($p < 0.05$) after the introduction of the fish into the recirculation system. This variation in the blood count may be related to the physiological responses of tilapia after stress and were similar to those observed by Fujimoto et al. (2009), who found an increase in neutrophils and a decrease in lymphocyte concentrations, but without observing a decrease in the percentage of hematocrit in parasitized camurim (*Centropomus undecimalis*). It was possible to verify through hematology that fish in the recirculation system suffered some type of stress, probably related to the stocking density in the RAS or even the presence of parasites.

Parasitological analysis of the fish before and after the experiment indicated the presence of parasites. It was possible to verify the occurrence of ectoparasites from the Monogenea class. Most common sites parasitized by Monogenea are the gills, nostrils, eyes, and body surface of fish. All these characteristics accentuate its pathogenicity, causing tissue damage and altering the behavior of the fish. Anorexia, increased mucus production, skin and branchial hemorrhages, hyperplasia in the gill filaments, weight loss and death may occur. Less intense infestations and small injuries are source of secondary infections, and in this case prophylaxis is essential (Jerônimo et al. 2011).

Although we carried out fish prophylaxis before entering the RAS, it was possible to verify that after a period of 54 days of cultivation under RAS technology, there was a significant increase ($p < 0.05$) in the number of parasitized fish, going from 25% to 63% of the prevalence, while the average abundance increased significantly ($p < 0.05$) from 3.55 ± 6.44 before stocking fish into the RAS to 9.37 ± 9.99 after 54 days of experiment. Regarding the average intensity of parasitism, no significant differences ($p > 0.05$) were observed between periods. Before stocking fish, the average intensity was 14.2 ± 6.31, while after 54 days it was 14.79 ± 8.74. Treatment and prevention of monogenetic infections involves the use of various anthelmintic parasiticides. However, prolonged, and frequent use of these medications in incorrect doses leads to the evolution of drug resistance (Zhang et al. 2014), a factor that may have limited the effectiveness of the treatment applied in the prophylactic bath in the present study.

Table 2. Hematological parameters (mean ± SD) of Nile tilapia juvenile before and after 54 days of stocking into the RAS. (*) Asterisk indicates significant difference.

Hematological parameters	Initial	Final	p-value
Erythrocytes ($\times 10^6$ μl^{-1})*	1.81 ± 0.24	2.13 ± 0.14	0.014
Leukocytes ($\times 10^3$ μl^{-1})	12.48 ± 1.62	10.40 ± 5.59	0.382
Thrombocytes ($\times 10^3$ μl^{-1})	52.65 ± 14.68	49.83 ± 9.99	0.594
Lymphocytes ($\times 10^3$ μl^{-1})*	22.4 ± 2.66	13.67 ± 3.38	0.033
Monocytes ($\times 10^3$ μl^{-1})	12.8 ± 8.88	15.07 ± 6.99	0.159
Neutrophils ($\times 10^3$ μl^{-1})*	12.15 ± 6.66	21.43 ± 11.68	0.014
Hematocrit (%)	32.92 ± 3.17	31.33 ± 2.18	0.058

Table 3. Zootechnical performance of Nile tilapia after 54 days of cultivation in a recirculating aquaculture system (RAS). Values are presented as mean ± SD.

Zootechnical indexes	Initial	Final
Weight (g)	91.05 ± 27.08	211.11 ± 29.16
Length (cm)	17.45 ± 1.91	22.74 ± 1.00
Survival (%)	100	98.27 ± 0.52
Biomass weight (kg)	70.98 ± 11.57	161.88 ± 45.12
Condition factor	1.85 ± 0.12	1.79 ± 0.15
Specific growth rate (g day^{-1})	*	2.25 ± 0.33
Feed conversion rate	*	1.46 ± 0.56

Final survival reached 98% (Table 3). Sampaio and Braga (2005) and Marengoni (2006) observed survival rates of Nile tilapia of 78% to 95% when cultivated in cages. This fact suggests that RAS can considerably improve survival rates, and consequently improve productivity. Furthermore, we herein observed that biomass almost tripled in 54 days and daily weight gain was similar to that reported by Moraes et al. (2022) for tilapia cultivated in RAS, showing a probable growth pattern under the same cultivation model. The daily specific growth rate is a valuable indicator for checking weight gain. In the present study, values found after 54 days were higher than the 0.33 ± 0.01% found by Owatari et al. (2022b) in the RAS. However, in the present study the average water temperature remained close to 27.99 ± 0.19 °C, while in the study of Owatari et. al. (2022b) the water in the tanks remained at an average temperature of 24.08 ± 2.30 °C, a factor that was probably advantageous for the fish in the present study.

CONCLUSIONS

Hematological analysis showed a possible stressor in the RAS, probably caused by the fish stocking density or the presence of parasites. The cultivation model with RAS technology increased parasitism among fish. However, Nile tilapia showed good growth indexes in RAS when compared to other studies.

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Declaration of interest

The authors declare that they have no conflict of interest in the present manuscript.

Author contributions

Rodrigo Stallbohm: conceptualization, investigation, and writing-original draft. Marco Shizuo Owatari: formal analysis, writing-review and ed-

iting. Evoy Zaniboni-Filho: writing-review and editing, visualization, supervision, and project administration. Maurício Laterça Martins: conceptualization, methodology, investigation, writing-review and editing, visualization, supervision, and project administration.

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