Int Aquat Res (2022) 14:139-146
https://doi.org/10.22034/IAR.2022.1957766.1272

ORIGINAL RESEARCH

Salinity in the culture of Nile tilapia in a biofloc system: Influence on growth and hematological parameters

Julianna Paula do Vale Figueiredo . Ana Paula Mariane de Morais . Weverson Ailton da Silva . Ramires Eloíse Queiroz Rafae . Ivanilson de Lima Santos . Maurício Laterça Martins . Felipe do Nascimento Vieira . Frank Belettini . Edemar Roberto Andreatta

Received: 29 March 2022 / Accepted: 09 June 2022 / Published online: 16 June 2022

Abstract The present study aimed to evaluate the influence of different salinities on water quality, growth performance, and hematology of the Nile tilapia *Oreochromis niloticus* cultured in a biofloc system. Four different salinities with four replicates were evaluated: 2, 8, 15, and 22 PSU. The fish (200 ± 10 g) were stocked at a density of 15 fish m^{-3} in tanks with 800 L of useful volume for 56 days. Fish were fed with a 32 % crude protein commercial feed. Water quality remained stable throughout the study. The growth parameters differed, with the salinities of 2 and 8 PSU presenting higher growth and survival. The treatment of 15 PSU showed high survival, however, with reduced growth. The salinity of 22 PSU had lower results than the others for all parameters analyzed. The hematological evaluation showed differences in RBC, hematocrit, and glucose counts, indicating that salinity may have caused stress to animals cultured at higher salinities, thus, causing low growth and survival. These results indicate that Nile tilapia can tolerate high salinities when cultured in a biofloc system. However, salinities above 15 PSU may compromise its growth and weight gain.

Keywords BFT . *Oreochromis niloticus* . Growth performance . Water quality

Introduction

Aquaculture is an increasingly developed activity with a great potential to meet the worldwide demand for food, reaching 114.5 million tons in 2018, of which 47.4% came from fish production (FAO 2020). New technologies have been studied over the years to make the development of this activity more sustainable. In this context, biofloc technology (Biofloc Technology System – BFT) has been widely used aiming to increase productivity and improve cost-effectiveness due to the reuse of water for several cycles, thus, reducing the effluents generated. In addition, this technology is composed of a microbial community that works to maintain water quality through removing nitrogen compounds, allows high stocking densities, and can be used as an additional feed source for fish and crustaceans reared in it (Avnimelech 2015; Khanjani and Sharifinia 2020).

The Nile tilapia *Oreochromis niloticus* is one of the main fish species in aquaculture due to its rapid growth, rusticity, and salinity tolerance (El-Sayed 2006; Pereira et al. 2016). Rearing tilapia in different

Julianna Paula do Vale Figueiredo . Ana Paula Mariane de Morais . Weverson Ailton da Silva (✉). Ramires Eloíse Queiroz Rafael . Ivanilson de Lima Santos . Felipe do Nascimento Vieira Frank Belettini . Edemar Roberto Andreatta
Laboratório de Camarões Marinhos, Departamento de Aquicultura, Universidade Federal de Santa Catarina (UFSC), Beco dos Coroas, 593, Barra da Lagoa, 88061-600, Florianopolis, Brazil
e-mail: pescailton@gmail.com

Maurício Laterça Martins
Laboratório de Saúde de Organismos Aquáticos AQUOS, Departamento de Aquicultura, Universidade Federal de Santa Catarina (UFSC), Florianópolis, Brazil

salinity is a reality in several countries, and this parameter is a determining factor in its growth (FAO 2016). This species can tolerate a wide range of salinity, supporting up to 36 PSU when gradually acclimated and up to 18 PSU when stocked directly, with the optimum range from 5 to 10 PSU (El-Leithy et al. 2019; de Oliveira et al. 2020). However, salinity levels fluctuations can stress animals and alter their homeostatic state (Baldisseroto 2009). According to Bœuf and Payan (2001), osmoregulation can lead to greater energy consumption in the body fluids regulation in fish, around 10 to 50 % of the energy balance, which can have negative consequences in the oxygen consumption, feeding, and hormonal regulation, therefore, directly interfering in its performance.

Aiming to detect functional alterations in response to different stress conditions, Hesser (1960) proposed to use hematological parameters in the evaluation of fish physiology and has been used as an index to assess the health status of these animals. Fish have a close relationship with the environment in which they live and, thus, their blood may reveal the internal bodily condition even before there is a visible clinical manifestation (Barbieri and Bondioli 2015; Ali et al. 2022). Therefore, hematological parameters have been increasingly recognized as a valuable tool in monitoring fish health, with reliable results (Schütt et al. 1997; Katalay and Parlak 2004).

In this context, this study aimed to evaluate the influence of different salinities in the culture of Nile tilapia in a biofloc system on growth, water quality, and hematological aspects.

Materials and methods

Biological material

The Nile tilapia juveniles (*Oreochromis niloticus*) were provided from the Empresa de Pesquisa Agropecuária e Extensão Rural of Santa Catarina (Epagri) and were maintained in a biofloc system until the beginning of the experiment at the Laboratory of Marine Shrimps (LCM), which is part of the Aquaculture Department of the Federal University of Santa Catarina (UFSC), located in Florianópolis, SC. This work was approved by the Ethics Committee on Animal Use of the UFSC (Protocol 3491250619).

Experimental design

The experiment was carried out in a completely randomized design (DIC) with 4 treatments, and 4 independent replicates, for 56 days. The experimental units consisted of circular tanks of 1,000 L (800 L of useful volume), prepared with 20% of mature biofloc water from a shrimp culture (total ammonia nitrogen 0.58 mg L^{-1}, nitrite-nitrogen 1.75 mg L^{-1}, nitrate-nitrogen 10.7 mg L^{-1}, orthophosphate 1.17 mg L^{-1}, alkalinity 170 mg $CaCO_3$ L^{-1}, and total suspended solids 394 mg L^{-1}). Approximately 140 L of this inoculum was transferred to the experimental units and filled for 5 days with fresh and/or marine water to acclimate the fish to different salinities (control with salinity 2 and the others with salinities of 8, 15, and 22 PSU). The fish density used was 15 fish m^{-3}. Fish were transferred to the experimental units weighing an average of 200 ± 10 g. The aeration system used in the units was made through a central ring with micro-perforated hoses (Aero tubes®) to remain the solids in suspension and dissolved oxygen higher than 5 mg L^{-1}. The tanks had an 800W heater and a thermostat to maintain the temperature at 28.8 ± 0.6 °C. Fish were fed with a 32 % crude protein commercial feed (Guabi 6 mm) 4 times a day (at 8:00 am, 11:00 am, 2:00 pm, and 5:00 pm) according to the Silva and Marchiori (2018) feeding Table.

Water quality

Dissolved oxygen and temperature were measured with an oximeter (YSI Pro20, USA) and salinity with a multiparameter probe (Eco-Sense YSI EC3, USA) twice a day. Analyzes of pH (pH-meter Tecnal®, Brazil), alkalinity (APHA 2005), total ammonia nitrogen (TAN) (Grasshoff et al. 1983), nitrite-nitrogen (NO_2-N) (Strickland and Parsons 1972), and total suspended solids (TSS) (APHA 2005) were performed twice a week. Nitrate-nitrogen (NO_3-N) and orthophosphate (PO_4^{3-}) (APHA 2005) were analyzed at the beginning, middle, and end of the experiment.

When the alkalinity values were lower than 150 mg L^{-1}, there was an addition of hydrated lime following the methodology as described by Furtado et al. (2011). TSS were maintained below 600 mg L^{-1}, and when there was a higher concentration, conical settling tanks were used to remove the excess solids (Schveitzer 2013). There was no need for the addition of a carbon source because the system remained stable. Freshwater was only added to correct the salinity due to the evaporation losses.

Growth performance

The fish growth was estimated biweekly to adjust the feed rates, according to the adopted Table (Silva and Marchiori 2018). At the end of the experiment, all fish in each tank were anesthetized, and weighed individually. Weight gain, feed conversion ratio (FCR), specific growth rate (SGR), productivity, and survival were calculated according to the equations described in Mohammadi et al. (2021):

$$Weight\ gain(g) = final\ weight(g) - initial\ weight(g) \tag{1}$$

$$FCR = \frac{feed\ given(g)}{weight\ gain(g)} \tag{2}$$

$$Survival(\%) = \frac{Final\ number\ of\ fish}{Initial\ number\ of\ fish} x100 \tag{3}$$

$$SGR(\%) = \frac{\mathrm{Ln}(\text{final weight}(\mathrm{g})) - \mathrm{Ln}(\text{initial weight}(\mathrm{g}))}{\text{experiment duration}(\text{days})} x100 \tag{4}$$

$$Productivity(kg\ m^{-3}) = \frac{final\ biomass(kg)}{volume\ of\ the\ tank(m^3)} \tag{5}$$

Hematological analysis

At the end of the experiment, blood samples of 6 fish per experimental unit were collected after anesthesia with Eugenol (75 mg L^{-1}) to evaluate the stress of the different salinities. The blood was withdrawn from the caudal vein using a 3 mL syringe with a drop of 10% EDTA for further analyses. The total number of erythrocytes (RBC) was counted in a Neubauer chamber after dilution (1:200) in Dacie's fluid (sodium citrate), 37-40% formalin, and toluidine blue, according to Blaxhall and Daisley (1973). Blood aliquots were used to make blood smears in duplicates and staining with May-Grunwald-Giemsa-Wright for differential and total count of leukocytes and thrombocytes counts by the indirect method (Ishikawa et al. 2008). An aliquot of blood was used for determining the hematocrit percentage and another fraction for glucose analysis by Accu-Chek® Advantage portable glucometer (Ranzani-Paiva et al. 2013).

Statistical analysis

Normality and homoscedasticity were evaluated by the Shapiro-Wilk and Levene tests, respectively. If both assumptions were met, a one-way Analysis of Variance (ANOVA) was used and, when necessary, followed by the Tukey test to verify the differences among the treatments. Data that did not follow a normal distribution were subjected to the non-parametric Kruskal-Wallis test followed by Dunn's multiple comparison test (Zar 2010). The significance level adopted was 5%. Data have been presented in mean ± standard deviation.

Results

Water quality parameters

The parameters of alkalinity, TAN, nitrite, nitrate, orthophosphate, and TSS remained stable and showed no significant differences among the treatments (Table 1). The salinity remained close to the levels proposed

Table 1 Physical-chemical variables of water during 56 days of *Oreochromis niloticus* culture in a biofloc system at different salinities

Parameters	**Salinity (PSU)**			
	2	8	15	22
Salinity (PSU)	2.5 ± 1.5[a]	7.9 ± 1.3[b]	14.6 ± 1.7[c]	21.6 ± 1.6[d]
PH	8.47 ± 0.7	8.49 ± 0.1	8.42 ± 0.1	8.39 ± 0.1
Alkalinity (mg L^{-1})	183.4 ± 39.0	181.1 ± 25.7	192.9 ± 27.4	186.7 ± 20.0
TAN (mg L^{-1})	0.06 ± 0.1	0.04 ± 0.1	0.06 ± 0.1	0.1 ± 0.2
Nitrite-N (mg L^{-1})	0.17 ± 0.4	0.13 ± 0.1	0.19 ± 0.2	0.24 ± 0.2
Nitrate-N (mg L^{-1})	8.4 ± 0.3	15.5 ± 16.8	10.6 ± 5.9	10.9 ± 5.7
Orthophosphate (mg L^{-1})	3.2 ± 1.8	3.8 ± 2.4	4.1 ± 2.8	3.6 ± 2.3
TSS (mg L^{-1})	555 ± 233	603 ± 224	592 ± 194	618 ± 206

Data presented as mean ± standard deviation (n = 3). Different letters on the same line indicate statistical difference by Tukey's test ($P < 0.05$).
TAN – Total ammonia nitrogen; TSS – Total Suspended Solids.

for this study. Nitrate exhibited the higher oscillation between treatments, ranging from 8.4 ± 0.3 to 15.5 ± 16.8 mg L^{-1}, for salinities 2 and 8 PSU, respectively.

Growth performance

Final weight, total weight gain, SGR, survival, and productivity were significantly higher in the 2 and 8 PSU treatments (Table 2). The animals reared at 22 PSU showed lower ($P > 0.05$) survival and final weight and, consequently, lower productivity; these parameters contributed for a higher FCR (5.7 ± 1.7) at this salinity.

Hematological analysis

The hematological analysis showed higher values of RBC and hematocrit for the treatments with 2 and 8 PSU. Glucose was higher at the highest salinity of 22 PSU. Total and differential count of leukocytes did not differ among the treatments (Table 3).

Discussion

The water quality parameters of all treatments did not differ during the experiment, possibly due to the biofloc inoculum used from a mature tank. Nitrogen compounds remained within the recommended levels for the species (El-Sayed 2006). This is due to the bacterial community present in the water, which controls these compounds and allows greater stability of the system (Avnimelech 2015). This can show the stability in the use of biofloc system even at different salinities (Kishida et al. 2008; Silva et al. 2022).

Studies show that salinity is a determining factor in controlling the growth and survival of fish, directly interfering with the performance of cultured species (Bœuf and Payan 2001). Freshwater fish, when subjected to saline environments, need to use physiological mechanisms to maintain homeostasis. Thus, the energy of the feed offered, which would be destined for growth, is also used for osmoregulation, causing a reduction in its weight gain (Kubitza 2007). According to Al-Amoudi (1987) and Schofield et al. (2011), the Nile tilapia shows a great performance in a salinity range from 0 to 18 PSU without compromising its survival. However, when reared in seawater (> 30 PSU), its growth is reduced by 60% (Cnaani and Hulata 2011). However, the present study showed that the salinity of 15 PSU, despite the survival above 90%, had compromised weight gain, and the salinity of 22 PSU interfered in the growth and survival of the animals.

Research by Lawson and Anetekhai (2011) showed similar results for the Nile tilapia reared at salinities of 0 and 7 PSU, with 100% survival. The authors also showed that at these salinities the fish were able to

Table 2 Growth performance of *Oreochromis niloticus* during 56 days of culture in a biofloc system with different salinities

Parameters	**Salinity (PSU)**			
	2	8	15	22
Initial weight (g)	197.6 ± 2.0	199.5 ± 4.22	197.7 ± 3.0	199.4 ± 4.1
Final weight (g)	439.8 ± 15.5 ^{a}	416.6 ± 17.4 ^{a}	347.4 ± 20.9 ^{b}	321.8 ± 12.7 ^{c}
Weight gain (g)	242.2 ± 16.5 ^{a}	217.1 ± 16.3 ^{a}	149.7 ± 22.9 ^{b}	122.5 ± 16.6 ^{c}
FCR	2.4 ± 0.2^{a}	2.6 ± 0.2^{a}	3.9 ± 0.5^{b}	5.7 ± 1.7^{c}
Survival (%)	100 ± 0^{a}	100 ± 0^{a}	93.7 ± 7.9^{a}	66.67 ± 14.4^{b}
SGR (% day^{1})	4.3 ± 0.3 ^{a}	3.9 ± 0.3 ^{a}	2.7 ± 0.4 ^{b}	2.2 ± 0.3 ^{b}
Productivity (kg m^{-3})	3.6 ± 0.2 ^{a}	3.3 ± 0.2 ^{a}	1.9 ± 0.2 ^{b}	0.2 ± 0.8 ^{c}

Data presented as mean ± standard deviation (n = 3). Different letters on the same line indicate statistical difference by Tukey's test ($P < 0.05$).
SGR – Specific growth rate; FCR – Feed conversion ratio.

Table 3 Hematological parameters of *Oreochromis niloticus* during 56 days of culture in a biofloc system at different salinities

Parameters	Salinity (PSU)			
	02	08	15	22
RBC (10^{6} L^{-1})	2.47 ± 9.84^{a}	2.54 ± 1.50^{a}	1.90 ± 2.34^{b}	1.83 ± 4.32^{b}
Hematocrit (%)	36.38 ± 2.03^{a}	35.54 ± 2.01^{a}	28.65 ± 4.31^{b}	25.58 ± 1.73^{b}
Glucose (g dL^{-1})	43.88 ± 4.59^{a}	41.92 ± 9.75^{a}	64.46 ± 15.88^{a}	77.72 ± 20.39^{b}
Total leukocytes (×10^{4} μL^{-1})	8.87 ± 2.24^{a}	8.86 ± 2.90^{a}	9.42 ± 3.84^{a}	9.38 ± 2.50^{a}
Lymphocytes (10^{4} μL^{-1})	72.77 ± 4.76^{a}	71.94 ± 3.43^{a}	67.35 ± 10.03^{a}	71.50 ± 6.32^{a}
Neutrophils (10^{4} μL^{-1})	4.54 ± 2.25^{a}	6.56 ± 1.87^{a}	10.07 ± 4.95^{a}	4.13 ± 4.17^{a}
Monocytes (10^{4} μL^{-1})	11.47 ± 7.05^{a}	10.10 ± 4.48^{a}	16.85 ± 7.65^{a}	18.25 ± 8.41^{a}
Thrombocytes (10^{4} μL^{-1})	11.22 ± 2.24^{a}	11.39 ± 2.90^{a}	5.73 ± 3.84^{a}	6.12 ± 2.50^{a}

Data presented as mean ± standard deviation (n = 3). Different letters on the same line indicate statistical difference by Tukey's test ($P < 0.05$).
RBC – Total number of erythrocytes.
TAN – Total ammonia nitrogen; TSS – Total Suspended Solids.

regulate their body physiology. However, at salinities above 9 PSU, mortality was 100%. The size of the animals possibly was responsible for the mortality since they weighed 15 g at the beginning of the experiment. Different results were found by Souza et al. (2019), in which the growth and survival were equal for salinities of 0 and 16 PSU. However, the initial weight was 93.8 g. There are some differences among the different studies, possibly due to the experimental conditions, the strain used, the age, and the size of the animals because some authors have reported that salinities above 20 PSU are harmful to Nile tilapia (Suresh and Lin 1992; Kamal and Mair 2005). Whereas recent studies showed positive results at salinities up to 25 PSU (Pereira et al. 2016), or even higher salinities when gradually acclimated.

The hematological analysis allows understanding of the influence of nutrition and environmental conditions on fish health, which are crucial parameters to prevent pathogens arising from stresses due to environmental changes. Leukocytes play an important role in innate immunity during inflammation (high number of these cells indicates a healthy status in fish), since they are responsible for the immune response against pathogens (Laice et al. 2021). In the present study, the leukocyte count showed no differences among the treatments and indicated no infections and/or tissue injury (Silva et al. 2012), and that tilapia reared at a high salinity (i.e., 22 PSU) were also healthy.

However, the other results in this study showed a significant effect on different levels of salinity in some parameters analyzed. The RBC (red blood cell) counts at the salinity levels of 2 and 8 PSU were similar to studies carried out with Nile tilapia at low salinities by Azevedo et al. (2015). The authors found that the results showed a decrease in the counting with the increase of salinity. This reduction may be related to cell fragility resulting from the osmolarity process in which the cell loses water to the hypertonic environment and, on the other hand, gains an excessive salts amount (Emelike et al. 2008).

The percentage of hematocrit showed a significant effect on the different levels of salinity. The authors reported that the results indicated a decrease in the concentration in the treatments of 15 and 22 PSU, which are the highest salinities, whereas, in the treatments of 2 and 8 PSU, the values were higher. Elarabany et al. (2017) showed similar results, which leads us to relate the reduction in hematocrit to the stress caused by salinity and, consequently, to osmotic changes. Plasma glucose is also important in animal welfare, as it is considered a good stress indicator, its assessment is quick, and any alterations are easily detected (Simões and Gomes 2009). Glucose has been used to diagnose physiological stress in fish when found above 60 mg dL^{-1} (Oba et al. 2009). In the present study, the animals reared at higher salinity showed a significant difference and the glucose concentration was higher than the results reported by Oba et al. (2009). Glucose is the main source of energy to the central nervous system, so it is possible that a high glucose level is being required to supply the salt stress and carry out osmoregulation. Elarabany et al. (2017) observed a similar behavior, with increasing glucose as salinity increased.

Conclusion

In this study, it was possible to conclude that Nile tilapia reared in a biofloc system can tolerate salinities of up to 15 PSU, however, with reduced growth. The lower salinities (up to 8 PSU) were more suitable for the culture efficiency. The hematological analyses of the animals reared at the salinity of 22 PSU showed changes in some parameters, reflecting lower survival rate and weight gain. In addition, the biofloc system used maintained water quality stable without the need for water renewal.

Funding The study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior Brazil.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed with the animals were approved by the Ethics Committee on the Use of Animals of the Federal University of Santa Catarina - CEUA UFSC, protocol n° 3491250619.

Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions JPVF: Investigation, Formal analysis, Data curation, Writing - Original Draft. APMM: Investigation, Formal analysis, Data curation, Writing - Review and Editing. WAS: Investigation, Formal analysis, Data curation, Writing - Review and Editing. REQR: Data curation, Writing - Review and Editing. ILS: Data curation, Writing - Review and Editing. MLM: Data Curation, Writing - Review and Editing. FNV: Supervision, Project administration, Writing - Review and Editing. FB: Data Curation, Writing - Review and Editing. ERA: Supervision, Writing - Review and Editing.

References

Al-amoudi MM (1987) The effects of high salt diet on the direct transfer of the *Oreochromis mossambicus*, *O. spilurus*, *O. niloticus* hybrids to sea water. Aquaculture 64:333-338. https://doi.org/10.1016/0044-8486(87)90195-5

Ali A, Azom MG, Sarker BS, Rani H, Alam MS, Islam MS (2022) Repercussion of salinity on hematological parameters and tissue morphology of gill and kidney at early life of tilapia. Aquac Fish, in press. https://doi.org/10.1016/j.aaf.2022.04.006

APHA – American public health association (2005) Water works association, water environment federation. Standard methods for the examination of water and wastewater, twenty-first ed. APHA, Washington

Avnimelech Y (2015) Biofloc technology - A practical guidebook, 3th Ed. The world aquaculture society, Baton Rouge

Azevedo RV, de Oliveira KF, Flores-Lopes F, Teixeira-Lanna EA, Takishita SS, Tavares-Braga LG (2015) Responses of Nile tilapia to different levels of water salinity. Lat Am J Aquat Res 43:828-835. https://doi.org/10.3856/vol43-issue5-fulltext-3

Baldisserotto B (2009) Freshwater fish culture in Rio Grande do Sul State: actual situation, problems and future perspectives. Cienc Rural 39:291-299. https://doi.org/10.1590/S0103-84782008005000046

Barbieri E, Bondioli ACV (2015) Acute toxicity of ammonia in Pacu fish (*Piaractus mesopotamicus*, Holmberg, 1887) at different temperatures levels. Aquac Res 46:565-571. https://doi.org/10.1111/are.12203

Blaxhall PC, Daisley KW (1973) Routine haematological methods for use with fish blood. J Fish Biol 5:771-781. https://doi.org/10.1111/j.1095-8649.1973.tb04510.x

Bœuf G, Payan P (2001) How should salinity influence fish growth? Comp Biochem Physiol Part C: Toxicol Pharmacol 130:411-423. https://doi.org/10.1016/S1532-0456(01)00268-X

Cnaani A, Hulata G (2011) Improving salinity tolerance in tilapias: past experience and future prospects. Isr J Aquac 63:1-21. https://doi.org/10.46989/001c.20590

de Oliveira CYB, e Abreu JL, de Oliveira CDL, Lima PC, Gálvez AO, de Macedo Dantas DM (2020) Growth of *Chlorella vulgaris* using wastewater from Nile tilapia (*Oreochromis niloticus*) farming in a low-salinity biofloc system. Acta Sci Technol 42(1):e46232. https://doi.org/10.4025/actascitechnol.v42i1.46232

Elarabany N, Bahnasawy M, Edrees G, Alkazagli R (2017) Effects of salinity on some haematological and biochemical parameters in Nile tilapia, *Oreochromus niloticus*. Agri Forest Fish 6:200-205. https://doi.org/10.11648/j.aff.20170606.13

El-Leithy AA, Hemeda SA, Abd El Naby WS, El Nahas AF, Hassan SA, Awad ST, El-Deeb SI, Helmy ZA (2019) Optimum salinity for Nile tilapia (*Oreochromis niloticus*) growth and mRNA transcripts of ion-regulation, inflammatory, stress-and immune-related genes. Fish Physiol Biochem 45(4):1217-1232. https://doi.org/10.1007/s10695-019-00640-7

El-Sayed AFM (2006) Tilapia culture in salt water: environmental requirements, nutritional implications and economic potentials. In: Suárez EC, Marie DR, Salazar MT, López MGN, Cavazos DAV, Cruz ACP, Ortega AG (ed) Avances en Nutricion Acuicola. 8th edn, Universidad Autónoma de Nuevo León, Monterrey

Emelike FO, Odeyenuma C, Jeremiah ZA, Obigwe BU (2008) The use of anti-coagulated and defibrinated blood samples for the evaluation of red cell osmotic fragility. Int J Nat Appl Sci 4:204-208. https://doi.org/10.4314/ijonas.v4i2.36271

FAO (2016) The state of world fisheries and aquaculture 2016. Contributing to food security and nutrition for all, 200

FAO (2020) State of world fisheries and aquaculture. FAO, Rome

Furtado PS, Poersch LH, Wasielesky Jr W (2011) Effect of calcium hydroxide, carbonate and sodium bicarbonate on water quality and zootechnical performance of shrimp *Litopenaeus vannamei* reared in bio-flocs technology (BFT) systems. Aquaculture 321:130-135. https://doi.org/10.1016/j.aquaculture.2011.08.034

Grasshoff K, Ehrhardt M, Kremling K (1983) Methods of seawater analysis, Second ed. Verlag Chemie Weinhein, New York, pp.61-72. https://doi.org/10.1002/9783527613984

Hesser EF (1960) Methods for routine fish hematology. N Am J Aquac 22:164-171. https://doi.org/10.1577/1548-8659

Ishikawa NM, Ranzani-Paiva MJT, Lombardi JV (2008) Methodology for quantification of total leukocytes in Oreochromis Niloticus fish. Arch Vet Sci 13(1):54-63. [Text in Portuguese]

Kamal AHMM, Mair GC (2005) Salinity tolerance in superior genotypes of tilapia, *Oreochromis niloticus*, *Oreochromis mossambicus* and their hybrids. Aquaculture 247:189-201. https://doi.org/10.1016/j.aquacul ture.2005.02.008

Katalay S, Parlak H (2004) Su kirliliğinin, *Gobius niger* Linn., 1758 (Pisces: Gobiidae) in kan parametreleri üzerine etkileri. EU J Fish Aquat Sci 19(1-2):115-121

Khanjani MH, Sharifinia M (2020) Biofloc technology as a promising tool to improve aquaculture production. Rev Aquac 12(3):1836-1850. https://doi.org/10.1111/raq.12412

Kishida N, Tsuneda S, Sakakibara Y, Kim JH, Sudo R (2008) Real-time control strategy for simultaneous nitrogen and phosphorus removal using aerobic granular sludge. Water Sci Technol 58:445-450. https://doi.org/10.2166/wst.2008.410

Kubitza FO (2007) The versality of salt in fish production. Paranorama da Aquicultura 17:14-23. [Text in Portuguese]

Laice LM, Corrêa Filho RAC, Ventura AS, Farias KNN, do Nascimento Silva AL, Fernandes CE, Silva ACF, Barbosa PTL, Souza AI, Emerenciano MGC, Povh JA (2021) Use of symbiotics in biofloc (BFT)-based Nile tilapia culture: Production performance, intestinal morphometry and hematological parameters. Aquaculture 530:735715. https://doi.org/10.1016/j.aquaculture.2020.735715

Lawson EO, Anetekhai MA (2011) Salinity tolerance and preference of hatchery reared Nile tilapia, *Oreochromis niloticus* (Linneaus 1758). Asian J Agric Sci 3:104-110

Mohammadi G, Rafiee G, Tavabe KR, Abdel-Latif HM, Dawood MA (2021) The enrichment of diet with beneficial bacteria (single-or multi-strain) in biofloc system enhanced the water quality, growth performance, immune responses, and disease resistance of Nile tilapia (*Oreochromis niloticus*). Aquaculture 539:736640. https://doi.org/10.1016/j.aquaculture.2021.736640

Oba ET, Mariano WDS, Santos LD (2009) Stress in farmed fish: aggravating and mitigating factors for profitable management. In: Management and health of farmed fish. Embrapa, Macapá. [Text in Portuguese]

Pereira DSP, Guerra-Santos B, Moreira ELT, Albinati RCB, Ayres MCC (2016) Hematological and histological parameters of Nile tilapia in response to the challenge of different salinity levels. Bol Inst Pesca 42:635-647. https://doi.org/10.20950/1678-2305.2016v42n3p635 [Text in Portuguese]

Ranzani-Paiva MJT, de Pádua SB, Tavares-Dias M, Egami MI (2013) Methods for hematological analysis in fish. Editora da Universidade Estadual de Maringá-EDUEM

Schofield PJ, Peterson MS, Lowe MR, Brown-Peterson NJ, Slack WT (2011) Survival, growth and reproduction of non-indigenous Nile tilapia, *Oreochromis niloticus* (Linnaeus 1758). I. Physiological capabilities in various temperatures and salinities. Mar Freshw Res 62:439-449. https://doi.org/10.1071/MF10207

Schütt DA, Lehmann J, Goerlich R, Hamers R (1997) Haematology of swordtail, *Xiphophorus helleri*. I: blood parameters and light microscopy of blood cells. J Appl Ichthyol 13:83-89. https://doi.org/10.1111/j.1439-0426.1997.tb00106.x

Schveitzer R, Arantes R, Costódio PFS, do Espírito Santo CM, Arana LV, Seiffert WQ, Andreatta ER (2013) Effect of different biofloc levels on microbial activity, water quality and performance of *Litopenaeus vannamei* in a tank system operated with no water exchange. Aquac Eng 56:59-70. https://doi.org/10.1016/j.aquaeng.2013.04.006

Silva ASED, Lima TAX, Blanco BS (2012) Fish hematology. Revista Centauro 3:24-32. [Text in Portuguese]

Silva BC, Marchiori NC (2018) The importance of feed management in Tilapia farming. Florianópolis: Epagri. 16p. [Text in Portuguese]

Silva WAD, Silva JLD, Oliveira CYB, De Moraes APM, Shinozaki-Mendes RA, Silva, UL (2022) Effect of stocking density on water quality, plankton community structure, and growth performance of *Litopenaeus vannamei* (Boone, 1931) post-larvae cultured in low-salinity biofloc system. Int Aquat Res, in press. https://doi.org/10.22034/IAR.2022.1936674.1176

Simões LN, Gomes LC (2009) Efficacy of menthol as an anesthetic for juvenile Nile tilapia (*Oreochromis niloticus*). Arq Bras Med Vet 61:613-620. https://doi.org/10.1590/S0102-0935200900030001 [Text in Portuguese]

Souza RLD, Lima ECRD, Melo FPD, Ferreira MGP, Correia EDS (2019) The culture of Nile tilapia at different salinities using a

biofloc system. Rev Cienc Agronom 50:267-275. https://doi.org/10.5935/1806-6690.20190031
Strickland JDH, Parsons TR (1972) A practical handbook of seawater analysis. Fisheries research board of Canada, Ottawa
Suresh AV, Lin CK (1992) Tilapia culture in saline waters: a review. Aquaculture 106(3/4):201-226. https://doi.org/10.1016/0044-8486(92)90253-H
Zar JH (2010) Biostatistical Analysis, 5th ed. Prentice Hall, New Jersey

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