

Influence of the rearing system on phagocytic indexes and weight gain of the silver catfish (*Rhamdia quelen*, Quoy & Gaimard, 1824) Influencia del sistema de cultivo en los índices fagocíticos y ganancia de peso del bagre (*Rhamdia quelen*, Quoy & Gaimard, 1824)

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ABSTRACT | Fish consumption has been growing since 1961. Indeed, the fisheries sector is essential to prevent hunger and malnutrition. However, this sector has its challenges. Fish stocks are being depleted beyond biological sustainability, with diseases and biosecurity risks to production and harm to the environment and ecosystems. There is a clear need to improve fish farming with minimum occupation and environmental impact. Heterotrophic and aquaponics systems are being utilized and modified to address these needs. However, environmental factors can affect the innate immunity of fish raised in these systems. Understanding how these systems affect fish's immunity is critical to prevent potential hazards and the breakdown of these production methods. The goal of this research was to evaluate the performance, growth, and phagocytic indexes of silver catfish (*Rhamdia quelen*) juveniles reared in water recirculation, biofloc, and aquaponics systems in consortium with watercress seedlings (*Nasturtium officinale*). In each system, 12 juveniles weighing $15.2 \text{ g} \pm 7.6$ were randomly divided into three replicates, fed with commercial feed. The following physical-chemical parameters - temperature, total and toxic ammonia, nitrite, and water pH - were daily assessed. Fishes were weighed monthly for six months. It was observed that *R. quelen* juveniles reared in the aquaponic system achieved higher average weight, but no statistical significance was detected ($p > 0.05$) when compared to those reared in the recirculated and biofloc systems. At the end of the experiment, the phagocytic index was lower only in the recirculating system. The phagocytic capacity was lower in all systems. Although the aquaponic system presented low average weight gain/animal the performance of this system was effective in reducing the concentrations of total ammonia and nitrite in the water, in consortium with the production of vegetables.

Palabras clave

Índices fagocíticos
Acuaponia
Biofloc
Berro
Rhamdia quelen

RESUMEN | El consumo de pescado ha estado creciendo desde 1961. De hecho, el sector pesquero es esencial para prevenir el hambre y la desnutrición. Sin embargo, este sector tiene sus desafíos. Las poblaciones de peces se están agotando más allá de la sostenibilidad biológica, con enfermedades y riesgos de bioseguridad para la producción y daños al medio ambiente y los ecosistemas. Existe una clara necesidad de mejorar la piscicultura con la mínima ocupación e impacto ambiental. Los sistemas biofloc y acuapónicos se están utilizando y modificando para abordar estas necesidades. Sin embargo, los factores ambientales pueden afectar la inmunidad innata de los peces criados en estos sistemas. Comprender cómo estos sistemas afectan la inmunidad de los peces es fundamental para prevenir posibles peligros y fallas en estos métodos de producción. El objetivo de esta investigación fue evaluar el rendimiento, crecimiento e índices fagocíticos de juveniles de bagre (*Rhamdia quelen*) criados en sistemas de recirculación de agua, biofloc y acuaponía en consorcio con plántulas de berro (*Nasturtium officinale*). En cada sistema, 12 juveniles con un peso de $15,2 \text{ g} \pm 7,6$ se dividieron aleatoriamente en tres repeticiones, alimentados con alimento comercial. Diariamente se evaluaron los siguientes parámetros físico-químicos: temperatura, amoníaco total y tóxico, nitrito y pH del agua. Los peces se pesaron mensualmente durante seis meses. Se observó que los juveniles de *R. quelen* criados en el sistema acuapónico alcanzaron un mayor peso promedio, pero no se detectó significancia estadística ($p > 0.05$) en comparación con los criados en los sistemas de recirculación y biofloc. Al final del experimento, el índice fagocítico fue menor solo en el sistema de recirculación. La capacidad fagocítica fue menor en todos los sistemas. Aunque el sistema acuapónico presentó una baja ganancia de peso promedio/animal, el desempeño de este sistema fue efectivo en la reducción de las concentraciones de amoníaco total y nitrito en el agua, en consorcio con la producción de hortalizas.

INTRODUCTION

To meet the growing demand for fish (Naylor *et al.*, 2000), its farming increased by more than 527% in volume since 1990, and it is estimated that from 2018 until 2030 aquaculture production will increase by 32% (reaching 109 million tons). For that to happen, sustainable aquaculture is a key factor (FAO, 2020). The total area needed for inland aquaculture is also another restraint, and the search for consociation with other crop techniques is very welcome (Pasch *et al.*, 2021).

The increase in fish production combined with periodic management, sudden changes in temperature, poor nutrition, and poor water quality contribute to stress and immunosuppression in fish. Immunosuppression increases the risk of infection leaving aquaculture vulnerable to disease outbreaks and production failure (Reverter *et al.*, 2014). The systems used for small and medium-scale breeding are aquaponics and the heterotrophic system, in addition to the recirculated system. Heterotrophic systems, or biofloc, and aquaponics are two farming systems that aim to reduce the environmental impact of aquaculture. (Mansour and Esteban, 2017; FAO, 2006; Santos, 2016; Blidariu and Grozea, 2011; Graber and Junge, 2009).

A heterotrophic system was developed in the United States in the 1990s (Browdy *et al.*, 2001) which consists of a system with high stocking densities without water renewal, maximizing biosecurity and minimizing the environmental impacts caused by aquaculture (Avnimelech, 2011). In the heterotrophic system, there is an improvement in water quality, a decrease in the introduction of pathogens, stimulation of the immune system of the animals under culture, and an increase in their resistance to diseases (Mansour and Esteban, 2017). According to FAO (2006), this technique reduces pumping costs and the possibility of introducing toxic compounds, pathogens, disease vectors, or other undesirable organisms, in addition to reducing the disposal of nutrients and organic matter. In systems without water renewal, there is an accumulation of ammonia and organic matter and a consequent decrease in dissolved oxygen, requiring constant artificial aeration and stimulating the formation of a predominantly aerobic and heterotrophic biota, with fertilization with sources rich in organic carbon (Schryver *et al.*, 2008).

Aquaponics integrates aquaculture with hydroponics, using equipment and methods from both farming systems (Santos, 2016). Interest in aquaponics has been growing not only for its sustainability, but also for its potential to save water, allow food supply throughout the year, and its potential to be implemented near or in urban centers (Santos, 2016). Aquaponics respects the principle of sustainable agriculture, by using plants to filter aquaculture wastewater, with ammonia nitrogen removal efficiency of 86 to 98%, and increases the possibility of economic efficiency with the additional production of vegetables (Blidariu and Grozea, 2011; Graber and Junge, 2009), as well a more rational optimization of the space.

The silver catfish (*Rhamdia quelen*) is an omnivorous fish of the Pimelodidae family with a neotropical distribution, found from southern Mexico to central Argentina (Gomes *et al.*, 2000). It inhabits lakes and deep river ponds with clear waters with a sandy or mud bottom (Gomes *et al.*, 2000), with temperatures between 15 and 34 °C (Chippari-Gomes *et al.*, 2000). In adulthood, silver catfish can reach a maximum total length of 47.4 cm (Zaniboni-Filho *et al.*, 2004) and a weight of 4.0 kg (IGFA, 2001). In this way, the species has excellent potential due to its adaptability to cultivation, rusticity, and adaptation to cold temperatures (Baldisserotto and Radünz Neto, 2004).

This work aimed to evaluate the phagocytic activity of peritoneal macrophages and the weight gain of juvenile *Rhamdia quelen* reared in recirculated, aquaponics, and heterotrophic systems after six months of cultivation.

MATERIAL AND METHODS

Organisms and rearing conditions

Thirty-six juveniles of *R. quelen*, weighing 15.2 g±7.6, were acquired from Piscicultura Dinamarca LTDA, São Paulo/SP, kept in the vivarium for aquatic animals, and fed once a day (3% of live weight) with

feed for omnivorous fish (Ração Peixe Juvenil 360-AM, AMICIL S/A, Brazil) with 36% crude protein and a minimum of 4600 kcal/kg. They were acclimated in a 500 L tank, before transferring to the rearing tanks.

The rearing systems – recirculated, aquaponics and biofloc - comprised three tanks each, which received randomly four juvenile fish. All rearing systems were oxygenated by aerators with porous stones.

All procedures were approved by the Ethics Committee on the Use of Animals of the Institute of Biomedical Sciences, University of São Paulo, Brazil, # 8118271118.

Rearing systems

The recirculated system (Fig. 1) consisted of three tanks with outlets linked to a filter for mechanical (expanded clay and acrylic blanket), chemical (activated carbon), and biological filtration. The water in each tank was completely filtered every 1 hour.

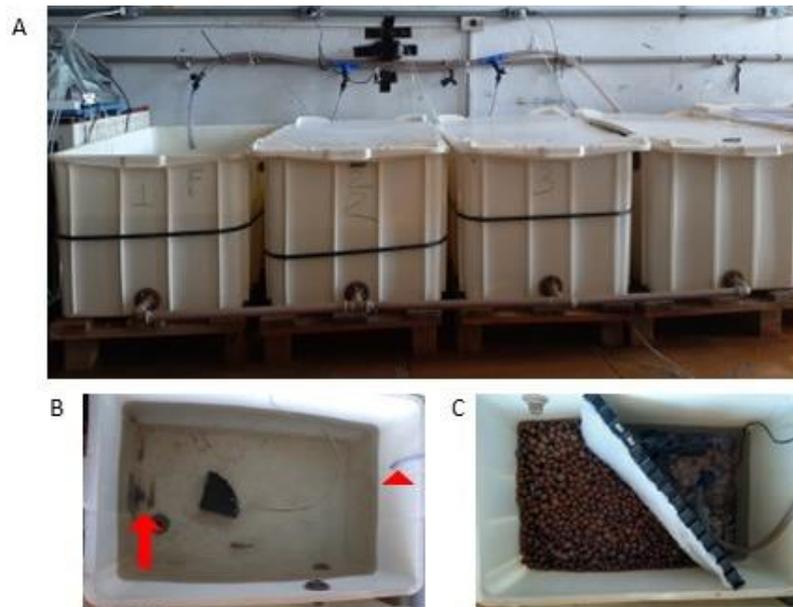


Figure 1. Recirculated system. A. The three tanks of the recirculated system with their identifications 1, 2, and 3, the box without identification is the filter. B. Detail of one of the tanks showing the water outlet (arrow) and water inlet (arrowhead). C. filter detail

In the aquaponics system (Fig. 2) a 600 L/h pump maintained a constant water flow between the fish tank and the watercress (*Nasturtium officinale*) cultivation boxes. The watercress cultivation boxes had a bell-type siphon through which the water returned to the fish cultivation pond. Each box was filled with expanded clay, acting as a support for watercress seedlings and a place for the colonization of nitrifying bacteria. The watercress was purchased from CEAGESP (*Companhia de Entrepósitos e Armazéns Gerais de São Paulo*) and placed in the culture boxes on the same day that the fish were distributed in the systems.

For the heterotrophic system (Fig. 3), probiotic bacteria (*Bacillus cereus*, *B. subtilis*, *Bifidobacterium bifidum*, *Enterococcus faecium*, and *Lactobacillus acidophilus*) and prebiotics (methionine, lysine, vitamins C and E, choline, mannan oligosaccharide, and dextrose) (DB Aqua, Imeve, Brazil) were added after distributing the fish in the tanks, following the manufacturers' specifications, for biofloc formation. Daily, molasses (3% of the feed provided) was added to the water as a carbon source for biofloc maintenance.

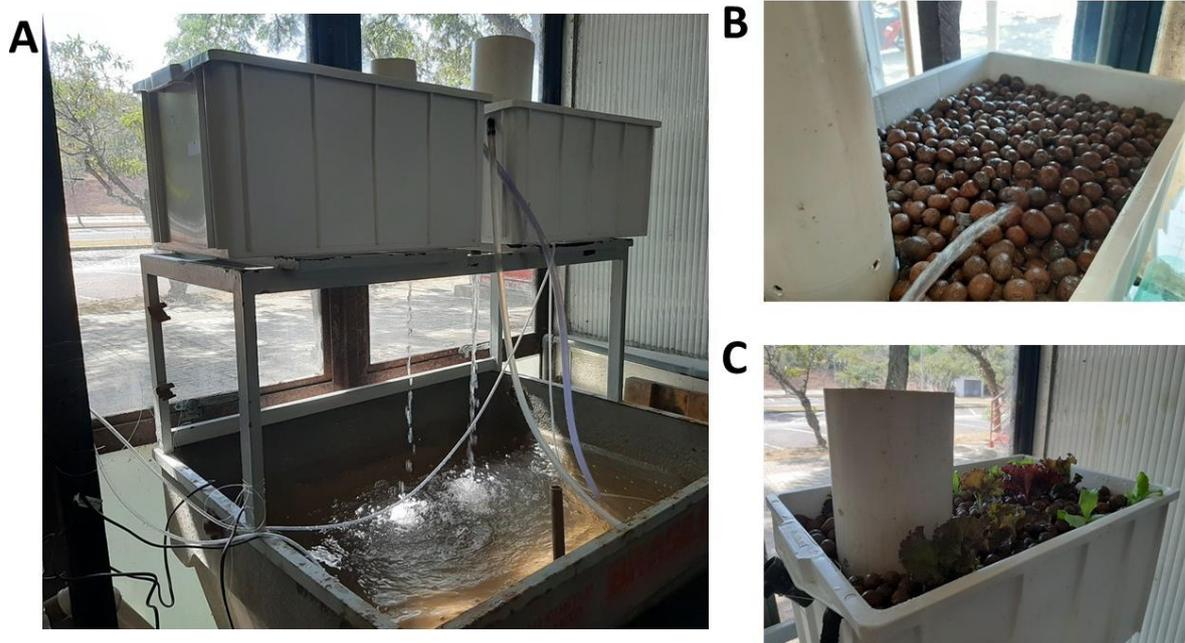


Figure 2. Aquaponics tank. A. Aquaponics tank with suspended cultivation box. B. Detail of the cultivation box ready to receive the water. C. Detail of the cultivation box with the water.

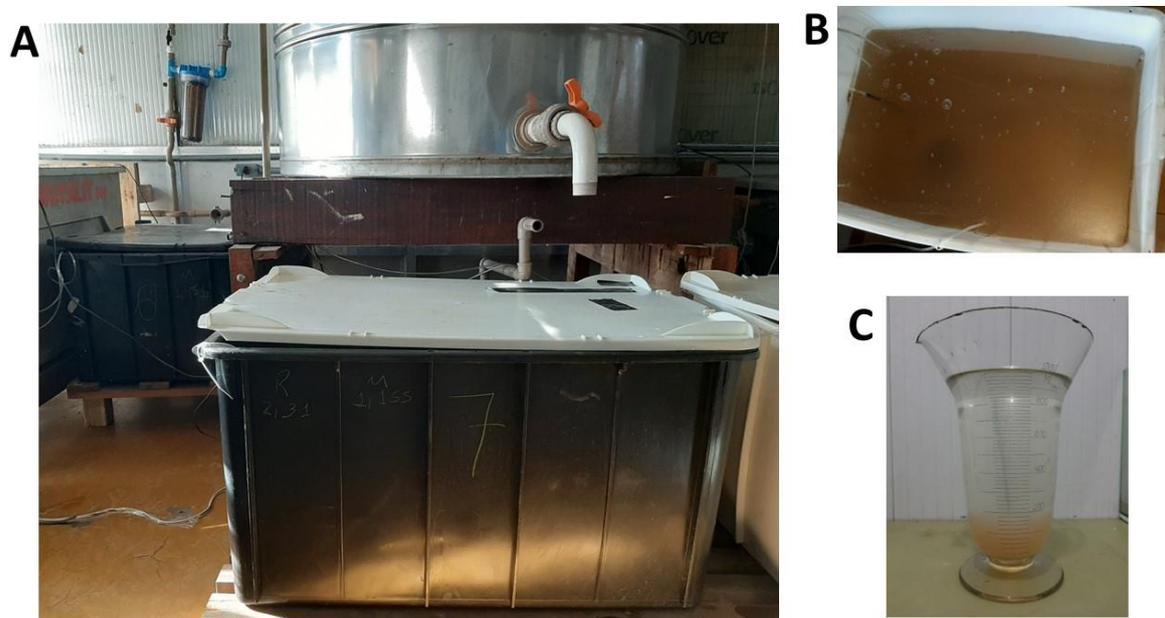


Figure 3. Heterotrophic system. A. two of the tanks of the heterotrophic system. B. Detail of one of the open heterotrophic tanks. C. Decanted Biofloc.

The removal of organic material was carried out by siphoning the bottom of the recirculated and aquaponic tanks on alternate days or when necessary. The water loss due to natural evaporation was replenished to keep its initial levels.

Water quality

To evaluate the water quality, the following physical-chemical parameters were measured: nitrite, total and toxic ammonia, dissolved oxygen, and pH (commercial kits, Alcon Pet, Brazil), temperature (with the aid of aquarium thermometers).

During acclimatization and stabilization of the systems, the water quality was daily checked. Whenever any of the parameters were close to toxic levels, partial water changes (1/3) were performed. After the stabilization of the systems, the water quality was weekly checked.

In order to keep the well-being of fish, physical-chemical parameters levels were maintained according to previous studies, as follows: water temperature of 23-24 °C (Piedras *et al.*, 2004), dissolved oxygen above 5.2 mg.L⁻¹ (Braun *et al.*, 2006), pH between 5.5 and 9.0 (Copatti *et al.*, 2011), total ammonia up to 2 mg.L⁻¹ (Miron *et al.*, 2008) and nitrite up to 1.1 mg.L⁻¹ (Lima *et al.*, 2011).

Weighing and collecting material

Each fish experimentally manipulated was previously anesthetized with a benzocaine solution (Benzocaine, Sigma-Aldrich, USA) at 40 mg.L⁻¹ (Cunha da Silva *et al.*, 2005) and weighed.

Based on weight values, the average daily gain/crop (Σ current month weight - Σ previous month weight/30) and average daily/animal gain (current month weight - previous month weight/30) were calculated.

For peritoneal macrophages collection, 1.5 ml of filtered Phosphate Buffered Saline (PBS) and 1.5 ml of air were injected into the peritoneal cavity using a 3 ml syringe attached to a 19x0.7 mm (24G) catheter (the catheter was not removed). The fish belly was massaged for 2 minutes and as much as possible PBS was removed (Afonso *et al.*, 1997) for phagocytosis assays.

Phagocytosis and phagocytic indices

Aliquots (200 μ L) of cell suspension collected from the peritoneal cavity were placed on round glass coverslips in a 24-well culture plate. After 2 h in a humid chamber, the wells were washed with PBS buffer to remove non-adhered cells, then a suspension of *Saccharomyces cerevisiae* yeasts in a culture medium at a concentration of 10 yeasts per macrophage was added.

After 2 h in a humid chamber at room temperature, the culture medium was discarded, the wells were washed with PBS buffer and the coverslips were fixed with methanol and stained with Rosenfeld (1947). Phagocytosis was quantified under light microscopy in a bright field, with a 40X objective (Standard 25 ICS-Carl Zeiss ® microscope) and 100 cells were counted. The phagocytosis indices were calculated using the following formulas:

Phagocytic Capacity: CF = No. of macrophages phagocytosing/No. of total macrophages

Phagocytic Index: IF = Total number of phagocytosed yeasts/Number of macrophages phagocytosing

Statistics

All data were analyzed using the GraphPad Prism 6.0 statistical package (GraphPad, USA) with parametric tests (t-test, ANOVA and Tukey's test) and non-parametric tests (Kruskal-Wallis) with a significance level of 95% (P<0.05).

RESULTS

Water quality

The physical-chemical parameters of water are described in Figure 4. It was observed that the highest values of total and toxic ammonia and dissolved nitrite were found in the recirculated system (P<0.0001). The pH, oxygen, and temperature values remained within the recommended values, with no difference among the rearing systems.

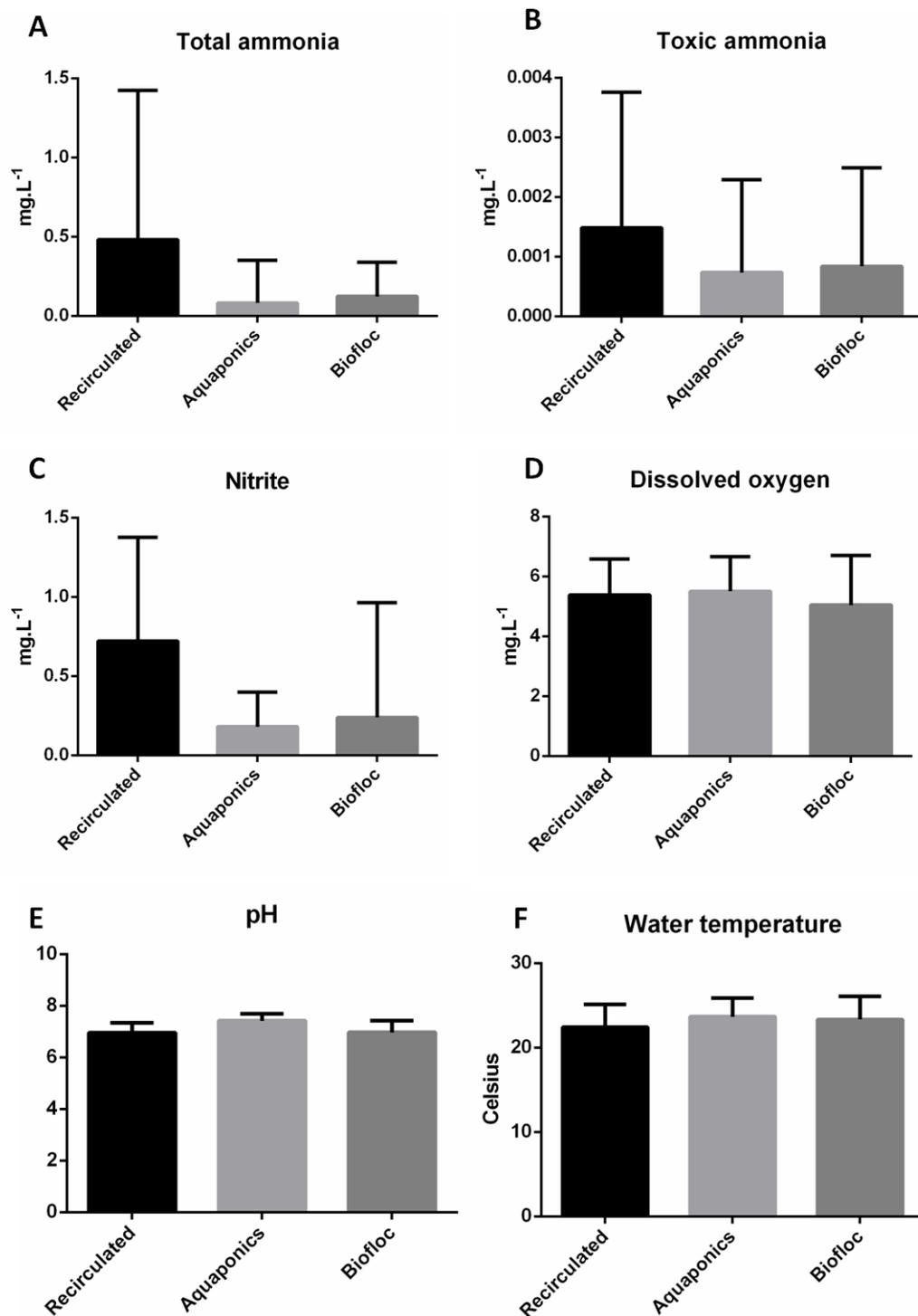


Figure 4. Water parameters of each rearing system for *R. quelen*. A, total ammonia; B, toxic ammonia; C, nitrite; D, dissolved oxygen; E, pH; F, temperature. The bars stand for standard deviation

Weight gain

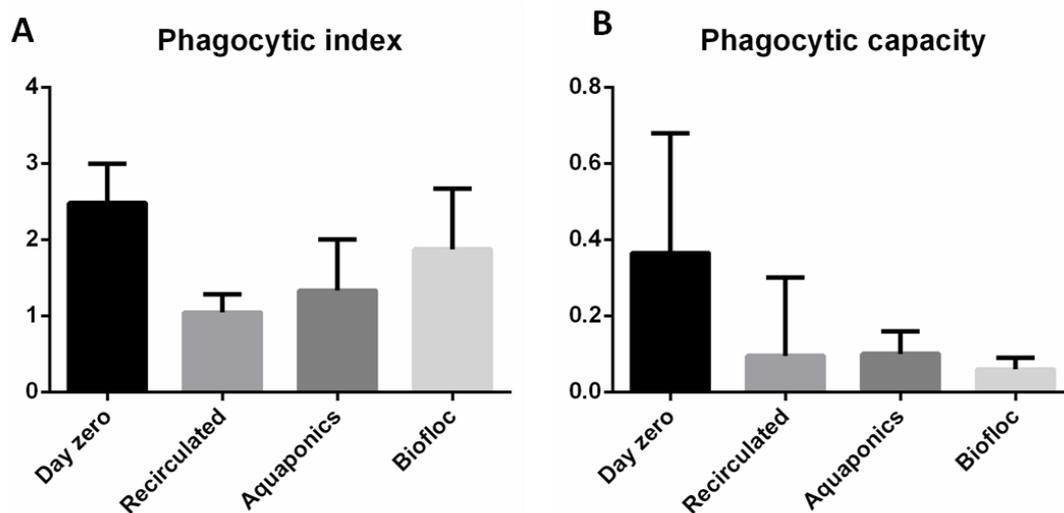
The average weight of fish in each system is shown in Table 1. Although the mean weight of fish reared in the aquaponic system was high, no significant difference was detected among the treatments in the periods evaluated. The average daily gain per crop and animal reflects the differences among individuals in each group.

Table 1. Average monthly weight, average daily gain per crop, and animal (in grams) of *Rhamdia quelen* juveniles kept in recirculation, aquaponics, and biofloc systems. Data are means and standard deviations

Months	Recirculated	Aquaponics	Biofloc
May	17.2±7.5	14.3±8.8	14.0±6.4
June	27.8±10.8	21.2±7.3	21.8±5.3
July	49.0±23.3	39.5±11.6	37.9±8.4
August	62.0±19.2	72.4±28.4	55.9±8.9
September	76.5±27.3	78.7±26.1	63.3±12.1
October	87.0±32.2	88.9±29.6	91.0±28.8
November	113.5±45.0	121.0±44.3	113.0±37.5
Mean daily gain/system	6.78	5.30	4.64
Mean daily gain/fish	0.56	0.44	0.38

Phagocytic indices

Both phagocytic index and phagocytic capacity decreased in all rearing systems after six months. The phagocytic index of *R. quelen* macrophages of the recirculated system phagocytized a smaller number of yeasts ($P<0.001$), but with lower efficiency demonstrated by the smaller phagocytic capacity in all systems ($P<0.001$) (Figure 5).

**Figure 5.** Phagocytic index (A) and phagocytic capacity (B) of peritoneal macrophages from *R. quelen* reared in recirculated, aquaponic, and biofloc systems. The bars stand for standard deviation.

DISCUSSION

None of the three systems was superior to the other in terms of final weight, with a large standard deviation in all systems. These data corroborate the final weights of *R. quelen* maintained in the three types of large-scale systems reported by da Rocha *et al.* (2017).

The results of the evaluation of phagocytosis by peritoneal macrophages show that the rearing systems have direct effects on *R. quelen* immune system, except for the biofloc/heterotrophic system. In this way, the heterotrophic system keeps the cellular immune system able to respond quickly to pathogens and with high efficiency.

The recirculated system requires routine cleaning of biological and physical filters, which can affect nitrifying bacteria and fish stress. Even so, the daily weight gain was higher in the recirculated system, even with lower water quality when compared to the aquaponics and heterotrophic systems. In the heterotrophic system, the major concern is with aeration for the fish and microbiota of this system, which can interfere with the development of both. When comparing the three types of rearing systems between African catfish (*Clarias*

garipepinus) and *Telfairia occidentalis*, Oladimeji et al. (2020) observed that the static system – similar to the heterotrophic – showed low levels of dissolved oxygen and high levels of total ammonia and nitrite when compared to the aquaponic and recirculated system, in addition to lower final weight gain. In both, the latter study and the present experiment, all rearing systems registered similar variations in the temperature of the cultured water.

Rhamdia quelen from the aquaponics system reached the highest average weight among the three systems. This result is linked to the efficiency of the nitrogen cycle bacteria combined with the use of nitrate by the *Nasturtium officinale* seedlings, favoring water quality and the growth of *R. quelen*. This fact was also observed by Oladimeji et al. (2020) in *C. garipepinus* kept in the aquaponic system. For *R. quelen* reared in an aquaponic system, a high specific growth rate, water pH above 7.3, and low concentrations of total ammonia and nitrite are observed (Araújo, 2015). Aquaponic systems with *C. garipepinus* and different plants (*Cucumis sativus*, *Mentha spicata*, and *Ocimum basilicum*) also showed low levels of dissolved ammonia and nitrite in the cultured water (Baßmann et al., 2017, 2020; Knaus et al., 2020; Pasch et al., 2021). The literature data and the results obtained demonstrate that the aquaponic system favors both the development of vegetables and fish, reducing the disposal of water with excess nitrogen compounds (Calone et al., 2019).

Few studies were found analyzing how the biofloc or aquaponics systems affect the catfish immune system. Popoola et al. (2021) observed an improvement in immune system parameters of *C. garipepinus* kept in biofloc system, such as the number of monocytes, serum lysozyme, and myeloperoxidase levels. For tilapias (*Oreochromis niloticus*) reared in aquaponics, the blood cell count and serum biochemistry were better than in tilapias raised in ponds (Osman et al., 2021). Those studies show that biofloc and aquaponics improved health or immune parameters, in opposition to what we observed. Thus, the decrease in phagocytic indices observed in the controls and the tested systems lead us to believe that this may be due to the stress of system-independent cultivation.

CONCLUSIONS

In conclusion, the aquaponics system is the most adequate due to the higher average final weight of each fish and the cultivation of vegetables, reducing the disposal of water with excess ammonia, even though the heterotrophic system maintains the highest activity of peritoneal macrophages, with the consequent protection against pathogens. Those techniques are very safe, saving space in the farm and leading to produce animal protein. With the better management of the systems, there is an expectation to improve in weight gain and in the immune parameters of the fish.

Declaration of conflict of interest of the authors

The authors declare that there is no conflict of interest

Declaration of good practices in the use of animals

The authors declare that they have followed all international, national, or institutional guidelines applicable to the care and use of animals

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