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Research Article

Evaluation of Lettuce Cultivation in an Aquaponic System, With and Without Bioflocs, Compared To a Hydroponic System, In a Greenhouse in Southern Brazil

Andréa Ferretto da Rocha[1]*, Aline Portella Cardoso[2], Rodrigo Favreto[1] and Marco Aurélio Rotta[1]

[1]Department of Agricultural Diagnosis and Research, Secretariat of Agriculture, Livestock, Sustainable Production and Irrigation, Rio Grande do Sul, Brazil

[2]Federal Institute of Education, Science and Technology of Rio Grande do Sul - IFRS. Rio Grande do Sul, Brazil

Abstract

A recirculation system was set up to evaluate lettuce production (floating) in aquaponics (Aqua) and aquaponics with bioflocs (AquaFloc) in comparison to hydroponics (Hydro), in triplicate, for 45 days. Juvenile tambacu into the aquaponics tanks were fed with a commercial feed. Water quality was monitored and vegetable growth was evaluated. Head diameter, height, fresh matter, dry matter, number of leaves, and chlorophyll a+b concentration were higher ($P<0.05$) in "Hydro" than "AquaFloc", even when the "AquaFloc" reached 60 days. The "AquaFloc" system presented 62% of fish survival, but presented lower ($P<0.05$) concentrations of ammonia and nitrite compared to "Hydro" and absence of nitrate. Electrical conductivity and concentration of total solids dissolved in water were lower ($P<0.05$) in "AquaFloc" than in "Hydro", while pH was higher ($P<0.05$) in "AquaFloc" in comparison with "Hydro", resulting in less availability of nutrients for plant growth. The "Aqua" system did not show lettuce growth and presented a complete fish mortality. The fish-vegetable ratio used did not meet the nutritional demand of the lettuces. New studies must be carried out with an appropriate fish-vegetable ratio that can provide sufficient nutrients to plants in aquaponics, with control of the system's pH.

Keywords: Green vegetable; Integrated systems; Multitrophic system

***Corresponding author:** Andréa Ferretto da Rocha, Department of Agricultural Diagnosis and Research, Secretariat of Agriculture, Livestock, Sustainable Production and Irrigation, Rio Grande do Sul, Brazil, E-mail: andrea-rocha@agricultura.rs.gov.br (or) andreadarocha@gmail.com

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Introduction

The development of technologies that promote the increase in food production with market demand and value, environmentally friendly production systems suitable for the social and environmental conditions, such as integrated food production systems, are essential for both rural areas and urban centers. Based on already applied hydroponics technologies, an integrated food production system model that has been growing and developing is the aquaponics system. According to Rakocy et al. [1], aquaponics is an integrated system in which vegetable production is carried out in a recirculation system integrated with fish production. In this system, plant cultivation is nourished by aquaculture effluent. There is a fusion between hydroponics and aquaculture, where one culture will benefit from the by-product of the other, considerably reducing the need for nutrient input as well as the production of effluents, unlike when the same systems are implemented individually [1].

Brazil has great possibilities for aquaponics, as it has numerous family rural properties producing vegetables, including production in greenhouses and hydroponic systems, and the increase in fish production from aquaculture. Brazil produced 860 thousand tons of farmed fish in 2022 [2] and 464 thousand tons of lettuce were sold in the country's main supply centers in the same year [3]. Much of the vegetable production in Brazil is carried out by family farmers. Family farming stands out as a segment of great economic and social importance through the agricultural production of vegetable foods, the number of rural establishments and the generation of employment in rural areas [4].

Hydroponics is already the way in which about 45% of the total number of greens in Brazil are produced, with 25 to 35 thousand hectares, mainly lettuce (Lactuca sativa L.) [5]. In producing greens in traditional hydroponics systems, fertilizer solutions are used to meet the nutritional requirements of plants grown in water. Theoretically, in the aquaponics system, this supplementation is not necessary since the nutrients required for plant growth will be available in the water through fish sludge and through microbiological activities on organic matter that occur in the aquatic environment [6]. Regarding fish, Shreejana et al. [7], point out that selecting fish species with high resistance to diseases and parasites and being adapted to local climatic and water quality conditions is essential for the successful application of the aquaponics system. A species of freshwater fish from South America with the potential to be raised in aquaponics, according to Pinho et al. [8], is the hybrid tambacu (Piaractus mesopotamicus Holmberg, 1887 x Colossoma macropomum Cuvier, 1818), an important native species farmed in Brazil with market value and nutritional quality for consumption. Furthermore, it is a fish with great acceptance in the consumer market, rusticity when handling, and good growth rate [9], and its performance in aquaponics has been evaluated [10]. The biofloc system (BFT - biofloc technology), an aquaculture system considered environmentally friendly with low water use, which promotes the recycling of nutrients in water through the handling of heterotrophic bacteria populations [11], has also been related to high productivity, water quality and control of bacterial infections [12]. Using biofloc and aquaponics systems together is considered promising, as it is possible to combine good water quality and better use of resources and inputs [13].

In this sense, the research objective is to evaluate the use of an aquaponics system with tambacu integrated into the lettuce crop, with and without bioflocs, comparing it with a hydroponics system in a greenhouse located in a high thermal amplitude region.

Material and Methods

Facilities and experimental conditions

The study was carried out at the State Center for Diagnosis and Family Agriculture Research (29°39’39” S, 50°12’38” W, 15 m, Cfa climate) between January and February 2020 for 45 days. Three lettuce cropping systems (aquaponics - Aqua; aquaponics with bioflocs - AquaFloc; and hydroponics - Hydro) were evaluated, with no control group, in a completely randomized design with three replicates for each system.

The crops were raised in a recirculation system combined with a floating raft subsystem. The system culture consisted of three storage tanks (1,000 L polyethylene tank capacity, 1.16 m base diameter, and 0.76 m height), one per treatment, supplied with underground water, in which the fish (Aqua system), fish + biofloc (AquaFloc system) and nutrient solution (Hydro system) were stored, and three trials tanks (1,000 L fiberglass tank capacity, 5.0 m length, 0.4 m height, and 0.5 m width) per treatment, totalling nine trials tanks, in which the lettuce seedlings (*Lactuca sativa*, Crespa Palmas, Isla®, 28 days old) were kept on floating trays (polystyrene; cells filled with commercial substrate - Bioplant®) at a density of about 40 plants m^{-2}. Three floating trays were placed in each experimental tank. All storage and trial tanks were well identified and covered by a mesh screen to reduce animal stress and sunlight into the water.

Juvenile tambacu (average: 1.0 g, 5.0 cm; no defined sex) [a hybrid from tambaqui (*Colossoma macropomum*) and pacu (*Piaractus mesopotamicus*)], purchased from a commercial fish farmer in the region, healthy-looking, were transported inside plastic bags filled with water and oxygen to the greenhouse, where they were acclimatized and randomly stocked into the two aquaponics storage tanks (Aqua and AquaFloc), 100 fish per tank, a density close to that recommended by Rodrigues [14] for tambacu in biofloc system. They were fed with commercial fish feed (42% crude protein - CP) at a rate of 15% of the total biomass per day, divided into two meals. In the Aqua storage tank, leftovers were removed after 30 minutes of the feed provided. The amount of feed placed daily in the storage tanks (Aqua and AquaFloc) remained the same until the end of the study despite fish mortality. Fish growth was not evaluated. Survivors were counted at the end of the study.

The study was conducted in a greenhouse (96 m^2 with an automatic temperature control system, with constant temperature and humidity monitoring by a data logger - RHT20, Extech Instruments®). Throughout the study, the average air temperature inside the greenhouse was 23.6 ± 4.8 ºC (minimum: 13.7 ºC and maximum: 40.6 ºC); the average relative humidity was 89.8% ± 12.8% (minimum 41.8% and maximum 99.9%), and the average dew point of the air was 21.6 ± 3.1 ºC (minimum 13.7 ºC and maximum 30.3 ºC).

System operation

All tanks of the crops systems (Hydro, Aqua and AquaFloc) were maintained in a recirculation system between the storage tanks and the assay tanks (with floating raft subsystem) of the corresponding treatments (Figure 1). Recirculation through plumbing and submersible pump (flow spf 8,000 - 7,500 L h^{-1}; Boyu®) was turned on for about one hour per day, and the assay tanks remained permanently filled with water from each corresponding treatment. The flow was conducted from each storage tank to the three assay tanks of the corresponding treatment subsystem through PVC piping and returning to the storage tank through hoses. All tanks were kept under constant aeration by oxygen diffusers from an air blower (10 Hp, 40 pcm, Chiaperini®).

For the Aqua treatment, the storage tank was maintained in recirculation also with a biofilter (300 L tank capacity) filled with plastic media - bio ring, with strong aeration all the time, with a submersible pump with lower flow and return by gravity. Before placing the fish and running the assay, pure (p.a.) ammonium sulfate and agricultural limestone were added to the biofilm tank. The bottom of the fish storage tank was cleaned daily to remove feces and food leftovers, and the water was exchanged (around 10 - 20% of the volume per day). In the Aqua storage tank, 10.0 ppm of 0.1 N hydrochloric acid was added at times to lower the pH.

Bioflocs were previously prepared in the AquaFloc system. The storage tank was fertilized with commercial fish feed (32% CP), wheat bran (purchased in bulk), and molasses (pasty, fresh, purchased from a sugarcane producer in the region) at approximately three-day intervals for about three months to increase the C: N ratio close to 15: 1 to promote heterotrophic growth for ammonia control, as recommended by Avnimelech (1999) and Hargreaves (2013). Vigorous aeration was kept to supply oxygen and turbulence to the bioflocs, in addition to being manually turned over two to three times during the week. With the study running, fertilization took place through the food offered daily to the fish, and once a week, 50 g of wheat bran was added, but with no addition of molasses. In the assay tanks, where the trays with lettuce were placed, the biofloc concentration did not exceed 10 mL L^{-1} (as settleable solids; 1.0 L for 15 min in Imhoff cone).

In the Hydro system, mineral fluid fertilizers for fertigation were added in the water of the storage tank, as follows: 5.0 L of solution 1 (N: 5.5%; Ca: 6.5%; Samo Fertilizantes®); 5.3 L of solution 2 (N: 4.75%; K: 9.5%; P: 6.0%; Mg: 1.3%; S: 1.0%; Samo Fertilizantes®) and 100 mL of solution 3 (N: 1.0%; P_2O_5: 30.0%; Agrosafra Sementes Ltda®) (Figure 1).

Lettuce crop

At the end of 45 days, in order to evaluate the development of lettuce in different treatments, the plants were collected and measured for head diameter and height (cm) and weighed (g) on an electronic scale (0.01 g, Bioscale®) for fresh matter and dry matter after 48 h at 60 ºC in a drying oven with air circulation (MA033, Marconi®).

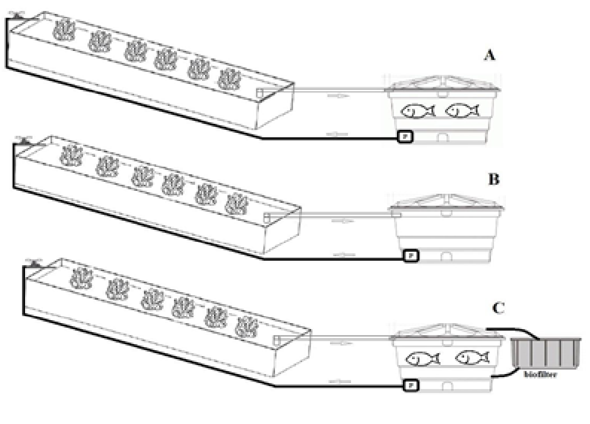

Figure 1: Schematic drawing shows the recirculating system used in the study. Storage (A, B, C) and assay tanks (3 per treatment). A: AquaFloc system; B: Hydro system; C: Aqua system. P: Pump. Source: Andréa Ferretto da Rocha; Marco Aurélio Rotta.

The concentration of chlorophyll *a+b* of the plants was also analyzed (ClorofiLog CFL1030, Falker®), and measurements were made on the first, second, and third leaves completely expanded in size of each plant, and the average of each plant was used for statistical analysis. Some plant samples from the AquaFloc treatment were kept in the system for another 15 days until they completed 60 days of cropping when they were collected for analysis.

Water quality monitoring

During the 45 days of the study, some water quality parameters relevant to fish and plants were monitored. Every two to three days, water temperature and dissolved oxygen were measured using portable equipment (YSI® 55), electrical conductivity (EC), and Total Dissolved Solids (TDS) using a portable conductivity meter (EC/TDS, HI 99300, Hanna Instruments®). The pH was measured daily with a portable device (HI 98183-02, Hanna Instruments®), as well as the concentration of ammonia-NH_3-N (LabconTest® and ProdacTest®, colorimetric kit). The parameters of total hardness-GH (LabconTest®, colorimetric kit), carbonate hardness-KH (LabconTest® colorimetric kit), nitrite-NO_2-N (LabconTest® and ProdacTest® colorimetric kit), and nitrate-NO_3-N (ProdacTest® colorimetric kit) were analyzed weekly, as well as the concentration of orthophosphate-PO_4-P (ProdacTest®, colorimetric kit) and iron (ProdacTest®, colorimetric kit) in addition to water turbidity (HI 98703-02, Hanna Instruments®).

Statistical analysis

All results were assessed for normality and homoscedasticity and subjected to one-way ANOVA analysis of variance, setting significance at $\alpha = 0.05$. The Student-Newman-Keuls (SNK) *post hoc* test highlighted the means when statistical differences were detected. The non-parametric Kruskal-Wallis test was run when the assumptions for the one-way ANOVA were not met. The test gave the ranking of the means when differences were observed. Percentage and pH values were transformed before analysis of variance (arcsine of the square root of x/1). Analyzes were run using Statistica 6.0 (StatSoft.Inc. Tulsa, OK, USA).

Ethics committee approval

The Animal Use Ethics Committee (CEUA-IPVDF) authorizes the study under protocol nº.19/2018.

Results and Discussion

Plant production

Significant differences ($P<0.05$) were observed in the development of lettuce (Table 1). It was observed that the Hydro and AquaFloc systems, in this line-up, were more productive than the Aqua system due to the fact that they presented higher averages for head diameter, height, total fresh weight, number of leaves, and value of chlorophyll *a + b* index. The value of total dry weight was significantly superior to the Hydro system than to the AquaFloc and AquaFloc + 15 days. The lettuce from the aquaponics treatment with no bioflocs (Aqua) did not develop sufficiently after 45 days of the trial. The chlorophyll *a + b concentration* was also higher in lettuce produced in the hydroponics system (Table 1).

Parameter	Hydro (n = 48)	AquaFloc (n = 44)	AquaFloc + 15 days (n = 44)	Aqua (n = 0)
Head diameter (cm plant^{-1})	25.9 ± 2.5^a (19.0 – 30.0)	15.8 ± 4.4^b (8.0 – 26.0)	18.8 ± 4.8^c (9.0 – 28.0)	NI
Height (cm plant^{-1})	28.5 ± 3.3^a (22.0 – 36.0)	16.8 ± 3.2^b (10.0 – 23.0)	20.6 ± 5.3^c (8.0 – 30.0)	NI
Total fresh weight (g plant^{-1})	95.5 ± 34.6^a (37.8 – 197)	16.9 ± 12.9^b (0.9 – 59.5)	44.9 ± 26.1^c (13.5 – 117)	NI
Total dry weight (g plant^{-1})	7.0 ± 2.6^a (2.4 – 13.9)	1.2 ± 0.9^b (0.1 – 3.9)	1.9 ± 1.3^b (0.2 – 5.2)	NI
Number of leaves (Unit)	19.1 ± 4.0^a (11 – 28)	9.2 ± 2.6^b (6 – 18)	13.4 ± 4.7^c (6 – 26)	NI
Chlorophyll ***a+b*** (ClF)	36.3 ± 2.9^a (31.0 – 43.8)	28.8 ± 2.9^b (22.5 – 36.8)	30.0 ± 2.5^c (24.2 – 35.2)	NI

Table 1: Mean values (± standard deviation) of lettuce (*Lactuca sativa*) crop over 45 days in hydroponics (Hydro), aquaponics with bioflocs (AquaFloc) with 45 and 60 days (AquaFloc + 15 days) and aquaponics (Aqua) systems.

Values in parentheses are minimum and maximum. Different letters on the same line indicate statistical differences after the Student Newman-Keuls (SNK) test ($P<0.05$). n = Number of plants. NI = No information.

The scarcity or absence of nutrients essential to plant development in aquaponics systems harmed the growth of vegetables (lack of nutrient solutions), which was not observed in the Hydro treatment. While the AquaFloc system still promoted plant development, albeit late (after 60 days), possibly due to the availability of other diverse ions, the same could not be observed in the Aqua system using a biofilter and water exchange since it showed no plant growth. In general, plant production is higher in hydroponics systems than in aquaponics [15], and it may be related to differences in nutrient concentrations in the water [16], since the necessary nutrients were fully supplied in the hydroponic system through the fertilizer suitable for hydroponics, while in the aquaponics system the availability of these nutrients was uncertain, and plants depended only on the nutrients provided by nitrogenous waste from fish farming. The absence of nitrogenous compounds in the Aqua system possibly indicates that the biofilter was not working properly to convert the ammonia into nitrite and subsequently into nitrate, the compound usually absorbed by the plants for N intake. In part, the damage to plant growth, through the unavailability of nutrients, can be attributed to the high pH of aquaponics systems, mainly because the high pH hinders the nitrification process. Although most hydroponic plants have an acceptable range of pH between 5.5 and 6.5 [17], their best development is at a pH of 5.8 to 6.2 [1]. However, lettuce still grows well at a pH of 7.0, although some iron deficiencies may appear due to reduced bioavailability of this nutrient above neutrality [18]. This is supported by Zou *et al*. [19], who obtained higher plant yields at a pH of 6.0 in aquaponics than at pHs of 7.5 and 9.0. No iron was detected in the water (Table 2), possibly due to its complete absence or at levels not detected by the colorimetric test used.

Parameter	Hydro	n	AquaFloc	n	Aqua	n
Temperature (°C)	25.7 ± 1.6^{a} (23.0 – 28.5)	15	26.6 ± 1.7^{a} (24.0 – 29.5)	15	25.8 ± 1.6^{a} (24.2 – 29.0)	15
Dissolved oxygen (mg L^{-1})	4.0 ± 1.9^{a} (1.8 – 7.4)	18	3.2 ± 1.7^{a} (0.7 – 6.6)	18	5.0 ± 1.1^{a} (3.0 – 7.0)	18
Turbidity (NTU)*	19.0 ± 8.0^{a} (7.9 – 32.1)	12	46.6 ± 22.6^{b} (19.1 – 81.8)	12	15.4 ± 9.0^{a} (4.9 – 34.7)	12
Total hardness - GH (mg L^{-1})*	223.3 ± 53.0^{a} (150 – 300)	15	100.0 ± 32.7^{b} (50 – 200)	15	60.0 ± 20.7^{b} (50 – 100)	15
Carbonate hardness - KH (mg L^{-1} $CaCO_3$)*	38.8 ± 7.3^{a} (35.8 – 53.7)	06	77.6 ± 21.7^{b} (53.7 – 107.4)	06	41.8 ± 9.2^{a} (35.8 – 53.7)	06
pH	7.3 ± 0.5^{a} (6.2 – 7.9)	15	8.5 ± 0.5^{b} (7.7 – 9.3)	15	9.4 ± 0.6^{c} (8.6 – 10.3)	15
Total dissolved solids - TDS (mg L^{-1})*	927.6 ± 141.7^{a} (776 – 1,200)	15	215.4 ± 25.3^{b} (184 – 260)	15	88.80 ± 10.29^{c} (70 – 106)	15
Electric conductivity - CE (µS cm^{-1})*	1,841 ± 290.2^{a} (1,502 – 2,400)	15	422.0 ± 43.88^{b} (364 – 516)	15	177.46 ± 20.29^{c} (140 – 206)	15
Toxic ammonia (NH_3-N mg L^{-1})	0.15 ± 0.1^{a} (0.04 – 0.29)	15	0.14 ± 0.13^{a} (0.03 – 0.44)	15	0.09 ± 0.06^{a} (0 – 0.22)	15
Nitrite (NO_2-N mg L^{-1})*	1.66 ± 0.97^{a} (0.25 – 2.80)	15	0.11 ± 0.11^{b} (0 – 0.25)	15	0.03 ± 0.09^{b} (0 – 0.25)	15
Nitrate (NO_3-N mg L^{-1})	3.0 ± 2.5 (0 – 5.0)	15	ND	15	ND	15
Orthophosphate (PO_4 mg L^{-1})	3.3 ± 1.5^{a} (2.0 – 5.0)	12	3.50 ± 2.42^{a} (2.0 – 10.0)	12	1.8 ± 0.5^{b} (0.5 – 2.0)	12
Iron - F (mg L^{-1})	ND	12	ND	12	ND	12

Table 2: Quality water mean values (± standard deviation) of hydroponics (Hydro), aquaponics with bioflocs (AquaFloc), and aquaponics (Aqua) systems used for the lettuce (*Lactuca sativa*) crop in a greenhouse over 45 days.

Values in parentheses are minimum and maximum. Different letters on the same line indicate statistical difference ($P<0.05$) in the Newman-Keuls (SNK) or in the *Kruskal-Wallis test. n = Number of analyzes. ND = Not detected.

In addition to a higher development of lettuce grown in the Hydro system, the plants also showed a dark green leaf color, with a significantly higher chlorophyll *a + b index* (Table 1). It was also possible to observe that at the end of 45 days, lettuces from the Hydro system had already surpassed the harvest period, while the lettuces from the AquaFloc system did not yet have satisfactory size or uniformity. Studies indicate the relationship between nitrogen and chlorophyll concentration in leaves and the relationship of chlorophyll with the nutritional status of plants concerning nitrogen, since nitrogen plays a structural role in determining the growth and development of plants and crop productivity [20,21].

Foliar fertilization is an option that can eventually be considered for the aquaponics system (MARIANO *et al*., 2021), given the faster correction of specific deficiencies, but it must be used with caution.

Water quality

The water quality parameters of the food production systems evaluated are presented in (Table 2) No significant differences ($P>0.05$) were observed for temperature, dissolved oxygen, and toxic ammonia. Significant differences ($P<0.05$) were observed for other parameters. AquaFloc shows higher values than the Hydro system and Aqua for carbonate hardness and turbidity. The pH was more acidic in the Hydro system, while the Aqua and AquaFloc systems were more basic, at worrying levels like this last one. A significant difference was observed between the three systems regarding TDS and electric conductivity. Nitrite mean levels were significantly higher in Hydro, and nitrate was detected only in this treatment. Orthophosphate and total hardness were observed at significantly low levels in the Aqua system, and no iron concentrations were detected in the water of any of the evaluated treatments.

During the study, the average water temperature of the treatments was 26.0 ± 1.6 °C (min. 23.0 °C and max. 29.5 °C), with no significant difference between the treatments evaluated (Table 2). This temperature is considered optimal for the growth of tropical fish raised in Brazil, such as round fish like tambacu [22,23], with tolerance to low temperatures being observed despite their thermal comfort being within the range of 27 and 30 °C [24] which makes it an interesting species to be bred in southern Brazil.

Lettuce's recommended daytime temperature range is 17–28 °C, although the optimum temperature is 15–22 °C [18]. The average concentration of dissolved oxygen in the water of the AquaFloc treatment was significantly lower than that of the Aqua treatment (Table 2), possibly due to the greater community of aerobic microorganisms present in the bioflocs, as reported by Hargreaves [25]. Nevertheless, the dissolved oxygen concentration was considered low in all evaluated systems, according to the average values and minimum concentrations observed (Table 2). Colliver and Stephenson [26] indicate a DO concentration between 3.0 and 4.0 ppm to promote the nitrification process, although Ruiz *et al.* [27], observed that DO concentrations above 1.7 ppm do not harm nitrification. Furthermore, concentrations between 1.0 and 1.5 ppm of DO can simultaneously promote nitrification and denitrification processes [28]. In aquaponics systems, the DO concentration in the water should be greater than 5.0 mg L^{-1} to benefit fish, microorganisms, and plant roots [29].

In addition to the insufficient supply of DO in all treatments, the development of microalgae in the Hydro system tanks was visually observed, which may also have negatively affected the DO at times since the proliferation of phytoplankton in tanks can cause fluctuations in dissolved oxygen concentration, ammonia, and pH [30]. The presence of phytoplankton observed in the Hydro tanks may have caused some competition for the uptake of nutrients available [31]. but without compromising plant growth in this treatment. In the AquaFloc treatment, the greater turbidity (Table 2) due to bioflocs had an inhibitory effect on the development of phytoplankton in the tanks of this system, and, in addition, it did not harm fish survival. In the Aqua system, phytoplankton development was controlled due to the biofiltration process and the daily exchange of a certain amount of water. Despite the variety of aquaponics systems, one of the most used involves passing the water through an additional filter before returning to the fish tanks [15]. Due to the filtration process, water exchange/replacement in aquaponics systems can be low, as plants can uptake nutrients from the water. However, occasionally, it may be necessary to exchange some of the water to maintain the health of the fish and their welfare, as the systems work with water recirculation. In the AquaFloc and Hydro systems, water was replaced only due to evaporation losses.

Regarding total hardness, which is represented by the sum of divalent cations, mainly Ca^{2+} and Mg^{2+} and expressed in mg L^{-1} of $CaCO_3$, waters can be classified as light (<50), moderately hard (50 - 150), hard (150 - 300) and very hard (>300), varying widely depending on the soil composition of the water source [32]. The average values of total water hardness of the Hydro system showed the significantly highest total hardness (Table 2). This wide range can be explained by the fact that Hydro received a supply of nutrient solutions for hydroponics with calcium and magnesium salts. As for water carbonate hardness, or alkalinity, in freshwater aquaculture systems, it is essential to maintain a stable pH throughout the day (buffering) and should generally be between 50 and 100 mg L^{-1} of $CaCO_3$ [32]. The highest ($P<0.05$) average value of carbonate hardness was observed in the AquaFloc treatment (Table 2). Alkalinity can be produced in the absence of DO in anoxic zones, such as inside bioflocs, due to the denitrification process [31]. This is supported by Zou *et al.* [19], who demonstrated that aquaponics in an alkaline environment has more denitrifying bacteria. It is important to point out that the nitrification and denitrification processes can happen simultaneously in the same reactor/tank under the same conditions [33,34], common in biofilms and bioflocs. Also, Boyd [35] reports that the combined presence of fish, feed and phytoplankton in tanks can increase inorganic carbon, carbonates, and bicarbonates in the water, increasing alkalinity. Proper alkalinity values are essential to avoid pH fluctuations in the water. Bicarbonates help maintain a stable pH in the water, but at high values, they require specific care to maintain an ideal pH (slightly acid) for growing lettuce. During the study, Aquaponics systems (Aqua; AquaFloc) showed significantly higher average pH values than the hydroponics system (Hydro). Although with a smaller range, the Hydro system still maintained an average pH higher than that suitable for aquaponics systems (Table 2). Rakocy *et al*. [1] recommend water with a pH of 5.8 to 6.2 for growing plants in aquaponics. It is possible that the development of greens in aquaponics treatments with bioflocs and, even more so, just aquaponics was mainly harmed by the high pH throughout the study period. This hypothesis is supported by Zou *et al*. [19], who obtained greater plant development in aquaponics at a pH of 6.0 than at a pH of 9.0. Rocha *et al*. [36], when cropping lettuce in aquaponics systems with Silver catfish - jundiá (*Rhamdia quelen*) juveniles with and without bioflocs, recorded an average pH of 7.0, close to that recommended by Rakocy *et al*. [1] for aquaponics and close to that recommended for fish maintenance by Baldisserotto and Radünz Neto [37].

Mean values of this study for Total Dissolved Solids (TDS) and Electrical Conductivity (EC) differed significantly between the three treatments (Table 2). The Hydro system had a higher concentration of dissolved ions from the nutrient solution for hydroponics, which resulted in a higher growth of lettuce. Rakocy *et al*. [1] recommend TDS to remain at around 1,000 to 1,500 mg L^{-1} in hydroponics and 200 to 400 mg L^{-1} in aquaponics, values close to those observed in the study for the Hydro and AquaFloc systems, respectively (Table 2). The average TDS value for the Aqua system could not meet the requirements for growing lettuce in aquaponics, possibly due to frequent water exchanges to maintain water quality for the fish, as the biofilter did not work properly. Carrijo *et al.* [38] recommend a solution for hydroponic lettuce crop with Electrical Conductivity (EC) between 1.5 and 2.3 mS cm^{-1}, and according to Martinez and Silva Filho [36], the maximum EC value tolerated by lettuce is 2.5 mS cm^{-1}. Only the Hydro treatment presented an EC suitable for lettuce production and despite having a lower EC than necessary, the lettuces in the AquaFloc treatment still developed. However, they reached a smaller size than those produced in Hydro during 45 days, even after remaining for another 15 days, totaling 60 days of the crop.

Regarding nitrogen compounds in water, the average values of toxic ammonia in water did not differ significantly between the treatments (Table 2), remaining below the LC50-96 h for tambacu fry, which is 1.63 mg L^{-1} [39]. Although it is not the usual way, ammonia is also one of the forms nitrogen plants take up [40], contributing to their development and growth, as does N in its other forms. Concerning nitrite, aquaponics treatments (Aqua and AquaFloc) presented lower average values when compared to the Hydro treatment (Table 2). Nitrate was just detected by the test used in the Hydro treatment (Table 2), indicating that, if present in the aquaponics systems, they were possibly in concentrations below the test's detection limit. Also, it is possible that the high pH level during the study, reaching 9.0 to 10.0, harmed the nitrification process, especially in the aquaponics treatment tanks, although Rakocy [29], stated that nitrification is more efficient at pH 7.5 or higher.

The average orthophosphate concentration in the water significantly differed between treatments (Table 2). A higher ($P<0.05$) average of orthophosphate was observed in the Hydro and AquaFloc systems, which can be justified due to the water exchanges in the Aqua system. Iron was not detected in any sample taken from any treatment throughout the trial period (Table 2). This may be related to the test's sensitivity or even to the absence of iron in the water, which could also have affected the growth of the plants.

Additionally, pH affects nutrient solubility. During the study, the pH level exceeded the appropriate level to maintain nutrient availability for growing plants, reaching 9.0 to 10.0. The optimum pH for nutrient solubility is 6.5 or slightly lower. Micronutrients such as iron, copper, zinc, boron, and manganese are unavailable at a pH above 6.5 [41]. If the pH is too high, nutrients precipitate from the solution, causing plants to experience nutrient deficiencies and promoting a growth and production decrease. Therefore, the aquaponics system should be maintained at pH 7.0 [29].

Fish survival

Using biofloc in the aquaponics system positively affected fish survival. Survival of juvenile tambacu in the AquaFloc tank was 62%. As Yu *et al.* [12] pointed out, the biofloc system probably promoted better water quality and control of bacterial infections, resulting in greater fish survival in this system. A similar study performed with juveniles of jundiá (*R. quelen*) in an aquaponics system with bioflocs resulted, after 46 days, 100% survival [36]. In this "Aqua" treatment, mortality reached 100% at the end of the study, even with the water exchange and using a biofilter. In this situation, all fish were affected by opportunistic diseases that led to their death. In contrast, different outcomes were found by Braga *et al.* [42], who observed a higher mortality of tambacu in the biofloc system, assuming that this species has not adapted as well to that system. However, a current review states that the immune system of several species of farmed aquatic animals, especially when in high densities, can be improved through the use of the biofloc system from microorganisms that develop in this environment and act with a probiotic function [43].

For fish farms with round fish like tambacu, the ideal is for the pH of the water to be within a range between 6.5 and 7.5 [44], and higher pH can cause mortality due to the increased concentration of toxic ammonia. This is supported by De Croux *et al.* [45], who evaluated the total ammonia toxicity of water for tambaqui juveniles (17 g), demonstrating that with a total ammonia concentration of 5.0 mg L^{-1} and a pH of 8.0, 10% to 20% mortality was observed, reaching 100% mortality at pH 9.0, while at pH 6.0 - 7.0, there was no mortality. On the other hand, tilapia, the fish species most commonly cultivated in aquaponics, both because it is resistant to diseases and tolerates a wide range of pH (3.0 - 11.0), has its growth rate best achieved when at a pH around 7.0 - 9.0 [7].

Additionally, very low DO levels were observed in the tanks of all treatments throughout the study. Tavares-Dias *et al.* [44-49] reported that DO levels below 3.0 mg L^{-1} negatively influence fish immunology, so it can be inferred that this fact may have negatively impacted fish health, leading to disease and mortality.

Final remarks

Preliminary results from the evaluation of three different systems demonstrated the possibility of producing lettuce in an integrated system with grow-out of tambacu and bioflocs combined. The hydroponics system had the best results for the growth of greens, besides the aquaponics system with bioflocs (AquaFloc) promoted the development of lettuces without chemical fertilizers, even not reaching a satisfactory size at the same time as those produced in the hydroponics system. Adding bioflocs to the aquaponics system improved the condition of the plants, fish, and water in the farming system compared to the aquaponics system without bioflocs. AquaFloc required no water exchanges or additional biofilters like a traditional aquaponics system. However, it is important to point out that the findings of this study should not be generalized to other species or experimental conditions.

Cropping leafy greens in aquaponics requires appropriate adjustments in the fish-vegetable ratio, with more fish or larger fish, taking into account that in aquaponics, nutrients are supplied daily to the plants from the fish's diet. Foliar fertilization can be considered in this system on an emergency basis but requires caution. Likewise, the pH of the water must be well managed in the aquaponics system, with or without bioflocs, to make essential nutrients available for plant growth.

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All people listed as authors have had sufficient participation in the execution of the project and writing of the article and agree to the publication, content, and transfer of publication rights of the article to ALL LIFE magazine.

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The authors declare that the research was conducted in the absence of any potential conflicts of interest, including financial and non-financial.

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Ethical statement

The authors confirm that the ethical guidelines adopted by the journal were followed in this work, and all authors agree with the submission, content, and transfer of the publication rights of the article to the journal. The authors also declare that the work has not been previously published, nor is it being considered for publication in another journal, assume full responsibility for the originality of the article, and may incur any charges arising from third-party claims concerning the article's authorship.

The Animal Use Ethics Committee (CEUA-IPVDF) authorizes the study under protocol nº.19/2018.

Author contribution statement

We state that all authors listed in this study contributed intensely for the design, working-out, data analysis, writing of the work and critical reading. With more details.

Andréa Ferretto da Rocha

contributed to the design of the project, carrying out the experiment, collecting data, statistical manipulation of data, analysis and interpretation of data, writing the article and critical review of the written work.

Aline Portella Cardoso

Contributed to the design of the project, carrying out the experiment, collecting data and writing the article.

Rodrigo Favreto

Contributed to the preparation of the project, data collection and critical review of the written work.

Marco Aurélio Rotta

Contributed to the writing and critical review of the written work.

We also state that all authors have approved the final version of the manuscript to be published and agree to be responsible for all aspects of the work.

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