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Comparison of length and weight gain, AGR, IGR, KM, and feeding efficiencies in *Oreochromis niloticus* grown at different densities in a Biofloc system in the laboratory

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Abstract
This study evaluated the effect obtained on feed efficiency in five treatments with Nile tilapia (*Oreochromis niloticus*) using a Biofloc system at different densities. The specimens were placed in five containers with 250 L of water each (50, 75, 100, 125, and 150 respectively), and every 15 days 30 organisms were taken, weighed, and measured. At the end of the experiment, the length and weight gain of the fish were evaluated, and the AGR, IGR, KM, FCR, and FCE were obtained. A one-factor analysis of variance was applied to the data obtained to find significant differences (p<0.05) between treatments, and the Tukey technique was applied. The organisms that obtained the highest weight were those grown in the 50 org 250 L^{-1} treatment, reaching 677.43±0.33 g in only 98 days of experimentation. The highest TCA of weight and length were presented in the 50 org 250 L^{-1} treatment (4.70 g day^{-1} and 0.43 cm day^{-1} respectively). In general, we can conclude that fish grew better and length at lower rather than higher stocking densities. However, this does not exclude the possibility of higher stocking densities, especially if good water quality is maintained to obtain a better final production.

Keywords: Tilapia, biofloc, culture, stocking densities

Introduction
The aquaculture industry has been identified as one of the main sources of pollution in the aquatic environment due to the chemicals and nutrients used for the growth, feeding, and health of cultured aquatic organisms [1]. Another problem is feed wastage, since up to 60% of the total amount supplied is not used by the organisms, which causes compounds such as phosphorus, carbon, and nitrogen to remain in the water as suspended matter, or as dissolved chemicals that are expelled from the system through gasification or water replacement, thus contaminating other bodies of water and nearby soils, causing ecological damage and economic losses to producers [2]. Such economic losses are significant and are because feed and feed costs generally represent the largest operating cost and can represent around 65% of production costs [3].
The dominance of this industry is due to the growing global demand for fish for human consumption. But today it is known that if seafood consumption is to continue to be maintained, farming techniques must evolve towards a sustainable and sustainable path through techniques that allow the optimization of space and less waste of inputs, which would in turn minimize the release of waste into the environment.
An environmentally friendly option that has already been studied and tested with promising results is the Biofloc system. This system consists of providing additional carbon sources to the aquaculture pond. Its addition stimulates the growth of heterotrophic bacteria that then promote nitrogen uptake through the production of microbial protein. Therefore, the ammonium concentration decreases more rapidly compared to the natural nitrification process. The feeding of dissolved organic matter in water in the presence of oxygen together with bacteria decomposes the organic matter into carbon dioxide and the water transforms a part of the organic matter into its biomass.

As a result, particulate matter in the culture system, such as fish excrement, unutilized food particles, and biofilms, can be easily removed. These solid particles are usually separated from the fish culture water by a physical filtration process and then discharged as sludge that can even be used later as fertilizer in vegetable crops [4].

One of the easiest fish species to cultivate for human consumption is the tilapia Oreochromis niloticus (Nile tilapia) due to its resilience and great trophic and ecological adaptations is considered a species that adapts very easily to any type of culture. That is why this experiment aims to find the adequate density in production systems, urban type, in tanks no larger than 250 L and that do not waste water by not performing partial water replacement constantly using the Biofloc technique.

Material and methods

Experimental design

In Rotoplas® tanks with a capacity of 250 L of water, each experimental treatment was placed: a) 50 fish; b) 75 fish; c) 100 fish; d) 125 fish; and e) 150 fish per m^3. Each tank was fitted with an aeration system with the aid of a 10 cm diameter porous aeration stone, with sufficient intensity to move all the water, allowing the food, carbon source, and waste to circulate throughout the water column (Fig. 1). The experiment lasted 120 days.

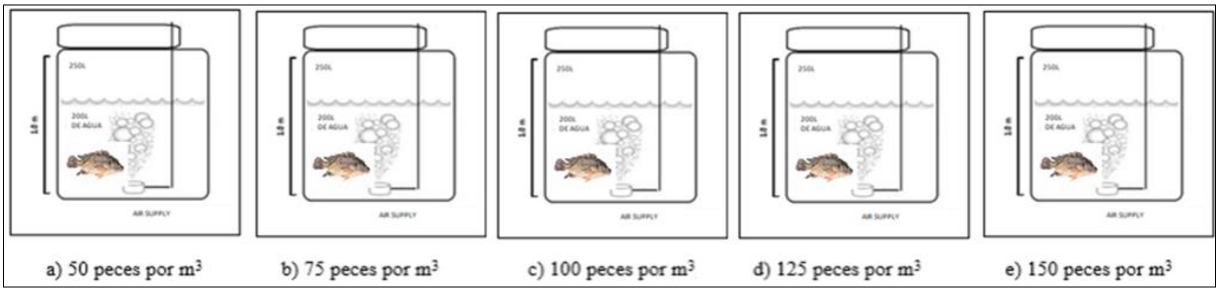

Fig 1: Culture system with the five experimental treatments.

To produce the Biofloc in the culture vessels, moringa powder (0.01% of the total weight of the organisms in the culture) was added once a day.

Biometry of the organisms

Every 15 days, 30 fish taken randomly from each experimental group were extracted to be weighed with the help of a Nimbus® digital scale with an accuracy of two decimal places. In addition, total length (LT) biometry was taken with the aid of a digital Vernier® with an accuracy of three decimal places.

Feeding of the organisms

All experimental treatments were fed with 2.0 mm dry tilapia feed at 10% of the total biomass of the cultured organisms. The feed was rationed in two applications 9:00 hrs and 16:00 hrs every day.

Water quality monitoring

The concentration of nitrites (NO_2- mgL^{-1}), nitrates (NO_3- mgL^{-1}), ammonium (NH_4- mgL^{-1}), and phosphates (PO_4^{-3}) was determined every 15 days in the system with the help of a HANNA® multiparameter meter.

Data processing

All values obtained were entered into a database in Excel 2019 to obtain the descriptive analysis. Growth trend curves were also obtained for each biometric variable. In addition, the following were obtained:

Length and weight gain.

Length = Final Length-Initial Length

Weight = Final Weight-Initial Weight

Absolute Growth Rate (AGR)

$$AGR = \frac{Final\ length\ or\ weight - Initial\ length\ or\ weight}{Number\ of\ days\ of\ experimentation}$$

Instantaneous Growth Rate (IGR)

$$IGR = \frac{LogN(Final\ length\ or\ weigth) - Log\ N(Initial\ length\ o\ weigth)}{Number\ of\ day\ of\ the\ experiment}$$

Degree of well-being (KM)

KM=Weight *Correlation coefficient (Weight: Length) * Length

Feed conversion factor (FCF)

$$FCF = \frac{Total\ amount\ of\ feed\ supplied}{Amount\ of\ final\ biomass\ obtained}$$

Feed Conversion Efficiency (FCE)

$$FCE = \frac{1}{FCA} * 100$$

Statistical analysis of data

A single-factor analysis of variance was applied to the data obtained to find significant differences (p<0.05) between treatments. When differences were found, a multiple mean comparison test was applied using Tukey's technique to determine between which treatments there were significant differences (p<0.05).

Results

In the first four treatments 50, 75, 100, and 125 fish in 250 L, there was a survival of over 90% of the cultured organisms, while in the treatment of 150 fish per m3, a survival of 78% was obtained. Table 1 shows the average length and weight of the organisms cultured with Biofloc.

Table 1: Mean values (±D.S.) of weight and length of cultured organisms in the five experimental treatments.

Sampling days	50 org 250L^{-1}		75 org 250L^{-1}		100 org 250 L^{-1}		125 org 250 L^{-1}		150 org 250 L^{-1}	
	Peso	**Longitud**	**Peso**	**Longitud**	**Peso**	**Longitud**	**Peso**	**Longitud**	**Peso**	**Longitud**
0	6.74±0.24	2.69±0.12	6.74±0.44	3.00±0.28	6.74±0.24	2.03±0.38	6.74±0.45	2.72±0.31	6.74±0.43	3.82±0.17
7	9.37±0.46	4.19±0.19	9.27±0.29	4.70±0.24	9.02±0.26	5.90±0.27	8.92±0.36	8.68±0.25	8.73±0.38	5.81±0.36
14	13.02±0.20	6.07±0.31	12.76±0.23	8.75±0.15	12.08±0.18	6.19±0.28	11.80±0.41	10.91±0.15	11.30±0.42	9.72±0.13
21	18.10±0.34	9.40±0.19	17.56±0.14	9.04±0.27	16.18±0.14	6.86±0.33	15.61±0.17	21.45±0.35	14.64±0.39	12.91±0.33
28	25.16±0.33	10.32±0.18	24.16±0.22	10.82±0.38	21.67±0.44	8.28±0.37	20.65±0.44	22.33±0.23	18.96±0.26	13.76±0.32
35	34.97±0.33	11.00±0.14	33.25±0.46	12.66±0.32	29.01±0.21	9.19±0.17	27.32±0.38	23.20±0.15	24.55±0.14	16.67±0.13
42	48.61±0.18	11.16±0.23	45.75±0.36	17.01±0.14	38.85±0.44	9.63±0.31	36.14±0.43	24.08±0.33	31.79±0.20	17.28±0.20
49	67.57±0.22	12.59±0.42	62.95±0.38	18.38±0.27	52.01±0.31	13.92±0.34	47.82±0.39	24.50±0.17	41.17±0.25	17.58±0.36
56	93.92±0.26	12.77±0.19	86.62±0.35	18.79±0.27	69.65±0.23	17.59±0.29	63.26±0.16	26.97±0.20	53.31±0.27	22.11±0.36
63	130.55±0.25	14.06±0.27	119.19±0.45	20.06±0.28	93.26±0.40	19.98±0.35	83.69±0.27	28.35±0.37	69.04±0.26	22.46±0.23
70	181.47±0.15	26.94±0.24	164.00±0.22	26.88±0.12	124.87±0.34	23.92±0.28	110.73±0.46	31.41±0.28	89.40±0.25	24.51±0.22
77	252.25±0.31	29.93±0.20	225.66±0.45	30.30±0.31	167.20±0.42	25.69±0.24	146.49±0.27	32.34±0.16	115.78±0.26	31.08±0.19
84	350.62±0.37	31.16±0.17	310.51±0.35	34.03±0.17	223.89±0.19	28.11±0.34	193.81±0.42	37.64±0.34	149.93±0.14	32.27±0.38
91	487.36±0.17	33.53±0.23	427.27±0.22	39.93±0.29	299.78±0.22	28.59±0.32	256.41±0.22	38.78±0.21	194.16±0.21	39.50±0.18
98	677.43±0.33	44.50±0.19	587.92±0.18	41.76±0.23	401.41±0.15	36.32±0.25	339.23±0.33	40.08±0.10	251.44±0.34	40.04±0.39
105					537.49±0.33	43.96±0.18	448.80±0.31	40.53±0.11	325.62±0.38	41.52±0.23
112							593.77±0.27	42.06±0.18	421.67±0.45	42.42±0.30
120									546.07±0.18	44.05±0.34

As can be seen, the organisms that obtained the highest weight were those grown in the 50 org 250 L^{-1} treatment, reaching 677.43±0.33 g in only 98 days of experimentation. While the lowest weight of the organisms was found in the treatment with 100 org 250L^{-1} with 537.49 ± 0.33 g. Regarding the length, the highest value was found in the 50 org 250 L^{-1} treatment at 44.50±0.19 cm and the lowest value at 41.76±0.23 cm in the 75 org 250 L^{-1} treatment.

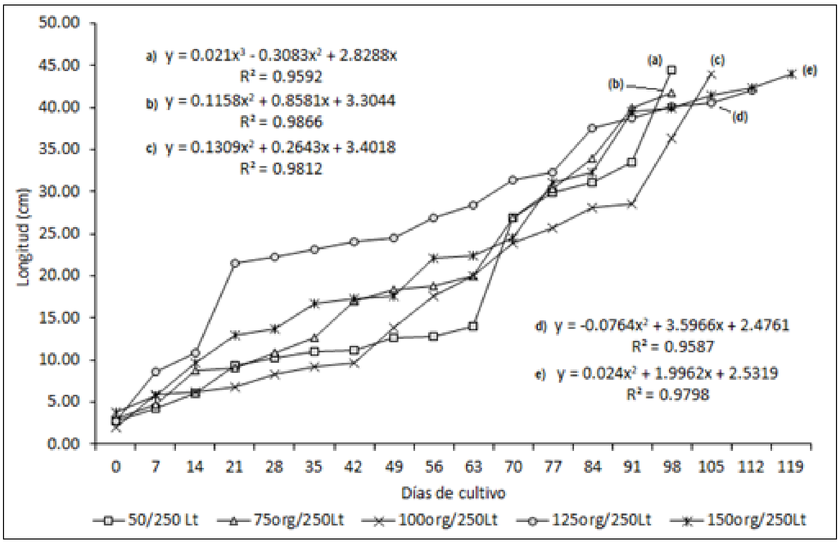

Fig 2: Shows the growth curves of the organisms in culture with Biofloc.

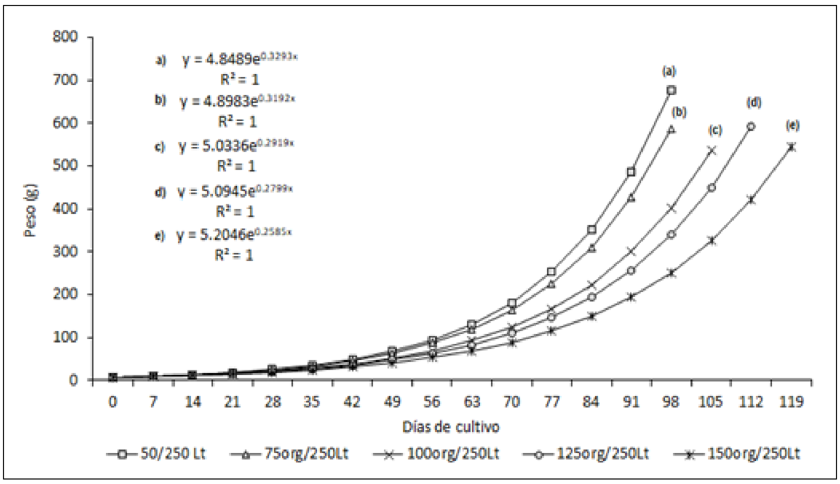

Fig 2: Length and weight growth curves of fish cultured in Biofloc.

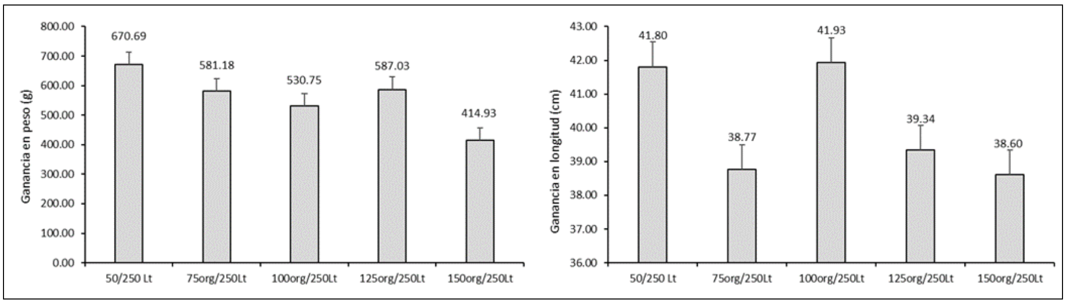

Fig 3: Length and weight gain of the organisms in culture in the experimental treatments.

Figure 3 shows the values of length and weight gain. It can be seen that all four species increased their biometric value.
The highest weight gain of the organisms was observed in the 50 org $250L^{-1}$ treatment with 670.69 g, while the lowest was for the 150 org $250L^{-1}$ treatment with 414.93 g. Regarding the gain in length, the 50 and 100 org $250L^{-1}$ treatments presented the highest value (41.80 and 41.93 cm respectively), not finding significant differences between both results (P<0.05).
The values of Absolute Growth Rate (AGR) and Instantaneous Growth Rate (IGR) are presented in Figure 4.

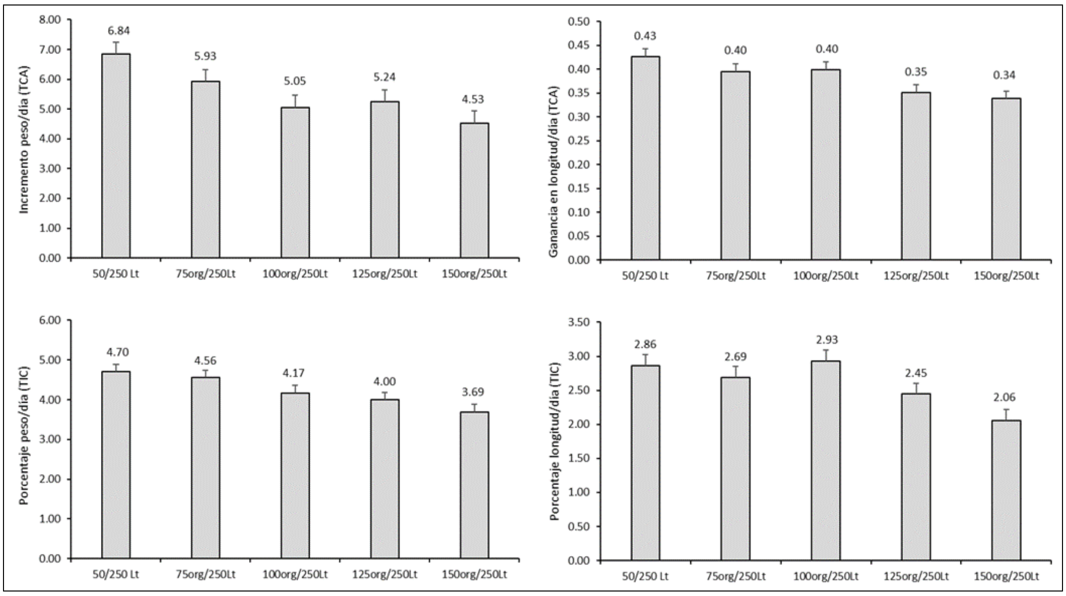

Fig 4: Absolute growth rate (AGR) and instantaneous growth rate (IGR) of the length and weight of organisms cultured with Biofloc.

The highest AGR for weight and length were presented in the 50 org $250L^{-1}$ treatment (4.70 g day^{-1} and 0.43 cm day^{-1} respectively), while the lowest value was observed in the 150 org $250L^{-1}$ treatment with 4.53 g day^{-1} and 0.34 cm day^{-1}. Concerning IGR, the highest value of daily increment was obtained in the 50 and 100 org 250 L^{-1} treatments with 4.70 % day^{-1} for weight and 2.93% day^{-1} respectively. The lowest value was for both cases (weight and length) in the 150 org $250L^{-1}$ treatment with 3.69 % and 2.06 % respectively.
Figure 5 shows the curves of the degree of well-being (KM) of the organisms, where it is observed that the diet and the conditions in Biofloc allow them to keep the length/weight ratio that each species in culture has.

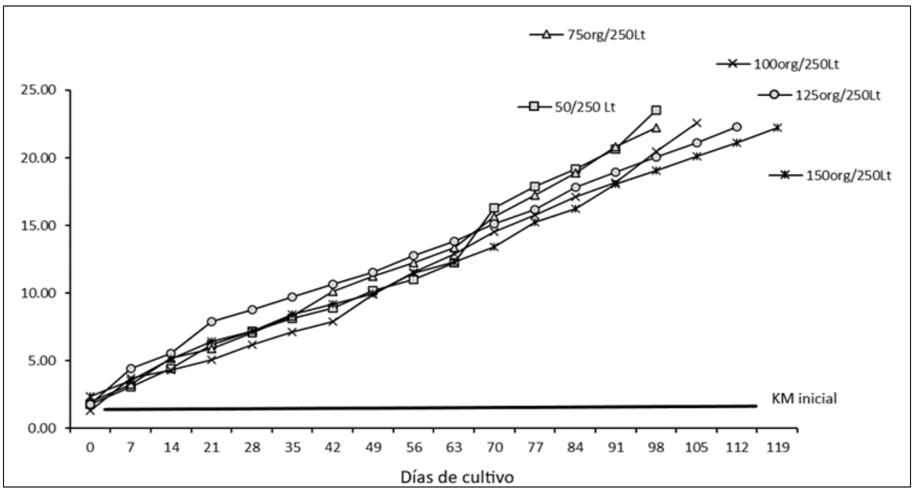

Fig 5: Degree of well-being of organisms cultured with Biofloc under the diets used in each of the treatments.

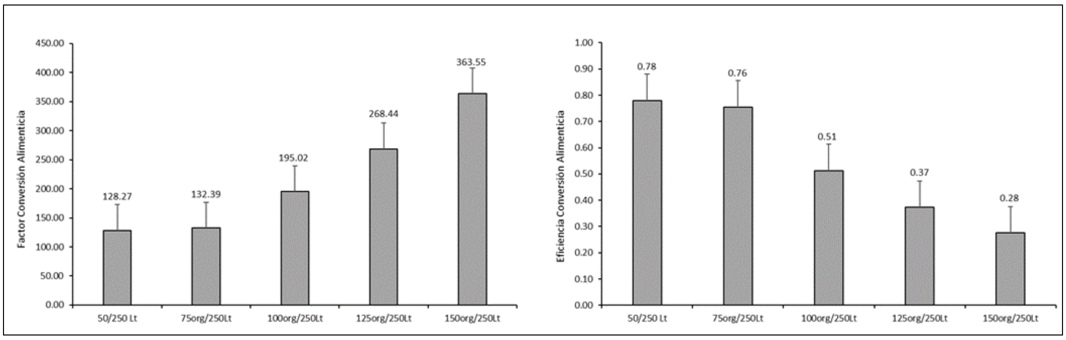

Fig 6: FCA and ECA values of the organisms in the different experimental treatments.

Figure 6 shows the values of Feed Conversion Factor (FCR) and Feed Conversion Efficiency (FCE). As can be seen, the 150 org 250 L^{-1} treatment presented the best FCA value with 363.55 and a better FCE with 0.28. While the lowest value of FCF (128.27) and FCE (0.78) was for the treatment of 50 org 250L^{-1}.

Discussions

Stocking density is important in both Biofloc and conventional tilapia culture systems. The stocking density can affect the growth in weight and length of the cultured fish, but it can also affect the organisms' survival, water quality, and health of the fish [5, 6]. Maintaining a fish culture close to its carrying capacity allows for more efficient management of space, more efficient feed supply, and ultimately maximizing production [7].

Planting fish at low density results in inefficient space utilization and low yields, while planting fish at densities above carrying capacity impairs fish growth performance due to the accumulation of metabolic wastes such as feces, and deterioration of fish social interaction and water quality [8].

Considering the study of Rayhan *et al*., [22] where they identified that stocking density was related to weight gain and mean length of tilapia it could be observed that in this experiment with higher weight were those grown in treatment 50 org 250 L^{-1}, reaching 677.43±0.33 g in only 98 days of experimentation. With this study, it can be suggested that fish weight decreased inversely proportional to the increase in tilapia planting density in the different treatments, which agrees with the results of Ferdous *et al*., [10] and Rayhan *et al*., [22]. However, recent studies have revealed that seeding at high densities could negatively affect the growth performance of fish due to competition for food and may even affect the reproductive development of fish as discussed in the works of Abaho *et al*., [11]; Mengsitu *et al*., [12]; Makori *et al*., [13]; Gindaba *et al*., [14]; Mapenzi & Mmochi[15]. In this experiment, it is observed that survival decreased with increasing population density. This may probably be due to the high stocking density even though the fish were fed with a sufficient amount of food, similar results were also reported by the works of Suresh & Lin [16] and Gibtan *et al*., [17].

Study by Maucieri *et al*.[6] observed that there was a reduction in AAR with increasing stocking density in juvenile tilapia reared in aquaponic systems, similar results are observed in this experiment, as the highest AAR of weight and length were presented in the treatment of 50 org 250 L^{-1} (4.70 g day^{-1} and 0.43 cm day^{-1} respectively), while the lowest value was observed in the treatment of 150 org 250 L^{-1} with 4.53 g day^{-1} and 0.34 cm day^{-1}. The FCA increased with increasing stocking density, as observed in the 150 org 250 L^{-1} treatment, which presented the best FCA value with 363.55 and a better ECA with 0.28 and this could probably be due to the amount of feed supplied in each treatment, a phenomenon also reported by Siddiqui & Al-Harbi [18]. Several studies report a negative effect as stocking density increases on the feed conversion rate of fish in both aquaponic and conventional systems [6, 19]. However, other authors still working in aquaponic systems report that there is no significant change in FCA by changes in the seeding density of the tilapia population [20, 6]. This is because the Biofloc is a conglomerate of nutrient flocs that allows the fish to have food all the time during the experiment.

It should be noted that the number of organisms planted in a conventional tilapia production system or a Biofloc and Aquaponics system will always influence water quality, so the lower the stocking density, the better the water quality in terms of pH, dissolved oxygen, ammonium, nitrite, nitrate, as shown by the work of Suresh & Lin [16] and Widanarni [21]. Regardless of the stocking density used, the measured water quality parameters were within the recommended concentrations.

In general, we can conclude that fish grew better, both in weight and length, at lower stocking densities than at higher ones. However, this does not exclude the possibility of using higher stocking densities, especially if good water quality is maintained and thus obtaining better production in the end.

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