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Castro Mejia Germán
Universidad Autónoma Metropolitana Unidad Xochimilco. Laboratorio de Producción de Alimento Vivo y Biofloc. Depto. El Hombre y su Ambiente. División de Ciencias Biológicas y de la Salud. Calz. del Hueso No. 1100. Col. Villa Quietud. Ciudad de México, 04960, Alcaldía Coyoacán, México

Castro Mejia Jorge
Universidad Autónoma Metropolitana Unidad Xochimilco. Laboratorio de Producción de Alimento Vivo y Biofloc. Depto. El Hombre y su Ambiente. División de Ciencias Biológicas y de la Salud. Calz. del Hueso No. 1100. Col. Villa Quietud. Ciudad de México, 04960, Alcaldía Coyoacán, México

Tinoco Pérez Laura Irlanda
Universidad Autónoma Metropolitana Unidad Xochimilco. Laboratorio de Producción de Alimento Vivo y Biofloc. Depto. El Hombre y su Ambiente. División de Ciencias Biológicas y de la Salud. Calz. del Hueso No. 1100. Col. Villa Quietud. Ciudad de México, 04960, Alcaldía Coyoacán, México

Salvat Navarrete Kenia Alejandra
Universidad Autónoma Metropolitana Unidad Xochimilco. Laboratorio de Producción de Alimento Vivo y Biofloc. Depto. El Hombre y su Ambiente. División de Ciencias Biológicas y de la Salud. Calz. del Hueso No. 1100. Col. Villa Quietud. Ciudad de México, 04960, Alcaldía Coyoacán, México

Corresponding Author:
Castro Mejia Germán
Universidad Autónoma Metropolitana Unidad Xochimilco. Laboratorio de Producción de Alimento Vivo y Biofloc. Depto. El Hombre y su Ambiente. División de Ciencias Biológicas y de la Salud. Calz. del Hueso No. 1100. Col. Villa Quietud. Ciudad de México, 04960, Alcaldía Coyoacán, México

Comparison of length gain, weight, AGR, ICT, KM, and feed efficiency of Pargo UNAM tilapia cultured in Biofloc inoculated with *Scenedesmus* sp and *Pinnularia* sp microalgae

Castro Mejia Germán, Castro Mejia Jorge, Tinoco Pérez Laura Irlanda and Salvat Navarrete Kenia Alejandra

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Abstract
In the present study, the efficiency of the microalgae *Scenedesmus* sp. and *Pinnularia* sp. as a supplement in the diet of tilapia was tested in the growth in size and weight of Pargo tilapia, UNAM, cultured in a Biofloc in 250 L containers, in a period of 120 days. Survival of the organisms in the three treatments ranged from 80-85%. Organisms in the 10 cells x 10^6 treatment obtained the highest weight with 598±0.60 g, although the highest length gain was found in the control treatment with 41.06 cm. The AGR for weight was higher in the 10 cells x 10^6 treatment with 4.74 g day^{-1}, while the highest IGR for length was presented in the control treatment with a 0.33% daily increase. For length, the highest AGR was for the 10 cells x 106 treatment with 4.58 g day^{-1} and the IGR was for the 2.5 cell x 10^6 treatment with 2.59% daily increment. Both FCF and FCE were for the 10 cells x 106 treatment with 0.247 and 404.5%, respectively. The incorporation of microalgae into the system provides nutrients that the Biofloc and bacterial biomass do not incorporate in the diet of the organisms.

Keywords: Snapper, UNAM, biofloc, *Scenedesmus*, *Pinnularia*, tilapia

Introduction
One of the main species produced in aquaculture worldwide is tilapia, due to its great adaptability and rapid growth. As a result, tilapia is the second most important fishery product in world aquaculture after carp production. In Mexico for the year 2020, a tilapia production of 75,290 tons was obtained, a figure below that obtained in 2019 due to the COVID-19 pandemic (FAO, 2021) [1], so it is important to solve, such as production costs and the search for profitable production systems, meet market demands.
In the search for more sustainable and productive strategies for aquaculture, several studies have found the efficiency of tilapia production in Biofloc (Hernández *et al*., 2019; Gaitán *et al*. 2022) [2, 3], since this system provides a mineral and vitamin supplement for the maintenance of high productivity of fish that reach 15 or 18 kg m^3, it also reduces labor and aeration costs, as well as other fixed costs of maintenance and operation of the fish farm.
Although Biofloc solves the problem of the system to be implemented, it is still necessary to implement nutritional additives that magnify weight and size gains in these organisms. Microalgae present high nutritional content with high levels of proteins, carbohydrates, fatty acids, and vitamins (Hernández *et al*., 2019; Gaitán *et al*., 2022) [2, 3]. However, it is important to note that adequate nutritional requirements are difficult to establish because the composition of microalgae can vary with culture conditions and their influence on the organism depends on the age, size, and reproductive condition of the fish, causing variation in their growth in size and weight.
Therefore, the objective of this study is to evaluate the efficacy of Scenedesmus sp. as the main source of proteins and pigments and of *Pinnularia* sp. a brown microalga rich in lipids and carbohydrates, together with the culture of tilapia in a Biofloc system, rich in bacterial biomass and zooplankton.

Material and Methods
Experimental design: Ten fish were placed per 250 L tank, which were 0.80 m high and

0.70 m wide (Fig. 1). The physicochemical parameters were kept constant during the 18 weeks with a temperature of 26 and 27 °C, dissolved oxygen of 6.7, and pH of 8.0 - 8.05. To produce the Biofloc, moringa powder (0.1% total weight of the organisms in culture) was added to the ponds every day and continuous aeration was used to keep the organic material suspended in the water in motion. The experiment lasted 120 days.

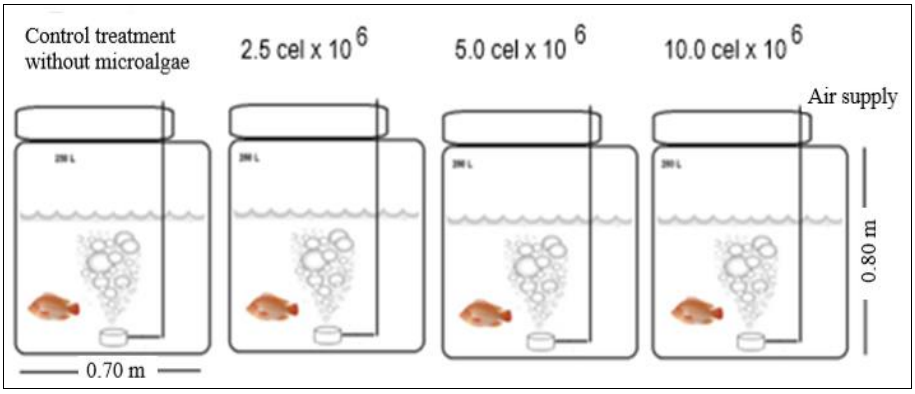

Fig 1: Distribution and capacity of fish containers.

Feeding of the organisms

In all treatments, fish were fed 3-4 mm inert feed (36% protein, 37% carbohydrates, 14% minerals, 8% fat, 5% fat, and 5% fiber). The feed was added to 10% of the total biomass of the organisms divided into two portions per day (9:00 hrs and 16:00 hrs). The treatments used were a) Control treatment; b) 2.5 cells x 10^6; c) 5.0 cells x 10^6; and d) 10.0 cells x 10^6. The microalgae were fed together in an amount of 1 L (500 mL *Scenedesmus* sp./500 mL *Pinnularia* sp.).

Biometry of the organisms

Fish obtained from a farm in the State of Morelos, Mexico were measured and weighed before being introduced to each of the ponds and subsequently every 15 days. These biometrics were performed with the help of a digital Vernier (0.001 precision) and an Ohaus analytical balance (0.001 precision).

Data processing

All values obtained were entered into a database in Excel 2019 to obtain the descriptive analysis. Growth 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)

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

Instantaneous Growth Rate (IGR)

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

Degree of well-being (KM)
KM = Weight * Correlation coefficient (Weight: Length) * Length

Feed conversion factor (FCF)

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

Feed Conversion Efficiency (FCE)

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

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 Control and 10 cells x 10^6 treatments, survival was 80%, while the highest value was 85% for the 5.0 cells x 10^6 treatment, and 82% for the 2.5 cells x 10^6 treatment.
Table 1. Shows the average values of the length and weight of the organisms cultured with Biofloc.
As can be observed, the organisms that obtained the highest weight were those cultivated in the treatment of incorporation of 10.0 cells x 10^6 of both microalgae with 598.53±0.60 g. This value is significantly different ($p<0.05$) from the other treatments. The lowest value (458.30±0.75 g), was found in the treatment of 2.5 cells x 10^6 of both microalgae, being significantly different ($p<0.05$) with the other treatments. It is worth mentioning that the organisms in the control diet reached a higher weight (540.41±0.64 g) than the treatments with 2.5 and 5.0 cells x 10^6. Regarding the length of the fish, the treatment that obtained the highest value was the Control with 44.76±0.13 cm, and the lowest value (29.67±0.26 cm) in the treatment with 5.0 cell x 10^6. In both cases, the difference is significant ($p<0.05$) concerning the other treatments.
Fig. 2 shows the growth curves of the organisms in culture with Biofloc.

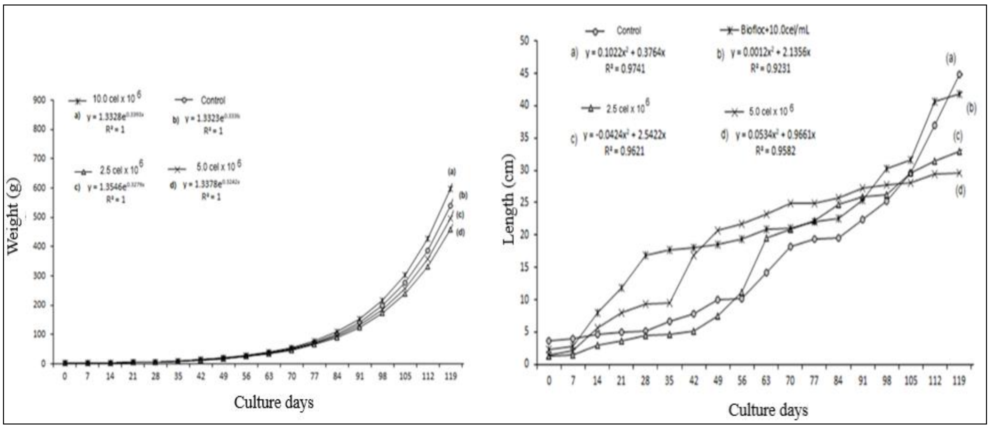

Fig 2: Weight and length growth curves of the organisms in the experimental treatments.

Figure 3 shows the values of height and weight gain.

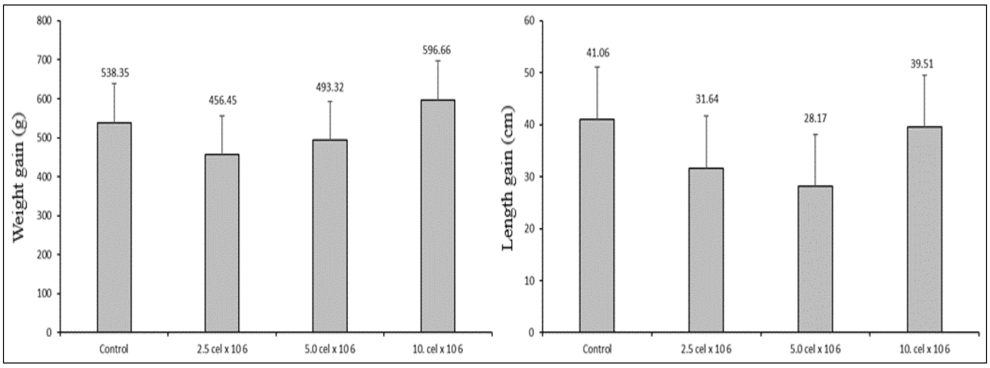

Fig 3: Length and weight gain of the organisms in culture in the experimental treatments.

The highest weight gain of the organisms was observed in the 10 cells x 10^6 org $250L^{-1}$ treatment with 596.66 g and the lowest value was for the 2.5 cells x 10^6 treatment with 456.45 cm. Regarding length, the highest gain was in the Control treatment with 41.06 cm and the lowest gain was in the 5.0 cells x 10^6 treatment with 28.17 cm.

The values of Absolute Growth Rate (AGR) and Instantaneous Growth Rate (IGR) are presented in Figure 4.

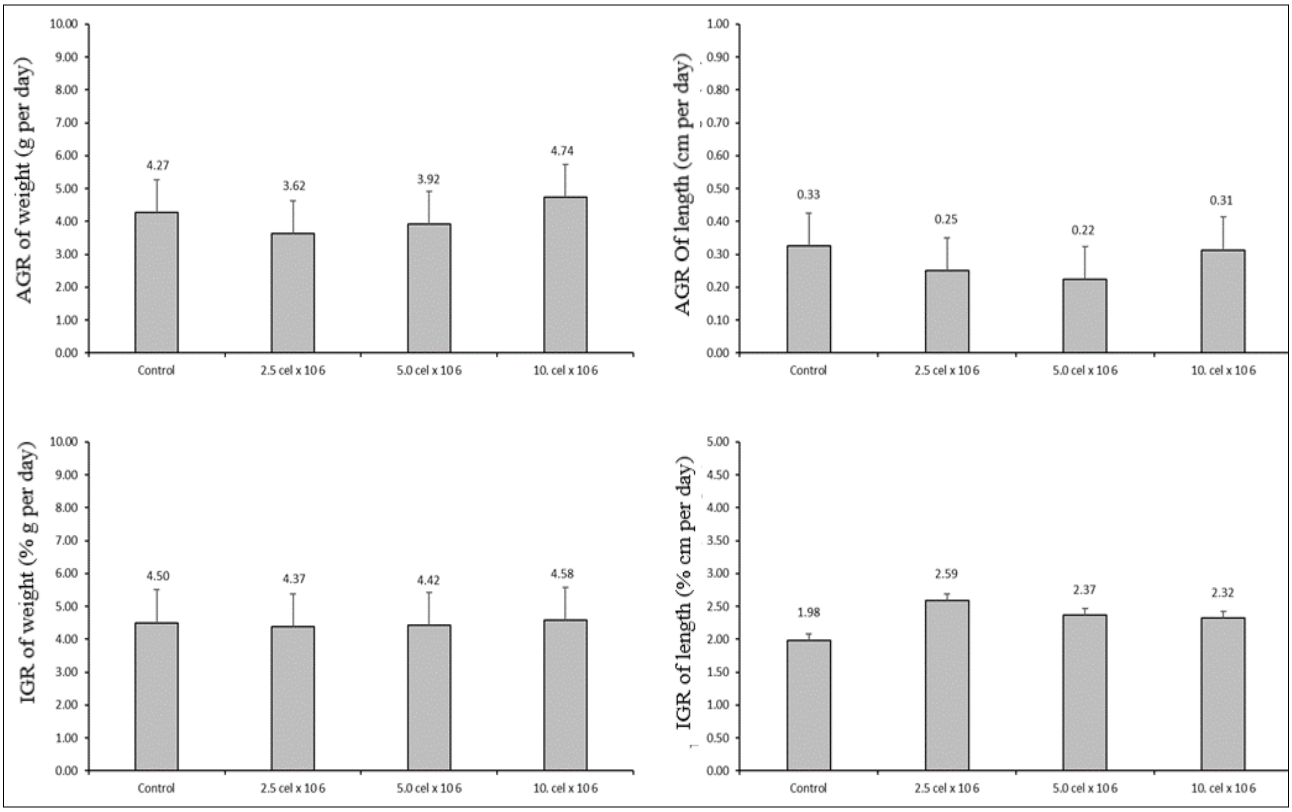

Fig 4: Absolute growth rate (AGR) and instantaneous growth rate (IGR) of the length and weight of organisms cultured with Biofloc

The highest AGR of weight and length were presented in the Control and 10.0 cells x 10^6 treatments (4.27 and 4.74 g day^{-1} and 0.33 and 0.31 cm day^{-1}) since they did not present significant differences between them ($p>0.05$). The same happens with the lowest AGR values in the 2.5 and 5.0 cells x 10^6 treatments (3.62, 3.92 g day^{-1} and 0.25, 0.22 cm day^{-1}).

Regarding IGR, the percentage increase in weight did not show significant differences ($p>0.05$) among treatments, presenting values between 4.37% and 4.58% of daily weight increase. For length, the Control treatment presented the lowest percentage increase with 1.98% daily, presenting significant differences ($p<0.05$) concerning the other three treatments that presented values between 2.32% and 2.59% daily increase.

Figure 5 shows the curves of the degree of well-being (KM) of the organisms, where it is observed that the culture conditions in all treatments allowed the organisms to obtain good growth in length and weight.

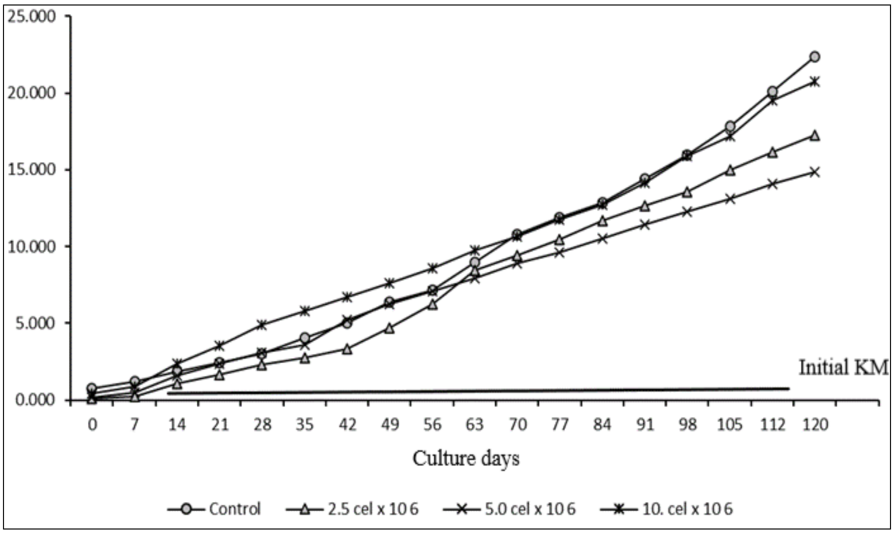

Fig 5: Degree of well-being of organisms cultured with Biofloc under the diets used in each of the treatments

Figure 6 shows the values of Feed Conversion Factor (FCF) and Feed Conversion Efficiency (FCE).

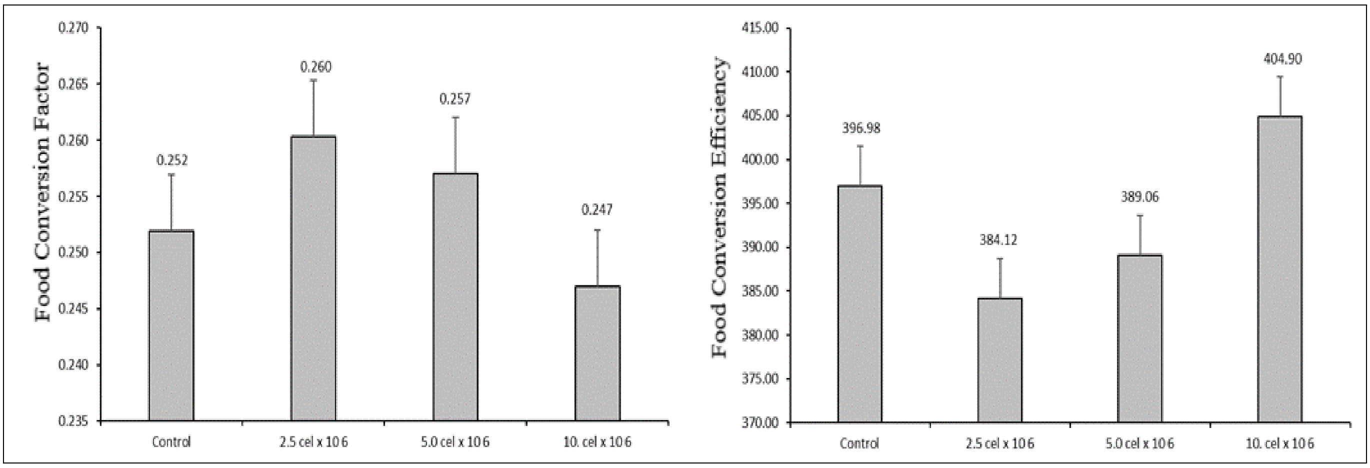

Fig 6: FCF and FCE values of the organisms in the different experimental treatments.

As can be seen, the 10.0 cell x 10^6 treatment was the one that presented the best FCF and FCE (0.247 and 404.90% respectively) and the lowest value was for the 2.5 cell x 10^6 treatment with 0.260 FCF and 384.12% for FCE.

Discussion

The production of tilapia using Biofloc technology has given adequate results in the reduction of water consumption in the system due to the few water replacements needed, due to the production of heterotrophic bacteria that decompose the nitrogenous compounds produced by the same organisms in cultures as well as the unconsumed food supplied due to the production of bioflocs that provide live food made up of microalgae, bacteria, ciliates, and even nematodes that are produced depending on the C:N source supplied to the system (Hernández *et al*. 2019; Gaitán *et al*. 2022) [2, 3]. However, in recent studies, it has been determined that the constituents of the Biofloc do not provide the necessary nutrients for the organisms in culture or these are not in sufficient concentration (proteins, lipids, carbohydrates, vitamins, and minerals) so that the incorporation of food rich in these compounds benefits the growth, in length and weight of organisms such as Tilapia.

The use of microalgae incorporation either in the larval part, as well as in the growth of tilapia, is starting to be a technique implemented to improve survival, welfare, and adequate weight and length growth in individuals (Araujo *et al*., 2016, 2017; Barraza-Guardado *et al*., 2019; Silva *et al*., 2022; Hossain *et al*., 2021; Rashid *et al*., 2022; Amaru and Yujura, 2023; Zaharan *et al*., 2023) [4-11]. Because many of the microalgae have adequate size and an easily digestible cell wall (Amaru and Yujura, 2023) [10], they are rich in both w3 (for freshwater fish) and w6 (for marine water fish) polyunsaturated fatty acids (Barraza-Guardado *et al*., 2019) [6], although these authors mention that the inclusion percentage should not exceed 10% since a palatability problem may be encountered, as well as being careful to use microalgae such as *Isochrysis* sp and *Nannochloropsis* sp due to their high concentration of cellulose in their cell wall and for having polysaccharides that are difficult to digest and for having antinutrient factors that alter the absorption function of enterocytes in the digestive system of fish. However, authors such as Zaharan *et al*. (2023) [11] mention that the microalgae *Nannochloropsis* sp which is rich in w3 fatty acids (20:5w3 and 22:6w3) if only 5% of them are included in the diet, can serve as precursors of growth hormones, have an antioxidant capacity, as well as an immunostimulant, anti-inflammatory and anticarcinogenic capacity due to the estrogens they contain.

Regarding the survival of tilapia by adding microalgae we have that Barraza-Guardado *et al*. (2019) [6] working with the incorporation of *Chlorella*, *Spirulina*, *Isochrysis*, and *Nannochloropsis* found an interval between 87 to 95%, values above those found in this research (80-85%). but in this work incorporating the microalga *Pinnularia* sp, which is rich in lipids and most likely rich in w3 fatty acids, which also contains vitamins, minerals, pigments, and glucans that have immunostimulant, oxidant, and anti-inflammatory properties that confer a better degree of well-being to the fish (Barraza-Guardado *et al*., 2019) [6]. However, using four microalgae with different nutritional compositions of proteins and lipids is not the same as using only two.

Regarding the weight gain of the individuals, it can be observed that tilapia show a greater gain in the treatments in which the microalgae are introduced. For example, Barraza-Guardado *et al*. (2019) [6], mention that the best weight gain in a 53-day culture was with *Nannochloropsis* sp. with 76.3±11.1 g, while Zahran *et al*. (2023) [11], using the same microalgae observed a higher weight gain (38.27±3.03 g) when it is included in a proportion of 10% in the diet and almost 10 g less (28.28±2.32) when only 5% of the microalgae is included. This was done in only two weeks of culture. With this experiment and its treatments with *Scenedesmus* and *Pinnularia* microalgae in 120 days of culture, weight gains of 596.66 g were obtained at a concentration of 10 cells x 10^6 and a difference of more than 100 grams (456.45 g) in the treatment of 2.5 cells x 10^6. It should be noted that the concentration of 5.0 cells x 10^6 was the one that presented the lowest gain in the experiment. Araujo *et al*. (2019) [12] mention that with *Chlorella vulgaris* as a supplement in tilapia culture, they obtained a gain of 21.55±3.61 g at a concentration of 10 cel x10^6 in 63 days of culture.

Concerning the AGR of the weight Zahran *et al*. (2023) [11] obtained values of 1.55±0.13 g day-1 in cultures where 10% of the microalga *Nannochloropsis oculata*, which is a marine species, is incorporated. However, Fernandez *et al*. (2022), who worked with *Nannochloropsis obliquus* at 50% inclusion obtained AGR values between 1.11-1.13 g day^{-1}. Araujo *et al*., (2019) [12], with *Chlorella vulgaris*, obtained an AGR of 0.30-0.34 g day^{-1}. All these experiments present a lower value than those found in this experiment whose highest AGR was found in the treatment of 10 cells x 106 (4.74 g day^{-1}), being the difference in a combination of a green alga (*Scenedesmus* sp.) and a brown alga (*Pinnularia* sp.), which more efficiently provide proteins, carbohydrates, lipids, vitamins, minerals, and pigments for better well-being of the organisms, together with the Biofloc system.

It is important to note that this experiment allows us to lay the foundations for the proper use of the Biofloc system with the incorporation of essential nutrients contained in the microalgae. Araujo *et al*., (2019) [12] mention that many times the lipid content in the Biofloc flocs is only 1 to 5%, so it is necessary to incorporate nutritional components that increase this concentration and that better the use of live food such as microalgae, which many times its lipid content goes from 20 to 50% in its composition. This increase in the concentration of lipids and above all of pigments contained in the microalgae will not only serve for better nutrition of the fish but also the presence of substances that serve as immunostimulants, anti-inflammatory, and oxidants, which intervene for the well-being of the fish and consequently the improvement of the utilization of the essential nutrients for the growth in weight and size.

Sauza *et al*., (2020) [13], mention that the incorporation of nutritional compounds such as microalgae can reduce the stress of the organisms, as well as, that all the components of the artificial diet supplied can be used to the maximum. In addition, it can increase the expression of the genetic potential that each of the cultivated species uses. These authors used the microalgae *Schizochytrium* sp. at 1.2% for its high content of polyunsaturated fatty acids and for its high production of vitamins that allow for regulating the immune system and the integrity of the digestive tissue of the fish. These authors also mention that in addition to this microalgae, *Schizochytrium* sp. increases the fish's resistance to pathogens, since it is known that there are also *Laminaria digitata*, *Ascophyllum nodosum*, *Ulva clathrata*, and *Gracilaria verrucosa*. It allows the intestinal microbiome of *Oncorhynchus mykiss* to stabilize and increases the concentration of essential fatty acids in both *Oreochromis niloticus* and *Ictalurus puntatus*. In this experiment, Sauza *et al*., (2020) [13], obtained organisms of 732.64 g, with a final size between 24.41 and 25.19 cm. These authors mention that the weight and size reached by the organisms in culture is mainly because saccharides (xylose and glucose), together with galactose and mannose, constitute part of the cell membrane of this microalga *Schizochytrium* sp. and therefore contribute strongly to the energetic contribution to the nutrition of the organisms, allowing the growth of heterotrophic bacteria with probiotic capacity that help in the well-being of the fish. In addition, these authors (Sauza *et al*., 2020), found in their study that this microalga can modulate blood cells (Including red blood cells and lymphocytes).

McDonald *et al*., (2020) [14], mention that the use of microalgae in fish culture is an opportunity to incorporate proteins and lipids, naturally, into the elaborated diet of the organisms in culture, allowing the costs of the diet to be reduced and promoting the sustainability of the culture system. Microalgae can be used as prebiotics to obtain substances that stimulate fish health by controlling the intestinal microbiome of the cultured species, as also mentioned by Sauza *et al*., (20202) [13]. McDonal *et al*., (2020) [14], found in their research that growth in tilapia is 100% higher than in the control diet.

Silva *et al*., (2022) [7], worked with *Scenedesmus obliquus* in a tilapia culture of 1.53 g and a density of 522 org m^3 and a salinity of 10 gL^{-1}, in tanks of 1000 L capacity with 800 L, during 62 days of culture, mention that the substitution of up to 50% of the food supplied by the microalgae, in this case *S. obliquus*, allows better growth, benefits the immune system of the fish and can reduce carbon, nitrogen, and phosphorus concentrations in the culture medium. Obliquus allows better growth, benefits the immune system of the fish, and can reduce the concentrations of carbon, nitrogen, and phosphorus in the culture medium. The supply of microalgae in tilapia cultures allows the formation of Biofloc, which allows the nutritional increase of the same flocs by their incorporation into them, increasing the natural consumption of these flocs by the fish and consequently the growth of the fish. These authors mention that the concentration of microalgae should be at 5 mg L^{-1} in the culture. Although *S. obliquus* is rich in protein and lipids, as well as its high concentration of carotenes in the form of lutein, the final weight of the organisms (13.77 g without microalgae; 14.55 g with microalgae); the feed conversion factor (1.97 without microalgae; 2.07 with microalgae), and survival (86% without microalgae; 93% with microalgae) did not show significant differences ($p<0.05$). These authors conclude that an inadequate diet of amino acids, lipids, and vitamins can cause immunosuppression and oxidative stress in fish, so it is necessary to choose the microalgae that meet these requirements and allow fish to produce antioxidant and immunostimulant compounds. These authors mention that it is important to consider the mixture of microalgae such as *Chlorella* sp. that allows them to supply, between them, all the necessary components to obtain a good diet and yields that reach over 100% in fish growth.

Zahran *et al*., (2023) [11], who worked with the microalgae *Nannochloropsis oculata* in dietary incorporation treatments of 5 and 10% during seven weeks of tilapia culture, found that the organisms obtained a final weight of 63.64 g (5%) and 72.27 g (10%), a daily growth rate of 1.25 g day^{-1} and 1.55 g day^{-1}, as well as a conversion factor of 1.36 and 0.76, respectively. *N. oculata* is rich in essential amino acids and rich in polyunsaturated fatty acids (C18, 20:5w3, and 22:6w3), which can be transformed into long-chain fatty acids (C20-C24), which are essential for growth and development. *N. oculata* can stimulate the production of growth hormones and present an immunostimulant efficacy due to the sterols it contains, as well as having anti-inflammatory and anticarcinogenic activity. This microalga is palatable and provides bioenergy to the organism's diet due to the active micro minerals it contains, as well as enzymatic cofactors involved in protein synthesis, antioxidants that help defend against diseases, and anti-inflammatory action. These authors conclude that a treatment that incorporates microalgae can stabilize lipid metabolism, activating lipase activity, and reducing problems with the fatty acids necessary for different functions.

Finally, Huang *et al*., (2023) [15] used the microalgae *Chlorella vulgaris* in concentrations between 0.5 and 2.0% in the diet, with tilapia in tanks of 300 L capacity. This microalgae contains high amounts of Lutein, beta-carotenes, astaxanthins, canthaxanthins, and a growth factor that has been determined as the growth factor of *Chlorella* sp (FCC), which allows fish in culture, whether shrimp, trout, tilapia or catfish, to present high growth rates, better utilization of the food consumed, increased digestive enzyme activity, antioxidant capacity, increased innate immune capacity of the organisms themselves, as well as resistance to diseases. It has also allowed protein and lipid concentrations to increase, as well as modifying the intestinal microbiome of the fish, which protects against pathogens that want to adhere to its walls. In addition, it allows the adhesion of probiotic bacteria that intervene in the nutritional metabolism and some important physiological functions of the animal.

Conclusion

For all of the above, the incorporation of microalgae in cultures, not only of tilapia but also of trout, catfish, ornamental fish, as well as crustaceans is important to be considered either as its incorporation in the dry diet in the form of flour or paste or used as an ingredient for the formation of floccules in the Biofloc system that allows the accumulation of filtering organisms and bacteria that form a biomass density usable as food available at all times. Greatly reducing the costs of feeding and improving the welfare and growth of fish and crustaceans in culture.

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

Day	Experimental treatments							
	Control		2.5 cells x 10^6		5.0 cells x 10^6		10 cells x 10^6	
	Weight	Length	Weight	Length	Weight	Length	Weight	Length
0	1.86±0.08	3.7±0.20	1.85±0.05	1.26±0.21	1.88±0.05	1.50±0.22	1.87±0.06	2.24±0.18
7	2.6±0.77	3.96±0.21	2.56±0.64	1.40±0.19	2.61±0.78	2.15±0.26	2.63±0.63	2.85±0.14
14	3.62±0.62	4.72±0.19	3.54±0.80	2.94±0.20	3.62±0.74	5.72±0.23	3.69±0.66	7.99±0.30
21	5.06±0.78	5.07±0.22	4.89±0.80	3.60±0.26	5.03±0.61	8.08±0.10	5.18±0.75	11.80±0.24
28	7.06±0.69	5.20±0.26	6.77±0.64	4.52±0.10	6.98±0.71	9.27±0.21	7.27±0.67	16.88±0.11
35	9.86±0.64	6.68±0.20	9.36±0.80	4.58±0.16	9.69±0.79	9.50±0.21	10.2±0.71	17.78±0.18
42	13.77±0.78	7.76±0.15	12.95±0.62	5.09±0.22	13.44±0.63	16.96±0.19	14.32±0.71	17.99±0.18
49	19.22±0.63	10.02±0.25	17.9±0.78	7.54±0.19	18.66±0.79	20.75±0.16	20.11±0.68	18.52±0.19
56	26.83±0.61	10.22±0.26	24.76±0.61	11.11±0.11	25.90±0.72	21.72±0.21	28.23±0.70	19.42±0.26
63	37.45±0.67	14.22±0.16	34.24±0.69	19.56±0.16	35.95±0.68	23.21±0.28	39.64±0.64	20.88±0.20
70	52.28±0.80	18.29±0.16	47.36±0.68	20.86±0.14	49.89±0.78	24.91±0.15	55.66±0.60	21.05±0.18
77	72.99±0.60	19.37±0.24	65.50±0.80	22.28±0.17	69.25±0.63	24.96±0.13	78.14±0.66	22.07±0.21
84	101.89±0.62	19.51±0.26	90.58±0.72	24.75±0.23	96.12±0.69	25.82±0.29	109.71±0.74	22.64±0.24
91	142.24±0.76	22.44±0.26	125.27±0.62	25.84±0.27	133.42±0.70	27.21±0.29	154.03±0.65	25.37±0.24
98	198.57±0.79	25.23±0.24	173.25±0.78	26.21±0.13	185.19±0.80	27.84±0.17	216.26±0.80	30.20±0.27
105	277.2±0.72	29.58±0.16	239.61±0.68	29.55±0.28	257.04±0.70	28.16±0.25	303.63±0.71	31.66±0.20
112	386.97±0.72	36.9±0.11	331.38±0.71	31.36±0.19	356.77±0.78	29.4±0.28	426.30±0.78	40.64±0.14
120	540.41±0.64	44.76±0.13	458.30±0.75	32.90±0.23	495.20±0.70	29.67±0.26	598.53±0.60	41.75±0.20

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