USE OF BIOFLOC TECHNOLOGY IN THE PRODUCTION OF THE *Colossoma macropomum* x *Piaractus brachypomus* HYBRID WITH A LOW PROTEIN DIET

UTILIZACIÓN DE LA TECNOLOGÍA DE BIOFLOC EN LA PRODUCCIÓN DEL HÍBRIDO DE *Colossoma macropomum* x *Piaractus brachypomus,* CON UNA DIETA BAJA EN PROTEÍNA

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ABSTRACT

The present study was carried out to observe the performance of the hybrid *Colossoma macropomum* x *Piaractus brachypomus*, when cultured in a Biofloc system. Six hundred fingerling with a mean weight of 3.3 ± 0.4 grams (g), were randomly distributed into six concrete tanks (22 fish/m^3). Three randomly selected tanks were routinely supplemented with molasses, as an extra source of carbohydrate (Mol) and the other three were only given feed (Feed) containing 28% protein, during 154 days. Water quality parameters such as: dissolved oxygen, total ammonia nitrogen, nitrite, nitrate, alkalinity, hardness and pH were in the accepted range for the specie in both treatments. Significance differences in final weigh, growth rate and final density were observed ($P < 0.05$) between treatments. In Mol treatment, fish grew to a final weight of 332.16 ± 79 g, a feed conversion ratio of 1.27 ± 0.06 and a survival rate of 97%. In Feed treatment, fish grew to a final weigh of 290.28 ± 72 g, a feed conversion ratio of 1.31 ± 0.06 and a survival rate of 93%. This work showed that the hybrid of *C. macropomum* x *P. brachypomus* can be grown in biolfoc systems without affecting its production yield.

Key words: Biofloc; *Colossoma macropomum*; *Piaractus brachypomus*; hybrid

RESUMEN

El presente estudio evaluó el desempeño del hibrido de *Colossoma macropomum x Piaractus brachypomus* cultivado en un sistema de Biofloc. Seiscientos alevines con un peso promedio de 3.3 ± 0.4 gramos (g), se distribuyeron al azar en seis tanques de concreto (22 peces/m^3) con un volumen de 4000 litros de agua. Duarte 154 días, tres tanques, seleccionados al azar, fueron suplementados con melaza, como fuente extra de carbohidratos (Mol) y los otros tres solo se les suministró alimento (Feed) que contenía 28% de proteína. Parámetros de la calidad del agua como: oxígeno disuelto, amonio total, nitritos, nitratos, alcalinidad, dureza y pH estuvieron en el rango aceptable para el híbrido de cachama. Se observaron diferencias significativas entre los tratamientos en el peso final, en la tasa de crecimiento y en la densidad final de cultivo ($P < 0.05$). En el tratamiento Mol, los peces crecieron hasta un peso final de 332.16 ± 79 g, una conversión alimenticia de 1.27 ± 0.06 y una tasa de sobrevivencia del 97%. En el tratamiento, Feed, los peces crecieron hasta un peso final de 290.28 ± 72 g, una conversión alimenticia de 1.31 ± 0.06 y tasa de sobrevivencia del 93%. Este trabajo mostró que el hibrido de *C. macropomum* x *P. brachypomus* puede ser producido en sistemas de biofloc sin afectar su rendimiento.

Palabras clave: Biofloc; *Colossoma macropomum*; *Piaractus brachypomus*; híbrido

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INTRODUCTION:

When an increase in production is required by rising density, fish growers face problems such as low concentrations of dissolved oxygen and high concentrations of toxic metabolites (NH_3, NO_2^- and NO_3^-). These metabolites can reach concentrations that could harm the normal development of cultured fish, impairing their growth and health. The advance of Recirculating Aquaculture Systems (RAS) has coped with many of these issues allowing a substantial increase in biomass production, reducing water use and space. However, one of the foes of RAS is their high initial installation and high energy cost which makes them unattractive for investors [4, 19] in developing Countries. In order to tackle these problems researchers have been working with different approaches to design closed systems which reduce the use of expensive biofiltration equipment and energy use, increasing biomass production and water reuse. In some of these systems, commonly named biofloc, the production of high quantities of phytoplankton in the water column is promoted in order to reduce nitrogen levels and produce oxygen (green systems) in others, the heterotrophic bacteria dominate over the phytoplankton and autotrophic bacteria. Biofloc technology (BFT) is based on a high load of heterotrophic bacteria in the water column which is stimulated with a high Carbon/Nitrogen ratio (20:1) and strong aeration [4, 6, 9, 19, 24, 28]. The heterotrophic bacteria assimilates the inorganic nitrogen produced, increasing the biomass that can be utilized by the fish as a supplement protein source [5]. But not all fishes can be grown in this kind of systems as they need to be resistance to low water quality parameters such as high levels of suspended solids and relative high rates of ammonia, nitrogen and nitrite. Other fish characteristics important when selecting the species for a biofloc system are their capacity to filter the flock and use it as a feed source [4, 8, 18, 19]. Among some of the species that could fit these characteristics are the South American fresh water fish: Piaractus brachypomus (Cuvier 1817) [7, 27], *Colossoma macropomum* (Cuvier 1818), *Piaractus mesopotamicus* (Holmberg, 1887) and their hybrids, known with different common names depending upon the country (Cachama blanca, Morocoto, Pacu, Pirapitinga, Cachama negra, Cherna, Gamitana, Tambaqui, Tambacu, Cachamay, Cachamoto and Tambatinga). This fish of the Caracids family are widely distributed in the Orinoco, Amazon River basin [3, 13, 23, 26] and Paraná and Paraguay Rivers, and are commercially important freshwater species, essential for the fisheries as well as the aquaculture economy of Brazil, Bolivia Colombia, Ecuador, Peru, North of Argentina, Paraguay and Venezuela [3, 23, 26, 29]. These fish outstand water with high concentration of suspended solids, low oxygen concentration 3 miligrams (mg) / liters (L) and relatively high concentrations of nitrogen metabolite. C. macropomum and its hybrid also have capacity of filter feed on zooplankton [16]. Traditionally, this fish are grown extensively in earthed ponds with low water exchange at a maximum density of 1 kilograms (kg)/ square meter (m^2), and in intensive way in cages [2, 13]. The objective of this work was to evaluate the behavior of the hybrid of Colossoma macropomum x Piaractus brachypomus grown in a bifloc system, contrasting both water and production parameters (growth, length, food conversion ration), between two treatments, one with an extra source of carbohydrate (Mol) and the other using just feed (Feed) to establish the microbial environment.

MATERIALS AND METHODS:

Location, fish and experimental design

This work was done at the Aquaculture Research Station of the Universidad Centroccidental "Lisandro Alvarado", located in Venezuela at 10°7'3"N and 69°6'48"W, and at 500 meters above sea level (m.a.s.l)(FIG. 1).

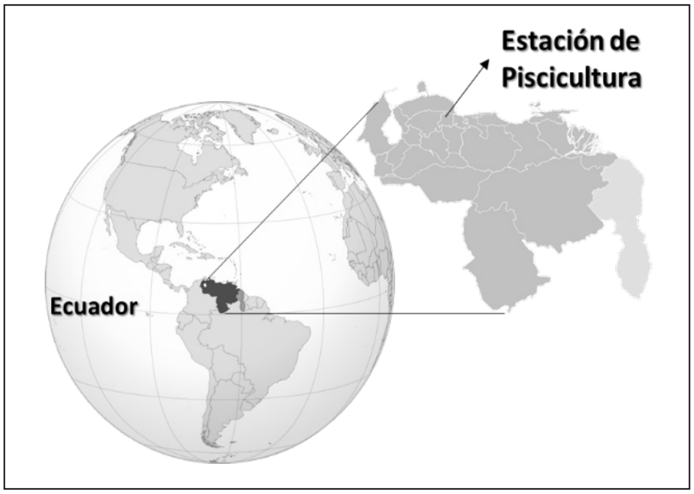

FIGURE 1. This work was carried out at the Aquaculture Research Station of the Universidad Centroccidental "Lisandro Alvarado", located in Venezuela at 10°7'3"N and 69°6'48"W, and at 500 meters above sea level, in the town of Yaritagua in Yaracuy State

Six hundred fingerlings of hybrid (C. macropomum x P. brachypomus) of 40 days (d) post hatching with a mean weigh of 3.3 ± 0.41 grams (g) and a standard length of 4.3 ± 0.03 centimeters (cm), were randomly distributed in six concrete tanks of 6 m^2 (2x3x1 meters), filled with 4.5 m^3 spring water (22.22 fish/ m^3). A 2.5 horse power (HP) regenerative blower (Sweetwater, model S51-AB, Apopka, FL, USA) was used to keep flowing air from the bottom in order to maintain the flock mixing and oxygen levels. Water was added to the tank only to reestablish level after evaporation or lost by removal of the settled sediment. Fish were fed to apparent satiety once a day with a commercial diet containing 28% crude protein (Purina, Puripargo28, Venezuela). Three randomly selected tanks were supplemented with molasses (Mol treatment), as an extra source of carbohydrate in order to keep a C:N ratio of 16.5:1, during week (w) 1 and 3, when the ammonia concentration started to rise during w 5, and then once a w until the end of the experiment. The other three tanks were only given feed (Feed treatment). Calculation of carbon nitrogen ratio was based on feed with 28% crude protein added to the system, assuming that protein is 15.5% nitrogen (N); approximately 75% of total N in feed ends up in water and 50% of feed material is carbon [4]. Molasses used contained about 20% weight/weight (W/W) carbon. Production rate of Total Ammonia Nitrogen (PTAN) was calculated by PTAN = Feed rate (g/d) x Percentage of protein in the feed x 0.15.

Water quality parameters

Dissolved oxygen (DO) data was obtained with a digital oximeter YSI, model 85 (YSI Inc., Yellow Spring, OH, USA) and

TABLE I

MEANS (± SD), MINIMUM/MAXIMUM AND P VALUES OF PHYSICAL-CHEMICAL PARAMETERS OBTAINED DURING 154 DAYS OF CULTURE OF THE HYBRID *C. macropomum* X *P. brachypomus*

Parameter	Mol			Feed			
	Mean ± SD	Min	Max	Mean ± SD	Min	Max	p
Alkalinity (mg/L)	118.3±24.89	66.00	150.00	96.60±27.70	47.30	149.30	0.01
pH	8.10±0.72	6.40	9.20	8.28±0.58	6.54	9.79	0.33
Hardness (mg/L)	534.00±143.99	64.00	754.70	437.90±116.59	70.70	613.30	0.02
DO (mg/L)	6.98±0.93	5.88	8.97	7.12±0.86	5.88	8.97	0.61
Ammonia (mg/L)	0.29±0.15	0.11	0.82	0.23±0.10	0.11	0.59	0.13
Nitrite (mg/L)	0.61±0.48	0.04	1.64	0.55±0.36	0.05	1.39	0.61
Nitrate (mg/L)	19.30±22.58	0.71	62.17	19.87± 22.70	0.76	64.94	0.94
Temperature (C)	26.47±0.74	22.86	30.71	26.53±0.74	22.86	33.17	0.78
Chlorophyll-a (mg/L)	3.42±1.93	0.30	8.39	3.21±1.64	0.40	5.48	1.64
Turbidity (NTU)	195.1±122.73	7.70	527.00	213.70±117.06	13.20	542.90	0.57
TSS (mg/L)*	777.07±425.74	180.00	1350.00	762.86±383.97	280.00	1300.00	
Daily water use (L/day)	13.70±0.73 (0.30%)				13.24±0.19 (0.29%)		

(NTU) Nephelometric Turbidity Units

*data was taken only during week 11,12,16,20 and 21

pH with a field pH-meter (pHtestr 3, Oakton Instruments, Vernon Hills, IL, USA), both parameters were obtained daily at 1,200 hours (h) during the course of the experiment. Water temperature was measured every h with a data logger submerged at 15 cm below surface (Onset Computer Co., Bourne, MA, USA). Total ammonia, nitrite, nitrates and turbidity were measured weekly following Alberro et al. [1] and alkalinity, hardness and chlorophyll-a following the Standard methods for water and wastewater examination [10], also once a w during the course of the experiment. The methods that used a colorimetric approach were quantified using a spectrophotometer model Aquamate (Thermo Electrón Co., Cambridge, UK). Total suspended solids (TSS) were measured from w 11 throughout w 21 as described by Alberro et al. [1]. Daily water used was calculated by estimating all the water added to each tank, in order to reestablish lost for evaporation and water removed with settable sediments, and divided by the numbers of d that the trial lasted. The water added by rain fall was not calculated.

Production parameters

Monthly, 20% of fish were captured from each tank, individually weighed using a field digital balance (Ohaus CO, model CS5000, Parsippany, NJ, USA) and the standard length measured using an ictiometer (Aquatic Biothecnology, model iK2, Cádiz, Spain）in order to get production parameters. The following formulas were used to obtain; Weight gain (WG =final weight − initial weight); Daily growth rate = final weight – initial weight/days; survival rate = remained fish x 100/ initial fish number, Food convention ration (FCR) = feed consumption/weight gain and final biomass (Biomass).

Statistical analysis

Results were expressed as mean ±standard deviation. Significant differences among means from the treatments were established through analyses of variance (ANOVA) and Tukey test used or post hoc mean comparisons using the statistic program PAST 2007 [17]. Differences were considered significant at $P< 0.05$.

RESULTS AND DISCUSSION

The use of BFT has been steadily growing all around the globe mostly to grow shrimp and tilapia because of the many benefits it has, from improving water used to reducing cost in feed. In this work, it show that the neotropical fish C. macropomum x P. brachypomus hybrid could be a good candidate to be grown using this technology. This fish is extensively grown in many South American Countries playing an important part in aquaculture; however, its production is achieved mainly in earthen ponds in semi-intensive systems

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Water quality parameters

Overall, all water quality parameters measured during the trial stayed in the safe range for the specie (TABLE I) [15].

After w 11, it can be observed that the alkalinity and pH went down in both settings. Addition of bicarbonate was necessary after w 18 in both systems to prevent the alkalinity from decreasing below 40 miligrams (mg) / Liters (L), as alkalinity concentrations of approximated 120 mg/L are required to keep a healthy nitrifying bacteria colonies which, in some biofloc systems form and important part of the inorganic nitrogen reduction capacity. Although nitrifyers are responsible for the majority of inorganic carbon removal (355 g of alkalinity as $CaCO_3$ kilograms (kg) $^{-1}$ of feed)[11, 12] the heterotrophic bacteria also utilize some inorganic carbon sources. The addition of water to keep evaporation lost and the characteristic of the tanks (concrete) were enough for keeping the amount of inorganic carbon needed by the autotrophic and heterotrophic bacteria, required to convert the small amount of nitrogen added with the feed in the first w of the experiment (FIG. 2).

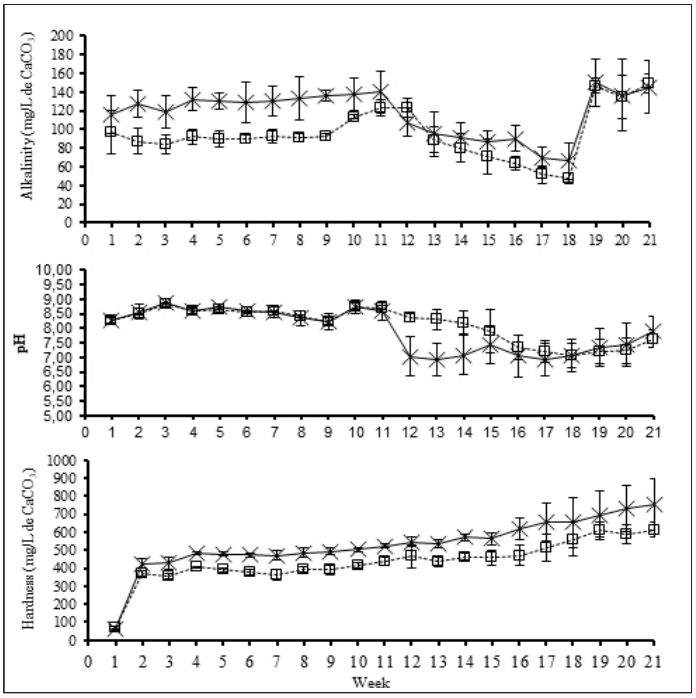

FIGURE 2. ALKALINITY, pH AND HARDNESS (MEAN± SD; N = 3) IN THE WATER OF TWO BIOFLOC SYSTEMS WHERE HYBRID, C. macropomum X P. brachypomus WERE CULTURED OVER 154 DAYS. One treatment had extra source of carbon (Mol)(crosses) and the other one only feed (open squares)

However, when an important population of autotrophic bacteria established after w 7 (FIG. 3) and the increase on feeding triggered heterotrophic bacterial growth, the alkalinity started to fall as well as the pH, this was stopped by adding bicarbonate to the water.

Hardness gradually increased during the trial in both treatment, showing signals of calcium accumulation probably due to the feed and the molasses added to the tanks (TABLE I, FIG. 3).

Inorganic N profiles from both treatments did not show significant differences but exhibited a sequential accumulation of TAN followed by nitrite and nitrate. Such profiles are common during the start-up of biofilters in recirculating aquaculture systems [22, 32] and are characteristics of nitrification [30]. TAN levels peaked in both treatments at w 7, followed by an increase in nitrite concentration (w 8) and accumulation of nitrate, showing the establishment of the nitrification process. This behavior has been seen in many experiments working in different intensively well-mixed production systems with shrimp [22, 31] or tilapia [25].

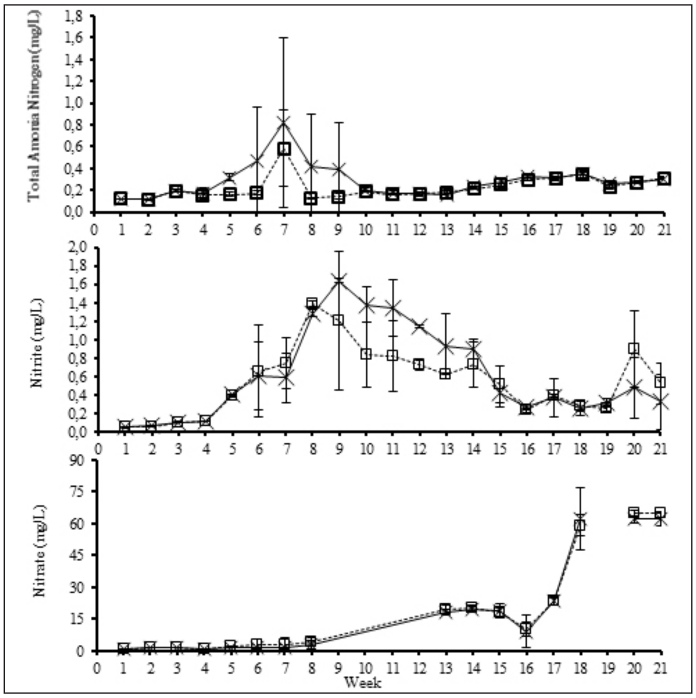

FIGURE 3. TOTAL AMMONIA NITROGEN (TOP), NITRITE-NITROGEN (MIDDLE) AND NITRATE CONCENTRATIONS (BOTTOM)(MEAN± SD; N = 3) IN THE WATER OF TWO BIOFLOC SYSTEMS WHERE HYBRID OF C. macropomum X P. brachypomus. One treatment had molasses as extra source of carbon (crosses) and the other one only feed (open squares)

Usually it takes about 2 w for the system to evolve a steady state [4], however, in the present study it took about 5 to 6 w (FIG. 2) in order to produce enough bacteria to star the nitrification process that begins with the oxidation of the ammonia. This process depends upon different parameters such as dissolved oxygen concentration, organic matter, temperature, pH, alkalinity, salinity and substrate. In the present study the amount of feed added in the first w was small and the amount of ammonia produced was probably assimilated mainly by the phytoplankton, because when fish biomass is below 0.5 kg/m^2, ponds are dominated by algae activity, although, bacteria are also present [4]. Nitrification, and assimilation by heterotrophic bacteria and phytoplankton, were both responsible for the effective control of inorganic nitrogen concentrations in the experiment as can be deduced in FIG. 4, were concentrations of accumulated TAN were lower than the PTAN during the course of the experiment.

The presence of phytoplankton in both treatments was witnessed by the color of the water (data not shown) as well as by the concentration of chlorophyll-a (FIG. 5), however, between w 9 and 10, water in tanks of the Mol treatment underwent an evident change in color from dark green to dark brown, corresponding with a change in feeding rate from 25 g/m d^{-1} to an average of 46.65 g/m d^{-1} and also with the increase of TSS. Although a similar change in the feeding rate took place in the Feed treatment, it stayed dark green during the rest of the experiment. Nitrate accumulation product of the nitrification process reached

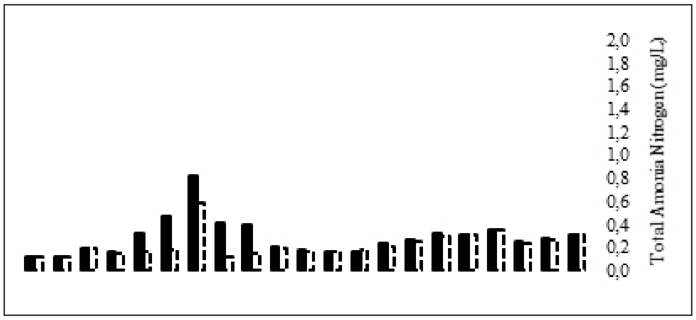

FIGURE 4. COMPARISON BETWEEN THE PTNA (LINES) AND THE ACTUAL CONCENTRATION OF TAN (BARS) IN THE SYSTEMS. PTAN = FXPCX0.155 G

up to 65 mg/L at the end of the experiment, however, unlike ammonia and nitrite, nitrate is relatively non-toxic to fish and the concentrations observed here was not a threat to the fish. The DO concentrations were above the ideal (4.0 mg/L) during the course of the experiment for the specie in both treatments and did not show significant differences among them (P = 0.61)(TABLE II).

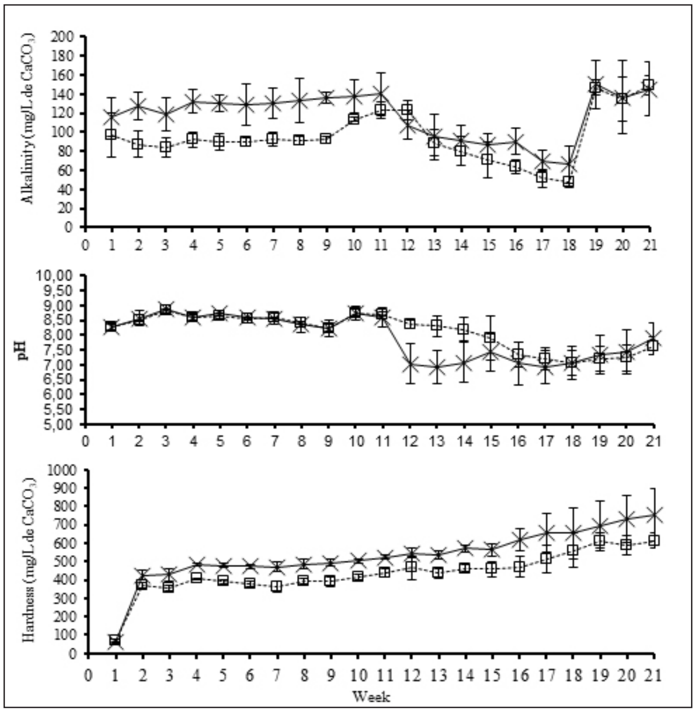

FIGURE 5. DISSOLVED OXYGEN AND CHLOROPHYLL CONCENTRATION (MEAN± SD; N = 3) IN THE WATER OF TWO BIOFLOC SYSTEMS WHERE HYBRID, C. MACROPOMUM X P. BRACHYPOMUS WERE CULTURED OVER 154 DAYS. A treatment had molasses as extra source of carbon (Mol) (crosses) and the other one only feed (open squares)

However, depending in the phase of the experiment, DO concentrations had variations that can be attributed to the phytoplankton population and to the increase of the biomass. In the first 7 w, DO fluctuations in both treatments (FIG. 5), were attributed to environmental changes, such as cloudiness. At the altitude and the temperatures at which the Station is located, water saturates oxygen at around 7.30 mg/L, nevertheless, concentrations of 9.0 mg/L were seen due to photosynthetic production (FIG. 5). After w 7, the observed decrease in DO concentrations were attributed to the increase of the biomass (fish plus bifloc) (FIG. 5 and 6) which surge oxygen consumption; however, aeration was capable of keeping oxygen concentrations above critical level during the entire experiment.

Production parameters

Fish grown with an extra source of carbon (Mol treatment) gained 13% more weight and had a better yield than the ones cultured only with feed (TABLE II, FIG. 6).

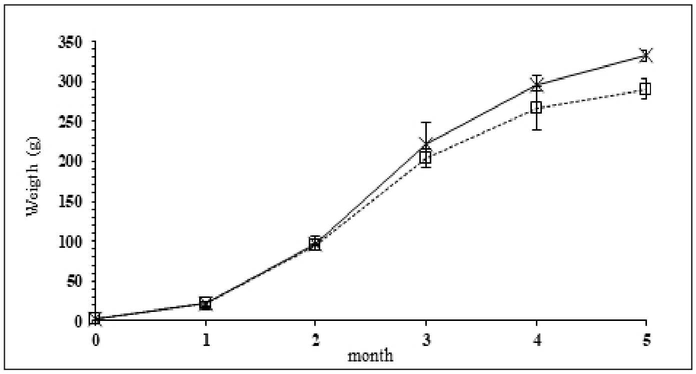

FIGURE 6. GROWTH OF C. macropomum X P. brachypomus (MEAN± SD; N = 3) CULTURED OVER 154 DAYS IN TWO BIOFLOC SYSTEMS. One treatment had molasses as extra source of carbon (Mol)(crosses) and the other one only feed (open squares)

Some evidences confirm that some fish cultivated in biofloc systems such as tilapia and carp, can utilize the bacteria aggregated as an extra source of protein, reducing formulated feed use up to 20% [4, 5]. Although, in this work the capacity of the hybrid on feeding in biofloc was not assessed, this fish has the ability to filter feed on zooplankton inherited from the C. macropomum [16], ability that could help explain the yields observed. The FCR in this work (FCR = 1.27)(TABLE II), was lower than the one found in tilapia grown in biofloc systems [6, 24, 33] or other experience with P brachypomus grown in a heterotrophic systems [27], or C. macropomum in semi-intensive conditions or in cages [14]. Fish did not stop growing during the experiment (FIG. 5), but in the last three months, a reduction of the growth rate was seen linked to a reduction of food intake (data not show), probably affected by the high quantity of TSS observed (1,300 mg/L), which were over the safe range for some fishes cultured in Biofloc (200 to 400 mg/L)[5, 6, 19]. Slow growth has been associated to high suspended solid concentrations in shrimp and tilapia cultures [20, 28, 32] as high concentration of suspended solids could negatively affect fish by clogging gills and producing lesions on them as was shown by Hattem *et al*. [21]
Both treatments worked with an average water exchange of 13.5 L/d, which meant 1 kg of fish per each 80 L of water used. This evidences a better use of the resource when compared with traditional earthed ponds culture, where approximately 21,000 L of water are used to produce 1 kg of fish [32].

TABLE II.
PRODUCTION CHARACTERISTICS OF HYBRID (*ColossomA macropomum* X *Piaractus brachypomus*)

Parameter	Mol	Feed	P
Fish number	300	300	
Initial weigh (g)	3.27±0.42	3.29±0.40	1.000
Final weigh (g)	332.16±79.45	290.28±71.70	0.020
Growth rate (g/d)	2.14±0.05	1.86±0.08	0.015
Final density (kg/m^3)	7.14±0.10	6.02±0.26	0.008
FCR	1.27±0.06	1.31±0.06	0.315
	385.87±8	383±5	
Feeding range (g)	4,044.64±527	3,500±60	
Survival (%)	97±2.1	93±2.9	0.180

CONCLUSIONS

The results in this work showed that the neotropical fish C. macropomum x P. brachypomus hybrid is a candidate to be grown in biofloc systems. Fish in the setting with extra source of carbon (Mol) showed better yield, however feed with relatively low protein content (28% Crude Protein) seems to be enough to start-up the biofloc system and keep a healthy combination of heterotrophic an autotrophic organisms. Further research will need to address the capacity of the hybrid to filter feed in the bifloc and how this could be improving fish performances and also which could be the maximum safe concentration of Total Suspended Solids (TSS) for the specie.

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