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Application of Biofloc Technology to the Production of *Andinoacara rivulatus* Fry using Different Carbon Sources and Protein Regimes

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ABSTRACT

Andinoacara rivulatus is a freshwater aquatic species native to South America with a high consumption rate in Ecuador. Despite its high consumption demand, scientific production related to the production systems and proximal composition of this species is limited. The present study evaluated the effect of Biofloc technology on water quality, the growth of old bluefish and the final quality of the flocs. For this purpose, seven treatments were applied with three levels of crude protein (24, 30, 35%) and two carbon sources (molasses and corn starch). The treatments were carried out in glass aquaria (50x34x34cm) with 57L capacity, under a factorial design in triplicate, including a control without carbon source. The results indicated that the treatment with molasses and 35% protein (TM-3) presented the best values in water quality parameters, such as lower biochemical oxygen demand (9.40 ± 0.11mg/ L) and lower levels of total ammonia nitrogen (0.97 ± 0.07mg/ L), suggesting higher microbial efficiency. In addition, TM-3 showed the highest specific growth rate (SGR), the highest protein efficiency index (PEI), and the highest crude protein content in the flocs (39.57 ± 1.18%). These results indicate that molasses is an effective carbon source for improving both water quality and zootechnical performance of *A. rivulatus*. In comparison, starch treatments also improved TCE, but to a lesser extent. The control presented the worst results in all parameters evaluated. Regarding floc analysis, molasses treatments, particularly TM-3, presented a higher crude protein (39.57 ± 1.18%) and crude fat (2.55 ± 0.27%) content, which makes them more suitable as a supplementary food source for fish. This superior composition of the flocs suggests a higher nutritional quality, favoring the zootechnical performance of *Andinoacara rivulatus*.

INTRODUCTION

Andinoacara rivulatus, commonly known as vieja azul, is a freshwater aquatic species native to South America, which naturally inhabits mainly rivers and slopes of the Pacific. In Ecuador, its natural distribution is from Esmeraldas to Huaquillas along the Pacific Ocean coast. This species, like others of aquaculture interest, has an omnivorous

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reproductive behavior and is characterized by depositing its eggs on immovable surfaces. However, it has not been observed that they provide oral shelter to their larvae or free-swimming young. Despite its high demand in consumption, scientific production related to the production systems and proximal composition of this species is limited **(González *et al*., 2016)**.

In many countries, the predominant method for fish production is the semi-intensive system, whose main limitations are the high requirement for continuous water replacement and the waste of large quantities of expensive feed. In search of improvements in the optimization of water quality management, new technologies have been proposed in the field of aquaculture, focused on reducing the dependence on constant water replacement and feed utilization. Biofloc technology (BFT) is an aquaculture production system that operates efficiently with minimal or no water exchange **(Zablon *et al*., 2022)**. This system is based on microbial aggregates, which play a key role in controlling toxic nitrogenous compounds, providing naturally available live food in the form of floc particles and strengthening disease resistance **(Durigon *et al*. 2020)**.

Bioflocs, or microbial aggregates, are made up of an inert portion, comprising food residues, fecal wastes and organic matter, and a living portion composed of heterotrophic bacteria, chemoautotrophic bacteria, phytoplankton, protozoa, nematodes, copepods and other microorganisms **(Raza *et al*., 2024)**. To stimulate microbial activity, it is necessary to ensure a balance in the carbon-nitrogen ratio and to guarantee adequate aeration. The structures function as a continuous source of natural food of high nutritional value, contributing to the development of fish in aquaculture systems. In addition, the efficacy of biofloc meal as a protein supplement in diets has been widely recognized **(Crab *et al*., 2007; Álvarez *et al*., 2023)**.

Biofloc systems in combination with conventional feeding have been analyzed in several species, reporting benefits in the increase of growth, fattening stage, larval development, fry production and broodstock formation in different tilapia stocking densities **(García *et al*., 2019)**. In addition, its implementation has been linked to the improvement in the carbon-nitrogen ratio, the efficient use of various carbon sources and its integration with aquaponic systems. In the specific case of *A. rivulatus* cultivation, optimal protein levels have not yet been precisely defined. Therefore, exploring these variables is important to optimize zootechnical performance of the old blue in an efficient and sustainable manner **(Brol *et al*., 2017; Espinoza *et al*., 2019)**.

The objective of this research was to analyze the effect of Biofloc technology on the regeneration of water quality in an aquaculture system integrating *A. rivulatus* fry. In addition, the interaction between different protein regimes and carbon sources in the improvement of zootechnical performance parameters and proximal characteristics of the flocs at the end of the experimental study was evaluated.

MATERIALS AND METHODS

Selection of fingerlings of *Andinoacara rivulatus*

The experimental research was developed during 12 weeks in the private property “Vladimir”, located in the city of San Isidro, province of Manabí, Ecuador. For the study, we used the blue old alevins selected under the criterion of size, with a length between 2.50 and 3.00cm, all free of any physical anomaly and visible diseases. The animals were acclimatized for one week until the beginning of the experiment.

Experimental design and conditions

A Biofloc experimental model was designed based on seven treatments in triplicate, one of them as a control treatment. Fifteen glass aquaria measuring 50x34x34cm, with a capacity of 57L, were used for the experiment.

The aquaculture systems were organized following a factorial design (crude protein and carbon source as factors), consisting of three levels of crude protein (24, 30, 35%) and two carbon sources (sugar cane molasses, corn starch). All the commercial feedstuffs used had a uniform composition: 5.0% crude fat, 5.0% crude fiber, 8.0% ash and 11.0% moisture. Details of the specific formulation of each treatment are described in Table (1).

Table 1. Specific formulation of the experimental treatments

TT	FC	PC (%)
TC-0	--	35%
TM-1	ME	24.0
TM-2	ME	30.0
TM-3	ME	35.0
TA-4	AM	24.0
TA-5	AM	30.0
TA-6	AM	35.0

Note. The following nomenclature was used to abbreviate the terms:TT= treatment, FC= carbon source, PC= crude protein, ME= molasses, AM= corn starch.

Each aquaculture system was initially inoculated with 5L of biofloc from a previous old blue culture. No water replacement was performed. For the development and maintenance of this community, 50 bluegill fry were planted for each system. The carbon-to-nitrogen ratio (C/N) was adjusted to a ratio of 20:1, following the procedures described by **Avnimelech (2009)**, and its calculation and maintenance were performed biweekly throughout the study.

Additionally, in accordance with the recommendations of **Karunaarachchi *et al*. (2018)**, aquatic organisms were fed twice daily, at 9:00 am and 4:30 pm. The daily ration corresponded to 5% of body weight, with adjustments made weekly to note changes in the biomass of the organisms.

In relation to aeration and water movement, additional aeration was provided to all aquaria using air diffuser stones connected to a centralized pump.

Water analytical procedure

Six sampling sites were established for each aquaponic system, and aliquots of water were collected at three specific stages: at the beginning of the experiment and at the end of the experiment.

Water quality was monitored using electronic equipment to measure parameters such as dissolved oxygen, biochemical oxygen demand, total ammonia nitrogen, total nitrogen, electrical conductivity and phosphates **(APHA, 2005)**.

Zootechnical performance parameters

Representative biometrics were performed on 30 fish per aquarium to evaluate body weight and total length. Additionally, water samples from the aquariums were analyzed using an optical microscope, with observations made in triplicate and using Lugol's solution to determine the density of microorganisms present as live food in the system. Systematic recording of total food consumption in the aquaria was also carried out during the entire experimental period **(Zablon *et al*., 2022)**. Zootechnical performance parameters were calculated as follows:

1. Specific growth rate (SGR)

The percentage increase in weight of the weights was measured, considering the relative change in weight during the growing period. It was calculated using the formula **(Weatherley, 1972)**:

$$\mathrm{SGR} = \frac{\ln \mathrm{W_t} - \ln \mathrm{W_0}}{\mathrm{t}} \times 100$$

Where, W_t is the final weight, W_0 is the initial weight and *t* is the length of the growing period in days.

2. Protein efficiency index (PER)

This index made it possible to measure the efficiency with which the fish converted the protein consumed into weight gain. The formula used was **(Bagenal, 1978):**

$$PER = \frac{W_t - W_0}{P_c}$$

Where, $W_t - W_0$ corresponds to the increase in fish weight and P_c refers to the total amount of protein ingested by the fish.

3. Feed conversion ratio (FCR)

The efficiency with which the fish converted the supplied balanced feed into biomass was evaluated. The parameter was calculated using the formula of **Cala (2021)**:

$$FCR = \frac{A_c}{W_t - W_0}$$

Where, A_c is the total amount of feed ingested during culture and $W_t - W_0$ corresponds to the increase in fish weight.

Proximal floc analysis

Floc samples were collected monthly using an 80µm mesh. Subsequently, moisture was removed from the samples by using an oven for 1 hour at a temperature of 35ºC. Finally, the samples were packaged, labeled and stored under refrigerated conditions for further processing at the Equahydrolysates Research and Development Laboratory, where crude protein, ash content, crude fiber and crude fat were quantified **(AOAC, 1990)**.

Statistical analysis

The evaluation of the experimental results was carried out by means of a one-way analysis of variance (ANOVA). In addition, Tukey's multiple comparisons of means test was applied using a significance level set at $P < 0.05$.

RESULTS AND DISCUSSION

1. Water analysis procedure

During the experiment, the fish showed growth, with no mortality reported. The water quality parameters corresponding to each aquaculture system are described in Table (2). The analysis of the parameters was carried out according to the reference values of **Boyd and Massaut (1999)**.

Table 2. Water quality parameters in experimental treatments

Parameters	Treatments						
	TC-0	**TM-1**	**TM-2**	**TM-3**	**TA-4**	**TA-5**	**TA-6**
OD (mg/L)	4.77 ± 0.14	5.12 ± 0.15	5.38 ± 0.15	5.49 ± 0.11	4.99 ± 0.13	5.06 ± 0.11	5.11 ± 0.12
DBO (mg/L)	12.49 ± 1.20	10.38 ± 1.14	11.77 ± 0.14	9.40 ± 0.11	10.85 ± 1.02	10.55 ± 0.88	10.20 ± 0.78
TAN (mg/L)	1.34 ± 0.09	1.03 ± 0.07	0.97 ± 0.06	0.97 ± 0.07	1.17 ± 0.08	1.14 ± 0.06	1.03 ± 0.10
CE (μScm^{-1})	164.11 ± 2.29	171.52 ± 1.63	174.92 ± 1.14	178.62 ± 0.87	167.22 ± 1.15	177.72 ± 1.36	173.01 ± 1.71

TDS (mg/L)	101.17 ± 2.18	107.26 ± 1.58	109.28 ± 1.41	112.17 ± 1.08	107.42 ± 1.16	122.34 ± 1.21	118.93 ± 2.06
NH_3 (ppm)	0.38 ± 0.04	0.29 ± 0.03	0.21 ± 0.02	0.18 ± 0.02	0.36 ± 0.04	0.27 ± 0.07	0.35 ± 0.02
NO_2^- (ppm)	0.09 ± 0.02	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.02	0.06 ± 0.01	0.04 ± 0.02	0.05 ± 0.02
NO_3^- (ppm)	1.66 ± 0.16	2.20 ± 0.14	2.45 ± 0.12	2.61 ± 0.11	2.10 ± 0.13	2.30 ± 0.09	1.79 ± 0.24

Note. The following nomenclatura was used to abbreviate the terms: OD= disolved oxygen, DBO: biochemical oxygen demand, TAN= total ammonia nitrogen, NT= total nitrogen, CE= electrical conductivity, TDS= total dissolved solids.

In general terms, all the treatments evaluated showed parameters within the permissible ranges of water quality for aquaculture systems. DO levels were higher in the treatments that used molasses and corn starch as carbon sources, particularly TM-3. This finding suggests that the inclusion of external carbon sources favors oxygenation of the systems, promoting greater microbial activity.

The biochemical oxygen demand was lower in TM-3 (9.40 ± 0.11mg/ L), which reflects a lower amount of biodegradable organic matter in the system, probably due to a higher efficiency of the microorganisms in the use of molasses. In contrast, the control presented the highest value (12.49 ± 1.20mg/ L), suggesting accumulation of unprocessed organic matter. The starch treatments showed intermediate values, with TA-6 standing out with 10.20mg/ L ± 0.78.

The lowest levels of total ammonia nitrogen were observed in TM-3 (0.97mg/ L ± 0.07), indicating higher nitrogen assimilation by the microbial biomass in this treatment. Starch treatments showed slightly higher levels, with a maximum value of 1.17 ± 0.08mg/ L.

In the study conducted by **Pinho *et al*. (2021)**, the bioremediation effect of a conventional aquaponics treatment was compared with another treatment in which aquaponics and biofloc technology were combined. Dissolved oxygen levels and water temperature were kept constant in both systems during each culture cycle. Initially, TAN levels in both systems were low, close to 0mg/ L. However, over time, TAN showed a gradual increase, being more accelerated in the conventional aquaponics system. This behavior indicated a lower efficiency in the management of total ammonia nitrogen compared to the combined aquaponics and biofloc system, possibly due to a lower microbial activity and an insufficiently balanced C/N ratio in the conventional system.

Electrical conductivity, an indicator of dissolved ion concentration and mineralization in aquaculture systems, was lowest in the control treatment (164.11 ± 2.29µS/ cm) and reached its highest value in TM-3 (178.62 ± 0.87µS/ cm), showing a greater accumulation of metabolites and products derived from microbial activity in this treatment.

Nitrite levels were low in all treatments, being lower in TM-3 (0.03 ± 0.02ppm) and TA-5 (0.04 ± 0.02ppm), reflecting an efficient nitrifying process. Nitrate levels were highest in TM-3 (2.61 ± 0.11ppm), indicating a higher conversion of nitrite to nitrate and consolidating TM-3 as the most efficient treatment.

In a previous investigation, fluctuations in the alkalinity of the tanks using Biofloc technology were recorded, with values ranging from 8 to 250mg/ L during the experimental period. The authors indicated that the system loses buffering capacity, requiring frequent additions of sodium bicarbonate to regulate alkalinity. In addition, they reported that the nitrification process in these systems requires approximately 4mg of oxygen and 8mg of bicarbonate to oxidize 1mg of TAN **(Azim *et al*., 2008)**.

Galvéz *et al*. (2022) recognized that the most commonly used carbon sources in biofloc systems are sugar, molasses and starch. However, they also mentioned acetates, glycerol and glucose as other opportunities for carbon utilization. However, the loss of carbon associated with certain carbon addition strategies has been rarely addressed.

Tinh *et al*. (2021) explored the potential of combining a carbon source, specifically corn starch, with pellet feed while maintaining a C/N ratio of 14.6:1 for shrimp culture under Biofloc technology. The findings indicated that, compared to a commercial diet with a C/N ratio of 7.6:1, the addition of supplemental carbon reduced carbon utilization efficiency, regardless of the method of incorporation. On the other hand, energy efficiency was higher when no additional carbon was added, regardless of the method of incorporation.

3.2. Zootechnical performance parameters

Fig. (1) presents the zootechnical performance parameters exhibited by *A. rivulatus* fish in the different experimental treatments.

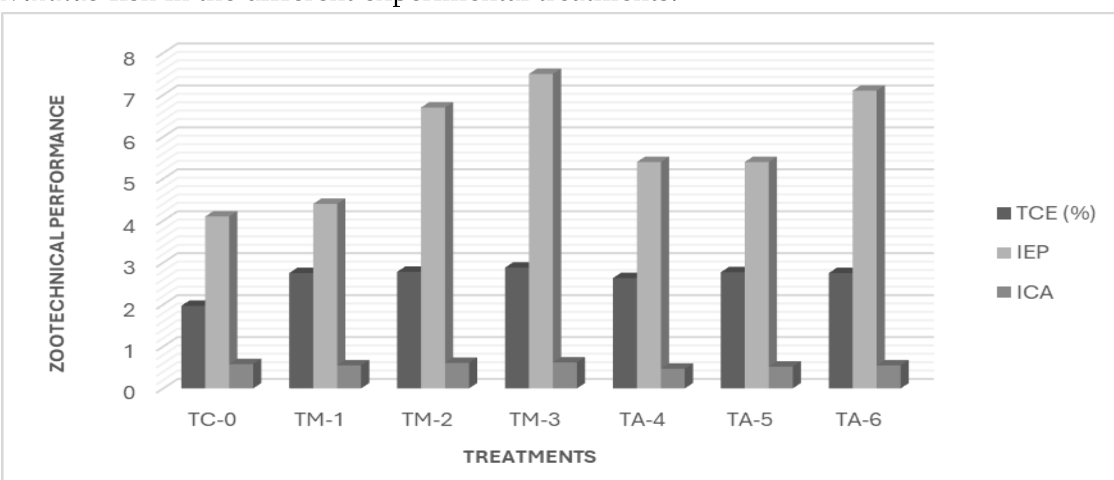

Fig. 1. Zootechnical performance parameters. Note: The following nomenclature was used to abbreviate the terms: TCE= specific growth parameters, IEP= protein efficiency index, ICA= feed conversion ratio

The specific growth rate showed a significant increase in the treatments that used molasses as a carbon source (TM-1 to TM-3), reaching its maximum value in TM-3. The starch-based treatments (TA-4 to TA-6) also improved TCE compared to the control (TC-0), although to a lesser extent in contrast to the molasses treatments. The control treatment had the lowest TCE, indicating that the absence of an additional carbon source limits the specific growth of the organisms.

In the study by **Zablon *et al*. (2022)**, the results indicated that the specific growth rate was significantly higher in fish cultured in biofloc units with glucose and molasses compared to the control, demonstrating that the carbon sources used in biofloc systems favor better growth. However, no significant effects related to protein level, carbon source or the interaction between the two were observed at a specific growth rate, suggesting that other factors may be more important determinants of this indicator.

Similarly, TM-3 showed the highest index of protein efficiency index, suggesting that this treatment promoted a better utilization of the protein consumed, associated to a higher microbial activity. In this case, the control showed the lowest IEP reflecting a less efficient protein conversion under conditions of no carbon addition.

In relation to the feed conversion index, whose indicator represented the amount of feed needed to produce one unit of biomass, the control treatment showed a limited feed efficiency (0.58) due to the absence of carbon supplementation. Although TM-3 presented the best values in other indicators such as TCE and IEP, its higher AQI suggests that faster growth may be associated with an increase in feed demand, partially affecting feed efficiency. On the other hand, TA-4 was consolidated as the most efficient treatment in terms of feed conversion, indicating that, although starch as a carbon source did not have the same impact on growth parameters as molasses, it contributed to the optimization of feed use.

3.3. Proximal analysis of flocs

Table (3) shows the results of the proximate analysis performed on the biofloc flocs in the different experimental treatments at the end of the experiment.

Table 3. Proximal analysis of flocs in experimental treatments

TT	FC	PB (%)	CN (%)	FB (%)	GC (%)
TC-0	--	32.04 ± 0.31	12.37 ± 2.10	8.02 ± 0.50	1.99 ± 1.14
TM-1	ME	35.75 ± 2.29	13.38 ± 0.54	6.27 ± 2.10	2.39 ± 0.24
TM-2		38.42 ± 1.32	11.65 ± 0.27	6.67 ± 0.22	2.21 ± 0.29
TM-3		39.57 ± 1.18	11.30 ± 0.27	5.77 ± 0.64	2.55 ± 0.27
TA-4	AM	37.17 ± 0.43	14.09 ± 0.22	6.48 ± 0.37	2.69 ± 0.17
TA-5		33.59 ± 2.88	14.88 ± 1.58	6.18 ± 0.77	2.18 ± 0.55
TA-6		34.57 ± 1.02	15.96 ± 0.33	6.71 ± 0.42	2.31 ± 0.49

Note. The following nomenclature was used to abbreviate terms: TT= treatment, FC= carbon source, ME= molasses, AM= corn starch, PB= crude protein, CC= ash, FB= crude fiber, GC= crude fat.

The molasses treatments (TM-1, TM-2 and TM-3) showed higher crude protein (PB) content, with TM-3 having the highest value (39.57 ± 1.18%), followed by TM-2 (38.42 ± 1.32%), while the corn starch treatments (TA-4, TA-5 and TA-6) showed intermediate levels, with the lowest in TA-5 (33.59 ± 2.88%).

According to **Jauncey (2000)**, it is suggested that diets for larger tilapia contain 35 to 30% crude protein, along with a crude lipid content of 6 to 8%.

Ash content (CN) was higher in starch treatments, reaching its maximum in TA-6 (15.96 ± 0.33%), which could reflect a greater accumulation of inorganic compounds in these systems. On the other hand, molasses treatments presented lower ash levels, with TM-3 being the lowest (11.30 ± 0.27%).

As for crude fiber (CF), starch treatments showed a tendency to higher values, with a maximum at TA-6 (6.71 ± 0.42%), while molasses treatments ranged from 5.77 ± 0.64% (TM-3) to 6.67 ± 0.22% (TM-2).

Crude fat (CG) was higher in the molasses treatments, with TM-3 standing out again (2.55 ± 0.27%), while the starch treatments showed lower values, the maximum of this group being in TA-6 (2.31 ± 0.49%). In comparison, the control treatment (TC-0), which did not include carbon sources, showed significantly lower values in crude protein (32.04 ± 0.31%) and crude fat (1.99 ± 1.14%), together with the highest level of crude fiber (8.02 ± 0.50%), implying a lower quality of the flocs generated in the absence of supplementation.

Chou and Shiau (1996) reported that a dietary lipid level of 5% is sufficient to meet the minimum requirements of juvenile hybrid tilapia. However, to achieve optimal growth, these authors concluded that a higher lipid level of about 12% is necessary.

The advantages associated with the implementation of Biofloc technology have been documented in previous studies. Benefits include low feed and water consumption, decreased risk of pathogen and disease introduction, increased biosecurity, as well as increased crop growth and survival **(Ahmad *et al*., 2016)**.

CONCLUSION

Biofloc technology proved to be a sustainable alternative for water treatment in aquatic crops, highlighting the treatment with molasses and 35% crude protein (TM-3) as the most effective. This treatment optimized key parameters such as dissolved oxygen, lower biochemical oxygen demand (BOD) and higher conversion of total ammonia nitrogen to nitrate, promoting efficient microbial activity and optimal development of Andinoacara rivulatus.

The TM-3 treatment presented the best results in terms of specific growth rate (SGR), protein efficiency (PE) and crude protein content in the flocs (39.57 ± 1.18%). In addition, the composition of the flocs generated with molasses showed a higher crude fat content (2.55 ± 0.27%) and lower ash content (11.30 ± 0.27%), which positions them as a high quality supplementary feed source.

The integration of molasses as a carbon source in Biofloc systems not only improved water quality and oxygenation but also maximized the zootechnical performance of A. rivulatus. These findings highlight the importance of using accessible and effective carbon sources to promote sustainability in aquaculture.

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