International Journal of Fisheries and Aquatic Studies

E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2020; 8(3): 321-326

www.fisheriesjournal.com
Received: 28-03-2020
Accepted: 30-04-2020

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Preliminary study of the growth of *Amatitlania nigrofasciata* (Günther, 1867) (Convict cichlid), fed with inert diets (dry and wet) in a Biofloc system, in laboratory conditions

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Abstract
Convict cichlid fish, *Amatitlania nigrofasciata*, is specie with a mean size of 13 cm of length that belongs to the family Cichlidae. They are omnivorous organisms, because its alimentation ranges from vegetable and organic matter, to small fish and crustaceans. This investigation aimed to analyze the performance obtained in the growth of juvenile individuals of *A. nigrofasciata* with an initial mean length between 2.22-2.68 cm and mean weight of 0.51-0.63 g. Every seven days the organisms were measured with a digital Vernier Truper® ± 0.001 mm and weighted with a digital balance Nimbus® ± 0.01 g. The weight of the organisms served to consider the 10% of the total biomass to give the experimental diets, the dry ones were: a) shrimp pellet of 0.8mm; b) TetraColor®; and the wet ones were: c) carrot and d) beetroot. Also, the 0.1% of the total biomass was given as the external carbon source, it was used moringa flour, to produce the Biofloc system. The organisms with the shrimp diet took 70 days to reach its maximum size (11.82±1.77 cm). The fish fed with TetraColor took 35 days to reach its maximum size (12.00 ± 1.29 cm), while the wet diets took 42 days (12.69±1.54 cm and 12.20±1.51 cm respectively). The multiple mean analysis showed significant differences (p>0.05) between them. It is mentioned that an excess in the quantity of supplied protein in the diet increases the ammonium levels in water, which can affect the growth and wellbeing of fishes. There is few information in the protein requirements for a good culture of cichlid convict, therefore this work contributes to finding optimal diets for this ornamental fish.

Keywords: *Amatitlania nigrofasciata,* convict cichlid, Biofloc system, laboratory conditions

1. Introduction
Convict cichlid fish, *A. nigrofasciata*, is specie with a mean size of 13 cm of length that belongs to the family Cichlidae. Its body is gray color with black strips, although in males there can be red, blue, or green spots, both at the sides and fins, which makes them a specie susceptible to the aquarium sector. It is an omnivorous organism, because its alimentation ranges from vegetable and organic matter, to small organisms like worms, crustaceans, insects, and smaller fish [1, 2]. This characteristic of its alimentation, allows to elaborate and/or supply diverse food to evaluate its growth and wellbeing, taking care of the diet formulation, which must cover the nutritional requirements of the organism, as well as, the way of to supply it in quantity and form [3].
But it is not only necessary to know and supply the quality and quantity that the fish requires, but also, it has to be considered that the way of culture is environmental friendly and do not allow the bad use of water and food lost, polluting the available source of water available for other functions [4]. One of the actual technologies that allow that, is Biofloc system that is based on the use of food residuals and organic matter produced in the culture system, which allows the production of bacteria capable to eliminate the toxic inorganic compounds and at the same time produce bacterial biomass with probiotic and nitrifying capacities that allow the elimination of nitrogenous compounds and become source of beneficial substances for the growth, reproduction and wellbeing of the organisms [5-10].
Biofloc technology has allowed to face the challenges presented not only by large-scale aquaculture, but its use in ponds or cultivation tubs of no more than 5,000 liters that allow to increase the produced biomass quantity, both in number and weight, but above all, the low use

of water to obtain a lower environmental cost [5].

Because of the above, the aim of this investigation is to analyze the obtained performance in the growth of juvenile *A. nigrofasciata* in a Biofloc system, fed with four diets: two dry diets and two wet diets.

2. Materials and Methods

2.1 Experimental design

Sixty juveniles of *A. nigrofasciata* were used, which had an initial mean length ranging from 2.22 to 2.68 cm and a mean weight of 0.51-0.63 g, which were placed in plastic cylinders of 200 L of capacity (0.80 x 1.0 m). with 150 L of water. The containers were maintained with strong and constant aeration to maintain all the water column in movement (Fig.1). The temperature was maintained at 23±2 °C and the physicochemical parameters of nitrites, nitrates, ammonium, phosphate, pH and chlorine were measured every 15 days.

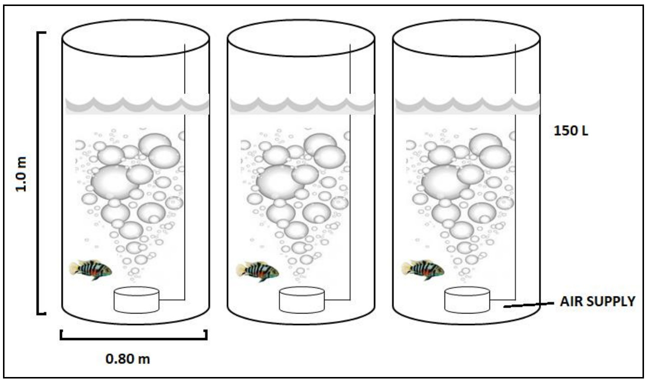

Fig 1: Culture system for *A. nigrofasciata*

At the beginning and every seven days the organisms were measured with a digital Vernier Truper ±0.001 mm, and weighted with a digital balance Nimbus ±0.01 g. The quantity of the diet was calculated according to the 10% of the total biomass in the containers. The dry diets were: a) Shrimp pellet of 0.8mm; b) TetraColor; and the wet ones were: c) carrot and d) beetroot. Also, the 0.1% of the total biomass was given as the external carbon source, it was used moringa flour, to produce the Biofloc system.

2.2 Experimental diets

Shrimp diet contained 55% of protein 15% lipids and 3% of fiber. TetraColor contained 47.5% of protein, 6.5% of lipids and 2% of fiber. The two wet diets based in carrot and beetroot contained 30% of protein and 10% of lipids proportionated by chicken gizzards, two vitamin E capsules, and 35% of fiber apported by using apple, banana and 250 g of oatmeal. Wet diets were agglomerated with gelatin (50 g). The four diets were supplied twice a day, while the carbon source (moringa) once per day.

2.3 Processing information

Every seven days, the biometric parameters of total length, height, width and weight were incorporated to an Excel 2010 data base to obtain the descriptive statistic, as well as its growth tendency curves. Also, it was obtained the gain, absolute growth rate (AGR) and instantaneous growth rate (IGR).

To obtain the gain values (G) of biometric values the following formula was used:

G = Final value - Initial value

To obtain AGR the following formula was used:

$$AGR = \frac{\text{Final value} - \text{Initial value}}{\text{Total culture days}}$$

To obtain IGR the following formula was used:

$$IGR = \frac{\text{Ln (Final value)} - \text{Ln (Initial value)}}{\text{Total culture days}} \times 100$$

2.4 Statistical analysis

Significant differences (p<0.05) in the four biometric measurements (total length, height, width and weight) between experiments were determined through an ANOVA test. When significant differences were obtained, it was made a multiple mean test through Tukey test.

3. Results

3.1 Chemical parameters of water

In the four experimental treatments, the pH maintained values of 7.13 to 7.37; chlorine between 0-0.4 mg L^{-1}; ammonium (NH_4) between 0.09 to 0.42 mg L^{-1}; nitrates (NO_3) were between 6.83-67.57 mg L^{-1}; nitrites (NO_2) from 3.67 to 31.5 mg L^{-1}; and phosphates (PO_4-) between 1.68 and 6.93 mg L^{-1}.

3.2 Survival

Shrimp diet presented a survival of organisms at the end of the experiment of 75%, while the other three diets presented values between 90 to 93%.

3.3 Total length

Mean values (±S.D.) are presented in Table 1. The organisms with shrimp diet took 70 days to reach its maximum size (11.82±1.77 cm), but it is the lowest length value. Fish fed with TetraColor took 35 days (12.00±1.29 cm) and wet diets with carrot and beetroot took 42 days (12.69±1.54 cm and 12.20±1.51 cm respectively), being the carrot diet the highest obtained value. Variance analysis (ANOVA) did not presented significant differences (p<0.05) in the final sizes of fish fed with the experimental diets. As it is observed in Table 1, the difference is on the time that the organisms took to reach maximum length.

Table 1: Mean values (±S.D.) of total length of fish fed with the four experimental diets

Culture days	Experimental diets			
	Shrimp	Tetracolor	Carrot	Beetroot
0	2.68±1.42	2.43±1.59	2.29±1.25	2.22±1.55
7	3.59±1.28	4.34±1.45	4.03±1.35	3.89±1.51
14	4.50±1.66	6.26±1.46	5.76±1.41	5.55±1.26
21	5.42±1.53	8.17±1.30	7.49±1.49	7.21±1.31
28	6.33±1.45	10.08±1.57	9.22±1.68	8.64±1.26
35	7.25±1.69	12.00±1.29	10.96±1.66	10.54±1.52
42	8.16±1.30	--	12.69±1.54	12.20±1.51
49	9.07±1.06	--	--	--
56	9.99±1.64	--	--	--
63	10.90±1.50	--	--	--
70	11.82±1.77	--	--	--

Total length growth tendency curves of the organisms in the four experimental diets are presented in Fig. 2, which were lineal type in all diets.

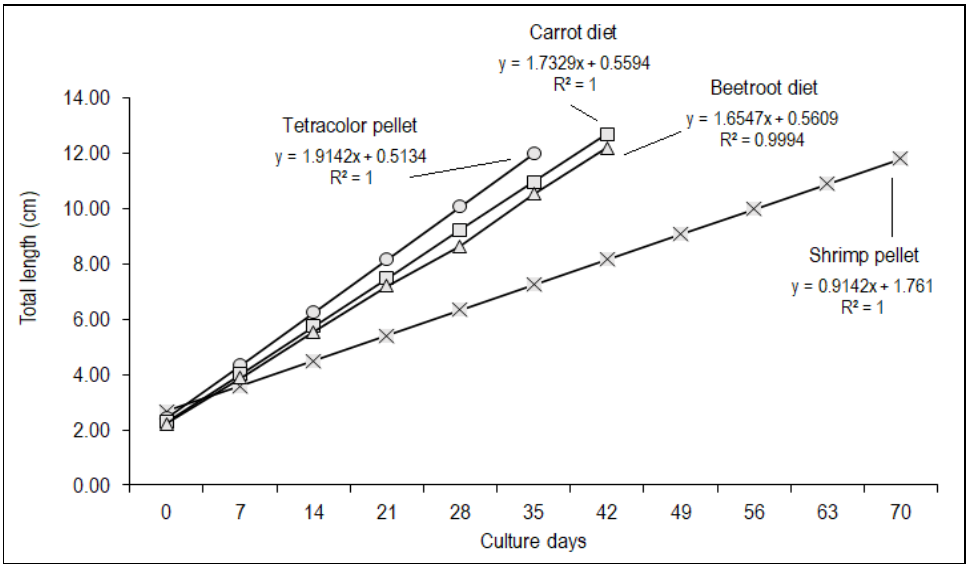

Fig 2: Growth tendency curves of total length of *A. nigrofasciata* with the four experimental diets

3.4 Height

Mean values (±S.D.) of height are presented in Table 2. Highest value was obtained in shrimp diet with 6.81±1.08 cm at 70 days of culture, while in the diet of carrot the fish obtained a maximum height of 2.46±0.85 cm in 42 days of culture, being the lowest obtained height value. Variance analysis (ANOVA) presented significant differences (p<0.05) between treatments, while the multiple mean comparison showed that these differences were found in the shrimp diet regarding the other three diets. TetraColor diet presented significant differences (p<0.05) regarding to carrot and beetroot diets. The height of fish did not present significant differences (p>0.05) between wet diets.

Table 2: Mean values (±S.D.) of the height of the fish fed with the four experimental diets.

Culture days	Experimental diets			
	Shrimp	Tetracolor	Carrot	Beetroot
0	0.88±0.89	0.83±0.85	1.06±0.79	0.70±0.60
7	1.47±0.98	1.58±0.84	1.30±0.66	1.07±0.67
14	2.06±1.11	2.34±0.87	1.53±0.78	1.44±0.79
21	2.66±1.11	3.09±0.82	1.76±0.84	1.82±0.67
28	3.25±0.80	3.84±0.86	1.99±0.87	2.19±0.52
35	3.84±0.84	4.59±0.78	2.23±0.76	2.56±0.64
42	4.44±0.84		2.46±0.85	2.93±0.57
49	5.03±0.96			
56	5.62±0.86			
63	6.21±1.02			
70	6.81±1.08			

Height growth tendency curves of the fish in the four experimental diets are presented in Fig. 3, which were lineal type in all diets.

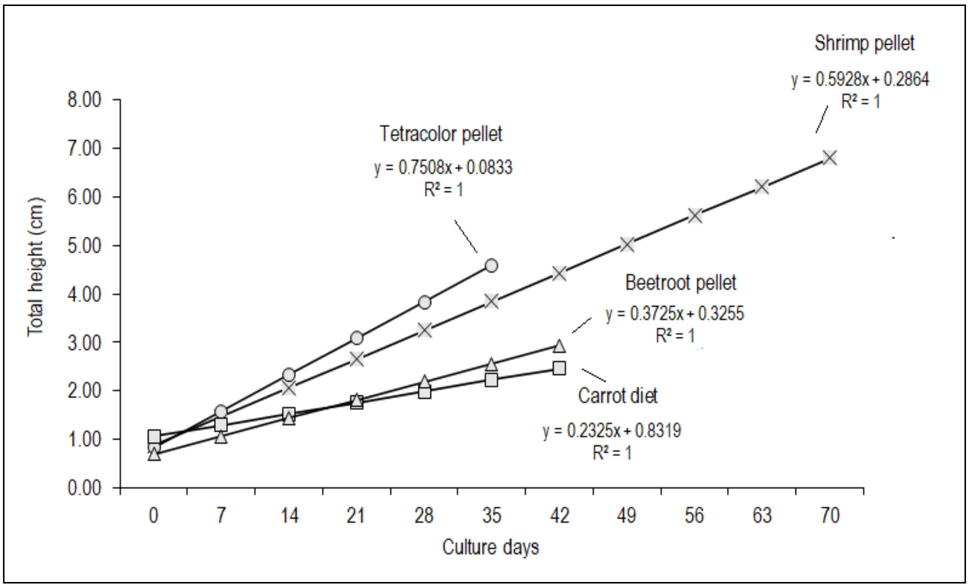

Fig 3: Height growth tendency curves of *A. nigrofasciata* in the four experimental diets

3.5 Width

Mean values (±S.D.) of width are presented in Table 3. Highest value was obtained in shrimp diet with 4.59±0.43 cm (at 70 days of culture), while the diet with beetroot obtained the lowest value with 1.31±0.43 cm (at 43 days of culture). The variance analysis (ANOVA) showed significant differences ($p<0.05$) between treatments, while the multiple mean comparison showed that these differences were found in the shrimp diet regarding the other three diets, as well as, beetroot diet regarding to TetraColor and carrot diet ($p<0.05$). The width of the fish did not present significant differences ($p>0.05$) between TetraColor and carrot diets.

Table 3: Mean values (±S.D.) of width of fish fed with the four experimental diets

Culture days	Experimental diets			
	Shrimp	Tetracolor	Carrot	Beetroot
0	0.41±0.45	0.26±0.52	0.39±0.44	0.47±0.43
7	0.83±0.34	0.77±0.46	0.76±0.36	0.51±0.44
14	1.25±0.36	1.28±0.58	1.14±0.37	0.60±0.38
21	1.66±0.50	1.79±0.50	1.51±0.51	0.72±0.46
28	2.08±0.42	2.30±0.59	1.88±0.51	0.88±0.38
35	2.50±0.45	2.81±0.43	2.25±0.36	1.07±0.41
42	2.92±0.36		2.63±0.42	1.31±0.43
49	3.33±0.52			
56	3.75±0.44			
63	4.17±0.40			
70	4.59±0.43			

Growth tendency curves of width of fish in the four experimental diets are presented in Fig. 4. Tetracolor, Shrimp and Carrot diets presented lineal type curves, only beetroot diet showed polynomic grade two curve type.

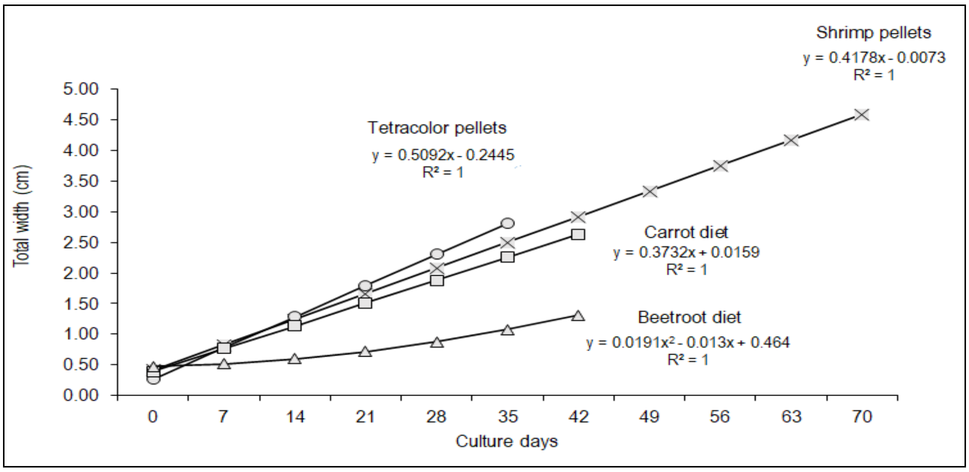

Fig 4: Growth tendency curves of width of *A. nigrofasciata* in the four experimental diets

3.6 Weight

Mean values (±S.D.) of weight are presented in Table 4. The highest values were presented in the wet diets (carrot with 25.12±0.12 and beetroot with 26.86±0.17 g at 42 days of culture), while in TetraColor diet it was observed a the lowest value with 11.44±0.12 g in 35 days of culture. Variance analysis (ANOVA) showed significant differences ($p<0.05$) between experimental diets. The multiple mean tests showed significant differences ($p<0.05$) between the wet diets (carrot and beetroot) regarding to shrimp and TetraColor diets, as well as between the dry diets. Wet diets did not present significant differences ($p<0.05$) between them.

Table 4: Mean values (±S.D.) of weight of fish in the four experimental diets

Culture days	Experimental diets			
	Shrimp	Tetracolor	Carrot	Beetroot
0	0.60±0.01	0.56±0.14	0.63±0.14	0.51±0.13
7	0.99±0.09	3.85±0.14	1.52±0.12	2.72±0.16
14	1.06±0.05	5.29±0.13	5.03±0.13	5.01±0.18
21	2.06±0.04	9.53±0.11	11.15±0.10	8.81±0.17
28	3.21±0.04	10.59±0.14	19.89±0.15	10.88±0.14
35	4.69±0.09	11.44±0.12	20.24±0.12	14.76±0.12
42	6.43±0.01		25.12±0.12	26.86±0.17
49	8.45±0.07			
56	10.74±0.01			
63	13.30±0.05			
70	16.12±0.10			

Growth tendency curves of fish's weight in the four experimental diets are presented in Fig. 5. All tendency curves were polynomic grade two curves type.

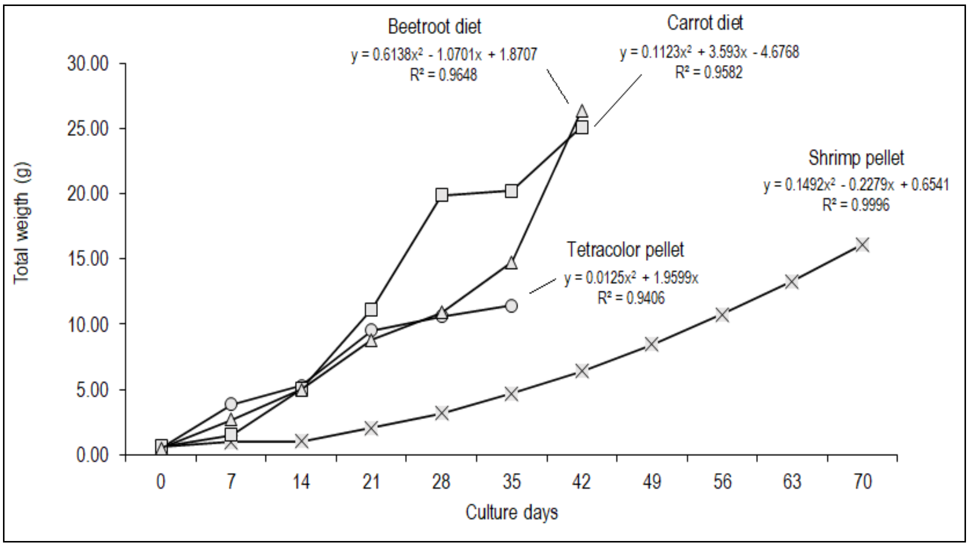

Fig 5: Weight growth tendency curves of *A. nigrofasciata* in the four experimental diets

3.7 Gain, AGR and IGR
In Table 5 the gain values and the AGR and IGR of the organisms in the four experimental diets are presented. The highest gain regarding total length and weight was found in carrot diet with 10.40 cm and 24.94 g. For height and width, the shrimp diet obtained the highest gains with 5.93 cm and 4.18 cm, respectively. The lowest value of gain in total length was found in shrimp diet with 9.14 cm, in height was in carrot diet with 1.39 cm, for width it was in beetroot diet with 0.84 cm, and in weight it was in TetraColor diet with 10.88 g.

Table 5: Gain, AGR, IGR (%) values of *A. nigrofasciatus* fed with four experimental diets

Biometric variables	Experimental diets											
	Shrimp			Tetracolor			Carrot			Beetroot		
	Gain	AGR	IGR (%)	Gain	AGR	IGR (%)	Gain	AGR	IGR (%)	Gain	AGR	IGR (%)
Total length (cm)	9.14	0.13	2.12	9.57	0.27	4.57	10.40	0.25	4.07	9.98	0.24	4.05
Height (cm)	5.93	0.08	2.92	3.75	0.11	4.87	1.39	0.03	1.99	2.24	0.05	3.42
Width (cm)	4.18	0.06	3.45	2.55	0.07	6.75	2.24	0.05	4.55	0.84	0.02	2.44
Weight (g)	15.52	0.22	4.70	10.88	0.31	8.61	24.49	0.58	8.76	25.87	0.62	9.38

4. Discussion
Ornamental fish production has gained a high interest due to its growing international demand and the growth of the market [11]. In Mexico are sold more than 43 million of ornamental fishes, which represent an income of approximately $1,650 million pesos at retail price. The 48% of fish are imported and the 52% are cultured in more than 250 units aquaculture production (UAP's) located in 20 states of the Mexican Republic [12, 13]. There are records that the cichlid *A. nigrofasciata* is used as ornamental fish since the 90's in all the world [14, 15]. Due to this it is important to find culture methods as Biofloc, that as observed in this work, helps in the culture of this ornamental fish, reducing the waste of water and obtaining a lower environmental cost.

Ergun *et al.* [16], Gullu *et al.* [17], Guroy *et al.* [18] and Gultepe *et al.* [15], mention that the importance of the nutritional requirements of ornamental fish and how it plays an important role in the health of the fish, its behavior and appearance (shape and color), which is very important in ornamental fish [19]. The NRC [20] mention that the protein given to fish in the diet allows a good growth in size and weight and wellbeing in the culture.

Gultepe *et al.* [15] worked with *A. nigrofascita* culturing it with four diets with different protein levels (30, 35, 40 and 45% RP), obtaining similar values to the ones in this experiment were carrot and beetroot diets (30%RP) obtained better weight results in AGR and IGR, while the other two diets with high protein content (Shrimp with 50% and TetraColor with 47.7%), obtained the lowest values regarding weight. Comparing the results with other omnivorous cichlid, the red head cichlid, with a diet with 41 % RP [21], the yellow tail cichlid with 35% RP [18] and the electric blue cichlid with 38.8% RP [17], it can be observed that the protein levels in diet to culture these cichlids does not pass the 40% RP. Olvera-Novoa *et al.* [21] found that with a diet with 40.81% RP the cichlid fish *Cichlasoma synspilium*, showed good results. Zehra y Khan [22], mention that it exists an optimal requirement of protein in the diet to supply the adequate aminoacids for a maximum growth. Increasing the quantity of protein in the diet can lead to a better growth in different species, bur increasing the protein in diet can also affect the good growth of fish and there may be a decrease in fish growth [23,24], which it was observed in the diet with 50% RP. If a lot of protein is added to the diet, only a part of it is used to form new protein and the excess is converted in energy, which means an increase in costs and an increase in the ammonium levels in water [22].

On the other hand, Gultepe *et al.* [15], mention that an excess in protein levels in diet, increases the ammonium levels in the culture water because of excretion, which can affect the growth and wellbeing of fish. Ergun *et al.* [16] and Guroy *et al.* [18] found out that the increase of protein in diet, increases the quantity of ammonium in water affecting the fish growth , which can be observed in this work, where shrimp diet with 50% RP, took more than 10 weeks to reach its maximum size, while the other three diets reached it in the sixth culture week. Gultepe *et al.* [15], mention that the water quality is very important for the health of the fish in culture, because the high levels of ammonium are toxic for fish.

Determining an optimum level of protein in diet will allow to obtain a better growth, health, and appearance in fish, also, decrease the cost of diet formulation and use more environmentally friendly diets [25].

5. Conclusion

There is very little information on protein requirements using low-cost diets and good growth of the convict cichlid *A. nigrofasciata*, therefore, this work helps to search optimal diets for this ornamental fish. As well as showing the viability of culture of this ornamental fish in a Biofloc system, to reduce the environmental impact that traditional culture mediums have. It is recommended to continue the research in the adequate protein levels in diet for the culture of ornamental fish.

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