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López García Evelyn
Universidad Autónoma Metropolitana- Xochimilco, Calzada del Hueso 1100, Villa Quietud 04960, CDMX, México

Cienfuegos Martínez Kathia
Universidad Autónoma Metropolitana- Xochimilco, Calzada del Hueso 1100, Villa Quietud 04960, CDMX, México

Vianney Ortíz Hidalgo
Universidad Autónoma Metropolitana- Xochimilco, Calzada del Hueso 1100, Villa Quietud 04960, CDMX, México

Aboytes Uribe Samantha del Carmen
Universidad Autónoma Metropolitana- Xochimilco, Calzada del Hueso 1100, Villa Quietud 04960, CDMX, México

Monroy Dosta María del Carmen
Universidad Autónoma Metropolitana- Xochimilco, Calzada del Hueso 1100, Villa Quietud 04960, CDMX, México

Corresponding Author:
López García Evelyn
Universidad Autónoma Metropolitana- Xochimilco, Calzada del Hueso 1100, Villa Quietud 04960, CDMX, México

Effect of orange peel flour in the growth and survival of Carassius auratus in a biofloc system

López García Evelyn, Cienfuegos Martínez Kathia, Vianney Ortíz Hidalgo, Aboytes Uribe Samantha del Carmen and Monroy Dosta María del Carmen

Abstract
Ornamental aquaculture is an important economic activity in Mexico and worldwide. Nevertheless, it is affected by infectious outbreaks, inadequate diets and excessive use and pollution of water. Therefore, different techniques have been developed to use less contaminants and obtain better benefits in nutrition, such as the Biofloc technology. This study aimed to evaluate the growth and survival of *Carassius auratus* cultured in a Biofloc system, using two different carbon sources. Ninety organisms were used with an initial weight and length of 1.7 ± 0.5 g and 4.5 ± 0.5 cm, respectively. Daily it was supplied with commercial balanced food with 38% of protein. Three treatments were installed: control, molasses (BFT1) and orange peel flour (BFT2), maintaining a relation C:N=20:1. At the end of the 45 days of culture, it was observed that survival of the fish in treatment BFT2 (orange peel flour) was the highest with 78%, while the treatments with molasses and control had 46 and 43% respectively. The same behavior was observed in the weight and total length because BFT2 treatment obtained the highest values (2.71 ± 0.61g and 5.59 ± 0.49 cm). Water parameters did not present significant differences in any treatment. According to obtained results, the use of orange peel flour promotes a better growth and survival of *Carassius auratus* cultured in Biofloc, due to its high content of vitamin C that works as antioxidant in fish, and immunostimulant that increase the survival and allows the production of microbial protein available for fish which improves its nutrition and thus its growth.

Keywords: Biofloc, water quality, *Carassius auratus*, growth, survival

1. Introduction
The ornamental fish trade is an expanding global market that since 1985 has shown a considerable increase, which moves high quantities of money, producing job opportunities and contributing to economic growth of underdeveloped countries [1, 2]. In Mexico, the production and commercialization of this organisms generate a high quantity of jobs and incomes, trading about 43 million ornamental fish primarily freshwater, which are produced in more than 400 aquaculture farms stablished in 20 States. Being Morelos, Veracruz, Yucatan, Mexico State and Jalisco the main States that produce ornamental fish, where production schemes vary according to the management of the different parameters that go from species selection, reproduction techniques, type of alimentation, production tanks, demand, among others [3]. Despite being an activity with an exponential growth in the last few years, ornamental aquaculture has different problems due to bad use of water, low nutritional food quality, difficulty in eradicating diseases, and produced wastes [4].
On the other hand, feeding represents one of the highest expenses in aquaculture production, due research has been carried out where they focus on the generation of diets for fish and crustaceans, but there is few information focused on ornamental fish, so stablished nutritional parameters for consumption species are used, which can affect the growth and water quality [4].
For the above described, it has been designed production systems for the culture of diverse organisms, that allow a better productive efficiency and decrease the environmental impact [5].
An example of this type of systems, is Biofloc technology (BFT) which consists in the development of microbial aggregates formed from the addition of external carbon sources as molasses (mainly), rice bran, wheat bran, among others, allowing the growth of bacteria, microalgae, protozoa, rotifers, fungi, oligochaetes, among others, as a result of the processes of microbial metabolism through which they use the produced nitrogen in the pond/tank, that is,

they carry out denitrification for the synthesis of microbial protein of high quality, easily usable by fish, which allows to have a better water quality. Also, it decreases the possibility of the action of pathogenic microorganisms and cause diseases to the pond, improving the biosecurity due to their activity as regulators of populations of pathogenic bacteria [6].

For Biofloc composition is required the addition of a carbon source of low cost and easy acquisition, which will determine the composition, structure and stability of flocs, as well as the development of some microorganisms and the variation of some water quality parameters [7, 8]. Although the most widely used carbon source in Biofloc systems is molasses, there are other available sources, in this sense some authors mention the necessity to experiment with other low cost carbon sources that allow the economic sustainability of production, such as food or fruit waste that can be considered as beneficial alternative sources in terms of cost, solubility and quantity of carbon [7, 9, 10].

In Mexico, the production of orange in 2017 was of 320,793.57 million of tons from which only the 50% of the biomass is usable and the other 50% is lost as seeds and peel [11, 12], the latter is a by-product with a high carbohydrate content, which allows it to be used as flour, but also it contains high levels of vitamin C that acts as antioxidants in fish and as immunostimulant, in addition to a high fiber content, protein and flavonoids. Therefore, the objective of this work was to compare the effect of the addition of molasses and orange peel flour used as carbon sources in the survival and growth of *Carassius auratus* cultured in a Biofloc system.

2. Materials and Methods

2.1 Experimental design

For the experimentation 9 cylinders of 80L capacity were conditioned with constant aeration. Three treatments were implemented: control (without Biofloc), Biofloc using molasses as carbon source (BFT1) and Biofloc with orange peel flour (BFT2), each treatment was made by triplicate. Were used 90 fishes of the specie *Carasius auratus*, which were distributed randomly.

Fish were fed daily with commercial food for the specie, making quantity adjustments every 15 days, after the obtainment of biometrical parameters.

2.2 Biofloc conformation

The carbon source corresponding to each treatment was supplied in a relation C:N= 20:1, making adjustments every 15 days and the quantity for every treatment was calculated according to the formulas used by Emerenciano [13]. To measure the volume of produced Biofloc per treatment, Imhoff cones were used, which were filled with 1 L of sample and after 20 the solids were sedimented; then the microbial composition of the treatments was observed.

2.3 Water quality

To determine the variations of the nitrogenous compounds in the culture system, once a week measurements of water quality parameters were made; using a multiparametric HANNA HI9829 to measure temperature and pH, and for nitrites (NO_2^-), nitrates (NO_3^-), and ammonium (NH_4^+) was used the photometer for aquaculture HANNA HI 83203.

2.4 Growth and survival

It was used the formula of Wang (2015), to obtain the survival rate (SR)

SR%= (Survival population/ initial population) (100%)

Also, it was obtained the total length (TL) of the fish with a Vernier (±0.05cm) as well as the weight with a digital balance OHAUS (0.01 g). The experiment had a duration of 45 days.

2.5 Statistical analysis

A data base was made in Excel 2010 to obtain the corresponding descriptive statistic and it was observed the normality of the data to later made a variance analysis of a completely randomized multi-factor model to determine significant differences ($P< 0.05$) of the growth of the fish regarding the three treatments during all the experiment.

3. Results

3.1 Survival

The highest survival rate was obtained in the BFT2 treatment with 78%, while in the BFT1 and control treatments obtained lower survival rates (46 and 43% respectively), which indicates that the best treatment was the one with orange peel flour.

3.2 Growth

Regarding to weight, in the first two measurements there were not significant differences, but in the third measurement there were significant differences between treatments, as it is shown in Table 1. Also, it was observed that the treatment with orange peel flour presented the highest weight gain at the end of the experiment (2.71 ± 0.61 g), followed by molasses (2.36 ± 0.59 g). Being control group the only with least gain (2.19 ± 0.53 g) (Figure 1).

Table 1: Fish's weight per treatment during the experiment

	CONTROL	MOLASSES	ORANGE
Initial weight (g)	1.77 ± 0.59	1.75 ± 0.58	1.75 ± 0.53
Middle weight (g)	$1.78^b \pm 0.54$	$1.93^a \pm 0.58$	$2.18^a \pm 0.54$
Final weight (g)	$2.19^b \pm 0.53$	$2.36^b \pm 0.59$	$2.71^a \pm 0.61$

Within the rows, the different superscript letters indicate significant differences ($P< 0.05$).

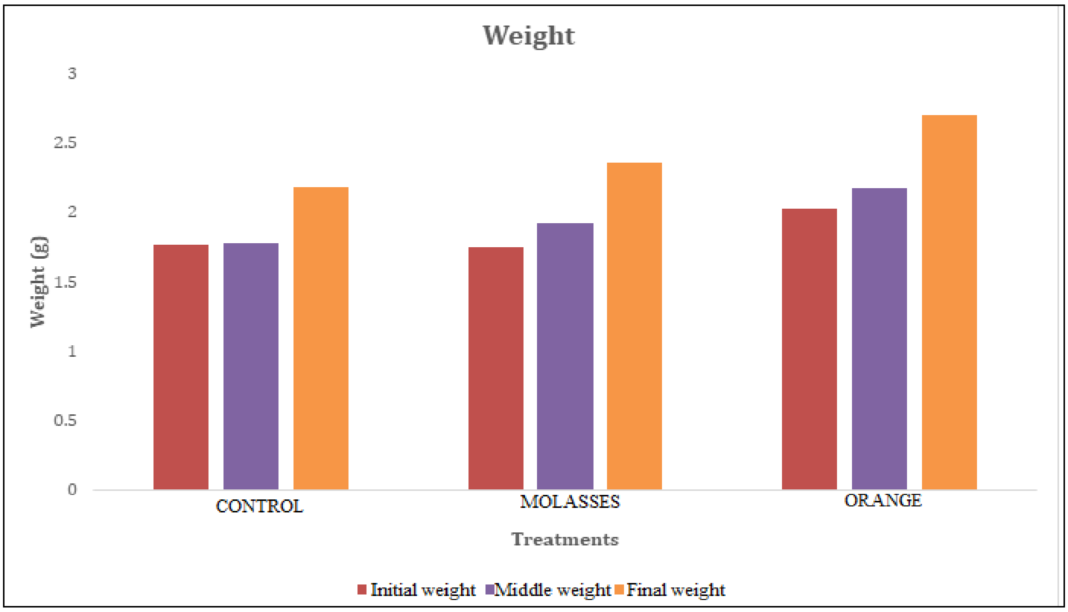

Fig 1: Initial, middle and final weight of fishes per treatment

Regarding to total length it was observed that the organisms of orange peel flour treatment, obtained better results (5.59 ± 0.49 cm), followed by molasses treatment with (5.51 ± 0.50 cm) and control with (5.47 ± 0.50 cm).

It was presented a significant difference between orange treatment and control with a value of $P < 0.05$; this comparing the first and last measurement (Table 2).

Table 2: Total length during the experiment per treatment

	Control	Molasses	Orange
Initial length (cm)	4.56 ± 0.65	4.53 ± 0.55	4.58 ± 0.43
Middle length (cm)	4.71^b ± 0.49	4.81^b ± 0.49	5.25^a ± 0.44
Final length (cm)	5.47^b ± 0.50	5.51^b ± 0.50	5.59^a ± 0.49

The organisms of the orange treatment obtained a higher growth, followed by molasses and control treatment respectively (Figure 2).

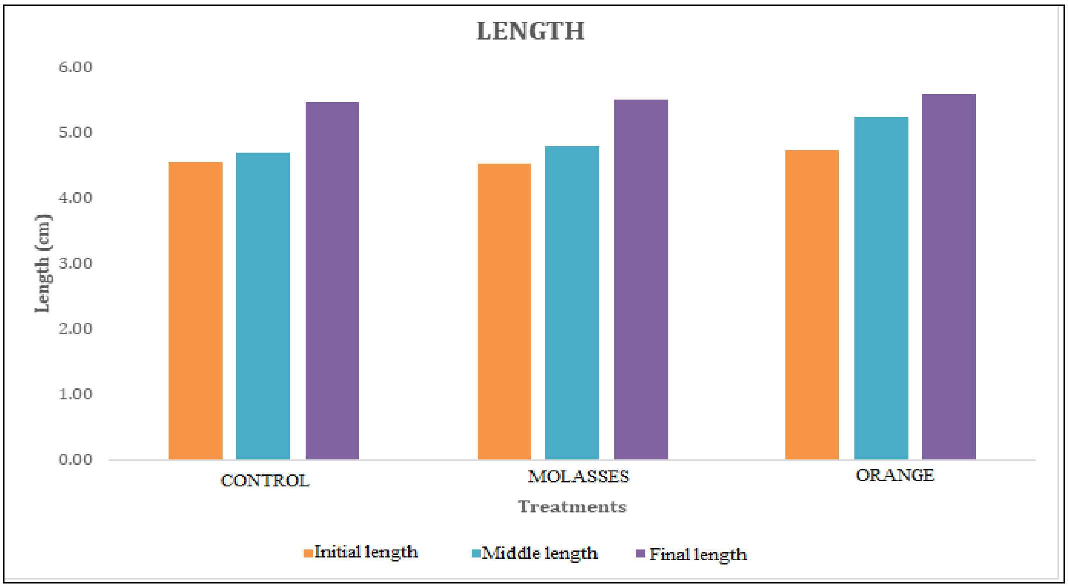

Fig 2: Initial, middle and final length of fish per treatment

3.3 Water quality

The results of the water quality parameters did not show significative variations during all the experiment. The obtained values of nitrites and nitrates, as well as the temperature and pH were maintained constant during the experiment in the three treatments and were within optimal ranges (Table 1).

Table 3: Water quality parameters

Parameter	Control	BFT 1	BFT 2	Optimal range
	M±SD	M±SD	M±SD	General*
pH	7.6	7.6	7.6	6.8 – 8.0
Temperature (ºC)	18.8±1.74	18.33±1.63	18.47±1.41	28- 30
NO_2- mg/ L	0.15±0.18	0.10±0.18	0.10±0.16	> 1
NO_3- mg/ L	10±5.67	6.33±2.29	7±4.14	0.5-20
NH_4^+ mg/ L	4.33±4.06	4.23±3.78	4.59±3.89	0.39-0.8

*Emerenciano *et al*., 2017
M= average
SD= Standard Deviation

4. Discussion

During the development of this study in different treatments (Molasses and orange peel flour) the highest survival rate was with orange peel flour, this is probably due to the antioxidants present in the orange peel as they neutralize the oxidative action of the free radicals with which cellular and tissue damages are avoided, also due to the high content of vitamin C that works as immunostimulant in fish [14].

According to some authors [15, 16] the immune system of fish is considered the first line of defense against a wide spectrum of pathogens and different stress factors, which causes greater well-being and better assimilation of nutrients, which is reflected in the weight and total length gain and increases the survival of the fish, this is similar to the reported by Rajkumar [17] who points out that by culturing shrimp in a Biofloc system using molasses as carbon source, it maintained a survival of 90.3% obtaining higher rates than conventional systems.

About weight the treatment with orange peel flour presented the highest weight gain at the end of the experiment. Kuhn [6] mentions that a diet complemented with flocs can increase the growth of the organisms by 65.1%, above diets in conventional systems. In the treatment evaluated by Moreno [18] it was added 80% of orange peel flour to 20% of commercial food to feed red tilapia hybrids, resulting the orange peel flour treatment more efficient in weight and growth gain regarding to control and the other treatments. Finally, Castro [19] observed in a Biofloc culture of *C. auratus* using molasses, coffee and moringa as carbon sources, the organisms with molasses reached higher weight values followed by coffee and moringa treatments during 120 days of experimental culture. Compared to our experiment, the gain was significative at 45 days of culture. Being the orange peel flour the one that obtained better results followed by molasses and control.

The orange peel flour, also, had better results in total length. The microorganisms present in the Biofloc system have an important role in the growth of organisms by being a natural protein source. Diverse works [20, 21, 22] indicate that maintaining high levels in the C:N relation promote the growth of heterotrophic microorganisms capable of consume diverse nitrogenous compounds like nitrites, nitrates, ammonium and organic matter, favoring the formation of microbial community that is *in situ* natural protein. By microscopically analyzing the water from the mature treatments in this investigation, were identified diverse planktonic microorganisms. The dominant groups that influence positively in fish alimentation in the Biofloc with molasses were *Lecane decipiens, Phacus, Thecamoeba, Euglena, Ankistrodesmus, Navicula sp. Microthorax, Actinophys* and *Paramecium*; and in Biofloc with orange peel flour: *Arcella, Euglena, Anabaena cylindrica, Spirostomum, Ankistrodesmus, Bosmina longirostris, Cladocera Chlorococcum sp., Micrasterias, Peranema* and *Actinastrum.* The identified planktonic groups have been reported as dominant groups in Biofloc systems, proving its positive effect in the maintenance of water quality and nutrition of fish in culture [23].

Water quality parameters were maintained in an adequate interval without significative variations, This results match with ones reported by Lovera [24] and Emerenciano [25], regarding to mean values of pH that goes from 7.17 to 8.26, also it supports the results of Becerril [26], which observed that when comparing different carbon sources (molasses, coffee residuals and rice flour) in a *Oreochromis niloticus* culture, the nitrogenous compounds and pH did not show significant differences. Serra [27] claim that the water quality is better when adding molasses than rice bran, because molasses allows faster immobilization of ammonia and convert it in microbial protein due to its simpler structure and is easy to use by microorganisms.

5. Conclusion

Orange peel flour is a good option for its use as carbon source in a Biofloc culture system of *C. auratus*, because it improves the growth, survival, and the nitrogenous compounds in the culture water. Nevertheless, it is important to keep obtaining results of its application in other species of commercial interest and evaluate other parameters that are of interest in ornamental aquaculture.

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