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Effect of biofloc on growth of genetically improved farmed tilapia juveniles in indoor condition

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Abstract
The experiment was focused to study the effect of biofloc on growth of Genetically Improved Farmed Tilapia (GIFT) juveniles. Two biofloc treatments; biofloc formed with added glucose (BFG) and molasses (BFM) by maintaining a C/N ratio at 15 were used. Water quality parameters, body weight and the standard body length were measured and specific growth rate (SGR) was calculated. Proximate analysis for biofloc was performed. Water quality parameters were within the favorable range for tilapia culture. The average body weight and length of tilapia were significantly higher ($P<0.05$) in both treatments from 6th week onwards. The significantly higher ($P<0.05$) SGR of fish was observed in BFG (2.5% d^{-1}). Crude protein content was higher ($P<0.05$) in BFG. Thus, it is concluded that biofloc enhances the growth of the tilapia.

Keywords: Biofloc, GIFT Tilapia, growth, water quality

1. Introduction

The biofloc technology is used for improving the water quality in aquaculture through balancing carbon and nitrogen in the system. The technology has recently gained attention as a suitable method to produce the extra protein rich feed while controlling the water quality. If carbon and nitrogen are balanced in the solution, ammonium in addition to organic nitrogenous waste will be converted into bacterial biomass [1]. This is due to the stimulation of bacterial growth and thereby production of microbiological protein by up taking the nitrogen from the culture [2].

In intensive fish culture systems, fish are fed with feeds that contain high amount of proteins. Avnimelech [3] indicated that, only 25% of fed protein is retained in the fish while rest is lost to the system as ammonia, organic nitrogen in faeces and feed reside. Hence, pond water contains a high load of nutrients which results feed wastage as well as accumulation of toxic residues, fish growth retardation and more importantly it will limit the intensification. These reasons lead to poor income and lower the rate of increasing the production. As an alternative in industrial level, it is possible to use Recycling Aquaculture Systems (RAS); however, it is more expensive.

Hence, it is important to find out the possible ecological sustainable solution for the above problems. Multi trophic aquaculture, integration of fish culture, periphyton technology and biofloc technology have gained much attention recently because those provide ecological solutions for the above problems. The biofloc technology can function under minimum water exchange and water usage in aquaculture systems through maintaining adequate water quality within the culture unit and while producing low cost bioflocs rich in protein and which in turn can serve as a feed for aquatic organisms. When considering the aquaculture sector in Sri Lanka, it is still at developing stage and contributes less than 20% to the total fish production [4]. Thus, it is possible to use biofloc technology, because it provides low cost and ecologically sustainable production. Therefore, this study was performed to investigate the suitability of different carbon sources to form the biofloc in indoor tanks for rearing of GIFT tilapia.

2. Materials and methods

2.1 Experimental setup: The experiment was conducted at the faculty farm premises, Faculty of Agriculture, Rajarata University of Sri Lanka.

The rectangular 12 glass tanks (60 cm x 30 cm x 30 cm) were arranged giving indoor conditions and total culture period was eight weeks.

2.2 Treatments: Two biofloc treatments and one control with four replicates were managed in 50 L indoor tanks: BF formed with added Glucose (BFG) and molasses (BFM) by maintaining a C/N ratio at 15 and clean water control without biofloc. The method described by the Crab *et al*. [5], the required carbon amounts was calculated. Carbon percentage of the glucose was calculated based on the atomic weights and the carbon percentage of the molasses was based on the Panjaitan [6]. Carbon was added to the tank at three days interval and it was split into three equal amounts and dissolved in the water.

2.3 Fish stocking and maintenance: Hormone treated 45-day old male Genetically Improved Farmed Tilapia (GIFT) juveniles with mean body weight 2.8 ± 0.2 g and standard length 1.8 ± 0.2 cm were used and they were acclimatized for 3 days prior to the experiment. The fish were stocked at a rate of 1 fish per litre for each treatment. Minimum water exchange (10%) was practiced for the biofloc treatment and daily 50% water exchange was followed for the control. Aeration was provided using an air blower throughout the experiment. Commercial feed (34.4 ± 0.8% protein) was provided daily in each tank at of 5% of the total body mass. Daily feed rations were split into four equal amounts and given at 0800, 1200, 1800 and 2200 h to all the tanks. Two types of commercial feeds were used based on their mouth size at initial and latter part of the study.

2.4 Fish growth measurements: The average body weight and length of tilapia were measured in 7-day intervals during the cultural period. Ten fish were randomly collected form each tank and measured the body weight using an electrical balance. For the standard body length measurement, it was netted five fishes and the length was measured using a ruler. The specific growth rate (SGR) was calculated according to the equation reported by Luo *et al.* [7].

2.5 Water quality parameter analysis: Some water quality parameters such as temperature, dissolved oxygen (DO) and pH were measured in situ each morning using a thermometer, pH meter and DO meter (EUTECH Instruments, Thermo Fisher Scientific, Singapore), respectively. Total ammonia nitrogen (TAN) and nitrate nitrogen (NO_3-N) were measured biweekly using phenate and salicylate method, respectively using a spectrophotometer (Spectro uv-vis spectrophotometer uvd-2, India).

2.6 Composition analysis: At the end of the experiment period, the compositions of the biofloc formed by both treatments were determined using proximate analysis method [8].

2.7 Statistical analysis: Growth performances of the fish and composition measurements were analyzed using analysis of variance (ANOVA) and student t test, respectively using SAS software, ver. 9.0 [9]. The means were separated using Tukey"s Studentized Range Test (TSRT). Statistical significance was declared at $P<0.05$.

3. Results and discussion

3.1 Biofloc formation by different carbon sources: Culture water of the control and two biofloc treatments were observed under the light microscope and it was found that biofloc water contains high number of microorganisms such as algae, rotifers, nematodes and other water dwelling organisms (Figure 1). Further, it was observed that abundance of the microorganisms was much higher in the culture water of the biofloc which formed by the addition of glucose. Moreover, most of the zooplankton like rotifers and copepods were grazed on the aggregates that were presence in the culture water. Azim and Little [10] identified that, taxonomically three types of organism were presence in the biofloc mainly: Protozoa, Rotifers and Oligocheta. However, during this study it was unable to identify the organism presence in the culture water by taxonomically. The organism presence in the culture water and the particle matters can be considered as the potential food sources for the cultured organism. In the water column, it is possible to occur a complex interaction between organic matter, physical substrate and large range of microorganisms such as phytoplankton, free and attached bacteria, aggregates of particulate organic matter and grazers, such as rotifers, ciliates and flagellates protozoa and copepods. Thus, natural food web and interactions play an important role on recycling nutrients and maintaining the water quality.

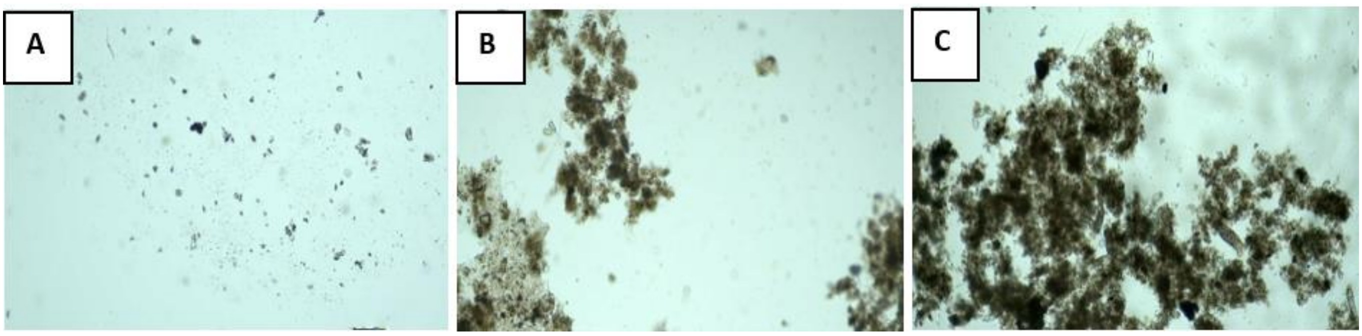

Fig 1: Micrographs; A - culture water from the control tank, B- biofloc formed by addition of glucose, C - biofloc formed by addition of molasses (×40 magnification)

3.2 Growth performances of Tilapia juveniles: The average body weight and the standard length were not significantly different ($P>0.05$) between the treatments during the 1st five weeks (Table 1 and 2). However, both parameters showed significantly higher values ($P<0.05$) in biofloc treatments during the 6th to 8th week. The significantly highest ($P<0.05$) SGR of fish was observed in biofloc glucose treatment (2.59% d^{-1}) followed by biofloc molasses treatment (2.23% d^{-1}) while the lowest was in control (1.75% d^{-1}, Fig 2).

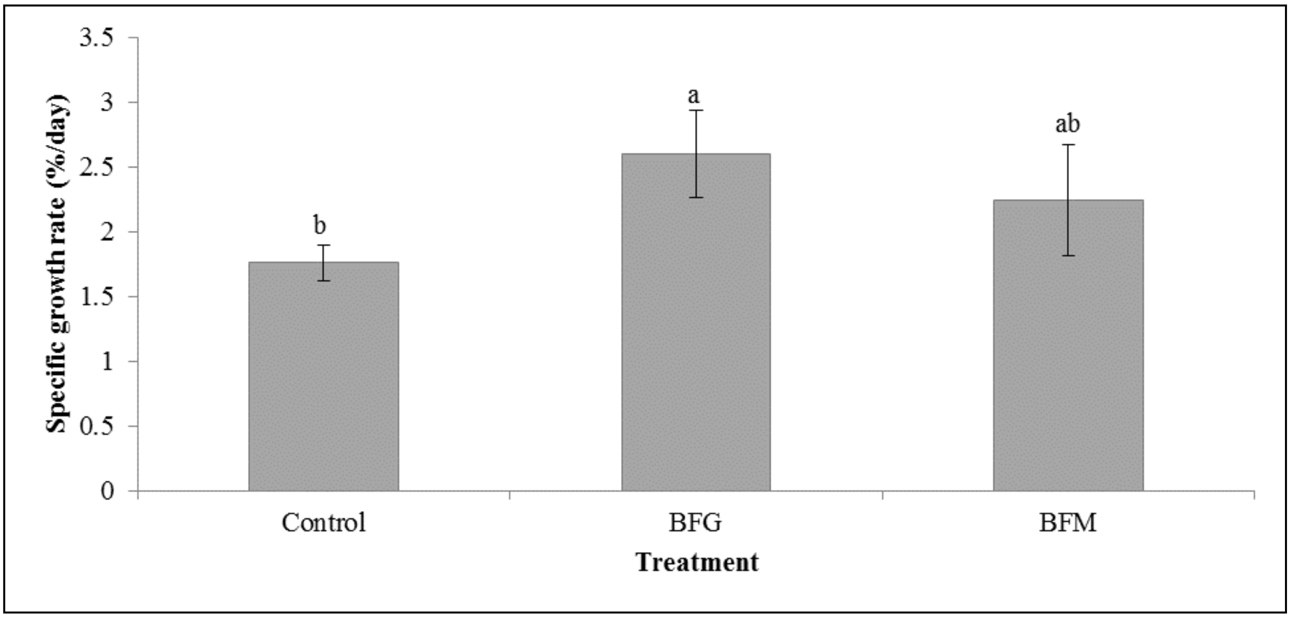

Fig 2: Comparisons of specific growth rates of tilapia juveniles in different treatments during total culture period
$^{a, b}$ means with different superscripts among the treatments are significantly different (P<0.05)

Growth performances results showed that biofloc enhances the growth performances of the tilapia juveniles in indoor condition. Azim and Little [10] and Luo *et al*. [7] also reported the similar findings with different cultured organisms. The higher growth performances with the biofloc treatments may be due to the high feed intake as compared to the control. It is well documented that microorganism and particles presences in the culture water contributed to the feed of the cultured organism [5, 1]. As it was observed, biofloc culture water was rich in microorganism and the aggregates (Fig.1). It is highly possible that they might have contributed to the feed intake of the tilapia juveniles and thereby increase the growth performances of the fish. The proximate analysis results revealed that biofloc have high nutritive value. Hence, it is logical to assume that additional feed intake with nutritive feed can enhance growth performances of the fish. Further, it was found that biofloc intake by the cultured species depends on the several factors, includes the species and feeding traits, animal size, floc size and floc density [3, 11, 12, 13]. Hence, it is expected that, the similar growth rate among the treatments during the initial period of the study due to a result of several factors including the floc size, density and the size of the fish. Moreover, the poor water quality also can play a role in reduction of the growth because fish were under the stressful condition with low DO values and elevated NH_3 levels.

Even though biofloc enhances the growth performances of the tilapia, a significant difference between the BFG and BFM treatment was observed. The researches have shown that water quality and the nutritional properties of the flocs are different with the carbon source used to produce the floc [11, 13]. Some authors revealed that different organic carbon sources stimulated specific bacteria, protozoa and algae, and hence influenced the microbial composition and community organization of the bioflocs and thereby also their nutritional properties [11]. Therefore, the differences observed in the growth performances between BFG and BFM may be due to results of the presence of different micro flora in the culture water. Further, Crab [11] and Crab *et al*. [13] found that the type of carbon source also influenced the availability, palatability and digestibility for the cultured organisms.

Table 1: Average body weight of the tilapia juveniles in different treatments throughout the culture period

Treatments	Weeks							
	1	2	3	4	5	6	7	8
Control	2.9 ±0.2	4.0 ±0.2	4 ±.0.2	4.5 ±0.1	6.0 ±0.5	7.1^{b} ±0.1	7.4^{b} ±0.3	7.9 $±0.6^{b}$
BFG	2.8 ±0.2	3.7 ±0.1	4.0 ±0.1	4.2 ±0.1	6.6 ±0.9	10.1^{a} ±1.0	10.6^{a} ±0.9	12.1^{a} ±1.1
BFM	2.8 ±0.3	3.5 ±0.1	4.0 ±0.2	4.2 ±0.1	6.5 ±0.2	7.8^{b} ±0.8	9.1^{ab} ±1.2	9.9^{ab} ±1.4

Data are presented as means ± SD in grams (g)
^{a,b} means within the same column with different superscripts are significantly different (P<0.05)

Table 2: Average standard body length of the tilapia juveniles in different treatments throughout the culture period

Treatments	Weeks							
	1	2	3	4	5	6	7	8
Control	1.7 ±0.1	2.5 ±0.0	3.2 ±0.1	3.7 ±0.1	3.8 ±0.0	4.2^{a} ±0.2	4.7^{a} ±0.3	5.2^{a} ±0.1
BFG	1.9 ±0.2	2.6 ±0.2	3.6 ±0.2	3.8 ±0.0	3.7 ±0.9	4.9^{b} ±0.1	5.3^{b} ±0.2	5.7^{b} ±0.2
BFM	1.9 ±0.1	3.0 ±0.1	3.4 ±0.3	3.8 ±0.1	4.3 ±0.2	4.8^{b} ±0.3	5.3^{b} ±0.1	5.6^{b} ±0.1

Data are presented as means ± SD in grams (g)
^{a,b} means within the same column with different superscripts are significantly different (P<0.05)

3.3 Water quality parameters: Temperature, pH and DO in water of all treatments were maintained in optimal condition for fish culture which were ranged from 27 - 32 °C, 6.5 - 8.5 pH and 2.1 - 6.9 mg/L respectively. Higher DO value was observed in control tank while low values were observed in BFT molasses tank.

Dissolved oxygen was depleted during the culture period, especially in the BFM treatment. In order to keep the DO at optimal level, water was replaced several times. As discussed earlier, different carbon sources stimulate different microorganisms in the culture water and molasses might have boosted the heterotrophic bacteria instead of the algae. Therefore, ability to produce oxygen through photosynthesis may be limited in the BFM tank as compared to the BFG tank. According to the Ebeling *et al.* [14], photosynthesis and nitrification processes that likely to develop in the system possibly resulted in pH fluctuation, as these processes are likely to alter CO_2 concentration and buffering capacity in water. During this study period, pH value showed fluctuation and it might be affected by CO_2 concentration in cultured water, dissolved chemicals in cultured water and assimilated nitrogenous waste in water.

During total culture period, the range between nitrate nitrogen and ammonia nitrogen were 0 – 15, 0 - 0.025 mg/L respectively. The nitrate concentration among the biofloc treatments were increased over the time while it remains approximately constant in the control treatment. Further, nitrate accumulation in BFM is higher than the BFG during the initial period of the study while at the latter part, it reached to the similar value. The similar results were obtained by Azim and Little [10]. According to the Ebeling *et al.* [14], nitrogen can be removed biologically from an aquaculture system mainly by algae through photoautotrophic pathway, by heterotrophic bacteria through immobilization or by nitrifying bacteria through chemo-autotrophic oxidation. In the present experiment, accumulation of nitrate in the culture water may result the dominant nitrifying bacteria populations compared to the other two types of organisms. Therefore, it is suggested that further research is required to identify the type of microorganisms found in the culture water. Even though it was observed the fluctuation of the ammonia concentration in the culture water, it remained within a suitable range for the tilapia growth in every treatment. The higher concentration was observed in the BFG while it remained less than 0.01 mg/L in the control treatment.

3.4 Proximate analysis: Proximate analysis was conducted at the end of the experiment to find out the nutritive values and the best locally available carbon source for biofloc formation in aquaculture farming.

The proximate analysis results of the two treatments (BFG and BFM) were significantly different ($P<0.05$) for three attributes. Results indicated that the nutritional condition of the fish kept in the BFT tank was generally better than that of the fish kept in the control tank. It has been reported that biofloc provides extra essential nutrients including proteins, lipids, essential fatty acids, minerals, vitamins, carotenoids and exogenous digestive enzymes [15, 16, 17]. Protein requirement for grow out culture of red tilapia seems to be varied from 20% to 42% [18, 19, 20]. High crude protein level was observed in biofloc glucose treatment. However, these values are lesser than the values reported by Azim and Little [10]; Kuhn *et al.* [21]; McIntosh [22] and Tacon *et al.* [23]. This may have attributed by the different composition of the microorganism presence in the culture water. Biofloc biological composition might influence its biochemical composition, whereas Shifrin and Chisholm [24] reported that diatom could contain lipid up to 25%. For that reason, it may be suggested that the high diatom density associated in bioflocs contributed to the high lipid content of biofloc. With regard to tilapia lipid requirement, it was noted that optimum dietary lipid requirement of tilapia is in a range of 5-12% suggesting that the lipid content of biofloc in this study was more than sufficient [25].

4. Conclusions

Based on the specific growth rate value, it is concluded that the biofloc enhances the growth of tilapia juveniles. Moreover, biofloc formed by addition of glucose has more crude protein than the biofloc formed by the addition of molasses. Careful observations are required in the biofloc tanks for further recommendations.

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