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Effect of biofloc on water quality and growth performance of *Etroplus suratensis* (Bloch, 1790)

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Abstract
This study evaluated the effect of biofloc on water quality and growth performance of *Etroplus suratensis* for 161 days at ICAR-CCARI, Goa. It included two treatments T1 (wheat flour + formulated feed (2% of fish biomass) and T2 (wheat flour) and a control (formulated feed) with triplicate in 312 L capacity indoor cement tanks filled with 25 L of pond water and 275 L of tap water. The tanks were left for 15 days to make it favorable for the growth of microbes to develop the biofloc. The fingerlings of Pearlspot, *Etroplus suratensis* with an average body weight of 9.2±0.2 g were stocked at a rate of 50 no. /tank and cultured for a period of 161 days. Specific growth rate (SGR) was recorded significantly ($P<0.05$) higher in T1 (1.06±0.19), followed by T2 (0.79±0.12) and control (0.46±0.09). Lower pH values were recorded in biofloc tanks may be attributed to the higher rate of nitrification and respiration by microbes. Besides, the addition of carbohydrate has significantly reduced the total ammonia nitrogen (TAN), and nitrate-nitrogen in water. The result of this study concluded that the use of wheat flour (carbohydrate source) along with formulated feed has effectively enhanced the biofloc production which contributed effectively in the maintenance of good water quality, resulting higher production of fish.

Keywords: Biofloc, Specific growth rate, water quality

Introduction

Fish is a rich source of high value protein, essential amino acids, minerals and poly-unsaturated omega 3 fatty acids. Moreover, it is considered as the cheapest source of protein among the various food sources. In general, the fish supply comes mainly from two sources i.e. capture fisheries and aquaculture. Interestingly, India is the second country after China which contributes about 6.3% to world aquaculture production

FAO [10] The total fish production in the world was estimated to be 158 million tonnes in 2012, to which aquaculture contributed about 42% FAO [10]. Globally, the demand for aquatic food is increasing year after year. In this regard, the huge gap in fish production projected for future can be addressed only through aquaculture. Thus, the country is looking for strategic approaches for improving the production from aquaculture systems. However, the major concern in the development of aquaculture is for identifying an effective, ecofriendly and low cost feed for the candidate species. Fish consumes only a small amount of feed and the remaining gets accumulated in a culture environment which affects the water quality Avnimelech [4]. Once feed is wasted, then it degrades and toxic metabolites are formed in the water such as ammonia, sulphide etc. Excessive accumulation of toxic metabolites in the pond causes deterioration of the surrounding environment. Hence, we need some technology to reduce, regulate or revert the metabolites. A novel approach called biofloc technology is a promising tool for addressing above issues. Biofloc technology is based on zero water exchange, subsequent development of dense microbial population through maintaining high C/N ratio to maximize biosecurity and minimize the external environmental effects Prajith, [16] The bacteria, forming bioflocs assimilate TAN (total ammonia nitrogen) to produce microbial proteins which facilitates the recycling of the unused feed Avnimelech [4] These microbial proteins form a good source of food for the cultured fishes. Hence, this technology can be recommended for the sustainable aquaculture production. Bioflocs are aggregates (flocs) of algae, bacteria, protozoans and other kinds of particulate organic matter such as feces and uneaten feed. Each floc is held together in a loose matrix of mucus that is secreted by bacteria, held by electrostatic attraction Hargreaves [13]. The bacterial cells are made up of proteins and they have to maintain a fixed carbon/nitrogen (C/N) ratio in their cell.

When the bacteria will be fed with organic matter which contains more carbon and less concentration of nitrogen; to synthesize the bacterial protein and to maintain a proper C/N ratio, it will have to take up more nitrogen from the water. Therefore, an increment in the C/N ratio by supplying an additional carbon source (carbohydrate) will increase the C/N ratio in the system. In order to balance the increased C/N ratio, bacteria assimilates more nitrogen from the nitrogenous compoundst present in water. Hence biofloc can convert unutilized nitrogen to produce microbial protein, rather than generating toxic compounds Avnimelech, [4]. These microbial protein will form a food source for fishes and indirectly, there will be a reduction in the load of organic matter in the biofloc system and the water quality will be maintained Avnimelech, [2] Biofloc based aquaculture system works best with fish species that are able to derive some nutritional benefits from the direct consumption of flocs. Pearlspot, *Etroplus suratensis*, is the largest among Indian cichlids, a high-valued food fish endemic to peninsular India and Sri Lanka. Because of their wide salinity tolerance, omnivorous feeding habit and high market price, pearlspot is considered ideal species for culturing in brackish and freshwaters. With its increasing demand, the price of the fish variety also soars. Apart from the growing demand from local market and domestic tourism sector, the fish is also exported in large quantity to foreign markets as well Padmakumar *et al*. [15]. In aquaculture, nearly 50 percent of the total cost is associated with feed, which is mainly due to the cost of the protein component in commercial diets by means of biofloc technology, a preferred fish species can be cultured in a closed system without the addition of feed also which will help maintaining water quality in a controlled aquaculture system. Compared to other treatments, biofloc system shows better growth of fishes as well as good quality and it more economical and viable. Aquaculture is an activity with a remarkable potential to provide protein rich food and to generate employment for human benefits. Its impacts deserve to be examined by using the technologies like biofloc system. Keeping in view the above factors, the present study aimed to generate information about the ecological impacts of biofloc on *Etroplus suratensis*. The study focused on evaluation of growth performance of the fish under biofloc-based culture system with a reference to the effects of biofloc on selected water quality parameters in the fish culture system.

Material and Methods

Duration of experiments

The experiments were conducted for 161 days to assess the effect of biofloc on water quality and growth performance of *Etroplus suratensis* fingerlings. Site of the Experiment The experiment was conducted in the hatchery yard of ICAR-CCARI, Goa. Subsequently, the laboratory work was carried out in the laboratory of Fisheries Section, ICAR-CCARI, Goa.

Experimental Animals

Fingerlings of *E. suratensis* were collected from the ponds of Curtorim farm, Goa. The initial average weight of the fishes was 9.2±0.2g. The fishes were acclimatized in the FRP tanks (500L) for 15 days and were fed with pelleted feed having a protein content of 35.6%. Feeding was carried out twice a day at 11.00 am and 4.30 pm during the acclimatization. The feed was provided at the rate of 2% of the body weight.

Experimental Diet

The ingredients were dried and powdered, thoroughly mixed and a dough was prepared by adding the required amount of salt, oil and water. The dough was autoclaved at 121 °C For 10 min. pellets of fish feed was prepared using the pelletizer and sun dried for 5-6 hour and stored in an airtight container.

Experimental design and setup Nine cement tanks of 312 liter capacity (1.0 x 0.48 x 0.65 m) were used for the experiment with 2 treatments and 1 control viz., T1 (wheat flour with+2%feed), T2 (wheat flour), and control (formulated feed). Tanks were cleaned thoroughly and filled with freshwater. Before floc formation procedure each tank was filled with 25 liter of pond water. The tanks were aerated well and mixed thoroughly. It was left as such for a few days to make the tank favorable for the growth of bacteria and other microbes, which resulted in generation of biofloc. Two treatments T1 (wheat flour with+2% feed), T2 (wheat flour) were used for the formation of bioflocs and were maintained for 161 days along with a control (C). A total of 450 fingerlings (9,2g) were equally and randomly distributed (50 in each tank, total body weight: 460g in each tank) in 3 experimental groups, each with 3 replicates. Two aeration pipes in each tank were put to meet the oxygen demand of the fish and to keep the flocs in continuous suspension. The fish were fed in the morning at 11:00am @2% body weight. The carbon sources were added weekly to their respective treatment to maintain a C/N ratio of 15:1.

Estimation of carbohydrate

Theoretically, C/N ratio of 15:1 was maintained by adding a carbon source to each treatment. The amount of carbon content depended on the added carbon source. Generally, it is assumed that the commercially available carbon sources contain about 50 % of C g-1. Calculations for maintaining a C/N ratio of 15:1 were done using Avnimelech [2]. method. 30% dietary protein pellet feed contained 4.8% N and assumed 50% of feed nitrogen into water directly by excretion or indirectly by microbial degradation of feed. 4,4g.day^{-1}of CHO was added to reduce total ammonia nitrogen (TAN) concentration by 1 mg l-1 N (i.e., 1 g N/m3) is 20 g/m3)

Water Quality Parameters

Water samples were collected at weekly interval from the experimental tanks after stocking during morning hours 8.00 and 9.00 am for a period of 5 months and 11 days. Temperature and pH was measured in the experimental unit itself. Estimation of dissolved oxygen and biochemical oxygen demand, water samples were collected separately in respective bottles following the guidelines for sample collection and preservation. For analyses of other water quality parameters, water samples were collected in 1L polyethylene bottles and stored at 4 °C. Water quality parameters were estimated in the laboratory using standard procedures.

Growth Parameters

The growth parameters of the *E. suratensis* were estimated by taking their body weight at every 28 days interval.

Results and Discussion

An experimental trial of 161 days was conducted to assess the effect of biofloc on water quality and growth performance of *Etroplus suratensis* fingerlings with two treatments: T1-(wheat flour with +2% formulated feed), T2 (wheat flour), and control (formulated feed). The various parameters observed during the experimental trial are presented below.

Water Quality Parameters

Water quality parameters of *E. suratensis* after 161 days of rearing in the biofloc based system showed a considerable variation. The water temperature of the different experimental groups ranged from 24.5 °C to 26.4 °C during the experimental period. There was a significant difference between treatments and control. The mean temperature ranged from 25.02 to 25.64 °C (Table 1). The total suspended solids (TSS) fluctuated from 1195 to 1425 mg L-1 in the experimental tanks. The TSS among treatment varied significantly. The TSS in T2 and T1 were significantly higher than the control (Table 1). According to Samocha *et al*. [19] TSS levels up to 500 mg/L are considered to be appropriate for the culture. The level of TSS could be reduced through the process of clarification, which helps to maintain dissolved oxygen at elevated levels throughout the culturing period, reducing the concentrations of phosphorus and nitrate and increasing alkalinity Gaona *et al*., [11] However, clarification to avoid removal of nitrate from the experimental units was not performed. The mean pH ranged from 7.66 to 8.32 (Table 1). The lower pH values in the biofloc tanks were possibly a result of high respiration rates by the large quantities of microorganisms, which might have increased CO2 concentrations. A similar trend was observed by Wasielesky *et al*. [21]. In addition, Chen *et al*. [6] reported a decrease in pH during the chemolithotrophic nitrification process as a result of $CaCO_3$ consumption and the release of CO_2 and H+ into the culture medium. The concentrations of dissolved oxygen in all experimental tanks were recorded within the range of 6-7.8 mg L^{-1} during the experimental period. Dissolved oxygen showed significant variation (P<0.05) among treatments. The T2 (7.65 mg L-1) had significantly higher DO than that of T1 (7.43 mg L-1) and control (Table 2). Dissolved oxygen (7.17-7.43 mg L-1) observed during the experimental period was in the optimal range and continuous vigorous (24 h) aeration was provided which ensured that DO is not a limiting factor. This revealed that biofloc technology (BFT) is positively affecting the system by improving the water quality BOD in T2 (1.95 mg L-1) was significantly higher than that in T1 and control (Table 1). Higher BOD in biofloc tanks than control is due to higher microbial density Azim *et al.* [5] The concentrations of dissolved organic matter in all the experimental tanks were recorded within the range of 2.4- 2.9 mg L^{-1} during the experimental period. The dissolved organic matter in T1 and T2 were significantly higher than that of control (Table 1). The average concentration of dissolved organic matter of 2.57, 2.65, and 2.68 mg L^{-1} was recorded in the control, T1 and T2, respectively. The dissolved organic matter constantly increased over the period of time and consequently nitrite concentrations also increased Hargreaves, [13] The mean chlorophyll ranged from 19.16 to 21.46 µg L^{-1} (Table 1). The concentration of available phosphorus ranged from 0.1 to 0.75 mg L^{-1}. The average concentration of chlorophyll a was recorded as 21.46, 19.59, 19.16 µg L^{-1} in the T1, and T2 and control, respectively. Higher chlorophyll-a content in BFT treatment indicates phytoplankton production, which is an indication of the positive effect on plankton nutritional quality Azim *et al*. [5] The concentration of TAN varied significantly (p<0.05) between the treatments. The highest mean values of 0.01 mg L^{-1} and the lowest mean value of 0.008 mg L^{-1} in was recorded T1 and T2, respectively (Table 1). The relationship between adding carbohydrates, reducing ammonium and producing microbial proteins depends on the microbial conversion coefficient, the C/N ratio in the microbial biomass and the carbon content of the added material Avnimelech, [2] Avnimelech [4] proved that addition of carbohydrate reduces the need of dietary protein concentration and also decrease the TAN level in the system. The average concentration of TAN observed was 0.01, 0.008, 0.008 mgL^{-1} in the control, T1 and T2 respectively. In this experiment, the wheat flour+2% feed (T1) and wheat flour (T2) effectively reduced the TAN level. The concentration of nitrate-N among the treatments varied significantly (Table 1). However, the mean value of higher concentration of NO_3-N (0.22 mg L-1) was observed in the control and lower concentration of 0.20 mg L^{-1} in T1 (Table 1) This low level probably relates to NO_3-N uptake by microbes in the treatments in particular when there was limited ammonia-nitrogen available in the water Hargreaves [13]

Table 1: Water quality parameters of different experimental group

Parameter	C	T1	T2
Temperature (°C)	25.6±0.01^a	25.3±0.02^b	25.0±0.05^c
Total suspended solids (mg L^{-1})	1249.57±6.02^c	1307±9.5^b	1318±9.39^a
Salinity(ppt)	0.094±0.001^b	0.095±0.001^a	0.095±0.0001^a
pH (no unit)	8.32±0.006^a	7.69±0.02^b	7.66±0.04^b
DO (mg L^{-1})	7.17±0.02^c	7.43±0.08^b	7.65±0.10^a
BOD (mg L^{-1})	1.33±0.01^c	1.77±0.03^b	1.95±0.05^a
Dissolved organic matter (mg L^{-1})	2.57±0.01^c	2.65±0.02^b	2.68±0.023^a
Chlorophyll a (µg L^{-1})	19.16±0.69^c	21.46±0.68^a	19.59±0.71^b
Available phosphorus (mg L^{-1})	0.37±0.02^b	0.36±0.02^b	0.39±0.03^a
TAN (mg L^{-1})	0.01±0.001^a	0.008±0.001^b	0.008±0.001^b
Nitrate-N (mg L^{-1})	0.22±0.02^a	0.20±0.02^b	0.21±0.023^a
Nitrite-N (mg L^{-1})	0.20±0.02^a	0.23±0.02^a	0.23±0.023^a

Values with same letter in superscript in each row do not vary significantly based on tukey HSD at p=0.05.
Values are presented as mean±SE

Growth performance among different treatment groups lowest SGR value was recorded for the control (0.46±0.09) group and a significantly higher (SGR) was found in the treatment T1 (1.06±0.19) (Table 2). The mean FER value was significantly different (P<0.05) among the treatments and control. A significantly higher FER value was recorded in T1 (11.27±1.09) as compared to control (5.21±0.24) (Table 2). FCR value recorded in control (0.20±0.014) was highest whereas a significantly lower FCR was noticed in T1 (0.125±0.020) (Table 2). In the present study, biofloc induced through supplementation of carbon source resulted in a significant increase in parameter viz. SGR, FER and a reduced FCR of *E. Suratensis* fingerlings when compared to control. In the BFT treatments, there was significant higher weight gain than the control confirming the utilization of bioflocs by fish as a supplementary food. In an earlier study, Mahanad *et al*. [14] studied the effectiveness of biofloc technology in *L. rohita* and reported that the fish showed an increase in growth performance under BFT system compared to control. Studies have indicated that that carbohydrate addition can result in the production and accumulation of bioflocs which could serve as an important food source for the zooplankton and thus could increase the growth of shrimp. Rostika *et al*. [18] reported that the microorganism present in the biofloc provide the food for the cat fish (*Clarius gariepinus*). Twenty to thirty percent of shrimp or tilapia growth is derived from the consumption and digestion of microbial protein Crab *et al.* [7] therefore, bio flocsoffer a cost effective solution for enhancing the production of shripms and fish species such as Clarius spp. The microorganism's community present in biofloc was mainly represented by protozoa grazers, rotifers, bacteria and diatoms that provides a continuous natural food source to fish. In the present study, specific growth rate (SGR) values recorded higher values compared to control Schryver *et al*. [21] demonstrated the consumption of biofloc by shrimp or fish has numerous benefits such as improvement in growth rate; decrease in FCR and associated costs in feed. FCR was significantly lower in biofloc systems compared to the control.

Table 2: Growth parameters of fish in different experimental groups

Growth parameter	Control	T1	T2
SGR	0.46 ± 0.09^{c}	1.06 ± 0.19^{a}	0.79 ± 0.12^{b}
FER	5.21 ± 0.24^{b}	11.27 ± 1.09^{a}	
FCR	0.20 ± 0.014^{a}	0.125 ± 0.020^{b}	

Values with same letter in superscript in each row do not vary significantly based on tukey HSD at p=0.05
Values are presented as mean±SE

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Conclusion
The study provides baseline data for validation of biofloc mediated enhancement of growth of *Etroplus suratensis.* Based on the experimental data it is concluded that induction of growth in above species is possible through biofloc formation in the culture tanks. Further, it is evident from the data that biofloc formation showed no deteriorating effect on water quality. Overall, the study establishes the fact that biofloc technology application to aquaculture is an ecofriendly and low-cost approach for the enhancement of aquacultural production. There is potential scope for large scale intensive culture of locally demanded finfishes in biofloc based systems which would be a new initiative in aquaculture systems.

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