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Growth performance of GIFT tilapia (*Oreochromis niloticus*) fry in biofloc system using different carbon sources

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Abstract
An experiment was conducted to find out the effect of locally available carbon sources such as sugar cane molasses (T_1), sugar (T_2), jaggery (T_3), wheat flour (T_4), wheat bran (T_5) and rice bran (T_6) along with control (T_0) on growth performance of GIFT tilapia fry in biofloc systems. The fishes (average initial weight 0.57±0.003 g, average initial length 2.84±0.04 cm) were stocked @250nos. m^{-3} in 110L capacity FRP tanks. The experiment was conducted as per completely randomized design with seven treatments and three replicates for 90 days. Results revealed that the water quality parameters were within suitable range for rearing of GIFT tilapia fry. A significantly higher length gain, weight gain (%) and SGR were recorded in fry reared in rice bran biofloc treatments at the end of 30, 60 and 90 days. One-way ANOVA showed no significant difference ($P>0.05$) between the survival of GIFT tilapia fry in different treatments. A significantly lower AFCR was found in rice bran biofloc treatment than that of control, but not significantly different from other treatments. Overall, results showed the rice bran as a better source of carbon for rearing of GIFT tilapia fry in biofloc systems.

Keywords: Carbon sources, biofloc, growth, tilapia fry

1. Introduction
Fisheries and aquaculture are important sources of food, nutrition, income and livelihoods for several hundred millions of people around the world [1]. Fish is a source of animal protein, which contains all essential amino acids, vitamins, minerals and omega-3 fatty acid and has good benefits for human health. In India, there is a decline in capture fisheries production and the efforts are being concentrated on culture-based fisheries. In this sector, tilapia is one of the potential fish species which is fast growing, can be one of the alternatives for fulfilling the nutritional requirements of under-nourished peoples in India.

Tilapia is commonly known as "aquatic chicken" and the consumption of tilapia has increased world over [2]. The most common species of tilapia farmed around the world is the Nile Tilapia, *Oreochromis niloticus*, which accounts for roughly 75% of farmed tilapia in addition to its broad market acceptance. Monosex tilapia culture is the most popular practice across the world as male tilapia grows faster than female tilapia. The existing GIFT strain (Genetically Improved Farmed Tilapia) of Nile tilapia (*O. niloticus* L.) is monosex (all male) tilapia, which was first introduced into Indian water bodies by the United Nations International Children Emergency Fund (UNICEF) in 1974 and later by the Bangladesh Fisheries Research Institute (BFRI) from Thailand. The farming technique of GIFT tilapia is well established in freshwater system and to fulfil the demand of rising population and local market, intensive and super intensive farming systems of tilapia have been practiced in different countries. Therefore, there are problems of the rapid accumulation of organic matter, feed residues, and toxic inorganic nitrogen due to high-intensity systems [3, 4, 5, 6]. To overcome these problems, there is an urgent demand for a relatively new and alternative aquaculture technology to ensure the continuous growth of aquaculture sector i.e. biofloc technology (BFT).

Fundamentally, BFT encourage the propagation of heterotrophic bacterial biomass to assimilate inorganic nitrogenous waste, this results in maintenance of the water quality at acceptable level and usage of floc as a feed source [7]. Positive effects of BFT on growth performance, water quality, digestive enzymes activity, and immune response in tilapia were reported in earlier studies[8, 9, 10]. There are limited studies related to the effect of locally available carbon sources in rearing GIFT fry in biofloc system. Therefore, the present study was undertaken to evaluate effect of locally available carbon sources on growth performance of GIFT strain of *O. niloticus* (Linnaeus, 1758) fry in biofloc system.

2. Materials and Methods

2.1.1 Experimental design

The experiment was carried out in the wet laboratory of College of Fisheries, Ratnagiri, Maharashtra. Locally available carbon sources such as sugar cane molasses (T_1), sugar (T_2), jaggery (T_3), wheat flour (T_4), wheat bran (T_5) and rice bran (T_6) were used as treatments, the control group (T_0) did not receive carbon source. The experiment was conducted as per Completely Randomized Design (CRD) with seven treatments and three replicates for 90 days.

2.1.2 Experimental animal

The fry of Genetically Improved Farmed Tilapia (GIFT) were procured from the RGCA, Andhra Pradesh and acclimatized at laboratory condition for 15 days. GIFT tilapia fry (average initial weight 0.57± 0.003g, average initial length 2.84±0.04cm) were stocked @ 250 nos. m^{-3} [11] in FRP circular tanks of 110L capacity. Feed was given @ 10% of body weight twice a day for first 15 days and then was adjusted fortnightly @ 8% and 6% of body weight for next 30 and for 45 days respectively twice a day [12].

2.1.3 Preparation of inoculum

Biofloc was developed separately for each carbon source using 2nos. of FRP tanks (TSS >400 mg L^{-1}) of 110L capacity[13]. Pond bottom soil was collected from brackish water farm of College of Fisheries, Ratnagiri for initial floc formation. A quantity of 20 g L^{-1} of pond bottom soil was added in 110 L capacity FRP tanks. Then 10 mg L^{-1} ammonium sulfate $(NH)_4SO_4$ and 200 mg L^{-1} of different carbon sources (Rice bran, wheat bran, sugar, jaggery, wheat flour and molasses) were added and biofloc was developed separately for each carbon source. Vigorous aeration was supplied to FRP tanks.

2.1.4 Biofloc development in experimental tank

A quantity of 60 L of biofloc and 40 L of freshwater were added to all the experimental tanks at the start of the experiment, whereas 100 L of freshwater was added in the control tank. Vigorous aeration was supplied to each tank to keep biofloc in suspension for 24hrs. Carbon sources were added at the rate of 15 times the TAN concentration to maintain a C/N ratio of 15 for optimum production of biofloc [7].

2.1.5 Sludge removal and water exchange

Sludge was removed to maintain TSS level below 400mgL^{-1}. In control tank, siphoning was carried out and 10% water was replaced with fresh water daily.

2.2 Water quality parameters

Water quality parameters such as pH, temperature, dissolved oxygen, carbon dioxide, total alkalinity, total hardness, total ammonia-N, nitrite-N and nitrate-N were recorded during the experiment [14]. Floc volume was measured by using Imhoff cone [15]. For TSS estimation, 100 ml of water sample was collected from each replicate and filtered through pre-dried and weighed Glass Fibre (GF/C) filter paper using Micropore vacuum filter.

2.3 Growth Parameters

Before stocking, length and weight of fry were recorded. After 30 days interval, 15 number of fishes were collected from each replicate and length and weight were recorded. The length gain (%), weight gain (%), Specific Growth Rate (SGR), and survival (%) were calculated [16].

$$1.\ \text{Length gain (\%)} = \frac{\text{Final length} - \text{Initial length}}{\text{Initial Length}} \times 100$$

$$2.\ \text{Weight gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

$$3.\ \text{Survival (\%)} = \frac{\text{final fish count}}{\text{Initial fish count}} \times 100$$

$$4.\ \text{Specific Growth Rate (\% day}^{-1}) = \frac{\ln(\text{final body weight}) - \ln(\text{initial body weight})}{\text{Days of experiment}} \times 100$$

$$5.\ \text{Apparent Feed Conversion Ratio (AFCR)} = \frac{\text{Feed offered}}{\text{Biomass increased}}$$

2.4 Statistical Analysis

The experimental data such as length gain, weight gain, SGR, AFCR and survival were analysed by One-way Analysis of Variance (ANOVA). The post-hoc analysis was done using Tukey's test. The statistical analysis was performed using SAS software ver. 9.3 [16].

3. Results

3.1 Water Quality Parameters

Water quality parameters such as average temperature (°C), average pH, average dissolved oxygen (mgL^{-1}), average total hardness (mgL^{-1}), average total alkalinity (mgL^{-1}), average free carbon dioxide (mgL^{-1}), average total ammonia-N (mgL^{-1}), average nitrite-N (mgL^{-1}), average nitrate-N (mgL^{-1}), average floc volume (mlL^{-1}) and average total suspended solids are presented in Table 1.

Table 1: Water quality parameters recorded during the rearing of GIFT tilapia fry

Parameters	T_0	T_1	T_2	T_3	T_4	T_5	T_6
Temperature (°C)	24.68 ±0.58	24.84 ±0.71	24.83 ±0.69	24.67 ±0.59	24.87 ±0.60	24.53 ±0.55	24.58 ±0.53
pH	8.03 ±0.01	7.88 ±0.03	7.83 ±0.03	7.80 ±0.04	7.80 ±0.03	7.79 ±0.04	7.74 ±0.03
DO (mgL^{-1})	7.16 ±0.09	6.10 ±0.17	6.16 ±0.20	6.01 ±0.19	5.92 ±0.18	5.91 ±0.10	6.01 ±0.10
CO_2 (mgL^{-1})	4.50 ±0.75	4.33 ±1.07	4.17 ±0.91	4.39 ±1.04	4.72 ±0.77	4.67 ±0.79	4.50 ±0.46
Alkalinity	48.89	70.00	56.11	81.95	108.06	46.95	78.61

(mgL^{-1})	±5.85	±5.87	±3.30	±3.45	±5.89	±3.90	±8.45
Hardness (mgL^{-1})	83.33 ±0.65	82.44 ±2.73	81.67 ±3.01	83.22 ±2.69	82.45 ±2.01	82.33 ±2.35	83.50 ±2.44
Ammonia (mgL^{-1})	0.34 ±0.04	0.35 ±0.08	0.34 ±0.09	0.42 ±0.08	0.37 ±0.09	0.47 ±0.11	0.45 ±0.11
Nitrite (mgL^{-1})	0.29 ±0.06	0.35 ±0.05	0.29 ±0.06	0.36 ±0.07	0.29 ±0.07	0.36 ±0.07	0.36 ±0.09
Nitrate (mgL^{-1})	3.03 ±0.64	5.42 ±1.03	5.67 ±1.01	5.68 ±0.97	5.63 ±0.94	5.87 ±0.99	5.75 ±1.10
Floc level (mlL^{-1})	<0.5	20.61 ±0.80	20.17 ±1.35	20.36 ±1.20	20.31 ±1.56	17.83 ±1.11	19.08 ±0.80
Total suspended solids (mgL^{-1})	0.83±0.09	227.83 ±46.55	211.89 ±38.19	202.72 ±35.04	209.39 ±37.82	205.45 ±34.32	202.33 ±36.04

The values are expressed as mean ± standard error (SE).

3.2 Growth and survival

The average length gain (%), average weight gain (%), average SGR(%day^{-1}), average survival (%) and average AFCR of GIFT tilapia fry reared in a biofloc system using different carbon sources and in control at the end of 30, 60 and 90 days are given in Table 2. Tukey's test showed that a significantly higher (p< 0.05) length gain (%) was recorded in T_6 which was not significantly different (p>0.05) from T_2, T_3 and T_5, but it was significantly different (p< 0.05) from other treatments at the end of 30 days of experimental period. After 60 days, the length gain (%) in T_6 was not significantly different (p> 0.05) from T_1, T_2, T_4, T_5, but it was significantly different (p< 0.05) from T_0 and T_3. At the end of the 90 days, the length gain (%) in T_6 was significantly higher (p< 0.05) than T_1, T_2, T_3, T_4, T_5 and T_0 (Table 2). A significantly higher (p< 0.05) weight gain (%) of fry was recorded in T_6 than T_0 treatment at the end of 30 and 60 days. At the end, the weight gain of fry in T_6 was significantly different (p< 0.05) than T_1, T_2, T_3, T_4, T_5, and T_0. The average weight gain (%) of GIFT tilapia fry reared in a biofloc system using different carbon sources and in control at the end 90 days is depicted in Fig. 1(a).

Tukey's test showed that average specific growth rate (%day^{-1}) of fry in T_6 was significantly higher (p< 0.05) than T_0 but not significantly different (p> 0.05) than other treatments after 30 and 60 days. After 90 days, the average specific growth rate of fry in T_6 was significantly higher (p< 0.05) than T_1, T_2, T_3, T_4, T_5 and T_0. The average specific growth rate (%day^{-1}) of GIFT tilapia fry reared in a biofloc system using different carbon sources and in control at the end 90 days is depicted in Fig. 1(b).

One-way ANOVA showed no significant difference (p>0.05) between the survival of GIFT tilapia fry in different treatments. One-way ANOVA showed a significant difference in AFCR *(p<0.05)* between the treatments. The average AFCR of GIFT tilapia fry reared in a biofloc system using different carbon sources and in control at the end 90 days is depicted in 163 Fig. 1(c).

Table 2: Average length gain (%), weight gain (%), SGR (% day-1) and survival (%) of GIFT tilapia fry after 30, 60 and 90 days.

Days	**Treatments**						
	T_0	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
	Average length gain (%)						
30	51.31 ±1.08 ^{d}	55.44 ±4.14^{cd}	65.85 ±2.29^{a}	64.66 ±2.53^{ab}	57.81 ±1.16bcd	59.27 ±3.10^{abc}	66.42 ±1.83^{a}
60	116.83 ±3.88^{c}	142.46 ±3.43^{ab}	144.82 ±2.42 ^{a}	128.70 ±1.25^{bc}	142.21 ±0.92^{ab}	142.82 ±3.75^{ab}	154.95 ±4.14 ^{a}
90	213.57 ±1.08 ^{c}	224.36 ±0.71 ^{b}	221.19 ±0.54 ^{b}	221.31 ±1.51 ^{b}	221.83 ±1.00 ^{b}	220.43 ±0.27 ^{b}	229.33 ±0.97 ^{a}
	Average weight gain (%)						
30	310.26 ±8.57^{b}	360.71 ±2.32^{ab}	362.19 ±15.38^{ab}	367.88 ±10.13^{ab}	365.58 ±21.03 ^{a}	363.56 ±12.16^{ab}	386.41 ±23.10 ^{a}
60	1477.99 ±24.99 ^{b}	1934.28 ±36.08 ^{a}	1942.07 ±57.73 ^{a}	1888.78 ±41.36 ^{a}	1917.56 ±19.91 ^{a}	1889.00 ±41.22 ^{a}	1965.07 ±48.85 ^{a}
90	2655.71 ±54.00 ^{c}	3333.37 ±54.23 ^{b}	3279.75 ±11.02 ^{b}	3281.32 ±58.26 ^{b}	3306.60 ±29.28 ^{b}	3283.36 ±10.75 ^{b}	3539.66 ±45.71^{a}
	Average survival (%)						
30	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00	100 ±0.00
60	94.67 ±1.33	96.00 ±0.00	97.33 ±1.33	97.33 ±1.33	96.00 ±0.00	97.33 ±1.33	97.33 ±1.33
90	88.00 ±0.00	93.33 ±1.33	96.00 ±2.31	94.67 ±1.33	94.67 ±3.53	94.67 ±1.33	96.00 ±0.00
	Average specific growth rate (% day^{-1})						
30	4.70 ±0.07^{b}	5.09 ±0.02^{ab}	5.10 ±0.11^{ab}	5.14 ±0.07^{ab}	5.12 ±0.15^{ab}	5.11 ±0.09^{ab}	5.27 ±0.16^{a}
60	4.60 ±0.03^{b}	5.02 ±0.03^{a}	5.03 ±0.05^{a}	4.98 ±0.03^{a}	5.01 ±0.02^{a}	4.98 ±0.03^{a}	5.05 ±0.04^{a}

90	3.68 ±0.02[c]	3.93 ±0.02[b]	3.91 ±0.01[b]	3.91 ±0.02[b]	3.92 ±0.01[b]	3.91 ±0.01[b]	4.00 ±0.01[a]

The values are expressed as mean ± standard error (SE). Values in the same row with different superscripts are significantly different at $p< 0.05$.

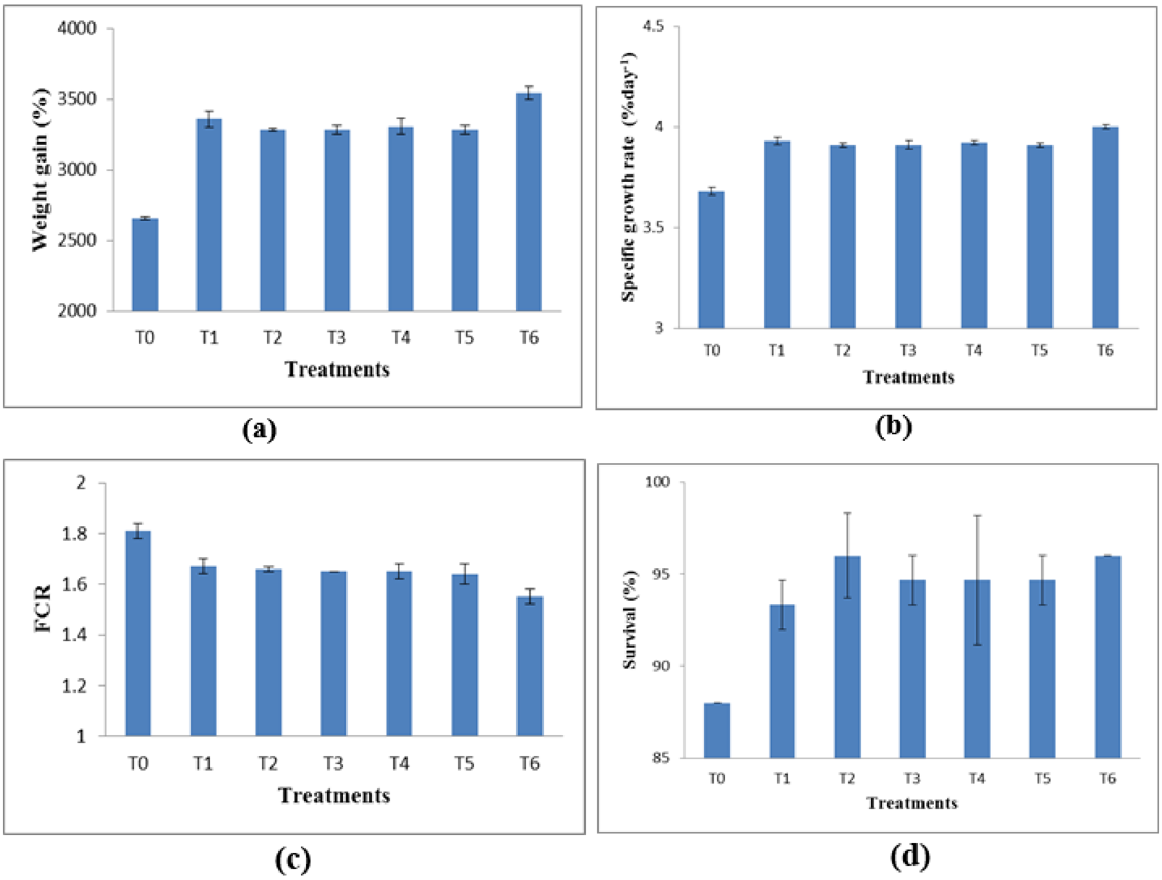

Fig 1: (a) Average weight gain (%) (b) Specific growth rate (% day^{-1}) (c) feed conversion ratio and (d) survival (%) in different treatments at the of 90 days

4. Discussion

4.1 Water quality parameters

The water quality parameters in the present study were within the recommended ranges and acceptable levels for tilapia rearing [17]. The observed values were similar to those of other BFT studies using tilapia.The average temperature values of different treatments observed in present study were similar to those of other BFT studies of tilapia [11]. The pH values were lower as compared to control in biofloc treatments [18]. This was probably due to the addition of carbon sources into the water, which reduced the pH and increased microbial metabolism for decomposition of organic matter in biofloc treatments. The dissolved oxygen was always above 5mgL^{-1} and the level of DO was appropriate because there was a 24h continuous aeration. If DO concentrations were above 5mgL^{-1}, carbon dioxide concentrations of at least 30mgL^{-1} could be tolerated by warmwater species [19]. In the present study, the average carbon dioxide (2.67±0.33–5.67±0.33mgL^{-1}) was suitable for tilapia fry. The alkalinity value more than 20 mgL^{-1} in water was desirable for tilapia culture [19]. In present study, the average total alkalinity (mgL^{-1}) was 48.89 ±5.85 in control, whereas, in different biofloc treatments, it was ranging from 46.95±3.90 to 108.06±5.89mgL^{-1}. The average ammonia was within the range of ammonia reported for Nile tilapia fry *O. niloticus* in biofloc [20]. The range of nitrite-N and nitrate-N was suitable for culture of tilapia [21]. The microbial flocs (sludge) were removed from the tanks and the TSS level was maintained below 400mgL^{-1} [10]. Floc volume was ranged from 17.83±1.11to 20.61 ±0.80 in BFT treatments, while average floc volume in control was below 0.5mlL^{-1} [20].

4.2 Growth performance

In previous studies, improved growth performance and FCR of tilapia in BFT was reported [5, 22, 10, 23, 11]. In the present study, improved growth performance and lower AFCR of fishes were recorded in all BFT treatments, with significantly higher growth performance in rice bran biofloc treatment. The nutritional properties of the flocs were depend on the carbon sources used [24]. The rice bran is a rich source of proteins, fats, minerals and micronutrients and in addition, it has higher amount silica and lysine content, which is essential amino acid for fishes [25]. This might have provided the better nutrition to floc in rice bran biofloc treatment, which might have contributed to higher growth performances in fishes. Similar to this, the improved growth performance and FCR of *O. niloticus* fish in wheat mealing by-product and rice bran based biofloc as compared to fish reared in clear water was reported [26].

The use of three different carbon sources; raw rice bran (RRB) or when incubated (24 hr) with *Bacillus* species under aeration (cellular respiration, ResRB) or without aeration (fermentation, FerRB) for rearing of African catfish juveniles in BFT was better than control [27]. Moreover, the result revealed that all biofloc treatments enhanced the growth performance of fishes than that of control. The higher growth of fishes in biofloc treatments showed the ability of fish to utilize microbial protein better way [28]. Also, the nutritive value of bioflocs in addition to supplied feed would have contributed for higher growth and lower FCR in biofloc treatments, as indicated by earlier studies [5, 29, 30,21].

5. Conclusions

The current study indicated that growth performance (length gain, weight gain and specific growth rate) of fishes was better in all biofloc treatment than that of control. Among the different carbon sources used, a significantly higher length gain, weight gain and specific growth rate were observed in rice bran based biofloc treatment, which suggested that rice bran is a better carbon source for rearing of GIFT tilapia fry in biofloc system.

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