COMPARATIVE EFFICIENCY OF BIOFLOC AND FEED BASED CULTURE OF COMMON CARP (*CYPRINUS CARPIO* L.)

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Biofloc technology is an environmentally sound aquaculture production system in which nutrients are continuously recycled and reused. In biofloc based production systems, integration of heterotrophic bacteria and algae is grown simultaneously forming flocs under controlled conditions *in situ*. The carbon sources of the culture system are proved to be limiting in biofloc formation, composition and its nutritive values. With the above background, the present study on comparative efficiency of biofloc and feed based culture of common carp (*Cyprinus carpio* L.) has been envisaged. The findings of the study indicated that biofloc alone was not sufficient enough for the comparable growth of the test fish under supplementary feeding regime. Biofloc alone resulted only in 2.79 times absolute weight gain against 9.27 times with only feed @ 6% body weight per day, however, mortality rate of the test fish was highest (33.33%) in biofloc treatment alone. It was clear that biofloc improved the performance of the supplementary feed both in terms FCR and FCE. Presence of biofloc improved the FCR from 1.75-2.09 to 2.31 in control. In spite of less absolute weight gain with feeding @ 3% body weight per day because of the most favourable FCR and less nutrient loading, the system was proved to be economically and ecologically more sustainable in presence of biofloc.

Key words: Biofloc, *Cyprinus carpio* L., FCE, FCR

Global aquaculture production continued to increase parallel with declining capture fishery production in recent times. Contribution of aquaculture increased from 13.4% to 42.2% during 1990 to 2012 (FAO, 2014) and the role of aquaculture in supplying animal protein to the global population is well documented (Tidwell and Allan, 2001; Sugiyama *et al.*, 2004). Animal husbandry and fisheries are the two sources of animal protein for the world population while fish and allied aquaculture products are considered to be the safest among the proteins from animal sources (MPEDA, 1992)

containing all essential amino acids, and are excellent source of unsaturated fatty acids (de Deckere *et al.*, 1998; Horrocks and Yeo, 1999; Connor, 2000; Ruxton *et al.*, 2005).

Aquaculture production depends largely upon the ambient nutrients as proper nutrient balance is important in maintaining growth and fish health towards higher production. The costs of the ingredients used in fish feed escalated between 20% and 92% during 2007 - 2008 (FAO, 2010). Aquafeed, being considered the most vital input cost, occupies almost 50-70%

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of the budget allocation for production (Anon, 1983; Veerina *et al.*, 1993). Moreover, with the increasing demand of seafood for human consumption, the demand for such used by the industrial sector for processing of fishmeal and fish oil products also increased (Péron *et al.*, 2010). About 5-6 million tonne of low-value trash fish are used as feed in aquaculture (FAO, 2010) and an estimated 1.9 kg of wild fish is required for production of one kilogram of fish (Naylor *et al.*, 2000). Therefore, studies on the development of feed substitution strategies with a minimal input of fishmeal and fish oil has been emphasized where fish products are being replaced by alternative, cheaper plant protein sources. Such studies on alternative protein inputs have resulted in considerable reduction in production costs (Albuquerque *et al.*, 2014) and contributed higher economic viability in animal production including aquaculture (Santos *et al.*, 2013).

The biofloc technology involves an unconventional way of thinking which deviates from the common mind-set that clear water is best for aquaculture (Avnimelech, 2012; Hargreaves, 2013). It is basically the recycling of waste to protein enriched biofloc as a natural live food within the culture system. Biofloc technology encourages suspended growth of flocs consisting of phytoplankton, microbes, and aggregates of living and dead particulate organic matter and grazers of bacteria (Hargreaves, 2006) with a view to recycle feed residues and to increase feed efficiencies. It enhances the use of a microbial community to produce a supplementary microbial food source, high protein bioflocs, which can be utilized by species capable of filter-feeding (Avnimelech, 2012). By adding cheap carbohydrate sources, heterotrophic bacterial growth within the pond is stimulated and

microbial mobilization of nitrogen through the production of microbial proteins are encouraged (Avnimelech, 1999). Sufficient aeration and mixing is required to keep the floc in suspension in the water, which allows the water quality to be controlled (Hargreaves, 2013). The microbial community, predominantly heterotrophic bacteria, within the water acts as a biofilter, resulting in nitrogen uptake and reducing ammonia (NH_4) levels faster and more efficiently than the nitrification process (Avnimelech, 1999; Crab *et al.*, 2012). Ammonium immobilization by heterotrophic bacteria takes place rapidly because the growth rate and microbial biomass yield per unit substrate of heterotrophs are higher by an order of magnitude compared to that of nitrifying bacteria (Hargreaves, 2006). Biofloc has been focused internally as an alternative protein for fish meal, mainly produced as microbial meal (Kuhn *et al.*, 2009). They are irregular in shape, having variably distributed particle size, fine, easily compressible and highly porous (>99% porosity) and are highly permeable (Chu and Lee, 2004). These systems have very low water exchange, and thus have a significantly lower impact on the surrounding environment through lower water requirements and minimal to no effluent water discharge (Avnimelech, 2007; Avnimelech, 2012; Hargreaves, 2013). Degradation of the environment is further reduced by the lower amount of pelleted feed needed and the efficiency in recycling nutrients from the fish waste and uneaten feed (Hargreaves, 2013). The success of this technology primarily depends upon the species of culture as the cultured species should have the ability to graze the bacterial flocculates efficiently and should have the ability to digest and incorporate the microbial protein (Tacon *et al.*, 2002; Burford *et al.*, 2004).

With this background the present research was conducted to evaluate the potential of biofloc in supporting the growth of benthivorous common carp (*Cyprinus carpio* L.) in experimental culture units.

MATERIALS AND METHODS

The present study was conducted in the Department of Aquaculture, Faculty of Fishery Sciences, West Bengal University of Animal and Fishery Sciences, Chakgaria, Panchasayar, Kolkata-94 (22°28'46"N 88°24'4"E). The duration of the study lasted for a period of about three months from April-June, 2015.

Experimental set up: Fifteen (~180 L) outdoor experimental cylindrical cement tanks (diameter: ~ 48 cm, length: 100 cm) were selected for the present investigation. After thorough washing and sun drying the tanks were provided with agriculture soil base of 15 cm and then filled with ground water (pH- 7.5, alkalinity- 124 mg L⁻¹, hardness- 631 mg L⁻¹). All the tanks were manured with cow dung @10,000 kg Ha⁻¹ as practiced in traditional pond preparation for fish farming. The estimated amount of cow dung was mixed with water in a bucket and dispensed as slurry into the tanks. All the tanks were treated with lime @ 200 kg Ha⁻¹ following one week of manure application and kept undisturbed for another one week. Then they were divided into five batches (T₁, T₂, T₃, C₁, C₂) in triplicate by complete randomized design and when the water colour turned to bluish green, 18 nos. of healthy fry of common carp (*Cyprinus carpio* L.) average wt. 0.841 g (0.845±0.855 g) and average length 4.8 cm (4.5±5.1 cm) collected from faculty pond were stocked in T₁, T₂, T₃ and C₁ batches of the tanks.

Preparation of biofloc: 150 L of boiled rice water was mixed with 3 kg molasses in a tub.

After that, yeast powder (250 g) and filtered fresh water (50 L) were added to the mixture and vigorously mixed together to make a solution and also kept for 72 hours in open air for aerobic fermentation. When the colour of solution turned into deep yellow the solution was transferred to another tub. Finally the solution was ready to use as a medium of biofloc growth enhancer.

Feeding: Fish in first (T₁) and second (T₂) batches of treatment tanks were fed with crumble feed (crude protein: 35%) once daily between 9.00 a.m. to 10 a.m. @ 6% and 3% of body weight respectively along with biofloc medium @ 15 mL/tank. Fish in the third batch of tanks (T₃) were applied only with biofloc medium @ 15 mL/tank daily without the above supplementary feed. Fish in the fourth batch of tanks (C₁) were supplied only with the above diet @ 6% of body weight but were not applied with biofloc medium and the last batch of treatment tanks without fish was applied only with biofloc medium @ 15 mL/tank daily acted as second control (C₂). The required amount of feed was broadcasted over the water surface in respective treatments (T₁, T₂ and C₁). The biofloc medium was also broadcasted by using a measuring cylinder over the water surface of respective treatments (T₁, T₂, T₃ and C₂). The biofloc medium was applied to the treatments once daily at early morning between 5.30 a.m. to 6 a.m. or late afternoon between 5 p.m. to 5.30 p.m. up to 45 days of the investigation period. All the tanks were aerated by two electromagnetic air pump (35 Watt and 220 Volt each), attached with two pumice stones in each tank which were placed 40 cm above the bottom surface and was continued for 12-14 hours per day depending upon the daily weather condition as during cloudy days duration of aeration was more.

Collection of samples: Water samples were collected at weekly intervals from each of the tanks at 9.00 a.m. from 15 cm depth for physico-chemical analyses. Collection of water samples were done carefully so as to prevent air bubbling. Soil samples were collected from each of the cisterns at fortnightly intervals. The soil samples were air dried, pulverized, sieved (150 µm mesh) and stored in labelled polythene packets for analyses.

Analysis of sample

Physico-chemical parameters of water and sediment: Selective water quality parameters like temperature, pH, total alkalinity, hardness, dissolved oxygen and BOD₁ were measured following the methods of APHA (1995). Surface water temperature was measured using a centigrade thermometer (Hanna HI98127) and pH of water samples was measured using a digital pH meter (Systronics VI). Nutrient parameters like ammonia–nitrogen (NH₃ N), nitrate–nitrogen (NO₃ N) (EPA, 2009) and ortho phosphate (APHA, 1995) were also measured using spectro photometric method through a double beam UV–Vis spectrophotometer (CECIL CE 4002).

Sediment physico-chemical parameters were measured following the procedures of Walkley and Black (1934) for organic carbon and available phosphorus by Jackson (1967) respectively and pH was measured using digital pH meter (Systronics VI) (APHA, 1995).

Fish growth: Fish growth was recorded at 15 days intervals from each cistern. Half of the stocked fish were caught randomly with a hand net and their weight (g) increments were recorded for estimation of average weight gain, specific growth rate (SGR), feed conversion ratio (FCR) and protein efficiency ratio (PER).

They were then released again as quickly as possible for further growth. The following estimates were done as:

Net B. Wt. gain = (Final - Initial) B. Wt

Body wet weight gain (%) = $\frac{(\text{Final} - \text{Initial}) \text{ B. Wt.} \times 100}{\text{Initial B. Wt.}}$

Feed conversion ratio (FCR) = $\frac{\text{Total dry feed fed}}{(\text{De silva and Anderson, 1995}) \text{ Wet weight gain}}$

Specific growth rate (%) = $\frac{\ln(\text{Final B. WT.}) - \ln(\text{Initial B. Wt.}) \times 100}{\text{Number of days}}$

Feed conversion efficiency (FCE) = $\frac{\text{Weight gain} \times 100}{\text{Food consumed}}$ (Goddard, 1996)

Mortality rate (%) = $\frac{(\text{Initial} - \text{Final}) \text{ no. of fish} \times 100}{\text{Initial no. of fish}}$

Statistical analyses: All the results were subjected to statistical analysis. One way analysis of variance (ANOVA) was applied to test the significance of difference among the treatments. Correlation coefficient (r) test was applied to establish relationship between selective parameters using appropriate software.

RESULTS

Biofloc characteristics: Qualitative examination of biofloc indicated that the flocs were principally composed of heterotrophic bacteria, phytoplankton, zooplankton and protozoa. The crude protein, crude lipid and ash content of the floc did not differ significantly and ranged from 18.5 - 19.22%, 0.55 - 0.62% and 49.56 - 51.45% respectively.

Growth of fish: Mortality of common carp fry was highest (33%) in only biofloc system without any supplements feeding. Weight of fish continued to increase over time in any case and the overall mean value was highest (4.65 g) in T₁ and lowest in T₃. Temporal trend of

weight gain (%) declined sharply as the period of rearing progressed (Fig. 1). The average value was maximal in T_1 (54.47%) followed by C_1 (50.15%), T_2 (43.88%) and lowest in T_3 (27.73%) (Fig. 2). Temporal trend in SGR (specific growth rate) of fish among the treatments was distinctly different from one treatment to another (Fig. 3). The overall mean was highest in T_1 (9.27%) and lowest in T_3 (2.59%). Absolute growth of fish in T_1 and C_1 was distinctly higher by 62.41% and 29.97% compared to T_2 and T_3 respectively (Fig. 4).

Water and soil quality: Surface water temperature did not differ among the treatments and ranged from 31-36 °C. Water pH remained alkaline (7.1 - 8.2) in all the treatments with strong significant difference ($F_{4,13} = 11.57$, $P \leq 0.01$) among them (Table 1). Total alkalinity remained congenial and the highest mean value was encountered in T_3 (134.28 ± 2.78 mg L^{-1}) and lowest in T_2 with marginal significant difference among the treatments ($F_{4,13} = 3.186$, $P \leq 0.05$). However, highest mean value of water hardness was encountered in T_1 and lowest in T_3 (Table. 1) where treatment difference was significant ($F_{4,13} = 7.85$, $P < 0.01$). Dissolved oxygen of water ranged from 6.2-9.3 mg L^{-1} in the treatments during the study period with insignificant treatment difference ($P \leq 0.05$) (Table 1). However, strong significant difference ($F_{4,13} = 15.4$, $P < 0.001$) of BOD_1 was observed among the treatments with highest mean value (3.54 ± 0.22 mg L^{-1}) in T_1 and lowest (2.36 ± 0.126 mg L^{-1}) in C_2 . The highest mean values of ammonia-N and nitrate-N was observed in C_1 and T_1 respectively (Table 1) and in both the cases treatment differences were significant ($F_{4,13} = 17.6$, $P < 0.05$ and $F_{4,13} =$

16.64, $P < 0.001$). Nitrate-N was conspicuous by its absence during most part of the investigation period in all the treatments. Ortho phosphate concentration was highest in T_1 and lowest in C_2 with significant difference ($F_{4,13} = 33.18$, $P < 0.001$) among the treatments (Table 1).

Physicochemical parameters of sediment indicated alkaline condition in all the treatments (Table 1). Organic carbon content of sediment ($F_{4,13} = 27.63$, $P < 0.001$), available-P content of sediment differed significantly ($F_{4,13} = 41.6$, $P < 0.001$) (Table 1).

DISCUSSION

Absolute weight gain of the test fish (*Cyprinus carpio* L.) decreased polynomially ($y = -0.562x^2 + 0.745x + 8.255$; $R^2 = 0.991$) (Fig. 5) from T_1 to T_3 either with decreasing ration size from 6% to 3% or with and without biofloc supplementation. Therefore, it was established that both the rate of supplementary feed application and nutritionally rich biofloc exerted a definite role in growth of the test fish. Moreover, such intensity of impact upon growth of the fish was extremely discernible among the treatments where in presence of biofloc, feed application @ 6% body weight (T_1) resulted in absolute weight gain (%) by an order of magnitude (Fig. 6) against 6.07% in T_2 (@ 3% feed). However, comparing an iso-nutrient regime in T_1 and C_1 effect of biofloc in increasing the absolute weight gain was only 6.42% in T_1 . Again, biofloc in T_3 resulted only in 2.79 times absolute weight gain against 9.27 times with only feed @ 6% body weight. Therefore, biofloc alone was not sufficient, for the comparable growth of the test fish under supplementary feeding regime. This was further evidenced from the mortality of the test fish

Table 1. Range and Mean ($\pm$ SE) of selected physiochemical parameters of water and sediment in different treatments

	T1 Mean((Range)	T2 Mean (Range)	T3 Mean (Range)	C1 Mean (Range)	C2 Mean (Range)
Water					
Temp. (p C)	(31-36)	(31-36)	(31-36)	(31-36)	(31-36)
Water pH	(7.1-7.64)	(7.29-7.6)	(7.17-8.2)	(7.2-7.6)	(7.6-8.15)
Alkalinity (mg L ⁻¹)	124.21 (108-132)	123.71 (102-148)	134.28 (116-146)	126.36 (102-148)	131.14 (120-142)
Hardness (mg L ⁻¹)	375.57 (250-640)	329.28 (216-612)	319.43 (228-595)	326.86 (212-648)	348.5 (212-660)
DO (mg L ⁻¹)	7.62 (6.4-8.4)	7.58 (6.4-9.6)	7.96 (6.4-8.8)	7.86 (6.3-9.2)	8.13 (6.2-9.2)
BOD ₁ (mg L ⁻¹)	3.54 (1.2-4.6)	2.97 (1-4.2)	2.38 (1.2-3.2)	2.74 (1-3.8)	2.36 (1.2-3.2)
Ammonia-N (mg L ⁻¹)	0.272 (0.075-0.335)	0.267 (0.115-0.392)	0.165 (0.052-0.235)	0.273 (0.085-0.4)	0.182 (0.067-0.33)
Nitrite-N	0.024 (0-0.05)	0.019 (0-0.05)	0.023 (0-0.05)	0.022 (0-0.05)	0.02 (0-0.041)
Nitrate-N	0.241 (0.102-0.305)	0.212 (0.125-0.26)	0.205 (0.135-0.23)	0.222 (0.12-0.28)	0.182 (0.117-0.2)
Ortho-P	0.082 (0.055-0.095)	0.073 (0.054-0.083)	0.067 (0.055-0.076)	0.074 (0.055-0.085)	0.062 (0.055-0.07)
Sediment					
pH	(7.31-7.99)	(7.3-7.95)	7.27-7.92	(7.2-8.04)	(7.27-7.71)
Organic carbon	2.022 (1.57-2.17)	2.026 (1.54-2.28)	2.007 (1.56-2.32)	2.041 (1.55-2.25)	1.782 (1.54-1.9)
Available phosphorus	0.27 (0.22-0.29)	0.263 (0.22-0.28)	0.222 (0.2-0.25)	0.27 (0.22-0.28)	0.22 (0.2-0.25)

Fig. 1. Temporal trend in growth of common carp in different treatments employed

Fig. 2. Temporal trend in live weight gain (%) of common carp in different treatments employed

Fig. 3. Temporal trend in specific growth rate (%) of common carp in different treatments employed

Fig. 4. Absolute growth of common carp in different treatments employed

which was highest (33.33%) in the biofloc alone treatment (T_3) and such rate declined by a modular pattern ($y = 30.68x^{-0.60}$; $R^2 = 0.89$) either with feeding support alone or both with feeding and biofloc support (Fig. 7).

However, presence of biofloc improved the FCR both in T_1 (2.09) and T_2 (1.75) against C_1 (2.31). Therefore, in spite of decrease in absolute weight gain in T_2 because of the most favourable FCR (Fig. 8) and less nutrient loading, the system was proved to be economically and ecologically more supportive. This was further corroborated by

the fact that FCE decreased polynomially ($y = 2.288x^2 - 16.04x + 70.81$; $R^2 = 1$) with an identical reverse trend in FCR with identical fit ($y = -0.057X^2 + 0.506x + 1.30$; $R^2 = 1$) (Fig. 9). So, it was clear that biofloc improved the performance of feed both in terms FCR and FCE.

The physicochemical conditions of the biofloc treatments remained congenial all throughout. Increase in the concentration of all the major nutrients like ortho-phosphate, ammonia-nitrogen and nitrate-nitrogen in the feed supplemented treatments was because of the uneaten feed and metabolic by-products as well.

Fig. 5. Modular trend in absolute weight gain in different treatments employed

Fig. 6. Absolute weight gain (%) of fish in different treatments employed

Fig. 7. Modular trend of mortality rate of fish in different treatments employed

Fig. 8. Modular trend of FCR in different treatments employed

Fig. 9. Modular trend of FCE in different treatments employed

Moreover, the resultant increase in N and P was subsequently manifested in higher growth of fish in the biofloc treated systems as

mobilization of nitrogen under suitable carbon reserve was effected in microbial protein synthesis as evidenced from the proximate composition of the floc. Again, higher presence of nutrients in the treatment with biofloc with fish (T₃) compared to its counterpart without fish (C₂) was because of the bioturbation effect of the test fish. Jana and Das (1992) and Jana *et al.* (2001) conclusively observed that benthivorous fish like *Cyprinus carpio* L. was effective in increasing the nutrient status of water through bioturbation of the sediment.

From the results of the present study it was

indicated that *in-situ* biofloc production has the potential of reducing the cost towards feed and overall production. Moreover, the ecological health of the culture units with regards to physicochemical properties will also be improved with this technology.

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