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The possibilities of *Cirrhinus reba* culture under biofloc technology

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Abstract Scarcity of water and shortage of land remain major constrain to fish production in inland aquaculture. Bioflocs technology is a technique that may solve this problem. A 60-day growth trial was evaluated water quality parameters and the survival rate of *Cirrhunus reba* in a biofloc culture system. In this study, Molasses or sugar as a carbon source was converted to nutritional composition. Optimum water quality parameters improved growth and survival rate. After 60 days the specific growth rate was satisfied and that was 1.74±0.014%/day (p<0.05). At the end of the experiment, the percent weight gain was 187.74±2.45% (p<0.05). The specific growth rate and percent weight gain were significantly increased in this experiment. The survival rate of the *C. reba* was 86±1.25%. Results suggested that biofloc system could improve the nutritional requirement contributing to the survival rate of *C. reba.*

Keywords: Biofloc technology, Growth rate, Survival rate, *Cirrhinus reba*

Introduction

Biofloc technology (BFT) is an aquaculture system which allows high stocking density culture with limited or zero water exchange. In this system, high levels of microbial particularly bacterial floc are maintained with constant air circulation. To facilitate the aerobic decomposition of organic matter, carbohydrates need to be added to maintain the carbon and nitrogen ratio. (Avnimelech and Weber, 1986). Aquaculture produces different waste particles including residual feed, faecal materials and metabolic products. These waste materials are used by heterotrophic bacteria with the presence of organic carbon sources including molasses, wheat flour, and tapioca to produce new biomass which is subsequently used by cultured fish (De Schryver *et al*., 2008). The Biofloc aquaculture system is a relatively simple, straightforward, and robust technology which not only provides an economical alternative to use land and water but also additional microbial protein source for the fish species (Crab *et*

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al., 2012). The level of possible waste materials discharged from the aquaculture system is mainly dependent on nutrient retention by the cultured species. According to FAO 2014, 66.7 million metric tons of aquaculture products in 2012 produced a total of 1.3 million metric tons of N and 0.20 million metric tons of P wastes. In 2008, waste from finfish and crustacean aquaculture production represented 0.9% of the human input to the N-cycle and 0.4% of the global N-cycle (Verdegem, 2013).

Waste nutrient recycling is the main principle of the Biofloc technology particularly nitrogen content to beneficial microbial biomass to be used by the culture fish species or to be used to prepare feed ingredients (Kuhn *et al*., 2010). The growth of heterotrophic bacteria can be stimulated by steering C/N ratio through the altering carbohydrate content in the feed or by the addition of different carbon sources in culture water. As a result, bacteria assimilate the waste ammonium and produce a high density of new biomass (Avnimelech, 2007) and maintain the water quality. Therefore, water replacement is not required. The biomass of heterotrophic bacteria provides numerous beneficial health to the fish and shellfish including supply of essential nutrients and digestive enzymes (Xu and Pan, 2012) and higher resistance to diseases (Ekasari *et al.,* 2014). Some researchers found that when tilapia broodstock culture in a biofloc system, they showed enhanced immune defence, resulting in larval robustness against diseases and environmental stress (Ekasari *et al.,* 2015).

Cirrhinus reba belonging to the family Cyprinidae of the order Cypriniformes is one of the commercially important freshwater minor carps in Bangladesh and is mainly distributed in India, Bangladesh, Nepal, Pakistan, Myanmar and Thailand (Gupta and Banerjee, 2016). In Bangladesh, *C. reba* is locally known as bhagan bata, raik or tatkini which is predominantly found in all types of waterbodies specifically riverine systems (Sultana *et al.,* 2018). This species has been listed as vulnerable in both India and Bangladesh and reported as the least concern under IUCN Red List of threatened species (Gupta and Banerjee, 2016). It has a high amount of protein, calcium and low fatty acid compared to its counterparts (Gupta and Banerjee, 2016). However, the natural production of *C. reba* has been hampered due to several man-made activities including urbanisation, increasing fishing pressure, siltation, aquatic pollution, and loss of natural habitat for spawning and growth (Keer *et al*., 2018; Ahmad *et al.,* 2017). Therefore, the new technology should try to be applied in aquaculture. The objectives were to identify the optimum water quality parameters of biofloc technology of *C. reba* and to observe the survival rate of *C. reba* in biofloc technology.

Materials and methods

Experimental Site

The study was carried out for 60 days from 15 February to 14 April 2020 at the hatchery of Hajee Mohammad Danesh Science and Technology University (HSTU), Bangladesh.

Tank Preparation/Biofloc settle

The circular concrete tank of 5000 litre capacity were used for this study. Water was supplied into the tank by pump and the outlet of the tank was completely sealed. Tanks were cleaned and disinfected with 100 ppm chlorine before being rinsed thoroughly with water and filled with pumped water. The tanks were aerated continuously through an air compressor and distributed in tanks using air hose and sinkers for continuous supply of oxygen. After 48 hrs of aeration, 5kg salt and 1kg molasses were added and the water quality parameters were checked after 3-6 hrs.

FCO (Fermented Carbon Organic) preparation

At first 10 liter of water was taken in a plastic drum with continuous aeration. Then 250g molasses, 100g salt, and 50g probiotic (pond care) were added to the plastic drum and left for one week.

Floc determination

The floc volume was measured by Imhoff cone. The biofloc water was taken in Imhoff from tank and drum both and observed for 30 min without disturbance in order to settle down the floc in the Imhoff cone. The floc volume was measured weekly.

Water quality parameters

The water samples were measured twice daily to check the temperature using thermometer (Hanna HI, Model-PT 582) and TDS using TDS meter (Model TDS-3). Electric heaters were used to maintain the temperature. The dissolved oxygen and pH were measured thrice daily using DO meter (Lutron model TDO-519) and pH meter (Hanna HI, Model 98107) respectively. Rectangular air stone was used to maintain dissolved oxygen and kept the aeration moderate for biofloc suspension without causing stress to the animals. The ammonia was measured once daily using total ammonia nitrogen (TAN) Kit (Hanna HI, Model: PA-536). Salt was added to maintain the suitable ammonia concentration in the water.

Experimental fish

The experimental fish was collected from "Easin Fish Hatchery", Bogra, Bangladesh, and kept for conditioning in the hatchery of HSTU. After preparation of biofloc and checking water quality parameters, the fish were stocked in the biofloc.

Growth parameters

Before stocked the fish were weighted and counted. Ten (10) fish were randomly sampled and monitored for growth parameters once every 15 days. The following growth parameters were calculated using the following equation:
The final average weight was used for calculating the mean weight gain (MWG), specific growth rate (%), and Percent weight gain (%WG).

Total weight gain = Final body weight – Initial body weight
Percent weight gain (%WG) = (Final body weight – Initial fish weight)/Initial body weight **×100**
Specific growth rate = $\frac{\text{ln (final body weight) – ln (initial body weight)}}{\text{Experimental duration (days)}} \times 100$
Survival rate (%) = $\frac{\text{Final fish count}}{\text{Initial fish count}} \times 100$

Data analysis

Data from this experiment were analysed using SPSS 25.0 software (SPSS< Chicago, USA). One-way ANOVA was performed on the growth parameters after conducting normality and homogeneity of variance tests. Differences were considered significant at P<0.05.

Results

Water quality parameter

The study was conducted for 60 days period and water quality parameters were tested regularly. Water quality parameters were checked (temperature, DO, and TDS) twice a day, however, pH and ammonia were tested once a day. The results of the water quality parameters were presented at 15-day intervals over the experimental period (15F-29F, 1M-15M, 16M-30M, 31M-14A where F = February, M = March, and A = April).

Temperature

The temperature varied between 23.007±0.63 to 28.47±0.31ºC. The average temperature was 25.95±2.38. The maximum temperature was 28.47±0.31ºC in April and the minimum temperature was 23.007±0.63 in February. At the start of the experiment, the temperature was low and then it increased gradually (Figure 1).

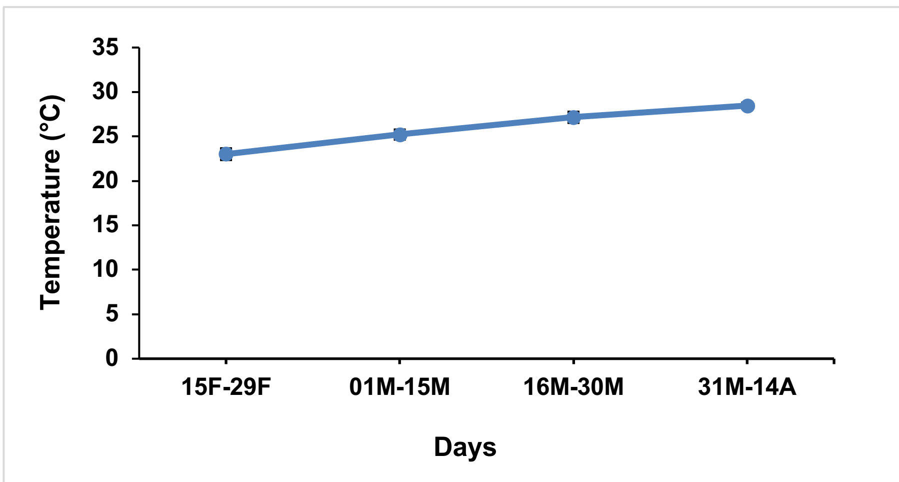

Figure 1. The temperature ranges of *Cirrhinus reba* culture in biofloc system

pH

The average pH was 7.46±0.15. The maximum pH was 7.61±0.62 in February and minimum pH was 7.26±0.47 in April. At the start of the experiment, the pH was high and over the experimental period it decreased (Figure 2).

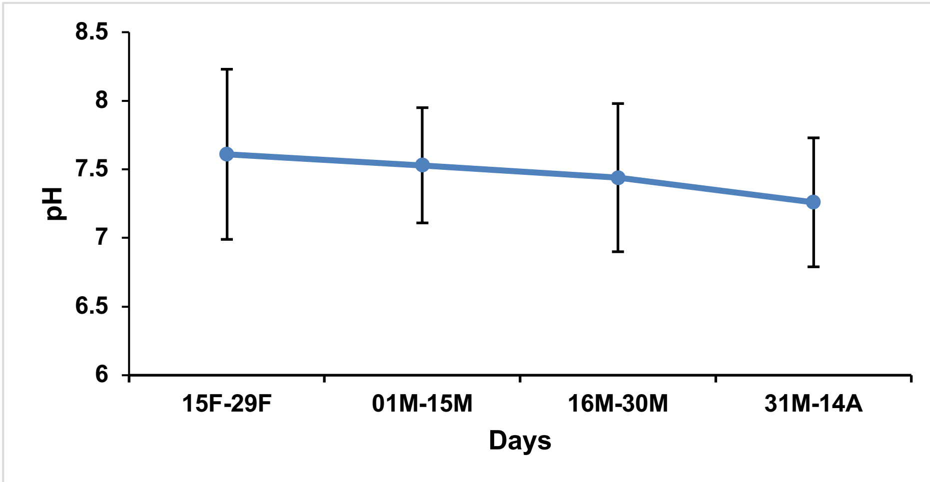

Figure 2. The pH changes of *Cirrhinus reba* culture in biofloc system

Dissolved oxygen (DO)

The dissolved oxygen of water during the experimental period of *C. reba* were decreased over the experimental period (Figure 3). During the culture period, the highest dissolved oxygen was 7.51±0.38 mg/L in February and the lowest was 6.19±0.82 mg/L in April. The average dissolved oxygen was 6.96±0.56mg/L.

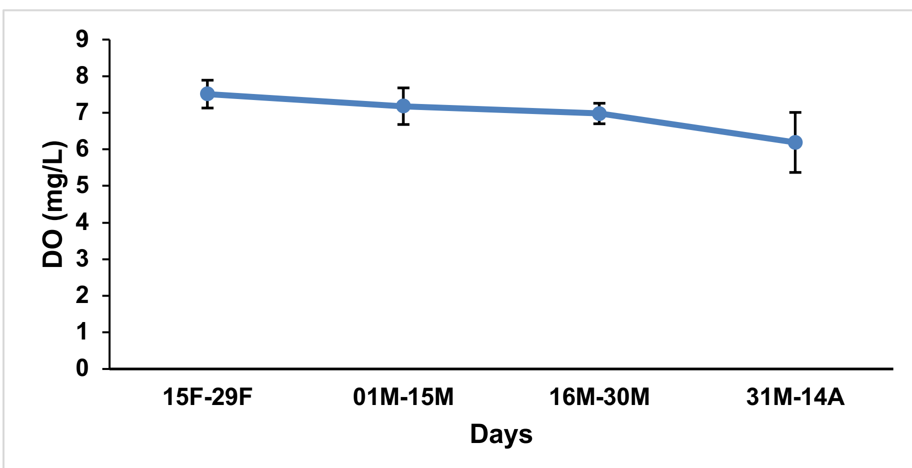

Figure 3. The dissolved oxygen changes of *C. reba* culture in biofloc system

Ammonia

During the observation period, subtle changes occur in ammonia concentration. The maximum value of ammonia was 0.38±0.24 ppm and the minimum was 0.23±0.14 ppm (Figure 4). The average ammonia over the culture period was 0.31±0.06 ppm.

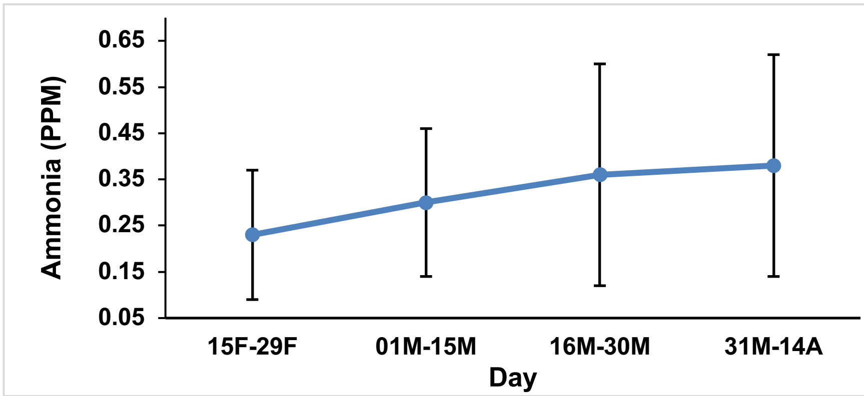

Figure 4. The changes of ammonia level of *C. reba* culture in biofloc system

TDS

The average TDS of water during the experimental period was 1069.013±67.95. The highest TDS was 1143.4±173.09 ppt in February and the lowest was 1002.66±122.98 ppt in March (Figure 5).

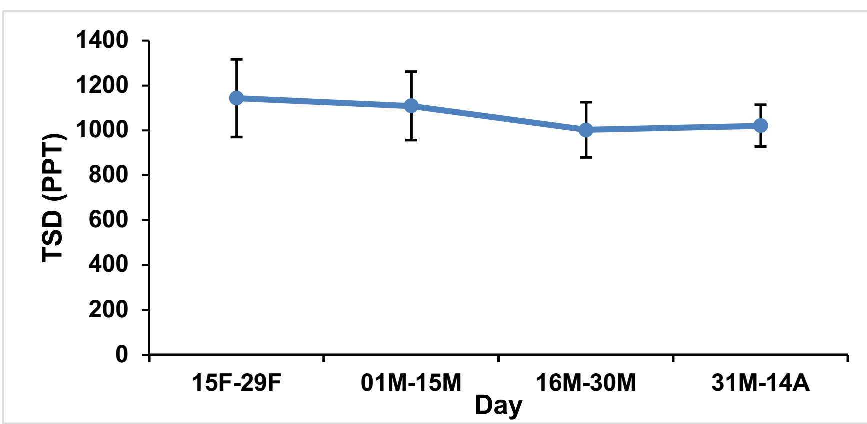

Figure 5. TDS changes of *C. reba* culture in biofloc system

Growth parameters

The growth of *C. reba* were evaluated. Sampling was done at 15 days intervals.

Final mean weight (g)

The initial weight of *C. reba was* 2.25±0.082 g and after 60 days the final mean weight increased to 6.43±0.055 g (Figure 6).

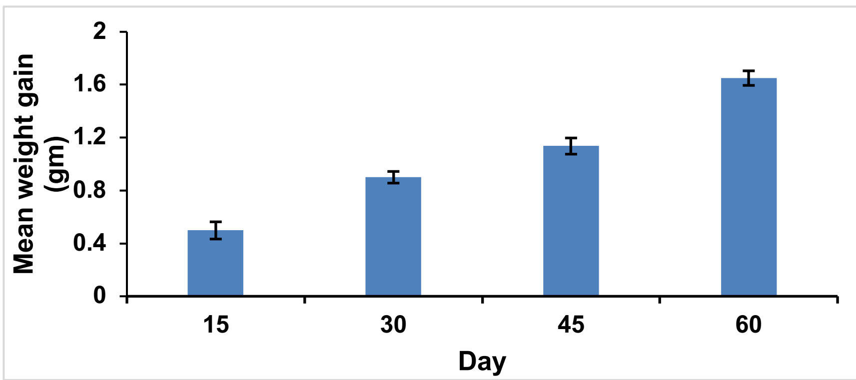

Figure 6. Final mean weight (g) of *C. reba*

Mean weight gain

The average mean weight gain was 1.04±0.48. After the first 15 days, the mean weight gain was 0.498±0.065 g and gradually increased to 1.65±0.055 g in the last 15 days (Figure 7).

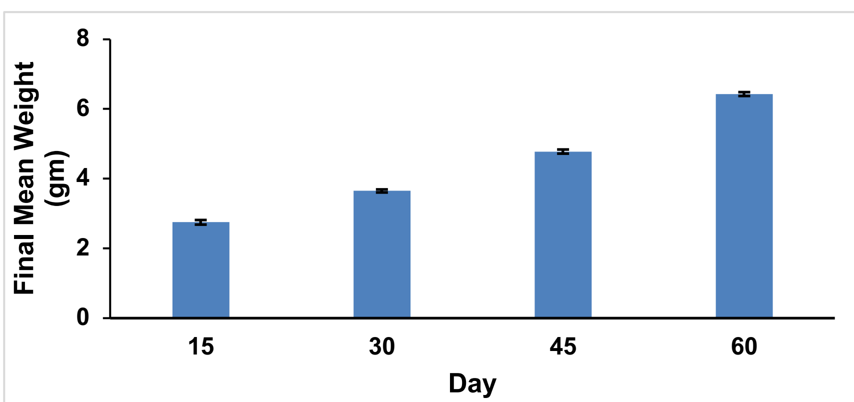

Figure 7. Mean weight gain of *C. reba*

Specific growth rate (%/day)

In the experiment, initial mean weight of *C. reba* was 2.25±0.082g. The specific growth rate was 1.33±0.16, 1.60±0.041, 1.67±0.027 1.74±0.014%/day after 15, 30, 45 and 60 days respectively (Figure 8).

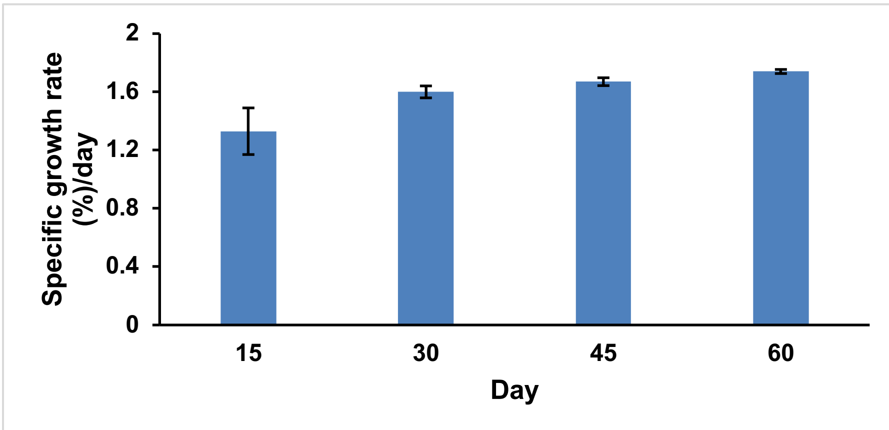

Figure 8. Specific growth rate of *C. reba*

Percent weight gain (%)

After 1st 2nd 3rd and 4th sampling percent weight gain was evaluated. The initial mean weight of *C. reba* was 2.25±0.082g. After 15, 30, 45, and 60 days, the percent weight gain was 22.113±2.95, 62.07±1.99 112.25±2.63 and 187.742±2.45 respectively (Figure 9).

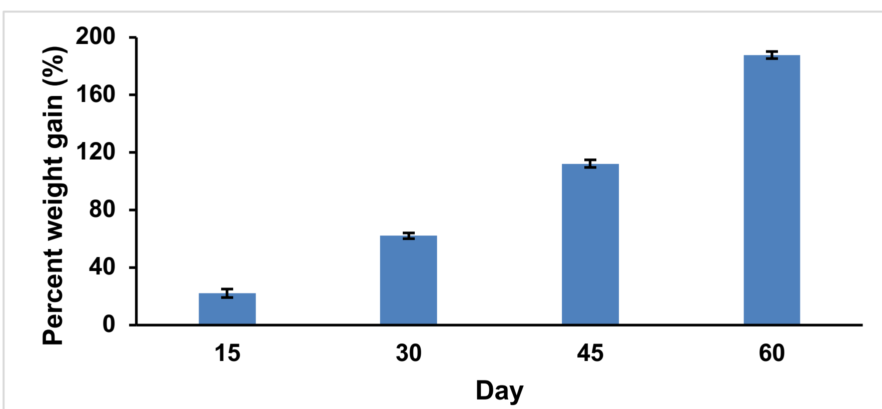

Figure 9. Percent weight gain of *C. reba*

Survival rate

At the end of the experiment, the survival rate of *Cirrhinus reba* was evaluated. Initially, we stocked a total of 2586 *C. reba* and after 60 days we found a total of 319 fish were died. Therefore, the survival rate of *C. reba* in this study was 87.66%.

Discussion

A new technique for fish culture is a biofloc technology. Fish are cultured at high stocking densities in this technique. This technique is based on the maintenance of high levels of microbial floc in suspension by continuous aeration. In this technique, molasses or sugar is used as a source of carbohydrates. Supplementation of carbohydrate sources create a mechanism of recycling fish culture by-products and converts it into a food source for the cultured organism (Crab *et al*., 2012). In this system, maintaining carbon-nitrogen balance is most important. Ammonia is consumed into bacterial biomass and an increase in C/N ratio stimulates heterotrophic bacterial production (Schneider *et al*., 2005). The success of this technology mainly depends on the selection of species, because the cultured animals should have the ability to harvest the bacterial floccules developed in the system, and should have the ability to digest and utilize the microbial protein.

During the experiment period, temperature, pH Dissolved oxygen, ammonia, and TDS were 25.95±2.38, 7.46±0.15, 6.96±0.56, 0.31±0.06, 1069.013±67.95 respectively. The water quality parameters were suitable for *C. reba* in this experiment. Moreover, the water quality parameters are necessary to maintain the biofloc technique. The previous study on *Anabas testudineus* showed that the temperature was 25.94±5.20ºC (Chakraborty and Nur, 2012) whereas research on *Oreochromis niloticus* showed that the temperature was 24.73±2.27 ºC (Gallardo-Collí *et al.,* 2019).

The average pH in this experiment was 7.46±0.15. Similarly, Putra *et al*. (2019) reported that pH was 7.5 when red tilapia was cultured in a biofloc system. Sukardi *et al*. (2019) showed that the pH was 7.5±0.16 in *Pangasius pangasius* biofloc technology using rice bran and with molasses, the pH was 7.5±0.12.

The average dissolved oxygen was 6.96±0.56 mg/L in this study. Putra *et al*. (2019) observed (Red tilapia) that dissolved oxygen was 6.2mg/L. Sukardi *et al*. (2019) showed (*Pangasius sp.*) that was 7.2±0.22 mg/L in rice bran and also found in molasses was 7.0±0.2 mg/L. Gallardo-Collí *et al*. (2019) showed that the dissolved oxygen was 6.49±0.83 mg/L.

The ammonia was observed at 0.31±0.06 in this experiment. Sontakke and Haridash (2018) (*Chanos chanos*) showed that the ammonia was 0.24±0.05ppm. Correia *et al*. (2018) experimented on red tilapia and found the ammonia was 0.34±0.03.

At the start of the experiment, the initial mean weight was 2.25±0.082g. The average mean weight gain was 1.046±0.48g. The specific growth rate was 1.74±0.014 in this experiment. Sharma *et al*. (2015) showed that the specific growth rate and percent weight gain of *Labeo rohita* were 1.77 ±0.01% and 187.742±2.45% respectively. A similar result was observed in other studies in which biofloc increased the percent weight gain of *C. auratus* and *O. niloticus* (Wang *et al.,* 2015).

The present study showed that biofloc improved the survival rate (87.66%) of *C. reba.* Chakraborti and Nur 2012 showed that the survival rate of *Anabas testudineus* and *Heteropneustes fossilis* was 90.55±2.24 and 88.82±2.45 respectively. Similar results were reported when *Pangasius* sp. (Meritha *et al*., 2018) Nile tilapia (Lima *et al*., 2018) and *Chanos chanos* (Sontakke and Haridash 2018) *were* cultured in a biofloc system. It is concluded that *C. reba* culture is possible in biofloc technology.

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