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CONTENTS

Evaluation of selected carbon sources in biofloc production and carps growth performance

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ABSTRACT: The effect of selected carbon sources (i.e., Mollasses, Potato & Wheat flour) on biofloc development and fish growth was evaluated during November 2019 to February, 2020 using four treatments in three replicates *viz.,* T_{-0} (Control), T_1 (Molasses as carbon source), T_2 (Potato as carbon source) and T_3 (Wheat flour as carbon source). Healthy and disease free fish fingerlings of *Cyprinus carpio haematopterus (*1.633±0.177 g*), Labeo catla* (10.725±0.177 g), *Ctenopharyngodon idella* (4.550±0.177 g), *Cirrhinus mrigala* (1.925±0.177 g), *Labeo rohita* (4.950±0.177 g) and *Hypophthalmichthys molitrix* (11.567±0.177 g) were stocked in circular FRP tanks (dia. 2.5 m, water volume 4.0 m^3) @ 200 fingerlings/tank (50 fingerlings/ m^3) in the ratio of 20:15:10:15:20:20, respectively. Selected water quality parameters (water temperature, electrical conductivity, total dissolved solids, dissolved oxygen, free carbon dioxide, pH, total alkalinity, ammonia and nitrate) were studied weekly. During the experimental period the range of various physico-chemical parameters was demarked from all the tanks *viz.,* temperature (13.3±0.2 to 25.4±0.25°C), electrical conductivity (302.03±0.16 to 462.69±6.71 µS cm^{-1}), total dissolved solids (171.17±0.450 to 243.48±11.16 mg L^{-1}), dissolved oxygen (3.95±1.13 to 10.05±0.33 mg L^{-1}), free carbon dioxide (0 to 2.96±1.84 mg L^{-1}), pH (2.527±0.047 to 8.84±0.300 mg L^{-1}), total alkalinity (50±2 to 193.333±4.16 mg L^{-1}), ammonia (0.015± 0.013 to 0.18±0.277 mg L^{-1}) and nitrate (0.733±0.208 to 3.8±0.6 mg L^{-1}). The recorded net fish weight gain in different treatments were 15.95 g (T_1), 13.64 g (T_2), 18.04 g (T_3) and 16.02 g (T_0). The highest SGR for *Cirrhinus mrigala* (2.14 % d^{-1}), *Cyprinus carpio haematopterus* (2.13 % d^{-1}), *Labeo catla* (1.41 % d^{-1}), *Ctenopharyngodon idella* (2.09 % d^{-1}) and *Hypophthalmychthys molitrix* (1.10 % d^{-1}) were in T_3 group while *Labeo rohita* performed best in T_1 group with highest SGR of 1.31 % d^{-1}. The results clearly indicated that the wheat flour is a good option for carbon supplementation in biofloc systems, which needs to be further tested in natural pond fish culture systems.

Key words: Biofloc, fingerlings, molasses, specific growth rate, wheat flour

Aquaculture has a tremendous scope in intensifying the food and protein requirement of the world's growing population. As the demand for fish has increased, it has devised new farm-based technologies that are efficient and inexpensive for the production of fish on commercial levels, in the open seas, inland farming systems, as well as along the coastal belts. It not only help in re-establishing some of the threatened fish base, but has also effectively restored the habitats of the fish. In India, fisheries as a subject of commercial value got its importance only after the independence from British and today, it is placed at the second position just after China in the total fish production as well as in inland fish production worldwide with an annual production of 14.16 million metric tonnes recorded during 2019-20 (DoF, 2020). There are many environment friendly closed fish culture systems *viz.,* intensive fish farming, recirculated aquaculture system, fish aquaponic system, fish culture in biofloc system *etc.* developed for enhanced fish production per unit area water and space. Among them the biofloc technology is hugely beneficial as a large extent it reduces the water usage. It is a technique of enhancing water quality by balancing carbon and nitrogen in the system. In this system, the retention of waste and its conversion to biofloc as a natural food within the culture system occur. Aquatic ecosystems recycle huge hoards of dissolved and particulate organic matter, playing one of the prime important roles in the food webs. In the biofloc set up, the bacteria surround the faeces, molts, dead organisms and surplus food to foster bacterial biomass, which is consumed by omnivores (Sunitha *et al.*, 2018). Biofloc technology has therefore proven to be a beneficial technique, reducing the expenses incurred on feed in the minimum space available with the maximum density of the fish cultured. With its limited use in the Indian state of Uttrakhand, it becomes imperative to study culture of cultivable carps in biofloc system, which can be used to benefit the commercial fisheries and lay a head way for further research and biofloc-farm practices for the farmers.

MATERIALS AND METHODS

The experiment was conducted in twelve circular FRP tanks (Size- 2.5 m diameter, 4000 litre water capacity) kept in indoor fish rearing unit (Poly carbonate house) of College of Fisheries, G.B. Pant University of Agriculture and Technology, Pantnagar, Distt. Udham Singh Nagar for a period of four months (November, 2019 to February, 2020). It is situated in the Tarai range of Himalayan foothills at the coordinates $28^0 58'$ N latitude, $79^0 30'$ E longitude and 243.84 m ASL altitude which has humid sub tropical climate characterized by hot and dry summer and very cold winter (Tiwari, 2008; IMD, 2015).

There were four triplicate treatment groups based on different carbon source and fixed nitrogen source (Ammonium sulphate) for maintaining C: N ratio of 15:1 *viz.* T_1 (Molasses), T_2 (Potato), T_3 (Wheat flour) and T_0 (Control- without biofloc). Healthy and disease-free fingerlings of six species of cultivable carp's *viz., Labeo catla, Labeo rohita, Cirrhinus mrigala, Ctenopharyngodon idella, Hypophthalmichthys molitrix* and *Cyprinus carpio haematopterus,* (Average weight 5.89 g) were stocked with 200 fingerlings in each tank @ 50 fingerlings/ m^3 in the ratio of 20:15:15:10:20:20, respectively (Sharma and Mishra, 2018). Before stocking in the FRP tanks, the fingerlings were kept in disinfected tanks and the water was well oxygenated to acclimatize the fish.

Each experimental tank was covered with a mesh cloth on the top, to thwart accidental escape of the fish under study. The water influx was controlled with the help of an electric motor and pipe system. An air blower was used to oxygenate the water in all the experimental tanks. The aeration was done continually to let the floc not settle down and, to maintain the optimum oxygen level. The inoculum was prepared in 40 litres capacity tank using 16 g carbon source, 0.4 g nitrogen source and 800 g pond bottom soil for adding there in the experimental tanks to create heterotrophic bacteria and other beneficial organisms. (Gaona *et al*. 2011; Sontakke, 2019).

The tanks were enriched with the prepared inoculum of biofloc every fortnight. The C: N ratio of 12 to 15:1 encourages the heterotrophic bacterial growth thus putting control on ammonia (Hargreaves, 2013). In addition to the biofloc materials the experimental fishes (Also in control tank) were fed daily with 2% of the pelleted, floating feed [24% protein feed made up of mustard oil cake (29%), soybean oil cake (15%), fish meal (10%), de-oiled rice bran (45%) and vitamin- mineral mixture (1%)] for the first 15 days, which was brought down and adjusted fortnightly to 1% of the body weight for the rest of the study period. The water quality parameters *viz.,* water temperature, electrical conductivity, total dissolved solids, dissolved oxygen, free CO_2, pH, total alkalinity, NH_3-N and NO_3^--N were monitored weekly following standard methods (APHA, 2017). Fortnightly sampling of the experimental fish was done swiftly to record the growth parameters avoiding fish mortality. To measure the value of important growth parameters *viz.,* Net weight gain and Specific growth rate, standard method (De Silva *et al*., 1995) was adopted.

RESULTS AND DISCUSSION

Water quality parameters play an important role in the production of biofloc organisms. Biofloc acts as natural probiotics. Heterotrophic bacteria, phytoplankton, zooplankton and protozoa create perfect conditions for microbial growth in biofloc system (Avnimelech *et al*., 1994). The variations in physico-chemical parameters (Water temperature, total dissolved solids, conductivity, dissolved oxygen, free CO_2, pH, total alkalinity, ammonia and nitrate) are depicted in Table 1. The value of water quality parameters showed a suitable range for fish survival and satisfactory growth in all experimented groups (Jhingran, 1991).

Water temperature is an important environmental parameter which affects different chemical and

Table 1: Variation in water quality parameters during experimental period

Parameters	Lowest value		Highest value	
	Value	Week and treatment	Value	Week and treatment
Water temperature (^{0}C)	13.3 ± 0.2	Dec.-IV, T_2	25.4±0.25	Nov-I, T_0
TDS (mgL^{-1})	171.14±0.45	Feb.-III, T_0	243.48±1.16	Feb.-III, T_3
Conductivity ($\mu S\ cm^{-1}$)	300.2±0.16	Nov.-II, T_1	462.29±6.07	Feb.-IV, T_0
DO (mgL^{-1})	3.94±1.13	Nov.-I, T_3	15.2±1.01	Jan.-IV, T_0
Free CO_2 (mg L^{-1})	0.00	Mostly in T_0	2.96±1.84	Jan.-I, T_1
pH	6.52±0.36	Feb-IV, T_0	8.84±0.03	Nov.-III, T_2
Total alkalinity (mgL^{-1})	50.0±2.0	Dec.-III, T_0	193.33±4.16	Nov.-II, T_0
NH_3- N (mgL^{-1})	0.015±0.013	Jan.-II, T_3	0.18±0.027	Jan.-III, T_3
NO_3^-- N (mg L^{-1})	0.733±0.020	Feb.-II, T_1	3.80±0.60	Dec.-II, T_0

Table 2: Growth performance of experimental fishes in different biofloc systems

Fish species	Initial weight (g)	Final weight (g)			
		T_0	T_1	T_2	T_3
Labeo rohita	4.50	17.83	21.80	20.00	20.30
Labeo catla	7.00	26.00	19.13	19.73	24.50
Cirrhinus mrigala	1.40	17.66	16.43	14.66	18.36
Ctenopharyngodon idella	1.50	17.66	16.07	16.64	18.46
Hypophthalmichthys molitrix	8.30	21.83	27.2	20.83	31.33
Cyprinus carpio haematopterus	1.50	17.34	19.27	14.2	19.5

Table 3: Two way ANOVA results of fish weight gain in experimental units

Source of Variation	SS	df	MS	F	P-value	F crit
Fish species	13.52139	5	2.704278	0.53751	0.745013	2.901295
Treatments	58.17255	3	19.39085	3.85418	0.031548	3.287382
Error	75.46683	15	5.031122			
Total	147.1608	23				

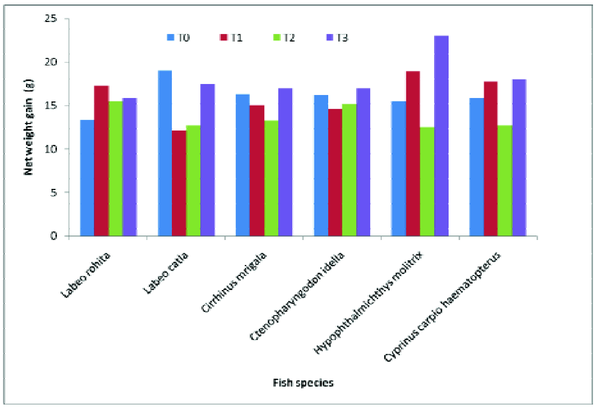

Fig. 1: Variation in net weight gain (g) of experimental fishes in biofloc system

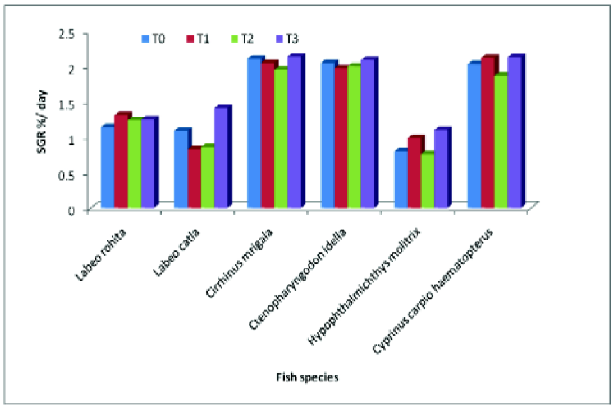

Fig. 2: Variation of specific growth rate of experimental fishes in biofloc system

biological processes in aquatic ecosystem (Jhingran, 1991). The water temperature recorded in experimental tanks varied from 13.3 °C (T_2 during fourth week of December) to 25.4 °C (T_0 during first week of November). Fluctuation in the total dissolved solids is mainly due to the variation in the ionic composition of water (Gupta and Paul, 2013). Ionic composition of water was different in different treatment groups which vary from 171.14 (T_0 during third week of February) to 243.48 mg L^{-1} (T_3 during third week of February) may be due to different carbon sources. The value of conductivity was observed in a range of 300.2 (T_1 during second week of November) and 462.29 µScm^{-1} (T_0 during fourth week of February). Dissolved Oxygen is one of the most important water quality parameters in fish culture. During the study period, the dissolved oxygen concentration varied from 3.94 (T_3 during first week of November) to 15.2 mgL^{-1} (T_0 during fourth week of January). The concentration of dissolved oxygen during the experimental period differs due to variation in photosynthetic and microbial activities. A high organic matter load may also decrease the DO values to a considerable level (Patil *et al.*, 2005). Free CO_2 in the experimental tanks varied from 0.0 (Mostly inT_0) to 2.96 mgL^{-1} (T_1 during first week of January). The free carbon dioxide was absent most of the time in T_0 may be due to its utilization in photosynthetic activities. The highest concentration of free CO_2 in T_1 may be due to deposition of organic matter. The source of CO_2 in these tanks is bacterial decomposition and respiration of fishes (Bregnballe, 2015). The concentration of CO_2 in the other tanks was considerably optimum due to proper utilization. During the study period, the pH value recorded in the range of 6.52 (T_0 during fourth week of February) and 8.84 (T_2 during third week of November). It was lower in tank T_0 (control condition) compared to the others may be due to high CO_2 production during night time and utilization by primary producers during day time. Optimum pH level for carp culture is 7.5 to 8.5 (Ayyappan *et al*, 2011). The total alkalinity varied from 50.0 (T_0 during third week of December) to 193.33 mgL^{-1} (T_0 during second week of November). Alkalinity acts as buffer against rapid pH change. The degradation of organic waste may also be one of the reasons for the increase in carbonates and bicarbonates there by the alkalinity (Chaurasia and Pandey, 2007). During the experimental period, the concentration of ammonia ranged from 0.015 (T_3 during second week of January) to 0.18 mgL^{-1} (T_3 during third week of January). Bhatnagar and Singh (2010) recommended that <0.2 mg L^{-1} ammonia concentration is not harmful for fishery. The amount of nitrate varied from 0.73 (T_1 during second week of February) to 3.8 mgL^{-1}(T_0 during second week of December). The level of nitrate is reduced by water exchange and by the use of denitrifying biological filtration (Bhatnagar and Devi, 2013).

After four months of rearing period, the growth performance of fish in different biofloc systems was studied and summarized in Table 2 and figures 1-2. Biofloc system is a low budget fish farming technology in which feed cost is reduced too much (Bauer *et al.,* 2012). At the end of the experiment the net weight gain of the experimental fishes ranged from 13.33 (T_0) to 15.8 g (T_3) for *Labeo rohita*; 12.13 (T_1) to 19.0 g (T_0) for *Labeo catla*, 13.26 (T_2) to 16.96 g (T_3) for *Cirrhinus mrigala*; 14.57 (T_0) to 16.96 g (T_3) for *Ctenopharyngodon idella*; 12.53 (T_2) to 23.03 g (T_3) for *Hypophthalmichthys molitrix* and 12.7 (T_2) to 18.0 g (T_1) for *Cyprinus carpio haematopterus* in different experimental tanks. With an overall net increase of 17.07g in weight, the fishes in polyculture-biofloc responded best in the T_3 with the net increase in weight of a 18.04 g followed by T_0 with a net rise in the weight of a 16.02 g and T_1 with a net rise in the weight of 15.95 g. The higher growth of experimental fishes in control (T_0) as compared to biofloc treatment (T_1) might be due to sufficient availability of suitable natural food (plankton) and their complete consumption. The difference in recorded weights in different carbon source was registered significant with the noted exception to potato. The more average net gain under controlled treatment compared to potato treatment may well be due to poor capability of potato to foam up strong flocs. Among the different biofloc treatments of polyculture, *Cyprinus carpio haematopterus*, *Cirrhinus mrigala* and *Ctenopharyngodon idella* responded best in T_3 with a net weight gain of 18.00, 16.96 and 16.96 g, respectively while *Labeo rohita* and *Hypophthalmichthys molitrix* showed best performance in T_2 with a net weight gain of 17.3 and 18.9 g, respectively and *Labeo catla* recorded best growth in T_0 treatment with a net weight gain of 19.0 g. The higher growth rates of *Cyprinus carpio haematopterus*, *Cirrhinus mrigala* and *Ctenopharyngodon idella* in T_3 might be due to the sufficient availability of benthic flocs and organic matter in the same treatment. *Labeo* *rohita* and *Hypophthalmichthys molitrix* can easily sweep off the flocs in the upper niche of the tanks as they occupy this area, even as *Labeo rohita* are column feeder thus complementing the suspended bioflocs in the system. These might be the possible reasons for better growth in the specific biofloc system. The two ways ANOVA analysis results of net growth of experimental fishes shows a non significant difference in individual fish species growth at 5% level of significance kept in various treatments but a significant difference has been observed within species (Tables 3). It may be due to variable consumption rate of biofloc materials by different fish species as their feeding habit is different (Jhingran, 1991). Also, the experiment conducted by Mannan *et al.* (2012) to assess the impact of water quality on fish growth in semi-intensively managed aquaculture farm revealed that the water quality parameters and species composition influence the growth and production of fishes.

The value of specific growth is used to know the comparative growth of experimental fishes. The higher values of specific growth indicate effective nature of the treatment introduced. The result of SGR in different treatment groups with six species of fish has been depicted in the Tables 2, 3, 4, 5 and figure 2. The result revealed that the SGR of experimental fishes varied from 1.14 (To) to 1.25 (T_3) in *Labeo rohita,* 0.83 (T_2) to 1.41 (T_3) in *Labeo catla,* 1.95 (T_2) to 2.14 (T_3) in *Cirrhinus mrigala*, 1.97 (T_1) to 2.09 (T_3) in *Ctenopharyngodon idella*, 0.76 (T_2) to 1.10 (T_3) in *Hypophthalmichthys molitrix* and 1.87 (T_2) to 2.13 (T_3) % day^{-1} in *Cyprinus carpio haematopterus* kept under different treatment groups. *Cirrhinus mrigala*, *Cyprinus carpio haematopterus*, *Labeo catla*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* recorded best SGR in the groupT_3 with the average SGR of 2.14, 2.13, 1.41, 2.09 and 1.1% d^{-1}, respectively while *Labeo rohita* registered best SGR in T_1 with the average value of 1.31% d^{-1}. Fabiane *et al.* (2015) reported molasses treatment to work better than rice bran due to easy dissolution of the former. Molasses can be used as a best carbon source for the biofloc culture of Tilapia (Ugo *et al.,* 2017). Anjali *et al.* (2019) delineated that biofloc treatments produced better response to growth than other treatments. Arnuparp *et al.* (2017) founded molasses with ground bread as the best source of carbon in their study. Deb *et al.* (2020) noted higher growth of Catla in the molasses treatment, in a polyculture biofloc system. The analysis result of specific growth rate (SGR) shows a significant difference at 5% level of significance in individual fish species kept in different treatment groups as well as within fish species. Most of the experimental fishes (except *Labeo rohita*) show better growth performance with T_3 (wheat flour as carbon source). It may be attributed with the better capacity to release the required nutrients for the proper growth of biofloc organisms. The result of present investigation was fairly similar to the findings of Enache *et al.* (2011) who found SGR range of 1.28% day^{-1} to 1.49% day^{-1} in different trials. The findings of SGR in the present investigation are different than the findings of Enache *et al.* (2011) and Kristan *et al.* (2019) while similar to the results of Dubey *et al.* (2017). Kamila *et al.* (2017) verified positive implications of biofloc on growth performance in common carp fingerlings. Deb *et al.* (2020) registered the highest SGR of Catla in biofloc system whereas as a corresponding lowest SGR of Mrigal in both biofloc and control system.

CONCLUSION

The present study done on the fish rearing in biofloc system with variable carbon source is an excellent technique for growing fish at low input cost. This technique can be used for growing fish in pond polyculture system at very high stocking density by maintaining proper aeration and C: N ratio using different carbon sources *viz*., molasses, potato, wheat flour, yam, sorghum etc. and enhance the productivity of water. Though the growth of experimental fishes was not satisfactory as the experiment was conducted mainly during winter season but most of the fishes performed better from weight gain and specific growth point of view in the unit where wheat flour was used as carbon source. This technique makes the suitable condition of the hydrographical parameters and does never cause any stress in the environment as well as growth of the fishes.

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