



Effect of different carbon sources on growth, non-specific immunity and digestive enzyme activity of amur carp (*Cyprinus rubrofuscus* Lacepede 1803) fingerlings in biofloc based rearing system using inland saline groundwater

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ABSTRACT

The study was conducted to evaluate the effect of different carbon sources in biofloc based system for rearing amur carp (*Cyprinus rubrofuscus* Lacepede 1803) fingerlings in inland saline groundwater. The study was undertaken in a complete randomised design (CRD) where each treatment was performed in triplicate. The experimental unit consisted of four different carbon sources viz., T1 (tapioca flour), T2 (wheat flour), T3 (rice bran), T4 (jaggery) and control (C) with water exchange for a duration of 45 days. Each tank (500 l) was stocked with 30 fingerlings with an average body weight of 11.17 ± 0.34 g. At the end of the rearing period, biofloc based treatments showed significantly better growth performance compared to control. Among the treatments, jaggery based biofloc system showed the highest biomass (629.4 ± 1.58 g), specific growth rate, SGR (1.32 ± 0.03 % day⁻¹), protein efficiency ratio, PER (0.29 ± 0.05), and lowest feed conversion ratio, FCR (0.56 ± 0.03). Digestive enzymes of the biofloc reared fishes showed enhanced activity compared to control group. Jaggery based biofloc (T4) showed significantly higher non-specific immune response in terms of respiratory burst activity (1.14 ± 0.01), superoxide dismutase (44.59 ± 0.19 U mg protein⁻¹) and catalase (1.59 ± 0.01 U mg protein⁻¹) activity compared to other biofloc treatments and the control. The present study concluded that jaggery is best as compared to other carbon sources tested (tapioca flour, wheat flour and rice bran) for better growth, non-specific immunity and digestive enzyme activity of amur carp fingerlings in biofloc based rearing system using inland saline groundwater.

Keywords: Amur carp, Biofloc, Carbon Sources, Inland saline groundwater, Jaggery, Non-specific immune parameters

Introduction

With the increasing global demand for food from aquatic sources, aquaculture needs expansion and intensification. Intensification with high inputs and high stocking densities, will inevitably lead to rapid and unregulated growth with irrevocable environmental destruction. Moreover, it also triggers the conflicts over the scant resources like land and water (Widanarni *et al.*, 2012). The allocation of land and water suitable for aquaculture are likely to reduce, while on the one hand, vast resources of land and water are left unutilised in inland areas due to secondary salinisation. In India, approximately 9.38 million ha area is engaged by salt-affected soils, out of which 5.50 million ha are saline soils (including coastal) and 3.88 million ha are alkali soils (IAB, 2000). Mostly, such lands are low-priced and comparatively unpolluted as these are located in remote areas. Hence, there is better scope for aquaculture in those areas and it will act as an additional income generating source from the unused infertile soil (Gong *et al.*, 2004). In Addition, there is a need to employ a culture system which uses limited natural resources, which is less polluting

and economically sustainable. Accordingly, the biofloc technology (BFT) based farming system has proved to ensure 40% lower water consumption than that of the recirculating aquaculture system (RAS) (Luo *et al.*, 2014).

Biofloc technology (BFT) is one among various technologies in which the nitrogenous waste is recycled through microbial assimilation (Avnimelech, 1999). Thus, a low cost proteinaceous *in situ* feed is made available as feed for the cultured organisms (Crab *et al.*, 2010). The feed ingestion by an aquatic organism depends primarily on palatability as well as grazing ability of an animal and also on the floc density. In order to achieve a high density of floc, C/N ratio has to be increased from 10-20:1 (Asaduzzaman *et al.*, 2010; Ballester *et al.*, 2010). An optimum C:N ratio can be maintained by adding locally available cheap carbon sources (Avnimelech, 1999; Hargreaves, 2006). The carbon source serves as a substrate for the operation and the production of microbial cells (Avnimelech, 1999). The consumption of microbial protein by shrimps and fishes has demonstrated numerous benefits such as improvement of growth rate

(Wasiolesky *et al.*, 2006; Ahmad *et al.*, 2017), decreased FCR, increased physiological health of a cultured organism owing to the presence of valuable bioactive compounds such as chlorophyll, carotenoids, phytosterols, bromophenols, amino sugars (Ju *et al.*, 2008) and anti-bacterial compounds (Crab *et al.*, 2010) and also it decreases the cost associated with feed (Burford *et al.*, 2004). BFT in inland saline groundwater is considered to be a new approach to increase production in aquaculture sector.

Inland saline aquaculture depends on production strategies such as growth and survival of salt-tolerant freshwater as well as estuarine/marine species. Salt tolerant freshwater species particularly, freshwater carps such as mrigal and common carp were extensively studied and proved to be candidate species for culture in inland saline area with salinity upto 10‰ (Garg, 1996; Dhawan *et al.*, 2009). Accordingly, the amur carp, *Cyprinus rubrofasciatus* Lacepede 1803 an improved strain of common carp, distributed worldwide was selected for the present study as it satisfies the characteristic requirement for inland saline aquaculture.

Amur carp was first introduced to India in the year 2000 for the genetic improvement programme in Karnataka (Basavaraju and Reddy, 2013). It is an omnivorous, fast growing species (27% faster growth than existing stock of common carp), with delayed sexual maturity, accepts supplementary feed and less susceptible to diseases as compared to the existing stock. However, so far no published information is available on the feasibility of BFT system for culture of amur carp in inland saline groundwater. So, the present study was designed to evaluate the growth performance and immune response of amur carp in biofloc based rearing units in inland saline water using different carbon sources.

Materials and methods

Biofloc inocula preparation

Primarily inocula were developed separately using four different carbon sources (*viz.*, tapioca flour, wheat flour, rice bran and jaggery) using 5‰ inland saline groundwater in 300 l FRP circular tank. For inocula development, 20 g l⁻¹ pond soil, 10 mg l⁻¹ ammonium sulphate and 200 mg l⁻¹ glucose were added to the respective treatments by following the procedure of Avnimelech (1999). The C:N ratio of 20:1 was maintained in the inocula tanks to achieve the development of heterotrophic bacteria in the microbial biofloc as it requires around 20 units of carbon to assimilate one unit of nitrogen (Emerenciano *et al.*, 2011). The heterotrophic microbial biomass development was observed after 48 h of incubation. The prepared inoculum was added to the treatment tanks, and sufficient aeration

was provided to keep the biofloc in suspension. The floc volume was measured by collecting one litre of water sample in an Imhoff cone. The volume was recorded after 30 min by allowing to settle in the cone, and the value is expressed as ml l⁻¹.

Experimental setup

The experiment was conducted for 45 days at the main wet laboratory unit, ICAR-Central Institute of Fisheries Education (I CAR-CIFE), Rohtak, Haryana, India. The experiment was designed following completely randomised design with one control and four treatments in triplicate *viz.*, C (control, clearwater without carbon addition), T1 (tapioca), T2 (wheat), T3 (rice bran) and T4 (jaggery). The amur carp (*Cyprinus rubrofasciatus*) fingerlings were procured from College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India. At the farm site, the fishes were disinfected using KMnO₄ and stocked in 0‰ water in 1200 l tank. Slowly, the fishes were acclimatised to inland saline groundwater by adding 5‰ water for 15 days. Fingerlings of amur carp having average body weight of 11.17±0.34 g were distributed randomly to all 15 tanks at a stocking density of 30 nos. per 500 l tank. To achieve the floc volume of 25 to 50 ml l⁻¹, vigorous aeration and C:N ratio of 20:1 was maintained in all experimental tanks with different carbon sources (Hargreaves, 2013). All the experimental fishes were fed with carp pellet feed having 32% crude protein.

Physico-chemical parameters

Water quality parameters like temperature (mercury thermometer) and pH (colorimetric method) were monitored daily. Other parameters such as dissolved oxygen (Winkler's method), total ammonia nitrogen, TAN (NH₃-N), Nitrite-N (NO₂-N), Nitrate-N (NO₃-N), total alkalinity and total hardness were analysed twice in a week, following standard methods (APHA, 2005).

Growth analysis

The growth performances were assessed at the end of the experiment in terms of specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER) and protein efficiency ratio (PER) using the following formulae:

$$\text{SGR day}^{-1} (\%) = \{(\ln \text{ Final weight} - \ln \text{ Initial weight}) / \text{Number of days}\} \times 100 \quad (1)$$

$$\text{FCR} = \text{Feed given (dry weight)} / \text{Body weight gain (wet weight)} \quad (2)$$

$$\text{FER} = \text{Body weight gain (wet weight)} / \text{Feed given (dry weight)} \quad (3)$$

$$\text{PER} = \text{Body weight gain (wet weight)} / \text{Crude protein fed} \quad (4)$$

Digestive enzyme analysis

At the end of 45 days of experimental trial, three fishes from each tank were sacrificed for analysing the digestive enzyme activity. The dissected tissues

(liver, gills, muscle, kidney and intestine) were homogenised with 0.25 M sucrose solution (pH 7.0, 1:20 W/V) using a hand held homogeniser. The homogenate was centrifuged at 5000 rpm for 10 min at 4°C in a refrigerated centrifuge. After centrifugation, the top lipid layer was removed and the supernatant solution was transferred to a 15 ml autoclaved tube. The samples were stored at -20°C until analysis.

The total protein content of various tissues was determined by Bradford (1976) method. It relies on the binding of the dye, coomassie blue G250 to protein. The amylase activity of samples was determined by Robyt and Whelan (1968) method using starch as a substrate. One unit of amylase activity was defined to hydrolyse 10 mg of starch in 30 min at 37°C. Protease activity was determined as per the method of Moore and Stein (1948), using bovine serum albumin as the substrate. The specific activity of protease was expressed as µg of leucine liberated mg⁻¹ of tissue protein h⁻¹ at 37°C. The lipase activity was determined by the method of Cherry and Crandall (1932). Enzyme activity was expressed as µmol oleic acid formed per ml of extract per min.

Non-specific immune parameters

The blood samples were collected using sterile 2 ml hypodermal syringe from three fishes in each replicate treatments at the end of 45 days of experimental trial. Before drawing blood, the syringes were pre-coated with EDTA (2.7%) and the fishes were anaesthetised using clove oil. The collected blood was then transferred to 1 ml EDTA coated vials. For serum preparation, blood was collected in syringe without anticoagulant, allowed to clot and the clot was spun down at 6000 rpm for 10 min, after 24 h the serum was collected in sterile tubes and stored at -20°C until analysis.

The respiratory burst activity was measured by nitroblue tetrazolium (NBT) assay following the method of Anderson and Siwiki (1995). Superoxide dismutase (SOD) activity in the liver was estimated by the method

of Mishra and Fridovich (1972) with slight modifications. Liver tissue was used for analysing SOD activity. The assay was based on the oxidation of epinephrine adrenochrome transition by the enzyme. One unit of SOD activity was the amount of protein required to give 50% inhibition to epinephrine auto oxidation. Catalase activity (CAT) was estimated in liver according to the method of Takahara *et al.* (1960). The activity was expressed as n moles of Hydrogen peroxide (H₂O₂) decomposed per min per mg protein.

Statistical analysis

Statistical analysis was done using SPSS Version 16 following one-way analysis of variance (ANOVA) and Duncan's multiple range test was used for *post hoc* comparison of mean (p<0.05) between different groups.

Results

Physico-chemical parameters

The water quality parameters recorded during the experimental trial are given in Table 1. Temperature (27.53±0.29 to 28.03±0.40°C) did not show any significant difference among various treatment groups. Mean pH value and total alkalinity ranged from 7.41±0.10 to 8.69±0.08 and 101.50±1.38 to 236.40±4.85 mg l⁻¹ with the lowest value observed in rice bran based biofloc unit. Dissolved oxygen (DO) was fluctuating throughout the experimental period with the lowest value observed in biofloc based treatments. TAN concentration and NO₂-N were lower in control compared to other biofloc treatments, while the NO₃-N was higher in tapioca (T1) than control and was much higher in rice bran (T3) based biofloc system.

Growth parameters recorded in various treatment groups are given in Table 2. Final body weight differed significantly among various treatment groups with the highest growth, and better FCR (1.77±0.09) at harvest were recorded in jaggery based biofloc system compared to control. SGR showed significant difference between rice bran (1.23±0.03) and wheat (1.12±0.02) based treatments, while jaggery (1.32±0.03) showed the highest SGR.

Table 1. Water quality parameters recorded in different treatment groups during the experimental period

Parameter	Control (C)	Tapioca (T1)	Wheat (T2)	Rice bran (T3)	Jaggery (T4)
Temperature T(°C)	27.53±0.29 ^a	28.20±0.55 ^a	27.89±0.26 ^a	27.96±0.27 ^a	28.03±0.40 ^a
DO (mg l ⁻¹)	6.4±0.12 ^b	6.21±0.04 ^b	7.87±0.15 ^a	7.86±0.45 ^a	6.39±0.08 ^b
pH	8.69±0.08 ^a	8.54±0.06 ^a	8.40±0.19 ^a	7.41±0.10 ^b	8.52±0.13 ^a
Alkalinity (mg l ⁻¹)	199.5±4.46 ^a	236.4±4.85 ^a	172.7±18.3 ^{ab}	101.5±1.38 ^b	213.7±44.98 ^a
Hardness (mg l ⁻¹)	1349.2±81.6 ^b	1799±61.2 ^a	1754.6±66.2 ^a	1898±12.6 ^a	1754.6±38.4 ^a
TAN (mg l ⁻¹)	0.02±0.00 ^d	0.43±0.04 ^a	0.19±0.02 ^c	0.09±0.01 ^d	0.29±0.02 ^b
NO ₃ -N(mgl ⁻¹)	1.80±0.05 ^d	7.52±0.87 ^c	16.02±0.97 ^b	21.2±0.27 ^a	7.95±0.26 ^c
NO ₂ -N (mg l ⁻¹)	0.01±0.00 ^d	0.44±0.04 ^b	0.60±0.03 ^a	0.31±0.03 ^c	0.34±0.05 ^{bc}

Values are expressed as Mean±SE. Values in the same row with different superscripts differ significantly (p<0.05) for each parameter. One way ANOVA was followed by Duncan's multiple range test.

Similarly, higher FER and PER were observed in biofloc based treatment groups while control group recorded the lowest FER and PER.

Digestive enzyme activity

At the end of the experimental trial, the digestive enzyme levels indicated significantly higher activity ($p < 0.05$) in fishes raised under different biofloc based treatments compared to control (Table 3). Significant difference ($p < 0.05$) was observed between the biofloc system, and the Jaggery based unit showed the highest amylase, protease and lipase activity and the control group registered the lowest (Table 3).

Non-specific immune parameters

The NBT activity (OD at 520 nm) was significantly higher ($p < 0.05$) in all biofloc based treatments compared to control. Fishes reared in jaggery based system showed improved performance (1.14 ± 0.01) at the end of the experiment followed by tapioca (1.13 ± 0.01) (Fig. 1). The SOD showed significant difference among different treatment groups. The fishes reared under biofloc based system exhibited a significantly higher SOD activity, and among them, jaggery unit showed the highest activity (44.59 ± 0.19 U mg protein⁻¹) compared to control

(42.62 ± 0.36 U mg protein⁻¹) (Fig. 2). The CAT activity also showed a similar trend (Fig. 3).

Discussion

The classical microbial loop by means of heterotrophic bacteria *via* organic carbon and inorganic nitrogen uptake, ameliorates water quality, together with it exerts, a controlling effect over pathogenic growth in biofloc based system (Avnimelech, 1999). Wilen and Balmer (1999) found that at lower temperature (40°C), deflocculation may happen when compared with higher temperature (18-20°C). Accordingly, the temperature was maintained above 27°C to achieve the optimum floc structure and to keep the microbial activity at an optimum level. Ebeling *et al.* (2006) also reported that the rate of microbial activity through nitrification will be affected below the neutral pH. As a result, the reduction of TAN and NO₂-N observed in the present experiment could be attributed to immobilisation by heterotrophic bacteria (Azim and Little, 2008) at a pH range of 7.96 to 8.73. In the present study, 100% survival and a conducive growth performance were observed in biofloc grown amur carp compared to control which indicated that, the biofloc not only reduces the N and P waste in the system but also

Table 2. Growth performance of *C. rubrofusculus* fingerlings reared in different biofloc based treatment groups

Parameter	Control (C)	Tapioca (T1)	Wheat (T2)	Rice bran (T3)	Jaggery (T4)
Biomass (g)	515.8±2.38 ^d	621.9±2.62 ^a	583.1±2.94 ^c	608±4.59 ^b	629.4±1.58 ^a
SGR	0.88±0.0 ^d	1.32±0.01 ^a	1.12±0.02 ^c	1.23±0.03 ^b	1.32±0.03 ^a
FCR	2.81±0.04 ^a	1.90±0.04 ^c	2.15±0.05 ^b	2.02±0.06 ^b	1.77±0.09 ^c
FER	0.35±0.00 ^c	0.52±0.01 ^a	0.46±0.01 ^b	0.49±0.01 ^b	0.56±0.03 ^a
PER	0.17±0.02 ^d	0.29±0.00 ^a	0.24±0.04 ^c	0.26±0.06 ^b	0.29±0.05 ^a
Survival (%)	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a	100±0.00 ^a

FCR- Feed conversion ratio, FER- Feed efficiency ratio, SGR- Specific growth rate, PER- Protein efficiency ratio. Values are expressed as Mean±SE. Values in the same row with different superscripts differ significantly ($p < 0.05$) for each parameter. One way ANOVA was followed by Duncan's multiple range test.

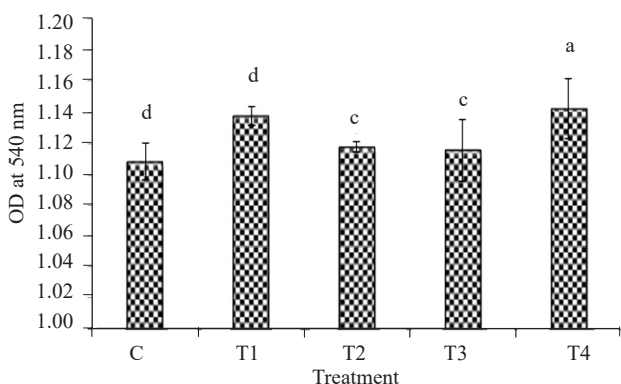


Fig. 1. Respiratory burst activity (OD at 540 nm) of *C. rubrofusculus* reared in biofloc based inland saline water supplemented with different carbon sources. Error bars represents mean±SE. The mean values with different superscript letters in common indicate significant difference ($p < 0.05$)

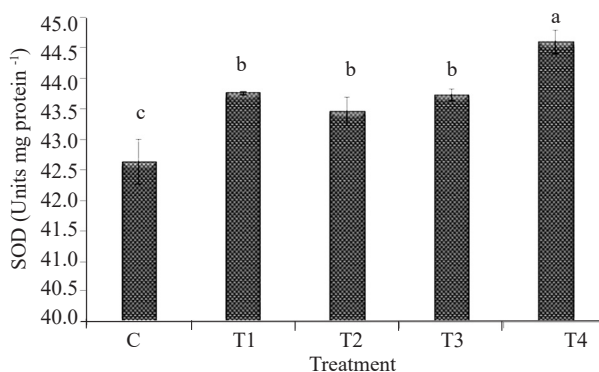


Fig. 2. Superoxide dismutase activity of *C. rubrofusculus* reared in biofloc based inland saline water supplemented with different carbon sources. Error bars represents mean±SE. The mean values with different superscript letter in common indicate significant difference ($p < 0.05$)

for the cultivated animals. Similarly, the related outcome earlier reported in biofloc reared fishes viz., tilapia (Azim and little 2008; Haridas *et al.*, 2017) and *Labeo rohita* (Mahanand *et al.*, 2013; Ahmad *et al.*, 2016) is consistent with the results of our present experiment.

Improved weight gain observed in jaggery based biofloc system; could be due to the continuous accessibility to the *in situ* biofloc. Tacon *et al.* (2002) stated that biofloc will boost the ingestion rate, nutrient absorption, assimilation rate, and also it will offer widespread source of cellular nutrition. Similarly, in earlier studies, higher growth rate was observed in biofloc raised tilapia (Azim and little 2008; Crab *et al.*, 2009), *L. rohita* (Mahanand *et al.*, 2013; Ahamd *et al.*, 2016), *Carassius auratus* (Wang *et al.*, 2015) and *Cyprinus carpio* (Najdegerami *et al.*, 2015), which clearly indicates that biofloc can substitute aquatic animal feed and can promote growth of diverse fish species. Sakkaravarthi and Sankar (2015) observed higher

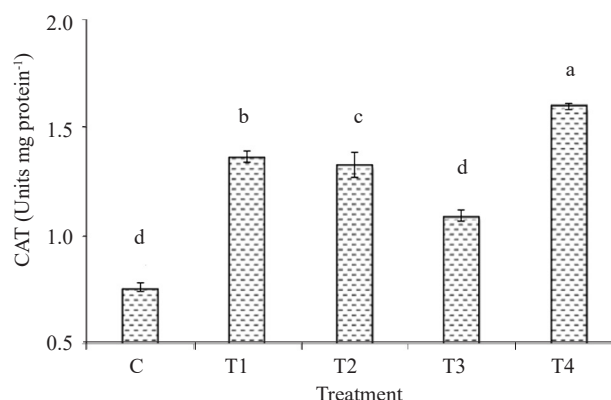


Fig. 3. Catalase activity of *C. rubrofusus* reared in biofloc based inland saline water supplemented with different carbon sources. Error bars represents mean± SE. The mean values with different superscript letter in common indicate significant difference ($p < 0.05$)

Table 3. Digestive enzymes activity of *C. rubrofusus* fingerlings reared in different biofloc based treatment groups

Treatment	Amylase (Units mg protein ⁻¹)	Protease (Units mg protein ⁻¹)	Lipase (Units mg protein ⁻¹)
Control	0.09±0.01 ^c	0.01±0.03 ^b	4.81±0.16 ^b
Tapioca	0.19±0.01 ^a	0.06±0.01 ^a	9.91±0.07 ^a
Wheat	0.13±0.02 ^b	0.03±0.02 ^c	9.22±0.71 ^a
Ricebran	0.12±0.04 ^b	0.04±0.02 ^{ab}	5.74±0.15 ^b
Jaggery	0.19±0.09 ^a	0.06±0.01 ^a	11.25±0.57 ^a

Values are expressed as Mean±SE. Values in the same column with different superscripts differ significantly ($p < 0.05$) for each parameter. One way ANOVA was followed by Duncan's multiple range test.

average body weight (ABW) in shrimp grown on jaggery based carbon source compared to molasses and sugar. Further, they suggested that jaggery based biofloc had comparatively more nutritive floc than other carbon sources based floc. In the present study also, jaggery produced more nutritive floc than the other treatments which may be the possible reason for higher body weight gain in the jaggery reared fishes. FCR and FER values of the present study showed that fishes reared under jaggery based biofloc system showed lowest FCR and highest FER compared to control fishes which showed the highest FCR and lowest FER. Previous study on jaggery based biofloc system revealed that this technology can reduce the FCR more effectively (Sakkaravarthi and Sankar, 2015). Jaggery has the capability to dissolve quickly in water and helps to develop more microbial load; especially, heterotrophic bacteria upto 13×10^6 counts (Singh *et al.*, 2009). Vijayendra *et al.* (2001) reported that jaggery is a promising carbon source for the economic production of pullulan (polysaccharide produced by the microorganism fungi). The results of present study clearly indicate that jaggery is the best carbon source for production of microbial floc at a faster rate with rich protein compared to other carbon sources. Hargreaves (2006) reported that,

consumption and regeneration of biofloc can increase feed utilisation efficiency. The results of our experiment also presented a similar higher FER and PER when jaggery was used as carbon source for rearing amur carp. Jaggery reared fishes showed significantly better growth performance compared to control and other experimental groups which discloses the highest (two times higher) protein utilisation by the fishes reared under BFT compared to conventionally fed intensive aquaculture ponds (Avnimelech, 1999).

The increased digestive enzyme activity and growth performance in the biofloc based system observed in the present study could be due to the microbial enzymes that help in the breakdown of proteins, carbohydrates and other nutritional ingredients of the feed into smaller units. Similarly, enhanced digestive enzyme activity was reported in *Penaeus vannamei* (Xu and Pan, 2012), tilapia (Long *et al.*, 2015) and *C. carpio* (Najdegerami *et al.*, 2015) when reared under biofloc based system.

Diseases remain a major limiting factor for the aquaculture industry (FAO, 2012). Since biofloc deals with the microbial environment of the culture system, it will be an ideal approach for health management in aquaculture by stimulating the innate immune system of the animals.

Studies have shown that biofloc improves the immune status of *L. rohita* against pathogens when reared in tapioca based biofloc (Kumari and Sahoo, 2006) and it also increased the disease resistance in brine shrimp, *Artemia franciscana* against *Vibrio harveyi* (Crab *et al.*, 2010). Similarly, Xu and Pan (2013) also stated that the biofloc technology is a system of microbial manipulation with beneficial bacteria such as *Bacillus* and *Lactobacillus* and so it may activate the digestive enzyme activity and the immune mechanism by colonising the gastrointestinal tract. In the present study, all the immune parameters tested like respiratory burst activity, SOD and catalase were found to be higher in fishes reared in jaggery based system compared to other experimental groups which may be due to the immunostimulatory effect of the biofloc specific to the carbon source. Ahmad *et al.* (2016) studied the non-specific immune parameters (total serum protein, NBT, myeloperoxidase) in *L. rohita* and reported an increased activity when reared under tapioca based biofloc treatment.

The antioxidant enzymes play a significant role in defence mechanism. Among them, SOD is an enzyme that catalyses the dismutation of superoxide (produced by respiratory burst activity) into O₂ and H₂O₂ and the catalase enzyme, catalases the composition of H₂O₂ (produced by dismutase of SOD) into water and oxygen (Mohankumar and Ramasamy, 2006). Dorame *et al.* (2014) identified that *P. vannamei* reared in biofloc-based systems showed an improved physiological performance as indicated by selected haemolymph parameters including SOD activity. Similarly, Haraz *et al.* (2018) reported an increased expression of SOD in BFT treatments compared to control which may be due to a development of an antioxidant defense system in Nile tilapia when reared under biofloc based system. Similarly, biofloc supplementation showed a significant higher SOD activity in biofloc raised Nile tilapia (Bairagi *et al.*, 2002; Long *et al.*, 2015) and crucian carp (Wang *et al.*, 2015). The results of the present experiment also showed an improved SOD level in biofloc treatment groups compared to control, which might be due to the bioactive compounds such as carotenoids, chlorophylls, phytosterols and bromophenols derived from biofloc (Ju *et al.*, 2008) and other immunostimulatory compounds (Crab *et al.*, 2012) that may stimulate the immune response of cultured fish. The results of the present study are in agreement with the assumption that, fishes will show increased antioxidant enzymes production such as SOD and CAT when reared under biofloc based system.

The objective of the present study was to evaluate the effects of BFT on water quality as well as on the growth, digestive enzyme activity and immune response of amur carp (*C. rubrofasciatus*) using different carbon sources in

inland saline groundwater. The results of the study clearly indicated that, the scarcity of water and the conflict for land usage for the expansion of aquacultural practices can be overcome by using the large unutilised inland saline resources of the country. BFT had beneficial effects on the maintenance of good water quality, improvement of feed utilisation, and the growth performance of amur carp. The enhanced digestive enzyme activity observed in fish reared in BFT based system suggests that BFT can influence the enzyme activity and also it can impart stimulatory effect on the immune response of the fish which consequently enhanced the feed utilisation and growth performance. From the present study, it can be concluded that jaggery is the best compared to other carbon sources (*i.e.*, tapioca flour, wheat flour and rice bran) for rearing amur carp fingerlings in biofloc system using inland saline groundwater.

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