Indian J. Fish., 71 (3): 78-87, 2024
doi:10.21077/ijf.2024.71.3.141622-09
Available online at: epubs.icar.org.in/index.php/IJF

Growth performance of critically endangered *Hypselobarbus pulchellus* in biofloc system: Influence of carbon sources on immune response, enzyme and antioxidant activity

Kanagaraja Anantharaja[1,2], Gangadhar Barlaya[1] C. H. Raghavendra[1], B. S. Anand Kumar[1], G. M. Siddaiah [3] Kalidoss Radhakrishnan[4], N. K. Chadha[4], Gopal Krishna[4], P. Routray[3,*] and K. P. Hemaprasanth[1]

[1]Regional Research Centre of ICAR-Central Institute of Freshwater Aquaculture, Bengaluru - 560 089, Karnataka, India
[2]ICAR-Central Institute of Brackishwater Aquaculture, Chennai - 600 028, Tamil Nadu, India
[3]ICAR-Central Institute of Freshwater Aquaculture, Bhubaneswar - 751 002, Odisha, India
[4]ICAR-Central Institute of Fisheries Education, Mumbai - 400 061, Maharashtra, India

Abstract

We investigated the effect of various carbon sources on the production performance of critically endangered *Hypselobarbus pulchellus* in biofloc system. The experimental setup comprised four biofloc treatment groups with various carbon sources (jaggery, tapioca, rice and corn flour) and a control group. Healthy early fry (0.0068 g) was randomly stocked at 100 individuals per m^3 into 15 circular fibre reinforced plastic (FRP) tanks (1000 l) and raised for 90 days. All the tested carbon sources significantly influenced weight gain, weight gain (%) and specific growth rate (SGR), with the jaggery treatment displaying the best growth performance ($p<0.05$). Fingerling survival rates varied from 93 to 95.67% ($p>0.05$). Compared to the control, the biofloc groups had significantly lower concentrations of nitrogen compounds, specifically total ammonia nitrogen, nitrite and nitrate concentrations were significantly lowered (0.29, 0.35 and 1.32 mg l^{-1}, respectively) in jaggery treatment ($p<0.05$). In addition, the treatment using jaggery had a higher amount of plankton biomass and an abundance of the Bacillariophyceae group (80%). All biofloc groups displayed enhanced immune and antioxidant activity compared to the control. The jaggery treatment had elevated levels of lysozyme activity than other treatments ($p<0.05$). Furthermore, fingerlings raised in BFT treatments had significantly higher amylase, protease and lipase activity ($p<0.05$). Overall, this study's findings suggest that the addition of various carbon sources significantly improves the production performance, with jaggery as the best carbon source for critically endangered *H. pulchellus* fingerling production in biofloc system to ensure sustainability.

*Correspondence e-mail:
routray30@yahoo.co.in

Keywords:
Antioxidant activity, Fingerling, Heterotrophic bacteria, Jaggery, Specific growth rate, Sustainability

Received : 04.09.2023
Accepted : 05.08.2024

Introduction

The present global population of 8 billion is anticipated to increase to 9.7 billion by 2050, that may pose a significant challenge for human nutrition in terms of meeting the growing demand for protein (Willett *et al*., 2019; UN, 2022). To counter this challenge, intensive aquaculture techniques like biofloc technology (BFT) are being considered as a sustainable solution to produce animal protein, using the minimum quantity of water by wastewater recycling, with biosecurity (Nisar *et al*., 2022). The technology operates by converting nutrient waste produced by unconsumed feed, faecal matter and metabolic by-products of cultured organisms into organism-usable microbial biomass (De Schryver *et al*., 2008; Avnimelech, 2009). To promote this conversion process, which is mainly facilitated by heterotrophic bacteria, a suitable ratio of carbon to nitrogen (C/N) must be sustained by applying a carbon source (Avnimelech, 1999). Bioflocs consist of heterotrophic bacteria, microalgae, flagellates, ciliates, fungi and micrometazoans (Avnimelech, 2009) and they are a great source of protein and bioactive components (Toledo *et al*., 2016). In addition, the availability of bioflocs as a food supply throughout the day helps to minimise feed expenditures (Avnimelech, 2007; Samocha *et al*., 2007; Khanjani *et al*., 2023). Biofloc technology (BFT) is a promising method to enhance the growth performance of fish and shrimp in aquaculture (Anand *et al*., 2014; Khanjani *et al*., 2017; Elayaraja *et al*., 2020; Aboseif *et al*., 2022). Among the commercially important fish groups under the Cyprinidae family, carps such as *Catla catla*, *Labeo rohita*, and *Cirrihinus mrigala* (Deb *et al*., 2020) *Cyprinus carpio* (Najdegerami *et al*., 2016) and *Carassius auratus* (Wang *et al*., 2015) have been investigated for production performance with BFT.

The carp species *Hypselobarbus pulchellus* (Day, 1870) is critically endangered as per IUCN Red List (Rema Devi and Ali, 2020). *H. pulchellus* is known locally as 'Haragi meenu' and is highly valued for its delicious flesh and it is a potential candidate species for aquaculture (Hemaprasanth *et al*., 2016). The ICAR-CIFA Regional Research Centre (RRC) at Bengaluru has developed brood stock and standardised breeding technology for *H. pulchellus* (ICAR-CIFA, 2019). However, the mass scale seed production has limitations as the relative fecundity of *H. pulchellus* is around 10, 000 number per kg female (Gangadhar *et al*., 2020). It is essential to produce healthy fingerlings of this species in large numbers under controlled environments to develop diversified aquaculture. Recent studies demonstrated that BFT-based nursery rearing promotes growth, survival, disease resistance, and immunological function (Panigrahi *et al*., 2020; Anantharaja *et al*., 2023). Enhanced growth performance of fish species such as *Oreochromis niloticus* (Elayaraja *et al*., 2020), *Cyprinus carpio* (Bakhshi *et al*., 2018) and *Carassius auratus* (Wang *et al*., 2015) was reported in the biofloc systems. Moreover, Hosain *et al*. (2021) found that BFT application enhanced the post-larval survival and growth of *Macrobrachium rosenbergii* during the nursery phase. To produce healthy *H. pulchellus* seed for aquaculture diversification, it is essential to investigate the peninsular carp's performance in BFT-based seed production systems.

It is important to choose the right carbon source for biofloc systems since it affects bioflocs' nutritional properties and water quality (Wang *et al*., 2015). Previous studies have examined the impact of using carbon sources like molasses, jaggery, glucose, sucrose, glycerol, tapioca, corn flour, rice bran, wheat bran and wheat flour in the BFT system for different species (Wang *et al*., 2015; Dauda *et al*., 2017; Khanjani *et al*., 2017; Elayaraja *et al*., 2020; Wei *et al*., 2020). However, currently no information is available on how different carbon sources affect critically endangered *H. pulchellus* seed production in BFT, which is crucial for developing package of practice of this species in BFT. Therefore, this research was conducted to investigate the effect of adding various carbon sources on the growth, quality of water, plankton community, digestive enzymes activity, immune response and antioxidant activity of critically endangered *H. pulchellus* during biofloc-based seed rearing.

Materials and methods

Experimental condition and monitoring

An experimental setup having four biofloc treatment groups and a control group, each with three replications, was used to examine the effect of various carbon sources (jaggery, tapioca, rice and corn flour) on the production performance of *H. pulchellus* for 90 days during the fry to fingerling phase (Fig. 1). The control group was maintained without adding carbon source. The critically endangered *H. pulchellus* early fry used in this study was produced in the peninsular carp hatchery at Regional Research Centre of

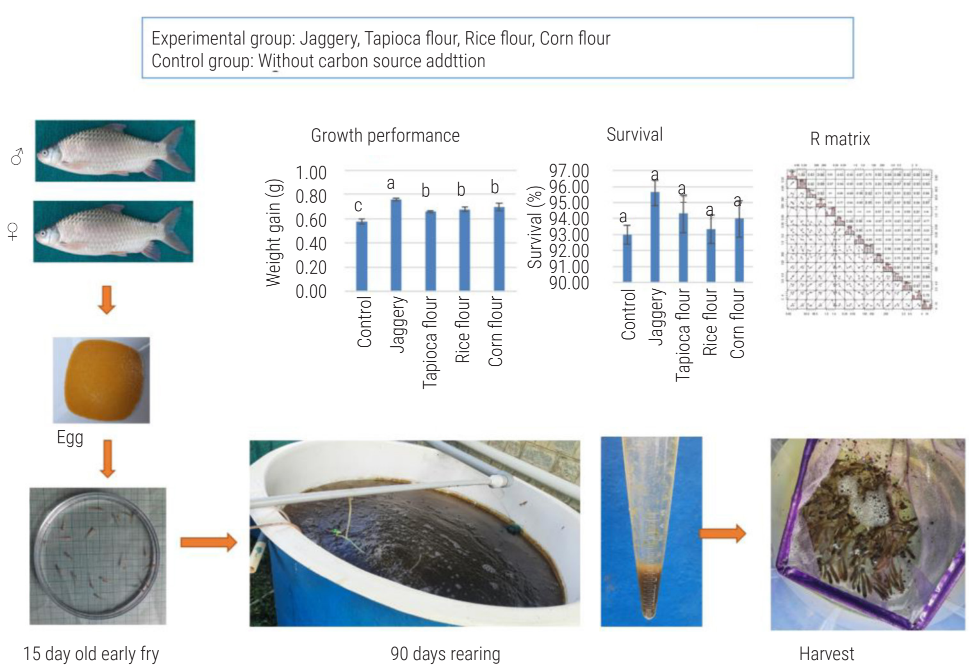

Fig. 1. A schematic representation of the biofloc-based seed rearing of critically endangered *H. pulchellus* using different carbon sources

ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA) in Bengaluru by breeding the stock maintained at the centre. The *pulchellus* hatchlings were raised to the early fry stage by feeding with mixed plankton initially, and then they were acclimatised to an experimental feed containing 35% crude protein a week later. The initial biofloc inoculum was developed based on the method of Avnimelech (1999). The hatchery produced 15-day-old *H. pulchellus* early fry weighing 0.0068 g were released into 1000 l circular fibreglass tanks at a stocking density of 100 per m^3. The feed was given 4 times the body weight initially and the feeding percentage was reduced according to body weight during the experimental period. The feeding was given two times a day at 09:00 and 15:00 hrs. The carbon source was added after mixing with water to maintain C/N ratio of 10:1 (Avnimelech, 1999) without considering the C content derived from the diets (Miao *et al*. 2020).

Calculation of growth parameters

The fish's weight and length from each replicate were taken into account to calculate growth parameters using the following equations:

$$\text{Weight gain (\%)} = \frac{[\text{Final weight (g)} - \text{Initial weight (g)}]}{\text{Initial weight (g)}} \times 100$$

$$\text{Specific growth rate (\% day}^{-1}) = \frac{[\text{Ln (Final weight (g))} - \text{Ln (Initial weight (g))}]}{\text{Duration (days)}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{Final count}}{\text{Initial count}} \times 100$$

Plankton community and proximate composition of biofloc

Plankton were sampled at monthly intervals by filtering 100 l of tank water *via* a 15-micron fabric mesh. The plankton-rich filtrate was preserved in 5% buffered formalin and the samples were counted using Sedgwick rafter cell (Jhingran *et al*., 1968) to determine the species composition. Plankton biomass was also estimated from the same sample after enumeration. Biofloc samples from each tank were assessed for crude protein, fat, fibre and ash, following AOAC protocols (AOAC, 2005). The NFE was determined following the equation: NFE = 100-(crude protein % + fat% + fibre% + ash%), in accordance with Hastings (1976).

Digestive enzyme analysis

The homogenate of intestines (1:19 w/v) (n=10) was made with a chilled solution of sucrose (0.25 M, pH 7) and centrifuged (10000 rpm at 4°C) for 10 min. For further studies, the supernatant was stored at -20°C. The activity of amylase was assessed by 3,5-dinitrosalicylic acid colorimetric technique (Rick and Stegbauer, 1974) using starch as the substrate. The activity of protease was determined by incubating samples with casein substrate at 25°C (Drapeau, 1976). The unit of amylase and protease activities were expressed in nanomoles of maltose and tyrosine release per minute, respectively. The activity of lipase activity was determined by measuring the fatty acids produced as a result of the enzymatic hydrolysis of triglycerides olive oil emulsion (Cherry and Crandall, 1932) and represented as unit per mg protein.

Analysis of immunological and antioxidant parameters

Kidney and gill tissue samples (n=10) were collected to determine immunological and antioxidant activity. The tissue homogenates prepared with a 0.25 M sucrose solution (1:19, w/v) were centrifuged (10,000 rpm at 4°C) for 10 minutes and the supernatant was preserved for analysis at -20°C. The technique developed by Quade and Roth (1997) was used to measure myeloperoxidase (MPO) activity, with 3',3'5',5'- tetramethylbenzidine hydrochloride and OD was read at 450 nm (Bio-Rad, Model-680). The lysozyme activity of kidney tissue was assessed by a turbidimetric assay methodology outlined by Ellis (1990) using *Micrococcus lysodeikticus* and the OD was read at 0 and 6 min. Lysozyme activity is expressed as the amount of enzyme that causes a 0.001 per minute drop in absorbance and is represented as U ml^{-1} min^{-1}. The superoxide dismutase (SOD) activity was assessed by following the Misra and Fridovich (1972) technique that is based on the inhibition of 50% of epinephrine's autooxidation. The assay for catalase (CAT) activity was conducted using hydrogen peroxide (H_2O_2) solution for the initiation of the reaction (Takahara *et al*., 1960) and represented as nanomoles of H_2O_2 decomposed per minute per milligram of protein.

Water quality

A portable oxygen metre (PDO-519, Lutron) and pH/temperature metre (PH-222, Lutron) was used to measure DO, pH and temperature. The total ammonia nitrogen (TAN), nitrite (NO_2), nitrate (NO_3) and carbonate alkalinity were analysed using standard methods (APHA, 1995). The biofloc volume was determined by measuring the settlement from 1 l of culture tank water for 15 min using an Imhoff cone. The tryptone soy agar was used for heterotrophic bacteria count. To calculate CFUs ml^{-1} for the water sample, the following equation was used: CFUs ml^{-1} = (Number of colonies × Dilution factor) / Volume (ml) of the sample.

Statistical analysis

All the data were analysed using one-way analysis of variance (ANOVA) followed by. Duncan's multiple range test (DMRT) to compare the significant difference between treatment mean using Statistical Package of Social Sciences (SPSS v 23). Pearson correlation matrix was plotted by R version 4.2.3.

Results

Growth and survival of *H. pulchellus*

The results indicated that carbon sources significantly ($p<0.05$) influenced weight gain (g), weight gain (%) and SGR. Significantly higher weight gain (0.76 g), weight gain percentage (11272%) and SGR (5.26% day^{-1}) was observed in the jaggery treatment compared to other treatments and control (Table 1). There were no significant differences in the survival rates of fingerlings between treatments, which ranged from 93 to 95.67% ($p>0.05$).

Table 1. Growth parameters (Mean±SE) of *H. pulchellus* during early fry to fingerling rearing under biofloc systems with various carbon sources

Parameters	Control	Jaggery	Tapioca flour	Rice flour	Corn flour
Initial length (cm)	1.20 ± 0.00^{a}	1.20 ± 0.00^{a}	1.20 ± 0.00^{a}	1.20 ± 0.00^{a}	1.20 ± 0.00^{a}
Initial weight (g)	0.0068 ± 0.00^{a}	0.0068 ± 0.00^{a}	0.0068 ± 0.00^{a}	0.0068 ± 0.00^{a}	0.0068 ± 0.00^{a}
Final length (cm)	3.98 ± 0.08^{c}	4.61 ± 0.05^{a}	4.21 ± 0.08^{b}	4.23 ± 0.04^{b}	4.28 ± 0.06^{b}
Final weight (g)	0.59 ± 0.02^{c}	0.77 ± 0.01^{a}	0.67 ± 0.01^{b}	0.69 ± 0.02^{b}	0.71 ± 0.03^{b}
Weight gain (g)	0.58 ± 0.02^{c}	0.76 ± 0.01^{a}	0.66 ± 0.01^{b}	0.68 ± 0.02^{b}	0.70 ± 0.03^{b}
Weight gain (%)	8527.45 ± 272.93^{c}	11272.55 ± 196.08^{a}	9703.92 ± 129.69^{b}	10096.08 ± 321.45^{b}	10292.15 ± 497.5^{b}
Specific growth rate (% day^{-1})	4.95 ± 0.04^{c}	5.26 ± 0.02^{a}	5.1 ± 0.01^{b}	5.14 ± 0.04^{b}	5.16 ± 0.06^{b}
Survival (%)	93 ± 0.58^{a}	95.67 ± 0.88^{a}	94.33 ± 1.2^{a}	93.33 ± 0.88^{a}	94 ± 1.15^{a}

Values in the same row with different superscripts are significantly different ($p<0.05$).

Water quality

The results of the water quality analysis indicated that pH, temperature and DO levels did not differ significantly between the carbon source group and control ($p>0.05$) (Table 2). Compared to the control group, the biofloc groups had substantially reduced concentrations of nitrogen compounds. Further, TAN (0.29 mg l^{-1}), NO_2 (0.35 mg l^{-1}) and NO_3 (1.32 mg l^{-1}) concentrations were lowered significantly in the jaggery treatment ($p<0.05$). There were no major changes among the treatments in alkalinity ($p>0.05$). THB count in the corn flour, jaggery and tapioca flour groups (3.5±0.15 × 10^8 CFU ml^{-1}, 1.63±0.22 × 10^8 CFU ml^{-1}, 1.15±0.02 × 10^8 CFU ml^{-1}, respectively) were noticeably higher level than rice and control ($p<0.05$). The jaggery treatment had the highest biofloc volume followed by corn, tapioca, rice flour and the control group.

Digestive enzymes analysis

The enzyme analysis showed higher amylase, protease, lipase and activity in BFT-raised fingerlings than in the control (Table 3). In the biofloc group, jaggery had the highest amylase activity (390.74 U mg protein^{-1}). In addition, fish grown in jaggery-based BFT tanks had significantly higher intestinal lipase activity (363.05 U mg protein^{-1}) than fish reared in tapioca and corn flour-based BFT groups ($p<0.05$). The corn flour group had higher intestinal protease activity (207.52 U mg protein^{-1}). However, the corn flour and jaggery groups did not differ significantly ($p>0.05$) in protease activity.

Immune and antioxidant activity

The immune and antioxidant activity of *H. pulchellus* is shown in Fig. 2. All biofloc groups had higher lysozyme and MPO activity than the control. Among the biofloc treatments, the jaggery group displayed a significantly higher activity of lysozyme than the other group ($p<0.05$). In contrast, no significant variations in MPO activity were found between biofloc groups ($p>0.05$). Similarly, higher SOD and CAT activity was found in the BFT group than in the control, with the highest activity in jaggery group.

Plankton and proximate analysis of biofloc

The jaggery treatment had the highest plankton biomass, whereas it was lowest in control (Fig. 3). During the study, the observed plankton species mainly belonged to Bacillariophyceae, Chlorophyceae, Conjugatophyceae, Cyanophyceae and Euglenophyceae, Trebouxiophyceae,

Table 2. Parameters of water quality (Mean±SE) observed in various treatments during the culture period

Parameters	Control	Jaggery	Tapioca flour	Rice flour	Corn flour
Dissolved oxygen (mg l^{-1})	7.95 ± 0.17^{a}	7.75 ± 0.18^{a}	7.87 ± 0.16^{a}	7.88 ± 0.14^{a}	7.76 ± 0.19^{a}
Temperature (^{0}C)	21.72 ± 0.19^{a}	21.74 ± 0.11^{a}	21.67 ± 0.15^{a}	21.9 ± 0.12^{a}	21.87 ± 0.13^{a}
pH	7.98 ± 0.06^{a}	7.88 ± 0.09^{a}	7.96 ± 0.07^{a}	7.97 ± 0.14^{a}	7.93 ± 0.09^{a}
Alkalinity (mg l^{-1} $CaCO_3$)	220.48 ± 3.12^{a}	211.9 ± 5.3^{a}	215.71 ± 3.78^{a}	217.14 ± 2.97^{a}	214.29 ± 4.59^{a}
TAN concentration (mg l^{-1})	0.53 ± 0.07^{a}	0.29 ± 0.04^{b}	0.38 ± 0.02^{ab}	0.41 ± 0.03^{ab}	0.31 ± 0.04^{b}
Nitrite concentration (mg l^{-l})	0.58 ± 0.05^{a}	0.35 ± 0.03^{c}	0.46 ± 0.03^{abc}	0.49 ± 0.02^{ab}	0.41 ± 0.05^{bc}
Nitrate concentration (mg l^{-1})	3.13 ± 0.39^{a}	1.32 ± 0.19^{b}	2.22 ± 0.27^{ab}	2.85 ± 0.4^{a}	2.16 ± 0.23^{ab}
THB (CFU ml^{-1})	0.16 ± 0.02^{d}	1.63 ± 0.22^{b}	1.15 ± 0.02^{c}	0.23 ± 0.01^{d}	3.5 ± 0.15^{a}
Floc volume (ml l^{-1}) at 90 days	3.50 ± 0.29^{c}	19.00 ± 2.02^{a}	14.50 ± 1.44^{ab}	13.83 ± 1.01^{b}	17.67 ± 1.59^{ab}

Values in the same row with different letters are significantly different ($p<0.05$).

Table 3. Specific activities of digestive enzymes (Mean±SE) in the intestine of *H. pulchellus* raised in biofloc system using various carbon sources

Digestive enzyme	Control	Jaggery	Tapioca flour	Rice flour	Corn flour
Amylase (U mg protein^{-1})	140.66±10.29^{d}	390.74±20.63^{a}	324.73±22.23^{b}	226.82±13.79^{c}	198.88±14.17^{c}
Protease (U mg protein^{-1})	122.35±5.91^{c}	193.83±6.61^{a}	172.17±3.78^{ab}	154.74±10.98^{bc}	207.52±21.5^{a}
Lipase (U mg protein^{-1})	208.9±14.08^{c}	363.05±17.64^{a}	283.88±23.4^{b}	306.66±18.5^{ab}	270.5±15.34^{b}

Values in the same row with different letters are significantly different ($p<0.05$).

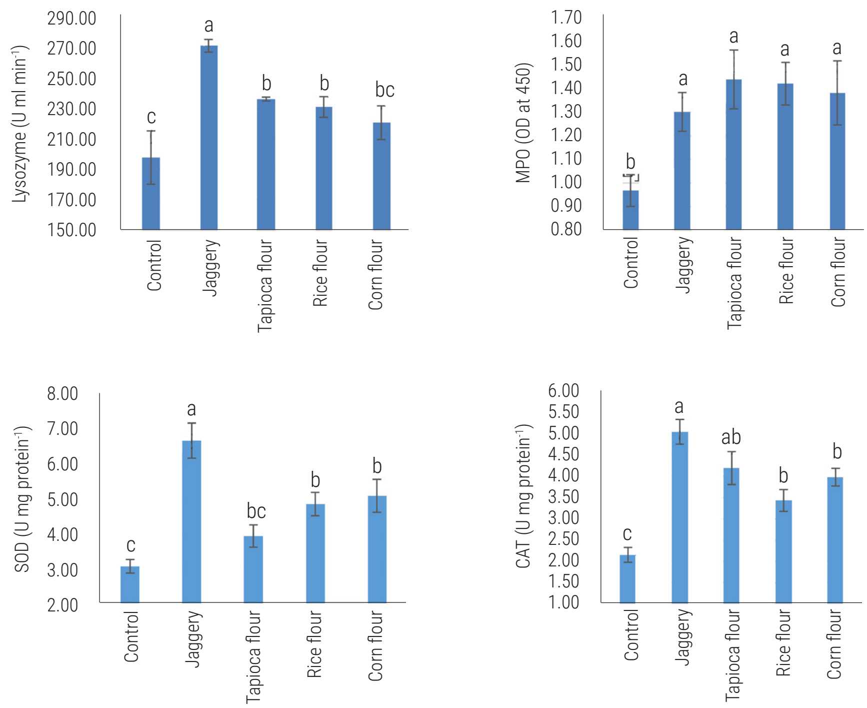

Fig. 2. Immune and antioxidant parameters (Mean±SE) of *H. pulchellus* under different treatments. Different letters denote significant differences ($p<0.05$) between treatments for each parameter

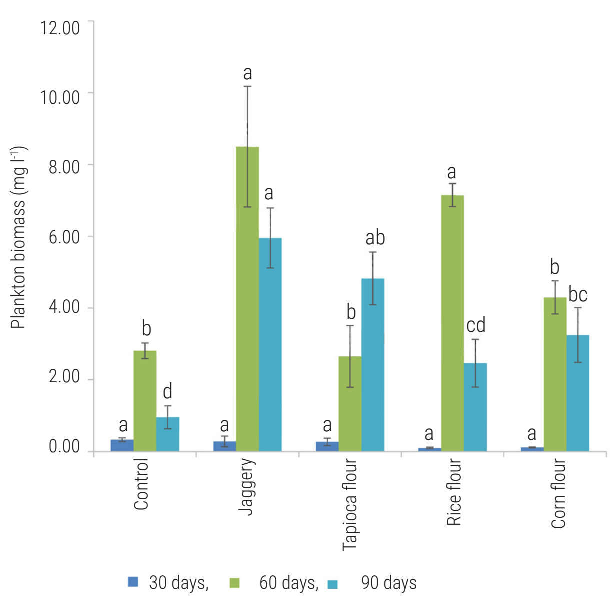

Fig. 3. Total plankton biomass (dry weight, mg l^{-1}) estimated on 30^{th}, 60^{th} and 90 days with different treatments (Mean±SE). Different superscripts denote significant differences ($p<0.05$) between treatments for each parameter

Rotifera and Crustacea. Higher density of planktonic diatom belonging to the Class Bacillariophyceae, and rotifers were found under jaggery treatment (Fig. 4). Carbon sources influenced the proximate composition of flocs (Table 4). Jaggery-based bioflocs had higher crude protein compared to other types.

Correlation matrix

The variables' relationships were analysed using the Pearson correlation matrix and is depicted in Fig. 5. The weight gain, an indicator of growth performance, had a very strong positive correlation with SOD (0.98), lipase (0.94), plankton (0.94), floc volume (0.92) and lysozyme (0.90) compared to other variables and conversely, TAN (-0.93) and Nitrite (-0.95) were highly negatively correlated. Further, there was a very strong negative correlation between TAN and Floc volume (-0.97).

Discussion

BFT improves production performance by providing natural food supply to cultured organisms (Avnimelech, 2009). In our study, all BFT groups outperformed the control in growth performance. Similarly, improved growth performance of fishes like *Oreochromis niloticus* (Elayaraja *et al*., 2020), *C. auratus* (Wang *et al*., 2015) and *C. carpio* (Bakhshi *et al*., 2018) were reported in the BFT system. Jaggery as a carbon source is highly soluble and showed better growth performance among the biofloc groups. According to Avnimelech (2012), simple carbohydrates such as sugars can remove ammonia nitrogen more efficiently than complex carbohydrates. Mirzakhani *et al*. (2019) and Putra *et al*. (2020) reported higher tilapia growth performance in the BFT system with soluble carbon source addition than starch. It is consistent with our findings and indicates that readily available carbon from jaggery is essential for improved growth performance.

Biofloc technology improves water quality by removing nitrogenous waste (Avnimelech, 1999; Dauda, 2019). In our study, the levels of TAN and nitrite were lowered in the BFT system due to the

Table 4. Proximate composition (dry wt. basis) of biofloc (Mean ± SE) in different biofloc groups

Treatment	Crude protein (%)	Crude fat (%)	Ash (%)	Crude fibre (%)	Nitrogen free extract (%)
Jaggery	20.03 ± 1.39^{a}	0.89 ± 0.14^{a}	31.66 ± 0.23^{d}	19.67 ± 0.53^{b}	17.62 ± 1.32^{a}
Tapioca	15.75 ± 0.14^{b}	0.85 ± 0.16^{a}	37.94 ± 0.52^{a}	23.55 ± 1.02^{b}	12.52 ± 0.98^{ab}
Rice flour	19.22 ± 1.45^{ab}	0.93 ± 0.17^{a}	34.49 ± 0.17^{b}	23.7 ± 2.86^{b}	11.66 ± 3.01^{b}
Corn flour	19.66 ± 0.79^{a}	0.96 ± 0.07^{a}	32.95 ± 0.34^{c}	31.94 ± 0.49^{a}	4.49 ± 0.51^{d}

Values in the same row with different letters are significantly different (p<0.05).

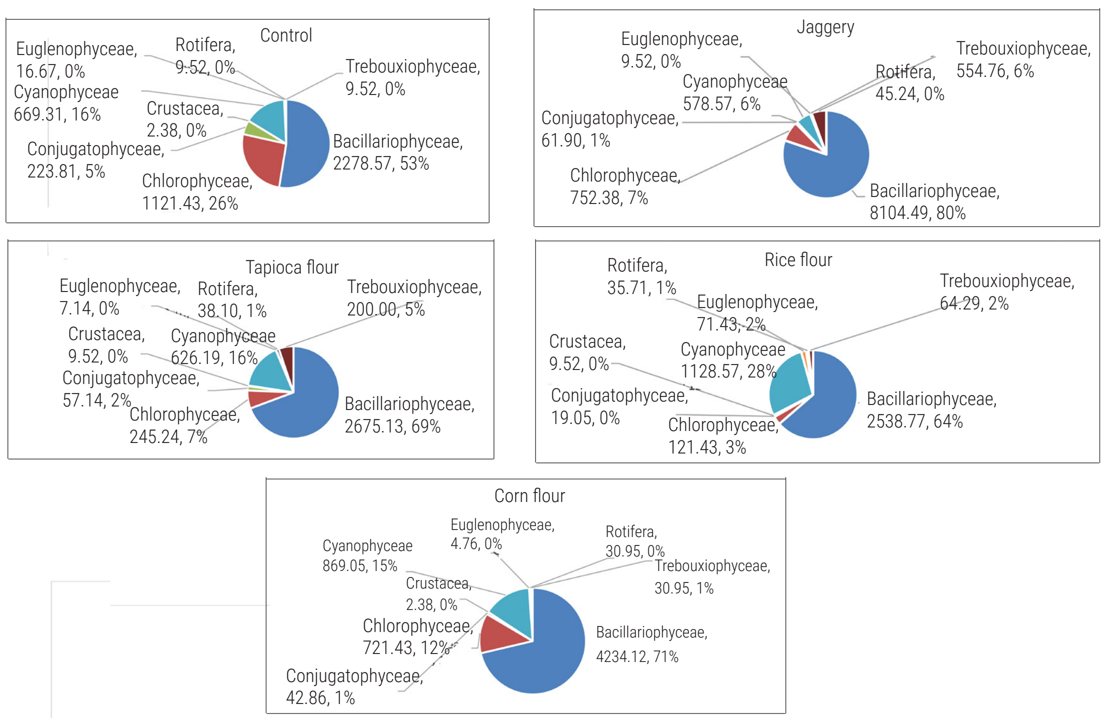

Fig. 4. Group-wise plankton proportion (average of 30, 60 and 90 days' sampling) in various treatments

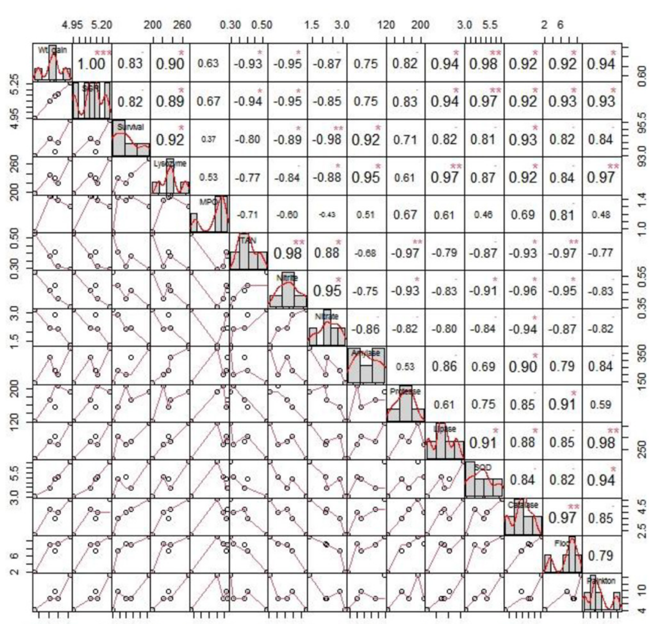

Fig. 5. Pearson correlation coefficient (r) matrix of different variables

proliferation of heterotrophic bacteria and higher floc formation, which improved water quality and created a favourable environment for *H. pulchellus*. Jaggery reduced N compounds in water better than the polysaccharides (tapioca, rice flour, and corn flour). Sugars eliminate ammonia-N faster than complex polysaccharides (Ekasari *et al*., 2014; Dauda *et al*., 2017; Wei *et al*., 2020). The slower breakdown of tapioca, rice and corn flour in our study influenced the floc development, leading to higher N-compounds in water, which in turn impeded *H. pulchellus* fingerling growth. Carbon source addition to BFT tanks increased THB count. However, the reason for the corn flour group's higher THB levels is unclear and needs further investigation. The volume of biofloc increased steadily in BFT treatments, with the jaggery group having the highest volume. The better growth performance in the jaggery group is associated with higher floc volume and improved water quality.

In this study, the bioflocs recorded protein content ranging from 15.75 to 20.03%, which was lower than the range 21.8 to 25% found by Bakhshi *et al*. (2018). The lipid level of biofloc ranged from 0.89 to 0.96%, with the corn flour group having the highest fat level. The higher growth performance observed in the jaggery and corn flour carbon source treatments compared to the tapioca and rice flour treatments may be attributed to the higher protein content and fat content of the biofloc. It indicates that *H. pulchellus* growth performance is highly dependent on the nutritional content of biofloc.

Digestive enzyme activity is an important measure that reflects the animal's digestion and metabolism abilities (Anand *et al*., 2014). BFT groups had increased digestive enzyme activity than the control group in our research. The cultured animals benefit from microbial flocs since they contain proteins, fatty acids and micronutrients (Ekasari *et al*., 2014; Wang *et al*., 2015). Further, the flocs may also contain enzymes like amylase and protease (Yu *et al*., 2007, 2009;), which may aid in the growth of cultivated organisms by breaking down carbohydrates and protein in the digestive tract. The enhanced enzymes activity in the BFT group, particularly in the jaggery-based BFT treatment, indicates that *H. pulchellus* benefits from feeding on flocs and it might be because of the combined effect of endogenous and exogenous enzymes for digestion found in fish and bioflocs.

The fingerlings in the BFT group showed higher lysozyme and MPO activities than the control, indicating that BFT enhances immunological activity in our study. Lysozyme is a nonspecific immune molecule that inhibits pathogens by hydrolysing their cell walls (Verschuere *et al*., 2000). MPO from neutrophil granules aids pathogen defence (Dalmo *et al*., 1997). The biofloc system, with its diverse carbon sources, can have varying effects on the immune enzyme activity of *H. pulchellus*. In BFT, the heterotrophic bacterial community may stimulate fish immune systems (Crab *et al*., 2012). Ahmad *et al*. (2016) reported elevated myeloperoxidase levels in biofloc-raised rohu. Mansour and Esteban (2017) observed significant improvements in lysozyme, and myeloperoxidase activity of *O. niloticus* reared in the BFT system. This indicates that the fish in the biofloc system develop an improved immune response, which can help them resist infectious outbreaks and environmental stressors.

Studies have shown that the antioxidant enzymes are crucial in mitigating oxidative stress and two key enzymes involved in this protective mechanism are SOD and CAT, both of which scavenge free radicals and play a role in preventing lipid peroxidation (Li *et al*., 2019). SOD prevents lipid peroxidation by converting superoxide anion into hydrogen peroxides (Tao *et al*., 2013), which is subsequently converted into oxygen and water by catalase (Tavares-Sanchez *et al*., 2004). *H. pulchellus* in the BFT group had enhanced SOD and CAT activities in gill tissue compared to the control in the current study. BFT contains a variety of naturally occurring organisms and bioactive compounds, such as chlorophyll, carotene, phytosterol, polysaccharides, polyphenols and taurine, which could assist fish to respond to stressful conditions (Ju *et al*., 2008). Further, research has shown that fish raised using biofloc technology (BFT) have increased levels of antioxidant enzymes (Mansour and Esteban, 2017). The present study's findings indicate that BFT may lower lipid peroxidation levels in *H. pulchellus* and enhance the fish's capacity to resist oxygen-free radicals. These positive effects may ultimately lead to improvements in overall fish health.

The treatment using jaggery had the highest amount of plankton biomass, while the control group had the lowest. This suggests that plankton abundance in the jaggery treatment enhanced fish growth, and the increased availability of plankton in the jaggery treatment might have played a vital role in the higher growth of fish in that group. It is worth noting that live food organisms, such as plankton, contain a variety of essential nutrients and are often referred to as "living capsules of nutrition" (El-Kassas *et al*., 2015). The major groups of plankton that were found in this research were Bacillariophyceae, Chlorophyceae, Conjugatophyceae, Cyanophyceae, Euglenophyceae, Trebouxiophyceae, Rotifera and crustacea, reflected the usual phytoplankton composition of tropical fish ponds (Gangadhar *et al*., 2018). Similar conditions were maintained for all the treatment groups except for the differences in the additional nutrients supplemented through carbon sources. Hence, the differences in the taxonomic composition of plankton between treatments can be attributed to the carbon source supplemented. Irrespective of the treatments, the density of diatoms (Bacillariophyceae and Coscinodiscophyceae) was the highest among all other classes of plankton encountered. During the study, a higher density of planktonic diatoms from the Bacillariophyceae class and rotifers was observed in the jaggery treatment. Diatoms are considered nutritionally valuable for many aquaculture species. The dominance of rotifers in the jaggery treatment suggests that this plankton community was able to utilise the newly grown diatoms. Rotifers are one of the preferred prey of fish and shrimp. The higher amount of plankton biomass in the jaggery treatment might have had a vital role in improved growth performance.

Pearson correlation matrix shows the strength and direction of relationships among variables. The correlation matrix findings suggest that SOD, lipase, plankton, floc volume, and lysozyme may be important factors contributing to growth performance. Conversely, high levels of TAN and nitrite may have a negative impact on growth. The strong negative correlation between TAN and floc volume suggests that TAN is assimilated during floc formation. Overall, these results provide valuable insights into the relationships among variables in the system and can be used to manage culture practices to optimise growth performance.

The current investigation demonstrated that the critically endangered *H. pulchellus* fingerling could be successfully produced using biofloc technology. The use of biofloc systems was found to have a positive impact on the *H. pulchellus* growth performance. The addition of jaggery as a carbon source resulted in better growth performance, enhanced digestive, antioxidant and immune activity, and better water quality, suggesting that jaggery is the best carbon source for critically endangered *H. pulchellus* in biofloc-based fingerling production. The positive outcomes observed with jaggery in this study also suggest its potential applications for other commercially important fish species in biofloc systems.

Acknowledgements

The authors thank the Director, ICAR-CIFA, Bhubaneswar and the Director, ICAR-CIFE, Mumbai for all the support extended to this study.

References

Aboseif, A. M., Flefil, N. S., Taha, M. K. S., Tahoun, U. M., Mola, H. R. A., El-Haroun, E., Van Doan, H. and Goda, A. M. S. A. 2022. Influence of dietary C: N: P ratios on Nile tilapia *Oreochromis niloticus* growth performance and formation of water biotic communities within a biofloc system containment. *Aquac. Rep.*, 24: 101136.

Ahmad, H. I., Verma, A. K., Babitha Rani, A. M., Rathore, G., Saharan, N. and Gora, A. H. 2016. Growth, non-specific immunity and disease resistance of *Labeo rohita* against *Aeromonas hydrophila* in biofloc systems using different carbon sources. *Aquaculture*, 457: 61-67. https://doi.org/10.1016/j.aquaculture.2016.02.011.

Anand, P. S. S., Kohli, M. P. S., Kumar, S., Sundaray, J. K., Roy, S. D., Venkateshwarlu, G., Sinha, A. and Pailan, G. H. 2014. Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. *Aquaculture*, 418-419: 108-115.

Anantharaja, K., Barlaya, G., Raghavendra, C. H., Kumar, B. S., Siddaiah, G. M., Chadha, N. K., Krishna, G., Routray, P. and Hemaprasanth, K. P. 2023. Growth performance of critically endangered *Hypselobarbus pulchellus* in biofloc systems with various C/N ratios. *Indian J. Anim. Res.,* 57(12): 1725-1732.

AOAC 2005. *Official method of analysis,* 18th edn. Association of Official Analytical Chemists, Washington DC, USA.

APHA 1995. *Standard methods for the examination of water and wastewater*. American Public Health Association, Washington DC, USA.

Avnimelech, Y. 2009. *Biofloc technology: A practical guide book.* World Aquaculture Society, Baton Rouge, Louisiana, USA.

Avnimelech, Y. 2012. *Biofloc technology: A practical guide book,* 2nd edn. World aquaculture society, Baton Rouge, Louisiana, USA.

Avnimelech, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*, 176(3-4): 227-235. https://doi.org/10.1016/S0044-8486(99)00085-X.

Avnimelech, Y. 2007. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture*, 264(1-4): 140-147. https://doi.org/10.1016/j.aquaculture.2006.11.025.

Bakhshi, F., Najdegerami, E. H., Manaffar, R., Tukmechi, A. and Farah, K. R. 2018. Use of different carbon sources for the biofloc system during the grow-out culture of common carp (*Cyprinus carpio L.*) fingerlings. *Aquaculture*, 484: 259-267.

Cherry, I. S. and Crandall, L. A. Jr. 1932. The specificity of pancreatic lipase: Its appearance in the blood after pancreatic injury. *Am. J. Physiol.*, 100(2): 266-273.

Crab, R., Defoirdt, T., Bossier, P. and Verstraete, W. 2012. Biofloc technology in aquaculture: Beneficial effects and future challenges. *Aquaculture*, 356-357: 351-356. https://doi.org/10.1016/j.aquaculture.2012.04.046.

Dalmo, R. A., Ingebrigtsen, K. and Bogwald, J. 1997. Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). *J. Fish Dis.*, 20(4): 241-273. https://doi.org/10.1046/j.1365-2761.1997.00302.x.

Dauda, A. B. 2019. Biofloc technology: A review on the microbial interactions, operational parameters and implications to disease and health management of cultured aquatic animals. *Rev. Aquac.,* 12(2): 1193-1210. https://doi.org/10.1111/raq.12379.

Dauda, A. B., Romano, N., Ebrahimi, M., Karim, M., Natrah, I., Kamarudin, M. S. and Ekasari, J. 2017. Different carbon sources affect biofloc volume, water quality and the survival and physiology of African catfish *Clarias gariepinus* fingerlings reared in an intensive biofloc technology system. *Fish. Sci.,* 83(6): 1037-1048. https://doi.org/10.1007/s12562-017-1144-7.

Deb, S., Noori, M. T. and Rao, P. S. 2020. Application of biofloc technology for Indian major carp culture (polyculture) along with water quality management. *Aquac. Eng.*, 91: 102106. https://doi.org/10.1016/j.aquaeng.2020.102106.

Drapeau, G. R. 1976. Protease from *Staphyloccus aureus*. *Methods in enzymology*, 45: 469-475. https://doi.org/10.1016/s0076-6879(76)45041-3.

Ekasari, J., Angela, D., Waluyo, S. H., Bachtiar, T., Surawidjaja, E. H., Bossier, P. and De Schryver, P. 2014. The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. *Aquaculture*, 426-427: 105-111. https://doi.org/10.1016/j.aquaculture.2014.01.023.

Elayaraja, S., Mabrok, M., Algammal, A., Sabitha, E., Rajeswari, M. V., Zagorsek, K., Ye, Z., Zhu, S. and Rodkhum, C. 2020. Potential influence of jaggery-based biofloc technology at different C:N ratios on water quality, growth performance, innate immunity, immune-related genes expression profiles, and disease resistance against *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol.*, 107: 118-128. https://doi.org/10.1016/j.fsi.2020.09.023.

El-Kassas, H. Y., Heneash, A. M. M. and Hussein, N. R. 2015. Cultivation of *Arthrospira* (*Spirulina*) *platensis* using confectionary wastes for aquaculture feeding. *J. Gen. Eng. Biotechnol.*, 13(2): 145-155. https://doi.org/10.1016/j.jgeb.2015.08.003.

Ellis, A. E. 1990. Lysozyme assays. In: Stolen, J. S., Fletcher, T. C., Anderson, D. P., Roberson, B. S. and Van Muiswinkel, W. B. (Eds.), *Techniques in fish immunology.* SOS Publications, Fair Haven, Connecticut, USA, pp. 101-103.

Gangadhar, B., Raghavendra, C. H., Ananda Kumar and Hemaprasanth, K. 2020. Induced breeding of farm-bred and pond-raised critically endangered peninsular carp, *Hypselobarbus pulchellus*. *Aquac. Asia*, 24(4): 10-13.

Gangadhar, B., Sridhar, N., Umalatha, H., Ganesh, H. and Jayasankar, P. 2018. Taxonomic and biochemical composition and digestive enzyme activity of periphyton and plankton: A comparative study. *Proc.Nat. Acad. Sci., India Section B – Biol. Sci.* 88(2): 715-720.

Hastings, W. H. 1976. Fish nutrition and fish feed manufacture. In: *FAO technical conference on aquaculture,* 26 May to 02 June 1976, Kyoto, Japan. *FAO Fish. Rep. No. 188.* Food and Agriculture Organization of the United Nations, 93 p.

Hemaprasanth, K. P., Raghunath, M. R., Gangadhar, B., Saurabh, S., Raghavendra, C. H., Sridhar, N. and Jayasankar, P. 2016. Polyculture of *Puntius pulchellus* with *Catla catla* and *Labeo rohita*. *J. Aquac. Trop.*, 31(1-2): 77-83.

Hosain, M. E., Amin, S. M. N., Arshad, A., Kamarudin, M. S. and Karim, M. 2021. Effects of carbon sources on the culture of giant river prawn in biofloc system during nursery phase. *Aquac. Rep.*, 19: 100607. https://doi.org/10.1016/j.aqrep.2021.100607.

ICAR-CIFA 2019. *Annual report 2019*. ICAR-Central Institute of Freshwater Aquaculture. Bhubaneswar, India.

Jhingran, V. G., Natarajan, A. V., Banerjea, S. M. and David, A. 1968. *Methodology on reservoir fisheries investigations in India*. *Bulletin No. 12.* ICAR-Central Inland Fisheries Research Institute, Barrackpore, India.

Ju, Z. Y., Forster, I., Conquest, L. and Dominy, W. 2008. Enhanced growth effects on shrimp (*Litopenaeus vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. *Aquac. Nutr.*, 14(6): 533-543. https://doi.org/10.1111/j.1365-2095.2007.00559.x.

Khanjani, M. H., Mozanzadeh, M. T., Sharifinia, M. and Emerenciano, M. G. C. 2023. Biofloc: A sustainable dietary supplement, nutritional value and functional properties. *Aquaculture*, 562: 738757. https://doi.org/10.1016/j.aquaculture.2022.738757.

Khanjani, M. H., Sajjadi, M. M., Alizadeh, M. and Sourinejad, I. 2017. Nursery performance of Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) cultivated in a biofloc system: The effect of adding different carbon sources. *Aquac.Res.*, 48(4): 1491-1501. https://doi.org/10.1111/are.12985.

Li, M. Y., Liu, X. Y., Xia, C. G., Wang, G. Q. and Zhang, D. M. 2019. Astaxanthin enhances hematology, antioxidant and immunological parameters, immune-related gene expression, and disease resistance against in Channa argus. *Aquac. Int.,* 3: 735-746. https://doi.org/10.1007/s 10499-019-00362-w.

Mansour, A. T. and Esteban, M. A. 2017. Effects of carbon sources and plant protein levels in a biofloc system on growth performance, and the immune and antioxidant status of Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol.*, 64: 202-209. https://doi.org/10.1016/j.fsi.2017.03.025.

Miao, S., Hu, J., Wan, W., Han, B., Zhou, Y., Xin, Z. and Sun, L. 2020. Biofloc technology with addition of different carbon sources altered the antibacterial and antioxidant response in *Macrobrachium rosenbergii* to acute stress. *Aquaculture*, 525: 735280.

Mirzakhani, N., Ebrahimi, E., Jalali, S. A. H. and Ekasari, J. 2019. Growth performance, intestinal morphology and nonspecific immunity response of Nile tilapia (*Oreochromis niloticus*) fry cultured in biofloc systems with different carbon sources and input C:N ratios. *Aquaculture*, 512: 734235. https://doi.org/10.1016/j.aquaculture.2019.734235.

Misra, H. P. and Fridovich, I. 1972. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.*, 247(10): 3170-3175. https://doi.org/10.1016/S0021-9258(19)45228-9.

Najdegerami, E. H., Bakhshi, F. and Lakani, F. B. 2016. Effects of biofloc on growth performance, digestive enzyme activities and liver histology of common carp (*Cyprinus carpio L.*) fingerlings in zero-water exchange system. *Fish Physiol. Biochem.,* 42(2): 457-465. https://doi.org/10.1007/s10695-015-0151-9.

Panigrahi, A., Saranya, C., Ambiganandam, K., Sundaram, M., Sivakumar, M. R. and Kumaraguru vasagam, K. P. 2020. Evaluation of biofloc generation protocols to adopt high density nursery rearing of *Penaeus vannamei* for better growth performances, protective responses and immunomodulation in bioflocbased technology. *Aquaculture*, 522: 735095. https://doi.org/10.1016/j.aquaculture.2020.735095.

Putra, I., Effendi, I., Lukistyowati, I., Tang, U. M., Fauzi, M., Suharman, I. and Muchlisin, Z. A. 2020. Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia *Oreochromis niloticus* system. *F1000Research*, 9: 293. https://doi.org/10.12688/f1000research.22977.3.

Quade, M. J. and Roth, J. A. 1997. A rapid, direct assay to measure degranulation of bovine neutrophil primary granules. *Vet. Immunol. Immunopathol.*, 58(3-4): 239-248. https://doi.org/10.1016/S0165-2427(97)00048-2.

Rema Devi, K. R. and Ali, A. 2020. *Hypselobarbus pulchellus* (amended version of 2011 assessment). *The IUCN Red list of threatened species 2020: e.T169654A176502336.* https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T169654A176502336.en.

Rick, W. and Stegbauer, H. P. 1974. α-Amylase measurement of reducing groups. In *Methods of enzymatic analysis,* 2nd edn. Academic Press, New York, USA, p. 885-890.

Samocha, T. M., Patnaik, S., Speed, M., Ali, A. M., Burger, J. M., Almeida, R. V., Ayub, Z., Harisanto, M., Horowitz, A. and Brock, D. L. 2007. Use of molasses as carbon source in limited discharge nursery and grow-out systems for *Litopenaeus vannamei*. *Aquac. Eng.,* 36(2): 184-191. https://doi.org/10.1016/j.aquaeng.2006.10.004.

De Schryver, P., Crab, R., Defoirdt, T., Boon, N. and Verstraete, W. 2008. The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture*, 277(3-4): 125-137. https://doi.org/10.1016/j.aquaculture.2008.02.019.

Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y. and Nishimura, E. T. 1960. Hypocatalasemia: A new genetic carrier state. *J. Clin. Invest.,* 39(4): 610-619. https://doi.org/10.1172/JCI104075.

Tao, Y., Pan, L., Zhang, H. and Tian, S. 2013. Assessment of the toxicity of organochlorine pesticide endosulfan in clams *Ruditapes philippinarum*. *Ecotoxicol. Environ. Saf.,* 93: 22-30. https://doi.org/10.1016/j.ecoenv.2013.03.036.

Tavares-Sanchez, O. L., Gómez-Anduro, G. A., Felipe-Ortega, X., Islas-Osuna, M. A., Sotelo-Mundo, R. R., Barillas-Mury, C. and Yepiz-Plascencia, G. 2004. Catalase from the white shrimp *Penaeus (Litopenaeus) vannamei*: molecular cloning and protein detection. *Comp. Biochem.Physiol. Part B: Biochem. Mol. Biol.*, 138(4): 331-337. https://doi.org/10.1016/j.cbpc.2004.03.005.

Toledo, T. M., Silva, B. C., do Nascimento Vieira, F. do N., Mouriño, J. L. P. and Seiffert, W. Q. 2016. Effects of different dietary lipid levels and fatty acids profile in the culture of white shrimp *Litopenaeus vannamei* (Boone) in biofloc technology: water quality, biofloc composition, growth and health. *Aquac.Res.*, 47(6): 1841-1851.

UN 2022. *Population.* United Nations, Geneva, Switzerland https://www.un.org/en/global-issues/population (Accessed 15 April 2023).

Verschuere, L., Rombaut, G., Sorgeloos, P. and Verstraete, W. 2000. Probiotic bacteria as biological control agents in aquaculture. *Microbiol. Mol.Biol. Rev.*, 64(4): 655-671. https://doi.org/10.1128/mmbr.64.4.655-671.2000.

Wang, C., Pan, L., Zhang, K., Xu, W., Zhao, D. and Mei, L. 2015. Effects of different carbon sources addition on nutrition composition and extracellular enzymes activity of bioflocs, and digestive enzymes activity and growth performance of *Litopenaeus vannamei* in zero-exchange culture tanks. *Aquac. Res.,* 47(10): 1-12. https://doi.org/10.1111/are.12784.

Wang, G., Yu, E., Xie, J., Yu, D., Li, Z., Luo, W., Qiu, L. and Zheng, Z. 2015. Effect of C/N ratio on water quality in zero-water exchange tanks and the biofloc supplementation in feed on the growth performance of crucian carp, *Carassius auratus*. *Aquaculture*, 443: 98-104. https://doi.org/10.1016/j.aquaculture.2015.03.015.

Wei, Y. fang, Wang, A. li and Liao, S. 2020. Effect of different carbon sources on microbial community structure and composition of ex-situ biofloc formation. *Aquaculture*, 515: 734492. https://doi.org/10.1016/j.aquaculture.2019.734492.

Willett, W., Rockström, J., Loken, B., Springmann, M., Lang, T., Vermeulen, S., Garnett, T., Tilman, D., DeClerck, F., Wood, A., Jonell, M., Clark, M., Gordon, L. J., Fanzo, J., Hawkes, C., Zurayk, R., Rivera, J. A., De Vries, W., Majele Sibanda, L., Afshin, A., Chaudhary, A., Herrero, M., Agustina, R., Branca, F., Lartey, A., Fan, S., Crona, B., Fox, E., Bignet, V., Troell, M., Lindahl, T., Singh, S., Cornell, S. E., Srinath Reddy, K., Narain, S., Nishtar, S. and Murray, C. J. L. 2019. Food in the Anthropocene: The EAT–Lancet Commission on healthy diets from sustainable food systems. *The Lancet*, 393(10170): 447-492. https://doi.org/10.1016/S0140-6736(18)31788-4.

Yu, G., He, P., Shao, L. and Zhu, Y. 2009. Enzyme extraction by ultrasound from sludge flocs. *J. Environ. Sci.,* 21(2): 204-210. https://doi.org/10.1016/S1001-0742(08)62252-4.

Yu, G. H., He, P. J., Shao, L. M. and Lee, D. J. 2007. Enzyme activities in activated sludge flocs. *Appl. Microbiol. Biotechnol.*, 77(3): 605-612. https://doi.org/10.1007/s00253-007-1204-5.