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Combined Effects of the Different Stocking Density and Biofloc on Growth Performance and Skin Pigmentation in Koi Carp (*Cyprinus carpio var. koi*)

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ABSTRACT

Background: The present study was conducted to evaluate the effect of stocking density in biofloc system on the water quality, growth performance, skin pigmentation and anti-oxidant activity of Koi carp (*Cyprinus carpio* var. *koi*).

Methods: The study was performed by three different stocking densities such as 100, 150 and 200 fish/m^3 in control (C) and biofloc system (B) (designated as C100 (100 fish/m^3), C150 (150 fish/m^3), C200 (200 fish/m^3), B100 (100 fish/m^3), B150 (150 fish/m^3) and B200 (200 fish/m^3). Koi carp juveniles (average body weight of 0.64±0.003 g) were stocked in 19 fiberglass-reinforced plastic (FRP) tanks of 500 L capacity and reared for 60 days.

Result: The water quality parameters, *viz*., ammonia, nitrite and nitrate, were significantly lower in B100. At the end of the experiment, a significantly higher weight gain (4.74±0.306 g) and survival rate (91.54±0.584%) were recorded in the B100 group and lower growth performance and survival rate (64.05±0.796%) were noticed in the C200 group. Lower anti-oxidant enzyme activity of superoxide dismutase (1.53±0.022 U/mg protein) and catalase activity (1.51±0.054 U/mg protein) was observed in the C200 group. The total carotenoid content of Koi carp was significantly higher (2.57±0.039 µg/g wet weight) in the B100 treatment. Hence, the present study concluded that the biofloc system stocked with 100 fish/m^3 enhanced growth performance, survival rate, water quality and coloraton in Koi carp.

Key words: Anti-oxidant enzyme, Biofloc system, Carotenoids, Ornamental fish, Stocking density.

INTRODUCTION

The ornamental fish industry is an expanding multi-billion-dollar industry with remarkable growth in the last two decades. In a global context, the ornamental fish market is expected to attain a market value from USD 6.18 billion in 2023 to USD 13.6 billion by 2032, with a compound annual growth rate (CAGR) of about 9.16% (Global Ornamental Fish Market Research Report, 2024). Despite being a big reservoir for ornamental fish resources, India's contribution to the global ornamental fish trade at a marginal level of only 1% (Rahman *et al.*, 2023). To improve the ornamental fish trade in India, it is essential to upgrade the infrastructure of the rearing method, improve feeding regimes, water quality management and effectively utilize the untapped resources.

The major problems associated with the rearing of young ones in ornamental aquaculture are the non-availability of fish seeds, lack of the right type of live feed, incidence of disease outbreaks and high production costs (Khanjani, 2020). Further to enhance ornamental fish production by using intensification practices with higher stocking densities that deteriorate water quality and result in mortality of fishes (Deocampo *et al.*, 2021). To address these problems, there is an urgent need for an alternative eco-friendly aquaculture practice, named biofloc systems, which can support higher stocking density of fish in a sustainable manner (Nazarpour and Mohammadiazarm, 2023).

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Biofloc systems recycle uneaten feed and feces by maintaining an ideal C: N ratio of 15:1 with continuous aeration to encourage the growth of heterotrophic bacteria that convert ammonia into microbial protein as biofloc (Lal *et al.*, 2024). Biofloc is composed of photosynthetic microorganisms, protozoa and rotifers (Khanjani *et al.*, 2023). Additionally, it comprises Proteins, lipids, carbohydrates and vitamin C and bioactive molecules such as bromophenols, carotenoids, phytosterols, free fatty acids, poly-hydroxybutyrate, lipopolysaccharides, peptidoglycans and Probiotic bacteria (*Bacillus sp*, *Lactobacillus sp*.) that improve overall health of biofloc reared fishes (da Cunha *et al.*, 2020; Khanjani *et al.*, 2023).

Koi carp (Family-Cyprinidae) has the highest commercial value that are selectively bred and domesticated for ornamental purposes (Sevak *et al.*, 2022). The market price of Koi carp varies depending on the coloration (Liu *et al.*, 2024). The Koi carp often shows faded coloration and depression of feeding when water quality deteriorates. Rearing of fishes under biofloc systems contain valuable bioactive compounds that can produce stunning-colored fish, maintain water quality, lower production cost maximize profit in ornamental fish farming and also uplift the export market of ornamental fishes in India (Menaga *et al.*, 2023). Therefore, this paper deals with the ideal stocking density of Koi carp (*Cyprinus carpio* var. *koi*), which promotes higher growth performance and skin pigmentation in a rice-starch-based biofloc system.

MATERIALS AND METHODS

The Koi carp juveniles were procured from the commercial ornamental fish farm located at Kolathur (Latitude: 13.1217°N, Longitude: 80.2154°E), Chennai, Tamil Nadu, India in 2023. Prior to transportation, fishes were allowed a fasting period of 12 hours to reduce the accumulation of ammonia and stress during transit. The Koi carp juveniles were packed in oxygen-packed polythene bags with a water-to-oxygen ratio of 1:3. To maintain the stable temperature, the sealed bag was placed in insulated containers. Then, the fishes were acclimatized gradually in a wet laboratory of the Advanced Research Farm Facility, Tamil Nadu Dr. J. Jayalalithaa Fisheries University-Madhavaram, Tamil Nadu, India, for 60 days. During the acclimatization, the fish were fed with 0.8 mm of floating commercial feed (crude protein - 36%) given twice a day at 5% of their body weight. The experiment was carried out in a 500-liter capacity FRP tank, following a 2×3 factorial design with two rearing systems of biofloc (B) and control systems (C) and three different stocking densities (100 fish/m^3, 150 fish/m^3 and 200 fish/m^3) and each were triplicated. The production of biofloc was performed based on the standard protocol of Avnimelech (2009). Under intensive aeration, the carbon source of rice starch was added to neutralize the total ammonia nitrogen (TAN) level and by manipulating the C: N ratio of 15:1. The rice starch was selected as a carbon source due to its affordability, higher proximity in the production area, ensured proper balance of C:N ratio, maintained water quality, enhanced beneficial bacterial biomass and biofloc formation, reduction in NH_3 accumulation in culture condition and required minimum processing and purification compared to other carbon sources and supported sustainable aquaculture practices (Sasikumar *et al.*, 2025). The quantity of carbon and nitrogen utilized in the biofloc system was calculated according to Lima *et al.* (2018). The acclimatized Koi carp juveniles with an average length of 2.24±0.005 cm and weight of 0.64±0.003 g (number of fish=2700) were randomly selected and stocked in biofloc and the control tanks as per stocking densities.

Physicochemical characteristics of water

The water quality parameters, such as water temperature and pH, were analyzed daily using a mercury thermometer and a pH meter, respectively. Other parameters such as dissolved oxygen (Winkler's titration method), total alkalinity, hardness, total ammonia nitrogen (TAN), nitrite-N and nitrate-N, were estimated once in a week during the experimental period using standard protocols (APHA, 2005).

Floc characteristics

The volume of floc was measured using an Imhoff cone, where the biofloc suspension tank water (1 L) was collected in the imhoff cone and allowed to settle for 30 minutes. The floc volume settled at the bottom of the cone was measured and expressed in mL/L (Avnimelech and Kochba, 2009). The floc volume, floc concentration and floc volume index were calculated using standard protocol (Mohlman, 1934).

According to Mueller *et al.* (1967), floc density was estimated using floc concentration and floc volume. FDI was calculated by the gram of floc occupied in a volume of 100 ml after settling for 30 minutes (WHO International Reference Centre, 1978). Floc porosity was analyzed using Smith and Coakley's method (1984).

Proximate composition of biofloc

The floc samples were collected and dried under shade, then stored in airtight containers for the proximate analysis of biofloc according to standard protocol (AOAC, 2005). The following parameters such as moisture (%), crude protein (%), crude lipid (%) and ash (%) of biofloc were analyzed.

Growth parameters

The growth performance of Koi carp was assessed by randomly selecting 10 fish from each tank (n=30 fish/treatment) during sampling at 15-day intervals. The growth parameters were calculated according to Raj *et al.* (2008).

Total carotenoid concentration

At the end of the experimental period, five fish from each tank (n=15 fish/treatment) were randomly selected and analyzed for skin pigmentation using Olson (1979) method.

Antioxidant enzyme activity

At the end of the experiment, fish blood samples were collected to assess the superoxide dismutase activity (Misra and Fridovich, 1972). The catalase activity was analyzed according to the protocol described by Takahara *et al.* (1960).

Statistical analysis

The statistical analysis was performed using SPSS software 16.0. Growth performance and total carotenoid content of Koi carp were analysed by Two-way ANOVA to examine the interaction between system and stocking density.

Water quality, floc parameters and proximate composition of biofloc were analysed by one-way ANOVA. Turkey test was used for post hoc comparison of mean values at a significance level of P<0.05 among the different groups.

RESULTS AND DISCUSSION

Water quality parameters

The water temperature shows a significant difference (p<0.05) among the different treatments and it was in the range of 26.2-28.4 in the control and biofloc tanks. Dissolved oxygen (DO), pH, alkalinity, hardness, total ammonia nitrogen (TAN), nitrite -N and nitrate -N values were significantly varied among the treatments (Table 1). TAN, nitrite and nitrate values were significantly higher in the control system and a lower value was recorded in the biofloc system. According to Avnimelech (2009), the temperature range of 28 to 31°C was favorable for floc production. In the present study, temperature was recorded in the range of 26.2-28.4°C to keep the microbial activity and floc formation at an optimum level in the biofloc system. Wilen and Balmer (1999) stated that dissolved oxygen is important for the metabolic activity of cells within biofloc. The DO range of above 5 mg/l was ideal for the optimum growth of fish and an oxygen level of below 2 mg/l led to the inefficient function of nitrifying bacteria. similarly in the present study also it was recorded. The floc stability was determined by the pH of the culture water in the biofloc system (Mikkelsen *et al.*, 1996). Nurhatijah *et al.* (2016) reported a pH range of 7.8-8.0 in the biofloc system. In the current study, pH range for the Koi carp reared in biofloc tanks was varied between 7.5-8.9. The addition of carbon sources in the biofloc system lowers ammonia and nitrite levels due to the dominance of heterotrophic bacteria via the mechanism of inorganic nitrogen immobilization supported the current study (Kuhn *et al.*, 2008).

Floc characteristics

Floc volume, floc concentration, porosity and pore volume were significantly varied (p<0.05) among the treatments (Table 2). Higher and lower values of floc volume (43.61±1.57 ml/l and, 20.51±1.22 ml/l), floc volume index (4.94±0.36 ml/g and 4.31± 0.01 ml/g) and floc concentration (9.75±1.232 g/m^3 and 4.64±1.531 g/m^3) were observed in B200 and B100 treatments, respectively. Lower and higher values of porosity of floc (0.019±0.014% and 0.029±0.003%) were recorded in the B200 and B100 groups, respectively. However, the floc volume was maintained at a range of less than 30 mg/l by the removal of settled material and water supply (Avnimelech, 2011). In the present study, the optimum floc volume favours the improved growth performance of Koi carp in B100 compared with other groups. The increase in floc volume at higher stocking density leads to the clogging of fish gills in the biofloc system (Minaz and Kubilay, 2021). According to Crab *et al.* (2010), biofloc, with a higher floc volume index, produces lower DO levels. In the current study, the higher and lower floc volume index was recorded in B200 and B100,

Table 1: Water quality parameters in control and biofloc system recorded during the experimental trial.

Parametrers	Experimenral groups						Two-way ANOVA		
	C100	C150	C200	B100	B150	B200	System	Stockin density	System × Stocking density
Temperature (°C)	27.84±0.05ab	27.94±0.03a	27.98±0.01a	27.13±0.11b	27.45±0.11c	27.72±0.11d	0.001	0.001	0.001
DO (mg/l)	5.91±0.12ab	5.41±0.16cd	5.23±0.12d	6.03±0.11a	5.74±0.11b	5.53±0.09c	0.001	0.001	0.257
pH	8.63±0.09b	8.84±0.08a	8.95±0.04a	8.24 ±0.12d	8.43±0.11c	8.54±0.09bc	0.001	0.001	0.986
Alkalinity (mg/l)	171.44±0.91b	179.84±1.42a	183.15±1.11a	148.63±1.57d	161.62±1.04c	169.24±0.36b	<0.001	<0.001	0.127
Hardness (mg/l)	256.42±0.41d	258.29±0.16b	259.25±0.08a	254.51±0.73e	257.39±0.26c	258.13±0.06b	<0.001	< 0.001	0.077
Ammonia (mg/l)	0.57±0.12b	0.76 ±0.11a	0.84±0.11a	0.21±0.06c	0.27±0.02c	0.46±0.09b	<0.001	<0.001	0.480
Nitrite (mg/l)	0.41±0.03 ab	0.44±0.04a	0.53±0.01a	0.18± 0.04c	0.24±0.13c	0.31±0.07bc	<0.001	<0.001	0.930
Nitrate (mg/l)	1.92±0.03c	2.34±0.13b	2.53±0.08a	1.17±0.09e	1.55±0.11d	2.15±0.15b	<0.001	<0.001	0.009

respectively. Floc porosity was inversely proportional to floc size, smaller flocs have higher porosity (Yuvarajan *et al.*, 2018). The porous structure of biofloc enhances the diffusion rate of nutrients, oxygen and water throughout the floc, efficient in adsorbing particulate matter and particles and promotes floc growth and microbial colonization (Smith and Coackley, 1984; Khanjani and Sharifina, 2020). In the present study, floc porosity was higher in B100 and lower in B200.

Proximate composition of biofloc

There was no significant difference ($p>0.05$) in the biochemical composition of biofloc formed in different stocking density treatments of biofloc systems (Table 3). The moisture, protein, fat, fibre and ash contents of the biofloc collected from different treatments were in the range of 10.71-10.79%, 16.04-16.38%, 3.41-3.47%, 6.21-6.53% and 23.62-23.72%, respectively. According to Solanki *et al.* (2023), *Gibelion catla* did not show any significant effect on the proximate composition of biofloc in different stocking densities. In the present study, proximate composition values were similar to the findings of Megahed and Mohamed (2014) and Azim *et al.* (2008) observed in biofloc systems reared with *Fenneropenaeus indicus* and *Oreochromis niloticus,* respectively.

Growth parameters and survival rate

Growth parameters and survival rate were significantly ($P<0.05$) influenced by the system and stocking densities (Table 4). A significant interaction effect ($p<0.05$) was recorded between the system and stocking density in the values of final weight and survival rate. Final weight (5.01±0.31 g), total weight gain (4.74±0.31 g), SGR (3.89±0.78 %/day) and survival rate (91.54± 0.58%) were significantly ($p<0.05$) higher in the biofloc system with a lower stocking density (B100). Significantly lower and higher FCR were observed in B100 (1.57±0.02) and C200 (1.98±0.01) groups, respectively. The growth parameters and survival rate decreased significantly ($p<0.05$) with increasing stocking densities. Whereas growth performance increased in lower stocking in the biofloc system (B100), Similar results were observed by Ruby *et al.* (2022), who recorded a better growth rate of Pearl spot (*Etroplus suratensis*) in the biofloc system at 100 fish/m^3. The better FCR in B100 was similar to the findings of Hussain *et al.* (2015) and Xu and Pan (2012), who studied in the *Cyprinus carpio* var. *koi* (*Cyprinus carpio*) in aquaponics and *Litopenaeus vannamei* in biofloc, respectively; their FCR values varied between 1.5 and 1.7. The specific growth rate and survival rate are inversely proportional to stocking density in the biofloc system (De Schreyer *et al.*, 2008; Wocher *et al.,* 2011). The survival rate of Koi carp is also decreased with increasing stocking densities. The lower survival rate in B150, B200 and control group is due to the competition for food and crowding stress.

Total carotenoid concentration

There was a significant effect ($p<0.05$) on coloration by system and stocking density (Fig 1). The interaction between system and stocking density had no significant difference on the final carotenoid concentration. The maximum carotenoid content was found in B100 (2.57±0.039 µg/g wet weight), followed by B150 (2.34±0.054 µg/g wet weight) and the lowest carotenoid content was observed in C200 (1.33±0.05 µg/g wet weight). Similarly, the skin carotenoid concentration of *Heros severus and Carrasius auratus* was improved using biofloc system compared to the control (da Cunha *et al.*, 2020; Castro-Castellon *et al.*, 2023). In the current study, Koi carp reared in the biofloc system at a lower stocking density (B100) showed significantly higher carotenoid concentrations and its value decrease with increasing stocking density found similar to the findings of Zeng *et al.* (2010) studied in darkbarbel catfish (*Pelteobagrus vachelli*).

Table 2: Floc characteristics observed in biofloc system stocked with koi carp at different stocking densities.

Treatment	Floc volume (ml/l)	Floc concentration (g/m^3)	Floc volume index (ml/g)	Floc density index(g/l)	Porosity pore (%)	Volume (ml/l)
B100	20.51±1.22^c	4.64±1.53^c	4.31±0.01^c	23.21±0.12^a	0.029±0.01^a	34.48±0.14^c
B150	34.21±0.51^b	6.88 ±1.13^b	4.43±0.54^b	22.57±0.21^b	0.023±0.01^b	43.35±0.13^b
B200	43.61±1.57^a	9.75±1.23^a	4.94±0.36^a	20.25±0.02^c	0.019±0.01^c	47.24±0.65^a

Values are represented as Mean±SE.

Table 3: Proximate composition of biofloc developed in the different rearing systems.

Treatment	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude fibre (%)	Ash content (%)	Gross energy
B100	10.79±0.12	16.38±0.17	3.47±0.18	6.21±0.24	23.62±0.05	2734.81±0.04
B150	10.74±0.02	16.15±0.09	3.45±0.01	6.32±0.05	23.68±0.01	2734.53±0.01
B200	10.71±0.07	16.04±0.03	3.41±0.18	6.53±0.02	23.72±0.03	2734.23±0.01

Values are represented as Mean±SE.

Anti-oxidant enzyme activity

There was a significant effect ($p<0.05$) on anti-oxidant enzymes treatment group (Fig 2). B100 group had significantly higher SOD (2.07±0.089 U/mg protein) and catalase activity (2.19±0.007 U/mg protein) and lower SOD (1.53±0.022 U/mg protein) and catalase activity (1.51±0.054 U/mg protein) were recorded in C200. Similarly higher levels of superoxide dismutase and catalase activity found in Nile tilapia and Crucian carp reared in biofloc system (Wang *et al.*, 2015; Long *et al.*, 2015). Further, Nageswari *et al.* (2022) also reported that *Pangasianodon hypophthalmus* fingerlings reared in millet-based biofloc at

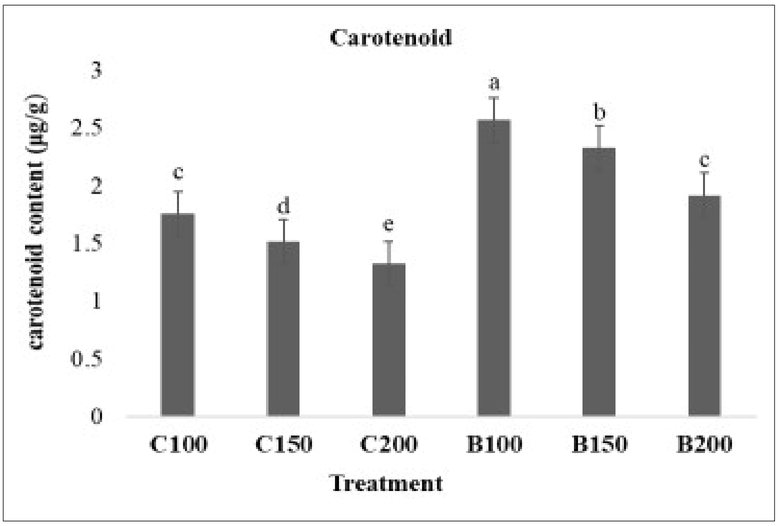
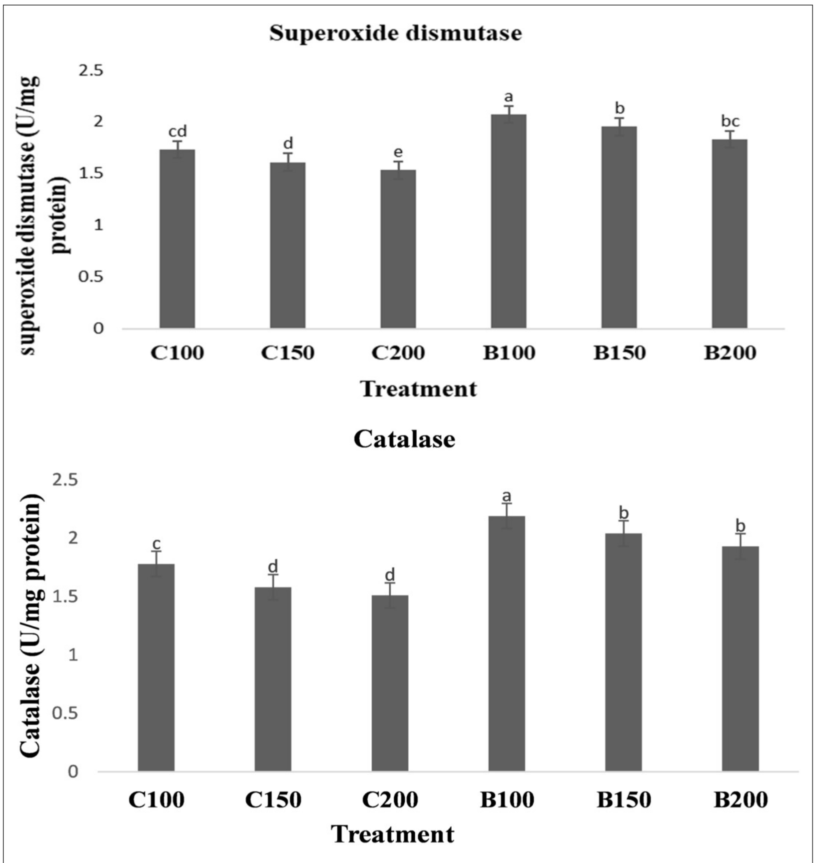


Fig 1: Total carotenoid content of koi carp reared in control and biofloc systems at different stocking densities.



(Bars with different superscripts differ significantly at p<0.05).

Fig 2: Anti-oxidant enzyme activity of koi carp reared in clear water and biofloc systems at different stocking densities.

Table 4: Growth parameters of koi carp reared in clear water and biofloc systems at different stocking densities.

Parameters	Experimental groups						Two-way Anova		
	C100	C150	C200	B100	B150	B200	System	SD	System × SD
Initial weight (g)	0.62 ± 0.01^{a}	0.59 ± 0.03^{a}	0.59 ± 0.01^{a}	0.64 ± 0.03^{a}	0.61 ± 0.01^{a}	0.62 ± 0.02^{a}	0.573	0.121	0.699
Final weight (g)	2.71 ± 0.65^{c}	1.63 ± 0.47^{c}	1.43 ± 0.46^{c}	5.01 ± 0.31^{a}	4.32 ± 0.63^{b}	2.36 ± 0.54^{c}	<0.001	<0.001	0.004
TWG (g)	2.45 ± 0.67^{c}	1.46 ± 0.45^{c}	1.07 ± 0.27^{c}	4.74 ± 0.31^{a}	4.01 ± 0.52^{b}	2.16 ± 0.52^{c}	<0.001	<0.001	0.153
ADG (g/day)	0.04 ± 0.01^{b}	0.03 ± 0.01^{b}	0.02 ± 0.04^{b}	0.08 ± 0.04^{a}	0.06 ± 0.09^{b}	0.04 ± 0.06^{b}	0.003	0.008	0.317
SGR (%/day)	2.46 ± 0.50^{bc}	1.95 ± 0.32^{bc}	1.49 ± 0.32^{c}	3.89 ± 0.78^{a}	3.33 ± 0.67^{ab}	2.41 ± 0.39^{bc}	0.009	<0.001	0.592
FCR	1.88 ± 0.03^{b}	1.95 ± 0.01^{a}	1.98 ± 0.01^{a}	1.57 ± 0.02^{d}	1.71 ± 0.04^{c}	1.82 ± 0.03^{b}	0.007	<0.001	0.041
FER	0.54 ± 0.01^{c}	0.52 ± 0.01^{d}	0.51 ± 0.01^{d}	0.64 ± 0.01^{a}	0.61 ± 0.01^{b}	0.56 ± 0.01^{c}	<0.001	<0.001	0.018
PER	0.27 ± 0.01^{c}	0.25 ± 0.04^{c}	0.24 ± 0.01^{d}	0.32 ± 0.02^{a}	0.28 ± 0.04^{b}	0.25 ± 0.01^{c}	<0.001	<0.001	<0.001
SR (%)	78.06 ± 0.79^{d}	69.67 ± 0.85^{e}	64.05 ± 0.80^{f}	91.54 ± 0.58^{a}	87.51 ± 0.62^{b}	79.42 ± 0.51^{c}	<0.001	<0.001	0.003

Note: Values are represented as Mean±SE. TWG- Total weight gain, ADG- Average daily growth, SGR- Specific growth rate, FCR- Feed conversion ratio, FER- Feed efficiency ratio, PER- Protein efficiency ratio, SR- Survival rate.

a lower stocking density of 150 fish/m³ showed enhanced anti-oxidant activity. The present study also showed higher anti-oxidant capacity of Koi carp in B100 and lower activity in the control group.

The limitation of the study includes that after 60 days of culturing koi carp in a biofloc system at higher stocking densities of 150-200 nos/m³, would deliberately affect the growth, coloration and physiological performance of fishes, but when these biofloc-reared koi carp fishes were cultured in an outdoor lined pond of 5 m × 3 m with a water depth of 1.5 m without biofloc for 30 days to attain a marketable size, showed higher growth performance**,** feed utilization, survival rate, skin pigmentation and enhanced overall health performance of fishes at stocking density of 50 nos/m³ similar to the findings of Nazari *et al.* (2023). Therefore, biofloc would be a viable alternative option for nursery rearing of koi carp and after that rearing them in a pond environment will show higher scalability and long-term performance in terms of sustainability, particularly the cost-effectiveness of the biofloc system in commercial koi carp farming.

CONCLUSION

This study concludes that rice starch is the cost-effective carbon source for rearing Koi carp in a biofloc system that will support ornamental farmers in a sustainable manner. The stocking density of Koi carp at 100 nos/m³ in the rice starch based biofloc system maintained the water quality, promoted better floc characteristics, enhanced the growth performance, survival rate, color enhancement and anti-oxidant activity. However, higher stocking densities of 150-200 nos/m³ had negative impacts on the fish health.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Ethical statement

All animal procedures for experiments were approved by the committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

There has been no conflict of interest among the authors. No sponsorship or funding was received for the study's design, data collection, analysis, manuscript preparation and decision to publish.

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