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Research Article

A comparison of growth performance and digestive enzyme responses in common carp (*Cyprinus carpio*) across different culture phases

Mohammadiam T.[1,2,7*], Valipour A.[3], Mokhtari M.R.[4], Mesbah M.[1,7], Alishahi M.[1,7], Dixson B.[5], Samiee S.[6]

1Department of Livestock, Poultry and Aquatic animal Health, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2Center for International Scientific Studies and collaboration (CISSC), of the Ministry of Science Research and Technology of Iran
3Department of Mechanical Engineering, Shahid Chamran University of Ahvaz, Ahvaz, Iran
4Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
5Department of Biology, University of Waterloo, Ontario, Canada
6Department of Chemistry, Shahid Chamran University of Ahvaz, Ahvaz, Iran
7Member of Excellence Center of Warm Water Fish Health, Shahid Chamran University of Ahvaz, Ahvaz, Iran
*Correspondence: t.mohammadian@scu.ac.ir

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Abstract

This study evaluated the effects of biofloc technology (BFT) on growth performance and digestive enzyme activity in common carp (*Cyprinus carpio*) across two consecutive culture phases: a 90-day nursery phase (250-L tanks) followed by a 120-day grow-out phase (100 m³ ponds). Three treatments were compared: a semi-closed recirculating system (control), a biofloc-recirculating system with indigenous *Bacillus* spp. (BFT+P), and a biofloc system without probiotics (BFT). Key growth parameters (*i.e.* specific growth rate, relative growth rate, feed conversion ratio (FCR), protein efficiency ratio, and daily weight gain) and intestinal digestive enzymes (*i.e.* trypsin, chymotrypsin, amylase, lipase, total protease, and alkaline phosphatase) were monitored. Results showed that BFT+P significantly improved growth and feed utilization. By day 90, FCR was lowest in BFT+P (1.10±0.10) versus control (1.36±0.20). Digestive enzyme activities were significantly higher in biofloc treatments (p<0.05), with total protease peaking at 215.53±4.31 U mg^{-1} in BFT+P at day 90. During the grow-out phase, BFT+P yielded the highest final average weight (1.4 kg), the highest harvested biomass (1228±123 kg), and the lowest FCR (1.27±0.24). Survival rate remained 100% across all treatments. Enzyme activities increased time-dependently under biofloc conditions, with the most stable and elevated responses in BFT+P. In conclusion, biofloc system, particularly when combined with indigenous probiotic supplementation, enhances digestive physiology and growth performance of common carp throughout sequential culture phases. These findings offer practical strategies for improving intensive carp aquaculture efficiency.

Introduction

Common carp (*Cyprinus carpio*) is one of the most economically important freshwater fish species worldwide and is extensively cultured in earthen pond systems. However, feed costs account for up to 84% of total freshwater aquaculture production expenses, representing a major economic constraint for sustainable fish farming (Mohammadian *et al*., 2022). Consequently, improving feed utilization efficiency has become a key objective in modern aquaculture systems.

Biofloc technology (BFT) has emerged as an innovative and cost-effective approach to enhance nutrient utilization while reducing feed input (Emerenciano *et al*., 2025). This system relies on the manipulation of microbial communities through carbon supplementation and continuous aeration, enabling the conversion of nitrogenous wastes into microbial biomass that can be directly consumed by cultured species (Mahmoudi *et al*., 2025). The microbial flocs formed in BFT systems not only improve water quality but also serve as a supplementary nutritional source rich in protein, lipids, vitamins, and digestive enzymes (Yang *et al*., 2011; Luo *et al*., 2014) .

Species that actively forage within the water column and possess well-developed chemosensory receptors, such as cyprinids, are particularly suitable for BFT systems. Common carp, considered a biomass-fed fish, can efficiently utilize biofloc particles, leading to improved feed conversion ratios and growth performance. In addition, microbial activity in biofloc systems has been reported to stimulate endogenous digestive enzyme secretion, thereby enhancing nutrient digestion and absorption.

Despite the recognized benefits of biofloc systems, information regarding the temporal responses of growth performance and digestive enzyme activities in common carp during different culture phases remains limited. Therefore, the present study aimed to evaluate the effects of biofloc-based rearing on growth indices and digestive enzyme activities of common carp across distinct culture phases (Tank and Geomembrane ponds). Despite the recognized advantages of biofloc technology, several knowledge gaps remain regarding its long-term effects on digestive physiology and growth responses in common carp. In particular, limited information is available on the temporal dynamics of digestive enzyme activities and growth performance during different culture phases and production scales. Understanding these responses is essential for optimizing biofloc-based rearing strategies and improving system efficiency under practical aquaculture conditions.

Therefore, the present study aimed to evaluate the effects of biofloc-based rearing systems on growth performance and digestive enzyme activities of common carp under different culture conditions. Specifically, the study compared semi-closed recirculating systems and biofloc-based systems, including treatments with and without indigenous bacterial communities, across two production phases (tank culture and geomembrane pond culture). The study also investigated temporal changes in growth indices and key digestive enzymes to better understand the physiological responses of fish to different

rearing environments. Also, the findings of this study provide important insights into the role of biofloc technology in improving feed efficiency, digestive capacity, and growth performance of common carp. By clarifying the physiological mechanisms underlying enhanced nutrient utilization in biofloc systems, this research contributes to the optimization of sustainable aquaculture practices. The results may support the development of cost-effective production strategies, reduce reliance on commercial feed inputs, and improve environmental management through enhanced nitrogen recycling. Furthermore, the application of biofloc technology has the potential to increase production efficiency, minimize environmental impacts, and enhance economic profitability in freshwater aquaculture systems. The outcomes of this research may therefore facilitate the wider adoption of biofloc-based culture systems for common carp and other freshwater species, contributing to sustainable intensification and long-term development of the aquaculture industry.

Materials and methods

Cultivation system

To carry out this research, three semi-closed systems, biofloc-rotating (with indigenous bacteria) and biofloc (without indigenous bacteria) were investigated in terms of water quality characteristics, activation and ability to remove inorganic nitrogen compounds. The probiotics used in the present study, indigenous quorum quencher bacteria, were used as heterotrophic auxiliary bacteria (Abdulaziz *et al*., 2025).

In the first phase, all systems had a 250-liter fish breeding tank. Each circulating system had a sedimentation tank, an airlift pump and a mineral pumice biofilter tank. In the biofloc system, aeration was performed by a perforated pipe in the bottom. In the biofloc-rotating system, a biofloc production tank, an airlift pump and a sedimentation tank were designed. After preparing the systems, juvenile common carp were stocked at a density of 10.25 kg/m3 in each system and reared for three months.

In the second phase, all systems had a 100 m^3 fish pond. Each recirculating system had a sedimentation tank, an airlift pump, and a pumice biofilter tank. In the biofloc system, aeration was performed by a perforated pipe (diffuser) in the bottom (although fungal aerators were replaced later in the experiment). In the biofloc-recirculating system, a biofloc production tank, an airlift pump, and a sedimentation tank were designed. After the systems were prepared, juvenile common carp were stocked at a density of 1,000 fish per 100 cubic meters in each system and reared for 4 months. Physicochemical factors such as temperature, pH, dissolved oxygen, and dissolved solids were measured daily with a portable multimeter (WTW, Multi 3630 IDS).

Experimental design

In the first phase, the fish will be randomly distributed into 3 treatments (each with three replications) in 12 tanks (30 fish per tank) equipped with aerators. 40% of the water in the tanks will be replaced daily (recirculation system). It is worth noting that before changing the water, the total

ammonia level in the water will be measured using the Nessler method. Before starting the experiment, the fish will be acclimatized to the laboratory conditions (water temperature 24 ± 2°C, photoperiod 16 hours light: 8 hours dark, oxygen 1±6 mg/L, pH: 7.6±0.2; salinity 1 g/L (Karun River water)) for 2 weeks. During the acclimatization period, the fish will be fed a commercial common carp diet three times a day, equivalent to 4% of body weight. The growth parameters of fish in the experimental groups were measured at the day 0, day 30, day 60 and day 90 study using the following formulas: Specific Growth Rate (SGR), Food Efficiency Ratio (FER), Food Conversion Ratio (FCR) and Protein Efficiency Ratio (PER). The calculations were performed using the following formulae: BWG %= 100× (FBW–IBW)/ IBW, SGR%= 100× (lnFBW–lnIBW)/ days, FCR=feed consumed/ (FBW–IBW), FCE%=(FBW–IBW)/feed consumed×100, PER=IBW/protein intake. IBW is initial body weight, FBW is final body weight and days are days of feeding.Where W_I is initial body weights; W_F is final body weights (g); and it is the trial duration in days (Mohammadian *et al.,* 2017).

The total amount of protein in the gut samples was determined by the standard (Bradford) method using BSA (bovine serum albumin) as the standard (Bradford, 1976). Chymotrypsin and trypsin activities were kinetically measured using N_α-Benzoyl-L-arginine ethyl ester hydrochloride (BAEE) and N-Benzoyl-L-tyrosine ethyl ester (BTEE) as substrates, respectively (Ghanei-Motlagh *et al*., 2019). The activity of intestinal alkaline phosphatase (ALP) was kinetically determined using the substrate 4-nitrophenyl phosphate using a commercial kit (Pars Azmoon Co., Tehran, Iran). α-amylase activity was quantified using soluble starch as the substrate hydrolysable to maltose reacting with 3,5-Dinitrosalicylic acid solution (Bernfeld, 1951). Standard solutions containing maltose was used to prepare the standard curves of total α-amylase (Areekijseree *et al*., 2004). Lipase activity was calculated titrimetrically using olive oil emulsion, and the fatty acids released were quantified after titration with sodium hydroxide solution using phenolphthalein as the color indicator (Borlongan, 1990). Enzyme activities were measured as the change in absorbance using a spectrophotometer (UV-2802S; Unico, Shanghai, China) and expressed as specific activity (U mg^1 protein) (Mohammadian *et al*., 2017).

In the second phase, the fish will be randomly distributed into 3 treatments (each with three replications) in 3 geomembrane ponds (1000 fish, 50±16 g per pond) equipped with aerators. 10% of the water in the tanks will be replaced daily. It should be noted that before the water is replaced, the total ammonia level in the water will be measured using the Nessler method. Before the experiment, the fish will be acclimatized to the laboratory conditions (water temperature 24±2°C, photoperiod 16 hours light: 8 hours dark, oxygen 6±1 mg L^{-1}, pH: 7.6±0.2; salinity 1 g L^{-1} (Karun River water)) for 2 weeks. During the acclimatization period, the fish will be fed a commercial common carp diet three times a day, equivalent to 3% of body weight. The growth parameters of fish in

the experimental groups were measured at the beginning (day 0) and the end (after 4 months) of study using the following formulas: Initial biomass = number of fish × initial weight (W_I, g); Final biomass = number of fish × final weight (W_F, g); Feed conversion rate (FCR) = feed intake (g) / weight gain ($W_F - W_I$, g).

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Statistical analysis

Data analysis was done using the IBM SPSS software. The data were expressed as means ± standard deviation (SD). The Shapiro-Wilk test was used to check the normality of the data. In order to determine the significant differences between the mean values, one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was performed. The significance level was considered at the 5% probability level.

Result

First phase

During the indoor and outdoor phases, fish were reared in 250-L tanks and 100-m^3 ponds, respectively, for 90 and 120 days under controlled environmental conditions (water temperature: 24±2°C; pH: 7.6±0.2; dissolved oxygen: 6±1 mg L^{-1}; salinity: 1 g L^{-1}; photoperiod: 16L:8D).

Growth performance

In the semi-closed recirculating system, the specific growth rate (SGR) decreased on day 90 compared with day 30. In contrast, fish reared in the biofloc-based recirculating system supplemented with indigenous *Bacillus* spp. exhibited a continuous increase in SGR, reaching its highest value on day 90. In the biofloc-based recirculating system without indigenous *Bacillus* supplementation, SGR increased until day 60 but subsequently declined by day 90**.** The RGR value in all treatments was higher on day 90 than on day 30, indicating an increase in growth over time. The circulating biofloc treatment with Indigenous bacilli showed the highest relative growth on day 90**.** In the semi-

closed circulation treatment, the FCR value was higher on day 90 than on day 30, indicating a decrease in feed efficiency. In the rotating biofloc treatment with Indigenous bacilli, the FCR value remained almost constant over time. In the rotating biofloc treatment without Indigenous bacilli, the FCR value decreased on day 60 but increased again on day 90**.** PER was relatively constant in the rotating biofloc treatments with Indigenous bacilli and without Indigenous bacilli on days 30 and 90. In the semi-closed circulation rotating treatment, PER did not differ significantly on days 30 and 90**.** DWG remained almost constant over time in the rotating biofloc treatment with Indigenous bacilli. In the semi-closed circulation rotating treatment, DWG decreased on day 90 compared to day 30. In the rotating biofloc treatment without Indigenous bacilli, the DWG value changed slightly on days 30, 60, and 90. In general, the trend of changes in growth indices shows that the rotating biofloc treatment with Indigenous bacilli had a more stable performance over different time periods and achieved better growth and higher feed efficiency compared to other treatments (Table 1).

Table 1: **Comparison of growth indices among different treatments over a 3-month period (Phase 1 of the experiment) under different aquaculture feeding systems (Results are reported as Means ± SD). Different lowercase letters indicate significant differences at the 0.05 level.**

Index	Treatment	Day 30	Day 60	Day 90
Specific growth rate (SGR)	Recirculating (Semi-Closed)	0.54±0.037 ^{Aa}	0.46±0.01 ^{Aa}	0.38±0.03 ^{Ab}
	Biofloc-Recirculating (With Indigenous *Bacillus*)	0.61±0.133 ^{Aa}	0.50±0.01 ^{Aa}	0.41±0.02 ^{Ab}
	Biofloc-Recirculating (Without Indigenous *Bacillus*)	0.55±0.064 ^{Aa}	0.53±0.03 ^{Aa}	0.39±0.02 ^{Ab}
Relative growth rate (RGR)	Recirculating (Semi-Closed)	40.1±3.8 ^{Ac}	91.5±4.3 ^{Ab}	121.5±7.4 ^{Aa}
	Biofloc-Recirculating (With Indigenous *Bacillus*)	49.5±2.8 ^{Ac}	106.4±4.3 ^{Ab}	139.04±13.7^{Aa}
	Biofloc-Recirculating (Without Indigenous *Bacillus*)	40.59±6.4 ^{Ac}	110.3±2.8 ^{Ab}	131.1±8.6 ^{Aa}
Feed conversion ratio (FCR)	Recirculating (Semi-Closed)	1.08±0.12 ^{Ab}	1.07±0.1 ^{Ab}	1.36±0.2 ^{Aa}
	Biofloc-Recirculating (With Indigenous *Bacillus*)	1.01±0.5 ^{Aa}	1.02±0.1 ^{Aa}	1.1±0.1 ^{Aa}
	Biofloc-Recirculating (Without Indigenous *Bacillus*)	1.034±0.3 ^{Aa}	0.9±0.1 ^{Aa}	1.28±0.1 ^{Ab}
Protein efficiency ratio (PER)	Recirculating (Semi-Closed)	2.95±0.3 ^{Aa}	2.9±0.3 ^{Ba}	2.58±0.5 ^{Ba}
	Biofloc-Recirculating (With Indigenous *Bacillus*)	3.10±0.7 ^{Aa}	3.03±0.3 ^{Ba}	3.36±0.3 ^{Aa}
	Biofloc-Recirculating (Without Indigenous *Bacillus*)	2.89±0.7 ^{Ab}	3.53±0.5 ^{Aa}	2.64±0.4 ^{Bb}
Daily weight gain (DWG)	Recirculating (Semi-Closed)	0.76±0.1 ^{Aa}	0.69±0.04 ^{Aa}	0.71±0.05 ^{Aa}
	Biofloc-Recirculating (With Indigenous *Bacillus*)	0.74±0.15 ^{Aa}	0.71±0.03 ^{Aa}	0.74±0.04 ^{Aa}
	Biofloc-Recirculating (Without Indigenous *Bacillus*)	0.73±0.1 ^{Aa}	0.72±0.06 ^{Aa}	0.71±0.15 ^{Aa}

*Different lowercase letters in each column indicate significant differences between the values ($p< 0.05$). Data were expressed as means ± SEM.

The activities of digestive enzymes, including chymotrypsin, trypsin, α-amylase, lipase, total protease, and alkaline phosphatase (ALP), were significantly affected by the rearing system and sampling time (p<0.05).

Digestive enzyme activity in phase I

Chymotrypsin activity showed no significant differences among treatments at day 0 (p>0.05). In the rotational semi-closed circulated system, enzyme activity remained relatively stable from day 0 to day 60, followed by a significant increase at day 90. In contrast, both biofloc systems exhibited a pronounced increase in chymotrypsin activity over time. The highest values at days 60 and 90 were recorded in the biofloc systems, particularly in the treatment without indigenous *Bacillus*, which showed significantly higher chymotrypsin activity compared to the rotational system (p<0.05)**.** At day 0 and day 30, trypsin activity did not differ significantly among treatments (p>0.05). However, from day 60 onward, a marked increase in trypsin activity was observed in both biofloc systems. The biofloc treatments exhibited significantly higher trypsin activity at days 60 and 90 compared to the rotational system (p<0.05). The highest trypsin activity was observed at day 90 in the biofloc system with indigenous *Bacillus*. α-Amylase activity varied significantly depending on the rearing system and sampling time (p<.05). In the rotational system, enzyme activity showed minor fluctuations without a clear increasing trend. Conversely, both biofloc treatments exhibited significantly higher α-amylase activity from day 30 onward. The biofloc system without indigenous *Bacillus* showed consistently elevated α-amylase activity at days 30, 60, and 90, while the biofloc system with indigenous *Bacillus* reached its peak at day 60, followed by a slight decline at day 90. Lipase activity remained relatively constant in the rotational system throughout the experimental period. In contrast, both biofloc systems showed a significant increase in lipase activity starting from day 30 (p<0.05). The highest lipase activities were recorded in the biofloc treatments at days 30 and 60, with no significant differences between the two biofloc systems at day 90. Overall, lipase activity in biofloc-reared fish was significantly higher than that in the rotational system at all sampling times after day 30. Total protease activity demonstrated a strong response to biofloc rearing conditions. While protease activity in the rotational system remained relatively low and unchanged over time, both biofloc systems showed a significant and progressive increase from day 30 to day 90 (p<0.05). The highest protease activities were observed at day 90 in the biofloc system without indigenous *Bacillus*, followed closely by the biofloc system with indigenous *Bacillus*. ALP activity did not differ significantly among treatments at day 0 (p>0.05). However, from day 30 onward, both biofloc systems exhibited significantly higher ALP activity compared to the rotational system (p<0.05). ALP activity peaked at day 30 in both biofloc treatments and remained elevated through day 90, whereas the rotational system showed comparatively lower and more variable ALP activity throughout the experiment (Table 2)**.**

Table 2: The activities of digestive enzymes in *C. carpio* fed during the first phase of the study. Values are based on the mean, bars indicate standard error and different letters indicate a significant difference between treatments at the time point (p<0.05).

Parameters	Groups	Day 0	Day 30	Day 60	Day 90
Chymotrypsin (U/mg)	Rotational (semi-closed circulated)	11.99±0.11 ^{Aa}	11.62±1.6 ^{Aa}	12.67±1.18 ^{Ba}	14.67±1.18 ^{Ba}
	Biofloc- circulated (with Indigenous *Bacillus*)	11.03±1.19 ^{Ab}	12.96±0.03 ^{Ab}	12.96±1.01 ^{Bb}	18.96±1.01 ^{Aa}
	Biofloc- circulated (without Indigenous *Bacillus*)	12.11±3.5 ^{Ab}	11.72±4.08 ^{Ab}	19.76±1.54 ^{Aa}	19.76±1.54 ^{Aa}
Trypsin (U/mg)	Rotational (semi-closed circulated)				
	Biofloc- circulated (with Indigenous *Bacillus*)	0.57±0.11 ^{Aa}	0.48±0.07 ^{Aa}	0.52±0.18 ^{Ba}	0.62±0.18 ^{Ba}
	Biofloc- circulated (without Indigenous *Bacillus*)	0.55±0.19 ^{Ab}	0.54±0.13 ^{Ab}	1.33±0.03 ^{Aa}	1.93±0.03 ^{Aa}
α-amylase (U/mg)	Rotational (semi-closed circulated)	0.5±3.5 ^{Ab}	0.63±0.08 ^{Ab}	1.22±0.34 ^{Aa}	1.72±0.34 ^{Aa}
	Biofloc- circulated (with Indigenous *Bacillus*)				
	Biofloc- circulated (without Indigenous *Bacillus*)	14.6±2.1 ^{Aa}	12.24±1.03 ^{Ba}	14.84±1.18 ^{Ba}	14.28±1.18 ^{Ba}
	Rotational (semi-closed circulated)	15.06±2.19 ^{Ab}	18.66±2.03 ^{ABb}	27.53±2.31 ^{Aa}	17.53±2.31 ^{Bb}
Lipase (U/mg)	Biofloc- circulated (with Indigenous *Bacillus*)	14.1±3.05 ^{Ab}	24.4±4.32 ^{Aa}	21.7±2.14 ^{Aa}	22.7±2.14 ^{Aa}
	Biofloc- circulated (without Indigenous *Bacillus*)				
	Rotational (semi-closed circulated)	113.6±20.1 ^{Aa}	118.24±9.03 ^{Ba}	112.84±31.18 ^{Ba}	119.8±31.18 ^{Ba}
	Biofloc- circulated (with Indigenous *Bacillus*)	119.06±2.19 ^{Ab}	224.6±17.3 ^{Aa}	193.53±2.31 ^{Aa}	180.53±2.31 ^{Aa}
	Biofloc- circulated (without Indigenous *Bacillus*)	111.1±13.05 ^{Ab}	208.4±14.32 ^{Aa}	187.7±19.14 ^{Aa}	208.7±19.14 ^{Aa}
Protease (U/mg)	Rotational (semi-closed circulated)				
	Biofloc- circulated (with Indigenous *Bacillus*)	50.6±1.1 ^{Aa}	47.65±3.03 ^{Ba}	59.84±11.18 ^{Ba}	48.84±11.18 ^{Ba}
	Biofloc- circulated (without Indigenous *Bacillus*)	49.0±0.06 ^{Ac}	149.6±17.3 ^{Ab}	152.53±4.31 ^{Ab}	215.53±4.31 ^{Aa}
	Rotational (semi-closed circulated)	49.1±3.05 ^{Ac}	173.4±14.32 ^{Ab}	166.7±9.4 ^{Ab}	231.7±9.4 ^{Aa}
ALP (U/mg)	Biofloc- circulated (with Indigenous *Bacillus*)				
	Biofloc- circulated (without Indigenous *Bacillus*)	16.6±1.1 ^{Aa}	18.24±2.03 ^{Ba}	15.84±31.18 ^{Ba}	21.84±31.18 ^{Ba}
	Rotational (semi-closed circulated)	15.06±4.19 ^{Ab}	48.6±6.3 ^{Aa}	39.53±2.31 ^{Aa}	47.53±2.31 ^{Aa}
	Biofloc- circulated (with Indigenous *Bacillus*)	17.1±3.05 ^{Ab}	54.4±7.32 ^{Aa}	33.7±19.14 ^{Aa}	53.7±19.14 ^{Aa}

Significant differences between values (means ± S.D n = 15), when compared with control groups, were characterized by alphabet symbol (p<0.05).

Phase II of the experiment in 100 cubic meter ponds

In the second phase of the experiment, the density per unit area was constant and equal

to 1000 in all three treatments. In the rotating biofloc treatment with Indigenous bacilli, the highest average weight (1.4 kg) was observed, while the lowest value was observed in the rotating biofloc treatment without Indigenous bacilli (1.1 kg). The survival rate in all three treatments was 100%, indicating that the treatments had no effect on the survival rate of the samples. In the rotating biofloc treatment with Indigenous bacilli, the highest harvested weight (1228) was observed, while the rotating biofloc treatment without Indigenous bacilli had the lowest harvested weight (1095). The feed conversion ratio in the rotating biofloc treatment with Indigenous bacilli (1.27) was the lowest, indicating better feed efficiency in this treatment (P<0.05). In contrast, the rotating biofloc treatment without Indigenous bacilli showed the highest FCR (1.51), indicating lower feed efficiency in this treatment. In general, the rotating biofloc treatment with Indigenous bacilli performed better than the other treatments in growth and feeding indices, as it showed both higher average weight, higher harvest weight, and lower feed conversion ratio. In contrast, the rotating biofloc treatment without Indigenous bacilli had the weakest performance and recorded higher feed conversion ratio (Table 3).

Table 3: Comparison of growth indices between the studied treatments during 3 months (phase 2 of the experiment) of rearing nutrition in different rearing systems (results are reported as Means±SD).

Treatments	Food Consumed	Survival Rate	Total Harvested Weight	Feed Conversion Ratio	Density per Unit Area	Avr. Weight (gr)
Rotational (semi-closed circulated)	1749	100%[a]	1173±205[a]	1.49±0.18[b]	1000	1/2±0.21[b]
Biofloc- circulated (with Indigenous *Bacillus*)	1568	100%[a]	1228±123[a]	1.27±0.24[a]	1000	1/4±0.38[a]
Biofloc- circulated (without Indigenous *Bacillus*)	1659	100%[a]	1095±85[b]	1.51±0.28[b]	1000	1/1±0.18[b]

*Values are based on the mean, bars indicate standard error, and different letters indicate significant differences between treatments at the time point (p<0.05).

Digestive enzyme activities in phase II

The activities of digestive enzymes, including chymotrypsin, trypsin, α-amylase, lipase, total protease, and alkaline phosphatase (ALP), were significantly affected by the rearing system and sampling time (p<0.05). At day 0, no significant differences in chymotrypsin activity were observed among treatments (p>0.05). In the rotational semi-closed circulated system, chymotrypsin activity showed a gradual but moderate increase over time, reaching the highest values at days 90 and 120. In contrast, both biofloc-circulated systems exhibited a pronounced time-dependent increase, particularly from day 60 onward (p<0.05). The highest chymotrypsin activity was recorded in the biofloc system without indigenous *Bacillus* at days 90 and 120, which was significantly higher than

the rotational system. Trypsin activity at day 0 did not differ significantly among treatments (p>0.05). In the rotational system, trypsin activity remained relatively stable throughout the experimental period. However, both biofloc treatments demonstrated a marked increase from day 60 onward (p<0.05). Maximum trypsin activity was observed at days 90 and 120 in both biofloc systems, with no significant differences between biofloc treatments with or without indigenous *Bacillus* at corresponding sampling times. α-Amylase activity showed no significant differences among treatments at day 0 (p>0.05). In the rotational system, α-amylase activity remained relatively constant during the experiment. In contrast, both biofloc systems exhibited significantly higher α-amylase activity compared to the rotational system from day 30 onward (p<0.05). The biofloc system with indigenous *Bacillus* showed a sharp increase at day 60, followed by a decline at days 90 and 120, while the biofloc system without indigenous *Bacillus* maintained consistently elevated α-amylase activity throughout the experimental period. Lipase activity did not differ significantly among treatments at day 0 (p>0.05). In the rotational system, lipase activity remained stable across all sampling times. In contrast, both biofloc-circulated systems exhibited a significant increase in lipase activity from day 30 onward (p<0.05). Elevated lipase activity was sustained through day 120 in both biofloc treatments, with no significant differences between systems with and without indigenous *Bacillus*. Protease activity at day 0 was similar among all treatments (p>0.05). In the rotational system, protease activity showed minor fluctuations but no consistent increasing trend. Conversely, both biofloc systems demonstrated a substantial and progressive increase in protease activity from day 30 to day 120 (p<0.05). The highest protease activity was recorded at day 120 in the biofloc system without indigenous *Bacillus*, followed closely by the biofloc system with indigenous *Bacillus*. ALP activity did not differ significantly among treatments at day 0 (p>0.05). In the rotational system, ALP activity showed only slight increases over time. In contrast, both biofloc treatments exhibited significantly higher ALP activity from day 30 onward compared to the rotational system (p<0.05). Elevated ALP levels were maintained until day 120, with no statistically significant differences between biofloc systems with and without indigenous *Bacillus* (Table 4).

Table 4: Digestive enzymes activity in *C. carpio* fed during the second phase of the study. Values are based on the mean, bars indicate standard error and different letters indicate a significant difference between treatments at the time point (p<0.05).

Parameters	Groups	Day 0	Day 30	Day 60	Day 90	Day 120
Chymotrypsin (U/mg)	Rotational (semi-closed circulated)	12.69±0.11 ^{Aa}	12.62±1.6 ^{Aa}	13.42±1.18 ^{Ba}	15.67±1.18 ^{Ba}	15.67±1.18 ^{Ba}
	Biofloc- circulated (with Indigenous *Bacillus*)	12.03±2.19 ^{Ab}	13.96±0.03 ^{Ab}	13.46±1.01 ^{Bb}	22.96±1.01 ^{Aa}	21.96±1.01 ^{Aa}
	Biofloc- circulated (without Indigenous *Bacillus*)	13.11±1.5 ^{Ab}	12.72±4.08 ^{Ab}	18.76±1.54 ^{Aa}	25.76±1.54 ^{Aa}	26.76±1.54 ^{Aa}

Table 4 (continued)

Parameters	Groups	Day 0	Day 30	Day 60	Day 90	Day 120
Trypsin (U/mg)	Rotational (semi-closed circulated)	0.59±0.11 ^{Aa}	0.49±0.07 ^{Aa}	0.53±0.18 ^{Ba}	0.58±0.18 ^{Ba}	0.56±0.18 ^{Ba}
	Biofloc- circulated (with Indigenous *Bacillus*)	0.57±0.19 ^{Ab}	0.53±0.13 ^{Ab}	1.45±0.03 ^{Aa}	2.06±0.03 ^{Aa}	1.98±0.03 ^{Aa}
	Biofloc- circulated (without Indigenous *Bacillus*)	0.51±3.5 ^{Ab}	0.65±0.18 ^{Ab}	1.39±0.34 ^{Aa}	1.97±0.34 ^{Aa}	1.84±0.34 ^{Aa}
α-amylase (U/mg)	Rotational (semi-closed circulated)	16.6±2.1 ^{Aa}	14.24±1.03 ^{Ba}	15.84±1.18 ^{Ba}	16.28±1.18 ^{Ba}	15.28±1.08 ^{Ba}
	Biofloc- circulated (with Indigenous *Bacillus*)	16.06±2.19 ^{Ab}	20.66±2.03 ^{ABb}	32.53±2.31 ^{Aa}	19.53±2.31 ^{Bb}	21.53±1.31 ^{Bb}
	Biofloc- circulated (without Indigenous *Bacillus*)	15.1±3.05 ^{Ab}	26.4±4.32 ^{Aa}	25.7±2.14 ^{Aa}	25.7±2.14 ^{Aa}	27.7±2.24 ^{Aa}
Lipase (U/mg)	Rotational (semi-closed circulated)	117.6±20.1 ^{Aa}	120.24±9.03 ^{Ba}	117.84±31.18 ^{Ba}	121.8±31.18 ^{Ba}	119.8±31.18 ^{Ba}
	Biofloc- circulated (with Indigenous *Bacillus*)	123.06±2.19 ^{Ab}	231.6±17.3 ^{Aa}	198.53±2.31 ^{Aa}	189.53±2.31 ^{Aa}	193.53±2.31 ^{Aa}
	Biofloc- circulated (without Indigenous *Bacillus*)	116.1±13.05 ^{Ab}	215.4±14.32 ^{Aa}	191.7±19.14 ^{Aa}	218.7±19.14 ^{Aa}	221.7±19.14 ^{Aa}
Protease (U/mg)	Rotational (semi-closed circulated)	52.6±1.1 ^{Aa}	48.65±3.03 ^{Ba}	61.84±11.18 ^{Ba}	58.84±11.18 ^{Ba}	52.84±11.18 ^{Ba}
	Biofloc- circulated (with Indigenous *Bacillus*)	51.0±0.06 ^{Ac}	159.6±17.3 ^{Ab}	162.53±4.31 ^{Ab}	237.53±4.31 ^{Aa}	241.53±4.31 ^{Aa}
	Biofloc- circulated (without Indigenous *Bacillus*)	50.1±3.05 ^{Ac}	176.4±14.32 ^{Ab}	171.7±9.4 ^{Ab}	251.7±9.4 ^{Aa}	258.7±9.4 ^{Aa}
ALP (U/mg)	Rotational (semi-closed circulated)	17.6±1.1 ^{Aa}	16.24±2.03 ^{Ba}	17.84±31.18 ^{Ba}	19.84±31.18 ^{Ba}	21.84±31.18 ^{Ba}
	Biofloc- circulated (with Indigenous *Bacillus*)	16.06±4.19 ^{Ab}	52.6±6.3 ^{Aa}	43.53±2.31 ^{Aa}	52.53±2.31 ^{Aa}	49.53±2.31 ^{Aa}
	Biofloc- circulated (without Indigenous *Bacillus*)	18.1±3.05 ^{Ab}	57.4±7.32 ^{Aa}	37.7±19.14 ^{Aa}	59.7±19.14 ^{Aa}	58.7±19.14 ^{Aa}

Significant differences between values (means±S.D n=15), when compared with control groups, were characterized by alphabet symbol (p<0.05).

Discussion

The present study demonstrated that rearing common carp (*C. carpio*) in biofloc-based systems significantly influenced both growth performance and digestive enzyme activities across different culture phases compared to conventional semi-closed recirculating conditions. These effects are consistent with previous findings showing that biofloc systems can enhance nutrient utilization, feed efficiency, and physiological responses in carp and other species (Avnimelech and Kochba 2009; Ekasari *et al*., 2014).

Our results indicate that fish reared in the biofloc–recirculating system with indigenous bacilli exhibited more stable specific growth rate (SGR), higher relative growth rate (RGR), and improved feed conversion ratio (FCR) compared with the semi-closed recirculating system, particularly during extended culture periods. Similar improvements in growth performance and FCR under biofloc culture have been reported in common carp and other culture species, where microbial flocs provided additional nutritional contributions and improved nutrient assimilation (*e.g.,* amylase and protease activities) relative to clear water controls or conventional systems (Long *et al*., 2015; Robles and Porchas *et al*., 2020). Biofloc systems also help maintain lower ammonia concentrations, reducing environmental stress and further supporting growth performance (Liu *et al*., 2018). The lower and more stable FCR observed in biofloc treatments suggests enhanced nutrient assimilation and reduced feed wastage. Biofloc systems recycle dissolved nitrogenous wastes into microbial biomass, which can be directly consumed by fish, thereby reducing dependency on formulated feeds and improving feed efficiency (Avnimelech and Kochba 2009; Hargreaves, 2013). In contrast, the semi-closed recirculating system showed a progressive decline in feed efficiency over time, likely due to increased metabolic stress and suboptimal nitrogen dynamics.

The activities of key digestive enzymes (chymotrypsin, trypsin, α-amylase, lipase, protease, and ALP) were generally higher in fish reared in biofloc systems compared with the semi-closed recirculating treatment. These enhancements in enzyme activity are likely linked to both increased endogenous enzyme secretion stimulated by microbial interactions and direct contributions of microbial extracellular enzymes present in bioflocs (Adineh *et al*., 2022). Elevated activities of amylase, protease, and lipase have been observed in common carp and other fish species reared in biofloc systems, suggesting improved digestion and nutrient assimilation under biofloc culture conditions (Haridas *et al*., 2021). Enhanced digestive enzyme activity is one mechanism by which biofloc systems improve feed utilization efficiency and growth performance, as reported in other aquaculture studies (Xu and Pan, 2014; Long *et al*., 2015; Robles and Porchas *et al*., 2020; Adineh *et al*., 2022; Mahmoudi *et al*., 2025).

The observed temporal increase in enzyme activities in biofloc treatments likely reflects adaptive responses of the digestive system to sustained exposure to microbial biomass and its associated extracellular enzymes. Biofloc-associated microorganisms produce proteases, amylases, and lipases that complement endogenous digestive processes, thereby improving macronutrient breakdown and absorption (Crab *et al*., 2012; Ekasari *et al*., 2014). In addition, biofloc systems can modulate gut microbiota composition, further enhancing digestive capacity and intestinal health.

The fish reared in biofloc systems supplemented with indigenous *Bacillus* spp. generally performed better in terms of growth and enzyme response compared with those without such probiotic supplementation. This suggests that

targeted microbial management within biofloc systems can further optimize the beneficial effects on host physiology. Probiotic bacteria such as *Bacillus* spp. are known to contribute to improved digestive enzyme activity as well as to enhanced immune and antioxidant responses in fish under biofloc conditions (*e.g.,* protease, amylase, and lipase) (Gullian-Klanian *et al*., 2023). The enhanced growth performance, improved feed efficiency, and elevated digestive enzyme activities observed in biofloc treatments highlight the potential of these systems to improve production sustainability in common carp culture. By recycling waste nitrogen into microbial biomass and enhancing in situ nutrient availability, biofloc systems can reduce reliance on external feed inputs and mitigate water quality deterioration two major challenges in intensive aquaculture. Their integration into biofloc systems appears to synergistically amplify the beneficial effects of microbial flocs on host physiology.

The combined improvements in growth performance, feed efficiency, and digestive enzyme activity observed in biofloc-based systems highlight the potential of BFT as a sustainable strategy for intensive common carp culture. By converting waste nutrients into valuable microbial biomass and reducing water exchange requirements, biofloc systems address critical challenges related to water scarcity, environmental impact, and production costs (Hargreaves, 2013). The present findings further suggest that targeted microbial management, including the use of indigenous probiotics, can optimize the efficiency and resilience of biofloc-based aquaculture systems.

Overall, this study demonstrates that the combination of biofloc technology and specific control of microbial population leads up to a substantial increase in common carp (*C. carpio*) productivity and physiological status using an intensive system. These results show that biofloc-skimmer RAS supplemented with native Bacillus spp. delivers better, and more consistent growth metrics, such as specific growth rate (SGR), relative growth rates (RGR) or feed conversion ratio (FCR), than conventional semi-closed recirculating systems. Mechanistically, these performance enhancements correlate with the biofloc phenomenon being used for two nutritional and physiological functions: as a constant allochthonous in-situ supplementary matrix of feed through nitrogen which is waste material converted into edible microbial biomass during consumption; and because it stimulates digestive function to yield better growth. In fact, the increase of important digestive enzymes (including protease, amylase and lipase) reported for biofloc treatments mirrors a dramatic change in the digestive ability of the fish. This improvement arises from the fish's adaptive physiological reaction to continuous exposure to microorganisms, as well as the direct action of foreign enzymes produced by the microbial population inside flocs. The inclusion of indigenous *Bacillus* spp. further amplified these benefits, suggesting a synergistic relationship where probiotics optimize the microbial ecosystem, leading to improved nutrient assimilation, gut health, and overall physiological resilience. From a sustainability perspective, the biofloc system addresses two of the most

pressing challenges in modern aquaculture: environmental pollution and feed costs. By maintaining lower ammonia concentrations, improving feed efficiency, and reducing waste output, biofloc technology minimizes the ecological footprint of farming operations. Concurrently, the enhanced utilization of dietary inputs and the direct nutritional contribution of the flocs reduce the dependency on expensive formulated feeds. Therefore, the integration of biofloc technology with probiotic supplementation represents a highly effective and sustainable strategy for intensive common carp culture. This strategy not only enhances production efficiency and fish health but also fosters environmental responsibility, laying the groundwork for more resilient and sustainable aquaculture methodologies.

Conclusion

The integration of biofloc technology with a probiotic supplementation represents a highly effective and sustainable strategy for intensive common carp culture. This strategy not only enhances production efficiency and fish health but also fosters environmental responsibility, laying the groundwork for more resilient and sustainable aquaculture systems.

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Conflicts of interest

The authors declare that they have no conflict of interest.

References

Abdulaziz, M.A., Mohammadian, T., Mesbah, M., Gharibi, D. and Jalali, S.M., 2025. Amelioration of hemato-serological parameters, antioxidant capacity, and resistance against *Aeromonas hydrophila* in common carp (*Cyprinus carpio*) fed with quorum quenching probiotics. *Iranian Journal of Fisheries Sciences*, 24(**6**), 1359-1380. DOI:10.22092/ijfs.2025.134574

Adineh, H., Naderi, M., Jafaryan, H., Khademi Hamidi, M., Yousefi, M., and Ahmadifar, E., 2022. Effect of stocking density and dietary protein level in biofloc system on the growth, digestive and antioxidant enzyme activities, health, and resistance to acute crowding stress in juvenile common carp (*Cyprinus carpio*). *Aquaculture Nutrition*, 1, 9344478. DOI:10.1155/2022/9344478

Areekijseree, M., Engkagul, A., Kovitvadhi, U., Thongpan, A., Mingmuang, M., Pakkong, P. and Rungruangsak-Torrissen, K., 2004. Temperature and pH characteristics of amylase and proteinase of adult freshwater pearl mussel, *Hyriopsis* (*Hyriopsis*) *bialatus* Simpson 1900. *Aquaculture,* 234, 575–587. DOI:10.1016/j.aquaculture.2003.12.008

Avnimelech, Y. and Kochba, M., 2009. Evaluation of nitrogen uptake and excretion by tilapia in bio floc tanks, using 15N tracing. *Aquaculture*, 287(**1-2**), 163-168. DOI:10.1016/j.aquaculture.2008.10.009

Bernfeld, P., 1951. Amylases α and β. In: Colowick, P. and Kaplan, N.O. (Eds.)

Methods in enzymology. Academic Press, New York. pp 149–157.

Borlongan, I., 1990. Studies on the digestive lipases of milkfish, *Chanos chanos*. *Aquaculture,* 89**,** 315-325. DOI:10.1016/0044-8486(90)90135-A

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry,* 72, 248-254.

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

Ekasari, J., Azhar, M. H., Surawidjaja, E.H., Nuryati, S., De Schryver, P. and Bossier, P., 2014. Immune response and disease resistance of shrimp fed biofloc grown on different carbon sources. *Fish & Shellfish Immunology*, 41(**2**), 332-339. DOI:10.1016/j.fsi.2014.09.004

Emerenciano, M. G.C., Khanjani, M. H., Sharifinia, M. and Miranda-Baeza, A., 2025. Could biofloc technology (BFT) pave the way toward a more sustainable aquaculture in line with the circular economy? *Aquaculture Research*, 1, 1020045. DOI:10.1155/are/1020045

Ghanei-Motlagh, R., Mohammadian, T., Gharibi, D., Menanteau-Ledouble, S., Mahmoudi, E., Khosravi, M., Zarea and El-Matbouli, M., 2019. Quorum quenching properties and probiotic potentials of intestinal associated bacteria in Asian sea bass Lates calcarifer. *Marine Drugs*, 18(**1**), 23. DOI:10.3390/md18010023

Gullian-Klanian, M., Quintanilla-Mena, M. and Hau, C.P., 2023. Influence of the biofloc bacterial community on the digestive activity of Nile tilapia (*Oreochromis niloticus*). *Aquaculture*, 562, 738774. DOI:10.1016/j.aquaculture.2022.738774

Hargreaves, J.A., 2013. Biofloc production systems for aquaculture. *Southern Regional Aquaculture Center Publication*, No. 4503.

Haridas, H., Chadha, N.K., Sawant, P.B. Deo, A.D., Ande, M.P., Syamala, K., Sontakke, R. and Lingam, S.S., 2021. Growth and digestive enzyme responses of grey mullet reared in biofloc systems. *Aquaculture Research*, 52, 4923–4933. DOI:10.1111/are.15319

Liu, G., Ye, Z., Liu, D Ye, Z., Liu, D., Zhao, J., Sivaramasamy, E., Deng, Y. and Zhu, S., 2018. Influence of stocking density on growth and digestive enzymes in biofloc systems. *Fish & Shellfish Immunology*, 81, 416–422. DOI:10.1016/j.fsi.2018.07.033

Long, L., Yang, J., Li, Y., Guan, C. and Wu, F., 2015. Effect of biofloc technology on growth and digestive enzyme activity of tilapia. *Aquaculture*, 448, 135–141. DOI:10.1016/j.aquaculture.2015.05.017

Luo, G., Gao, Q., Wang, C., Liu, W., Sun, D., Li, L. and Tan, H., 2014. Growth, digestive activity and cost-effectiveness of tilapia cultured in biofloc systems. *Aquaculture*, 422–423, 1–7. DOI:10.1016/j.aquaculture.2013.11.023

Mahmoudi, N., Saeedi, N., Fakharzadeh, S.M.E. and Khanjani, M.H., 2025. Biofloc application in carp polyculture: impacts on water quality, growth, and immune-antioxidant responses. *Aquaculture International*, 33(**6**), 576. DOI:10.1007/s10499-025-02243-x

Mohammadian, T., Alishahi, M., Tabandeh, M., Ghorbanpoor, M. and

Gharibi, D., 2017. Effects of *Lactobacillus* spp. on digestive enzymes in *Tor grypus*. *Iranian Journal of Fisheries Sciences*, 16, 296–317. DOI: 20.1001.1.15622916.2017.16.1.23.4

Mohammadian, T., Monjezi, N., Peyghan, R. and Mohammadian, B., 2022. Effects of dietary probiotics on growth and digestive enzymes of common carp. *Aquaculture*, 549, 737787. DOI:10.1016/j.aquaculture.2021.737787

Robles-Porchas, G.R., Gollas-Galván, T., Martínez-Porchas, M., Martínez-Cordova, L.R., Miranda-Baeza, A. and Vargas-Albores, F., 2020. Nitrification in biofloc aquaculture systems. *Reviews in Aquaculture*, 12, 2228–2249. DOI:10.1111/raq.12416

Xu, W.J. and Pan, L.Q., 2014. Evaluation of dietary probiotics in biofloc culture system for *Litopenaeus vannamei*. *Aquaculture*, 428–429, 36–40. DOI:10.1016/j.aquaculture.2014.02.032

Yang, W., Sun, H., Xiang, F., Yang, Z. and Chen, Y., 2011. Response of crucian carp to long-term ammonia exposure. *Journal of Freshwater Ecology*, 26, 563–570. DOI:10.1080/02705060.2011.596414