Research Article

Evaluation of Growth Indices, Feed Utilization, Survival and Body Composition of Climbing Perch (*Anabas testudineus*) Reared under Different Feeding Rates in a Biofloc Tank System

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Abstract | This study aimed to evaluate the impact of varying feeding rates on the growth performance, survival, feed utilization, and proximate composition of *Anabas testudineus* cultured in a biofloc system without sludge removal. Fingerlings (mean weight: 1.99±0.06 g) were stocked at a density of 450 fish/m^3 in twelve biofloc tanks (capacity: 480 L) and reared for 112 days with a commercial diet (36% crude protein) at four feeding rates: 0% (FR-0), 2% (FR-2), 4% (FR-4), and 6% (FR-6) of body weight per day (BW/day) in triplicates. The results showed that all growth metrics significantly improved ($p < 0.05$) with increased feeding rates, although the rate of increase was lower beyond FR-4. Linear and quadratic regressions ($R^2 > 0.95$) demonstrated strong correlations between feeding rates and growth indices. The feed conversion ratio increased, while the protein efficiency ratio declined at higher feeding levels. The survival rate and condition factor were significantly lower ($p < 0.05$) in the unfed group (FR-0), while body protein content increased with feeding rate. Based on broken-line regression estimation, a feeding level of 4.65-4.75% of BW/day is recommended as optimal for maximizing biological performance without compromising growth and feed efficiency in *A. testudineus* under biofloc conditions.

Keywords | Climbing perch, Feeding rate, Growth, FCR, biofloc, Optimization

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INTRODUCTION

The climbing perch (*Anabas testudineus*), locally known as koi, is a commercially important freshwater fish widely distributed in Southeast Asia and the Indian subcontinent, including Bangladesh (Shahariar *et al.*, 2025). This indigenous air-breathing fish has gained remarkable popularity due to its exceptional taste and flavor, high iron, copper, and protein content, and adaptability to diverse environmental conditions in stagnant or sluggish water bodies (Islam *et al.*, 2013; Debnath *et al.*, 2022). Aquaculture is increasingly evolving from traditional extensive methods to more intensive systems driven by the need for greater productivity and sustainability while using limited resources (Jega *et al.*, 2018). Most fish farming is still conducted in earthen ponds, but novel technologies such as biofloc technology (BFT) present a promising option that remains underutilized for many fish species (Islam *et al.*, 2020; Khan *et al.*, 2021). The climbing perch could be an excellent candidate for BFT due to its omnivorous feeding habits, foraging feeding behavior, accessory respiratory organs, and ability to survive in low-oxygen environments (Debnath *et al.*, 2025).

BFT is an emerging technique in which the addition of a carbon source promotes the growth of heterotrophic bacteria, facilitating the conversion of organic nitrogenous waste into protein-rich microbial floc particles (Avnimelech, 2009). The floc generated in this system not only helps preserve water quality but also serves as a supplementary food source containing essential micro- and macronutrients, thereby enhancing feed efficiency and growth (Emerenciano *et al.*, 2017; Mahanand and Pandey, 2022). The effects of BFT on growth, physiology, and overall welfare have been extensively studied in several species, such as tilapia (Ekasari, 2015; Azim and Little, 2008), shrimp (Liu *et al.*, 2017), carp (Habib *et al.*, 2022), and catfish (Putra *et al.*, 2017; Zafar *et al.*, 2021; Zaidy *et al.*, 2022). However, the application of BFT for climbing perch is limited; notably, Debnath *et al.* (2022) optimized its stocking density in a simple biofloc tank system, and Hanafie *et al.* (2023) compared the effectiveness of rearing the species in biofloc with a conventional system.

Feed and feeding management account for approximately 50–60% of total aquaculture production costs (Parvez *et al.*, 2024). Effective feed management attributes, such as feeding rate and frequency, timing of feeding, feeding methods, feed type, feed intake, and nutrient absorption capacity, significantly influence fish production efficiency (El-Sayed, 1999; Parvez *et al.*, 2013). Among these attributes, understanding appropriate feeding rate is crucial for enhancing production efficiency and reducing disease risks and mortality associated with overfeeding or underfeeding. Both overfeeding and underfeeding can negatively impact water quality, fish digestion, growth, immunity, and overall composition (Davies *et al.*, 2006; Cho *et al.*, 2024). Several studies have explored the impact of feeding rates on growth, survival, feed intake, and body composition in various culturable finfish species farmed in clear-water conventional systems (Abbas and Siddiqui, 2009; Abidi and Khan, 2014; Oberg *et al.*, 2014; Luo *et al.*, 2015; Tian *et al.*, 2015; Silva *et al.*, 2007).

Typical feeding rates for fish in conventional aquaculture range from 1% to 5% of their body weight per day (BW/day). Several factors, primarily habitat (pond, cage, or tank), culture technology (clear-water, biofloc, recirculatory, or flow-through), and water quality (temperature, pH, ammonia, etc.), can influence the feeding rate and amount of major farmed fish (Liu *et al.*, 2023; Villes *et al.*, 2024). Similar to conventional aquaculture systems, the feeding rate is critical in BFT systems for controlling growth performance, survival, health status, and nutrient utilization because the interactions between feed input, microbial community structure, and nutrient recycling are closely linked. Research on feeding rate optimization in BFT for Nile tilapia (Mohammady *et al.*, 2023), koi (Duan *et al.*, 2025), olive barb (Thoufeek *et al.*, 2025), and Manchurian trout (Liu *et al.*, 2023) indicates that fish growth improves with increasing feed levels up to a certain optimal point, after which further increases result in slower growth and reduced feed efficiency. However, there is limited knowledge regarding suitable feeding rates for *A. testudineus* in biofloc tank culture systems. Therefore, this study aims to investigate the effects of different feeding rates on the zootechnical performance and proximate composition of *A. testudineus* cultured in an indoor biofloc tank system with no water exchange.

MATERIALS AND METHODS

Experimental design

The experiment was conducted in the biofloc laboratory complex of the Fisheries and Marine Resource Technology Discipline at Khulna University, Khulna, Bangladesh. Fingerlings of *A. testudineus* were reared for 112 days in an indoor biofloc tank system employing a completely randomized design with four different feeding rates, *viz.*, 0%, 2%, 4%, and 6% of BW/day, designated as FR-0, FR-2, FR-4, and FR-6, respectively, in triplicates.

Biofloc tank preparation and FCO production

We employed a simple and user-friendly biofloc system without periodic sludge removal. Twelve rectangular plastic tanks of 480 L capacity each (dimension: 40 inch (length) × 20 inch (width) × 36.5 inch (height)) were used to conduct the study. Each tank was connected to a one-horsepower (HP) capacity aerator via water hose pipes and air stones to ensure continuous aeration in the culture system. Before fifteen (15) days of stocking, all tanks were thoroughly washed with detergent and then with bleaching powder (60 ppm; 35% active chlorine) for disinfection. After disinfection, the tanks were filled with deep tube-well water to a height of approximately three-quarters, totaling around 335 liters. Bleaching powder was added to the tank water at a concentration of 25 ppm for further disinfection. Residual chlorine in the water was removed by providing vigorous aeration in the tanks.

To develop floc in the culture tanks, fermented carbon organic (FCO) was continuously produced under vigorous aeration for five days in a separate plastic bucket. The FCO was produced by fermenting a mixture containing 10 L of chlorine-disinfected water, 200 g of molasses, 10 g of NaCl, 20 grams of fish feed (36% crude protein), and 30 g of a commercial probiotic blended with (*Bacillus subtilis, B. licheniformis, Nitrosomonas europaea, Nitrobacter winogradskyi, Aspergillus oryzae, Trichoderma viride, Pseudomonas denitrificans, P. oxalaticus*). FCO was inoculated in the culture tanks before the stocking and during the culture when floc volume became below 30 mg/L.

Fish collection, acclimatization, and stocking

A total number of 2000 fingerlings of *A. testudineus* (mean weight: 1.99±0.06 g) from the same batch were obtained from a fish hatchery located in Jashore, Bangladesh. After procurement, the fish were transported to the laboratory complex with minimum stress by ensuring proper oxygenation facilities. Upon arrival, the fish were disinfected for 30-40 seconds using dilute $KMnO_4$ and acclimated in the culture tanks for 5 days. During the acclimation period, the fish were fed a commercial diet containing 36% protein at a satiation level. Average water temperature, pH, dissolved oxygen (DO), and total ammonia nitrogen (TAN) were maintained at optimal levels throughout acclimation. Following acclimation, the fish were randomly distributed into the twelve culture tanks for stocking at a rate of 450 fish/m^3 following the optimum density as reported by Debnath *et al.* (2022).

Feeding management

The fish were fed with a commercial extruded pellet that contained the following proximate composition: Crude protein 36%, lipid 6.5%, ash 11.3%, and moisture 10.6%. The initial feeding amount was determined by weighing the fish stocked in each tank. Subsequent feeding amounts were adjusted based on sampling at 15-day intervals when at least 30% of the fish were weighed to adjust feeding amount. The total amount of feed calculated according to the experimental feeding rates for a day was offered to the fish three times at 9:00 am, 2:00 pm, and 7:00 pm, dividing the feed into three equal parts.

Water quality management

Approximately 100 mL of FCO was added into each culture tank before stocking to start flocculation. Molasses was used as an organic carbon source to achieve a 15:1 C/N ratio. The C/N ratio was maintained by calculating the nitrogen produced from the fish's excretion plus uneaten feed (with the assumption that 75% of the feed-N ends up in the water) and the quantity of organic matter (molasses) needed to remove the nitrogen. The nitrogen volume production and the quantity of molasses as organic carbon required to maintain the ratio were schematically calculated according to the procedure described by Crab *et al.* (2012) and Mahanand and Pandey (2022). The initial floc target was set at 30 mL/liter. A 1-L Imhoff cone was used to measure the floc volume; it was filled with water from the tank and allowed to settle for 30 minutes, after which the volume was assessed using a graduated scale. Floc volume was measured at 7-days intervals, and a concentration above 30 ml/L was maintained by effective use of feed, molasses, and FCO when necessary. The other key water quality parameters, such as total ammonia nitrogen (TAN) and pH, were measured every alternate day using API test kits (Mars Fishcare North America, Inc., USA), while temperature and TDS were recorded once a week with a digital thermometer and a TDS meter, respectively. All water quality parameters were measured between 8:30 and 9:00 am before offering the first feed to fish.

Sampling and analysis

Growth metrics

At the end of the experiment, the fish were starved for about 24 hours before final harvesting. At the final harvest, all surviving fish were collected and mildly anesthetized using 2-phenoxyethanol at a dose of 0.3 ml/L. The weight at stocking and the weight at harvest of the fish in each tank were recorded for analyzing growth indices according to the procedures followed by Zablon *et al.* (2022): mean initial weight (IW) (g) = total weight (g) of fish stocked/ no. of fish stocked; mean final weight (FW) (g) = total weight (g) of survived fish at harvest/no. of fish survived at harvest; mean weight gain (WG) (g) = mean final weight (FW) (g) at harvest – mean initial weight (g) at stocking; mean percent weight gain (WG) (%) = (mean final weight (FW) (g) at harvest – mean initial weight (g) at stocking) × 100/mean initial weight (g) at stocking; absolute growth rate (GRabs) (g/d) = (final weight (g) – initial weight (g)) × 100/days of culture; specific growth rate (SGR) (%/day) = (ln final weight (g) – ln initial weight (g)) × 100/days of culture; and total yield (TY) (kg/m^3) = {(mean final weight (g) × no. of fish survived at harvest)/1000}/ volume (m^3).

Feed utilization

The amount of feed and calculated protein offered to the fish in each tank and the weight of fish obtained from the tank were used to calculate feed utilization parameters as follows: feed conversion ratio (FCR) = total weight of feed offered (g)/total live weight gain of fish (g); and protein efficiency ratio (PER) = total live weight gain of fish (g)/ total protein offered (g).

Survival and condition factor

The number of fish that survived at harvest was counted for the determination of survival rate (SR). To determine condition factor (CF), all surviving fish in each tank were weighed (g) individually by using a sensitive weighing balance and measured for total length (cm) by using a measuring board. The calculations for SR and CF were as follows: SR (%) = (final number of fish harvested/initial number of fish stocked) × 100; CF = (final live individual weight (g)/(final individual length (cm)^3) × 100.

Proximate composition analysis

After harvest, five fish from each tank (a total of fifteen fish from each feeding rate group) were anesthetized and processed to collect body muscle. The floc produced in the culture tanks was filtered and dried in the oven for analysis. The crude protein, lipid, ash, and moisture contents of the fish body muscle, dried floc, and commercial feed that was fed to the fish were determined according to the guidelines of the Association of Official Analytical Chemists

International (AOAC, 2012) at the fish nutrition and feed formulation laboratory of FMRT Discipline, Khulna University.

Crude protein was determined following the Kjeldahl method using an auto digestion-distillation system (Velp, Italy) with the formula (0.1 × burette reading × 6.25)/(sample weight × 100%), while crude lipid was extracted using chloroform and methanol solvent according to the procedures described by Bligh and Dyer (1959) with the formula: (final weight - crucible weight)/(sample weight × 100%). Ash content is determined by igniting the dried sample at approximately 600 °C for 8 hours in a muffle furnace. The residue is weighed and recorded as ash using the formula: (final weight - crucible weight)/sample weight × 100%. Moisture content was determined by drying a sample at 105 °C for 20 hours until a constant weight was achieved using the formula: (initial weight - final weight)/sample weight × 100%.

Statistical analysis

The statistical software 'IBM SPSS Statistics (version 27) was used to analyze all the collected data. All data were tested for normality using Shapiro–Wilk test, while homogeneity of variances was tested using Levene's test. Data deviated from normality were transformed using square-root transformation. If the data met the assumptions of normality and homogeneity of variance ($p > 0.05$), they were subjected to perform one-way ANOVA to identify significant differences among the treatment means, followed by multiple comparison tests using Tukey HSD at a 95% confidence interval ($p < 0.05$). If data didn't improve normality after transformation ($p < 0.05$), the non-parametric 'Kruskal–Wallis' test with pairwise comparison among treatment means was performed. Regression curve estimation was used to analyze both linear and quadratic effects of different feeding rates. Moreover, to determine the optimal feeding level for *A. testudineus,* a broken-line regression model (Robbins *et al*., 2006) was applied to evaluate weight gain (WG), specific growth rate (SGR), feed conversion ratio (FCR), and protein efficiency ratio (PER) in relation to varying feeding rates.

RESULTS

Key water quality parameters

Water temperature, dissolved oxygen (DO), and total dissolved solids (TDS) did not significantly differ among the feeding rate (FR) groups ($p > 0.05$) (Table 1). However, pH values showed a significant reduction ($p < 0.05$) in FR-2-6 compared to FR-0. Total ammonia nitrogen (TAN) concentrations were notably lower ($p < 0.05$) in fed groups (FR-2, FR-4, FR-6), with the lowest value observed in the FR-4 treatment. The TAN value in FR-6 was significantly higher ($p < 0.05$) than those of FR-2 and FR-4. The floc volume was similar in FR-2, FR-4, and FR-6 but significantly higher ($p < 0.05$) in those groups compared to FR-0.

Growth metrics

The growth trend of *A. testudineus* displayed a clear dependence on feeding rate, with progressively improved body weight across the fed treatments (Figure 1). While the initial weight (IW) of the stocked fish remained statistically similar across all groups ($p > 0.05$), the final weight (FW), weight gain (WG), percent weight gain (WG%), absolute growth rate (GRabs), specific growth rate (SGR), and total yield (TY) significantly increased ($p < 0.05$) with increasing feeding rates after being reared for 112 days (Table 2). Though the growth increased significantly with increasing feeding rates, the rate at which it increased was decreasing from F-4 to F-6 feeding rates compared to from F-0 to F-2 and F-2 to F-4. The fish reared with FR-6 outperformed in all the growth indices, obtaining significantly higher ($p < 0.05$) indices. Both linear and quadratic regression analyses revealed strong and significant relationships ($p < 0.001$) between feeding rate and growth indices (Table 2).

Feed utilization

Feed utilization efficiency varied significantly among the treatments (Figure 2). The feed conversion ratio (FCR) increased significantly ($p < 0.05$) with increasing feeding rates, reaching the highest value in the FR-6 group (Figure 2A). In contrast, the protein efficiency ratio (PER) peaked

Table 1: Key water quality parameters recorded in the biofloc tanks reared to *A. testudineus* fed diets with different feeding rates (mean ± SD).

Parameters	FR-0	FR-2	FR-4	FR-6
Temperature (°C)	$30.86±1.79^a$	$31.02±1.70^a$	$30.87±1.85^a$	$30.83±1.74^a$
pH	$8.10±0.17^a$	$8.01±0.18^b$	$7.99±0.20^b$	$8.03±0.21^c$
DO (mg/L)	$5.56±0.54^a$	$5.31±0.75^a$	$5.25±0.56^a$	$5.33±0.66^a$
TAN (mg/L)	$0.84±0.45^a$	$0.42±0.35^b$	$0.39±0.28^b$	$0.50±0.39^c$
TDS (mg/L)	$463.61±39.23^a$	$460.31±29.86^a$	$469.88±42.57^a$	$478.73±52.67^a$
Floc volume (ml/L)	$24.05±7.67^a$	$47.62±20.43^b$	$45.62±26.29^b$	$48.03±25.56^b$

*means ± SD in the same row with different superscripts are significantly different ($p < 0.05$).

Table 2: Growth performance of *A. testudineus* fed diets with different feeding rates (mean ± SD).

Growth indices	FR-0	FR-2	FR-4	FR-6	LR	R^2	QR	R^2
IW (g)	1.98±0.04[a]	1.98±0.07[a]	1.99±0.09[a]	2.00±0.04[a]	0.645	*	0.738	*
FW (g)	5.11±0.22[a]	9.96±0.11[b]	17.41±1.10[c]	22.07±1.24[d]	0.844	*	0.959	*
WG (g)	3.13±0.21[a]	7.98±0.17[b]	15.42±1.08[c]	20.08±1.24[d]	0.979	*	0.983	*
WG (%)	157.7 ± 10.1[a]	404.4 ± 22.1[b]	775.9 ± 57.1[c]	1005.2 ± 62.4[d]	0.978	*	0.983	*
GRabs (g/d)	0.03±0.00[a]	0.07±0.00[b]	0.14±0.01[c]	0.18±0.01[d]	0.977	*	0.981	*
SGR (%/d)	0.84±0.03[a]	1.44±0.04[b]	1.94±0.06[c]	2.14±0.05[d]	0.884	*	0.934	*
TY (kg/m^3)	1.64±0.26[a]	13.02±0.29[b]	22.21±1.99[c]	29.26±1.70[d]	0.987	*	0.995	*

*means ± SD in the same row with different superscripts in feeding rates (FR) are significantly different ($p < 0.05$); IW, initial weight; FW, final weight; WG GRabs, absolute growth; specific growth rate, SGR; TY, total yield; LR, linear regression; QR, quadratic regression; NS, no significance; *$p < 0.001$

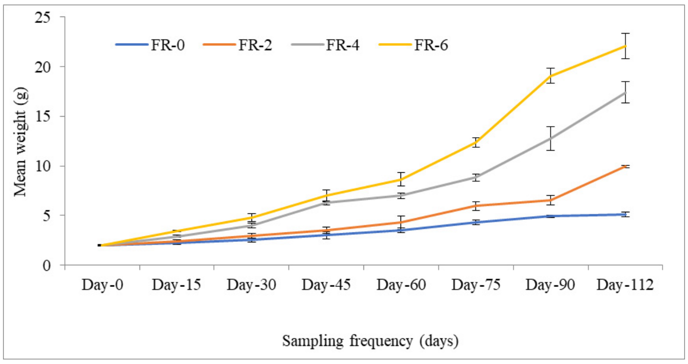

Figure 1: Growth pattern of *A. testudineus* fed diets with different feeding rates in the biofloc tank system during the culture period.

in the FR-2 group and declined gradually at the highest feeding level (FR-6) (Figure 2B). Regression analysis confirmed that FCR was significantly influenced ($p < 0.001$) by feeding rate (LR = 0.990; QR = 0.994), whereas the PER showed a significant quadratic relationship only (QR = 0.927; $p < 0.001$).

Survival and condition factor

The survival rate (SR) of *A. testudineus* significantly increased ($p < 0.05$) with increasing feeding rates, as shown in Figure 3. The highest survival was recorded in the FR-6 group, while the FR-0 group exhibited below 25% (Figure 3A). The condition factor (CF) of the fish in the FR-0 group was significantly lower than the fish in FR-2, FR-4, and FR-6, which varied insignificantly ($p > 0.05$) (Figure 3B). A strong linear and quadratic relationship was observed for survival (LR = 0.844; QR = 0.912; $p < 0.001$), while CF exhibited a lower regression fit (LR = 0.685; QR = 0.782; $p < 0.001$).

Proximate composition

The proximate composition of biofloc generated in the rearing tanks is presented in Table 3. Protein content in the biofloc significantly increased ($p < 0.05$) in all fed groups (FR-2 to FR-6) compared to the unfed group (FR-0), with the highest value recorded in the FR-6 group. However, lipid, ash, and moisture contents did not show significant variation ($p > 0.05$) across all FR groups.

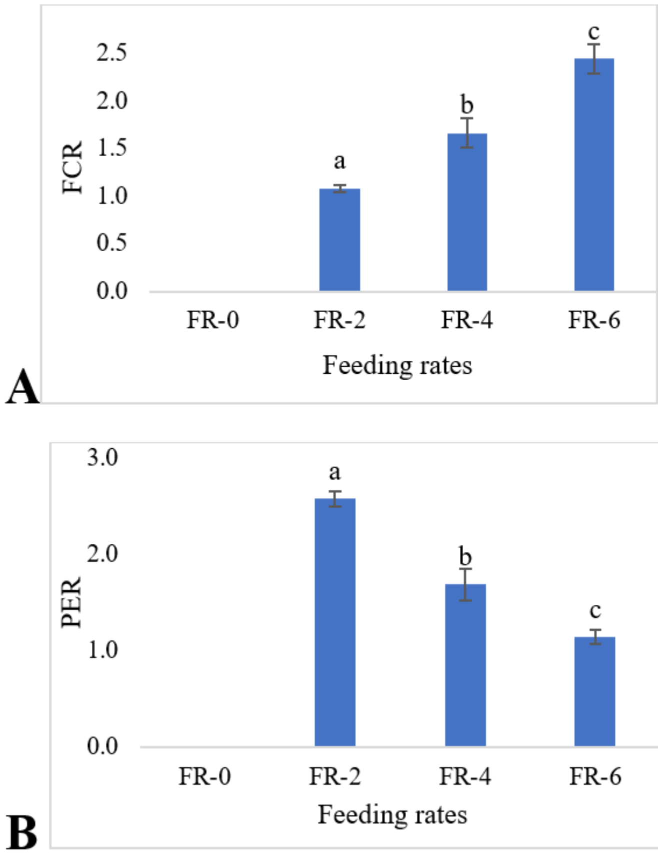

Figure 2: (a) Feed conversion ratio (FCR) and (b) protein efficiency ratio (PER) of *A. testudineus* fed diets with different feeding rates in the biofloc tank system (means ± SD in the same column with different superscripts are significantly different ($p < 0.05$); in FCR, LR = 0.990 (*) and QR = 0.994 (*), and in PER, LR = 0.581 (NS) and QR = 0.927 (*); NS, no significance; *$p < 0.001$).

The body composition of *A. testudineus* varied with feeding rate (Table 4). Body crude protein significantly increased ($p < 0.05$) in fish from FR-2 to FR-6 compared to FR-0, with the highest level observed in FR-4. Lipid and moisture content showed no significant differences among groups ($p > 0.05$). Ash content varied inconsistently, with a significant increase ($p < 0.05$) in the FR-4 group.

Table 3: Proximate composition (g/100g) of biofloc generated in the rearing tanks of *A. testudineus* fed diets with different feeding rates (mean ± SD).

Parameters	FR-0	FR-2	FR-4	FR-6
Protein	23.94±1.78^{a}	29.13±1.30^{b}	30.74±2.76^{b}	31.03±2.10^{b}
Lipid	7.07±0.59^{a}	6.68±2.03^{a}	7.13±0.87^{a}	6.06±4.42^{a}
Ash	26.37±3.35^{a}	24.81±2.07^{a}	28.78±3.10^{a}	27.89±1.35^{a}
Moisture	14.47±1.00^{a}	15.63±3.09^{a}	18.15±4.57^{a}	16.45±1.12^{a}

*means ± SD in the same row with different superscripts are significantly different ($p < 0.05$).

Table 4: Body composition (g/100g) of *A. testudineus* fed diets with different feeding rates (mean ± SD).

Parameters	FR-0	FR-2	FR-4	FR-6
Protein	13.27±1.65^{a}	16.39±2.03^{ab}	18.24±1.53^{b}	17.63±0.91^{b}
Lipid	6.51±1.12^{a}	8.86±1.77^{a}	7.96±1.33^{a}	8.15±1.94^{a}
Ash	4.59±0.58^{ab}	3.15±0.79^{a}	5.78±1.91^{b}	4.36±1.16^{ab}
Moisture	67.12±3.61^{a}	70.56±2.55^{a}	68.91±1.05^{a}	67.74±1.76^{a}

*means ± SD in the same row with different superscripts are significantly different ($p < 0.05$).

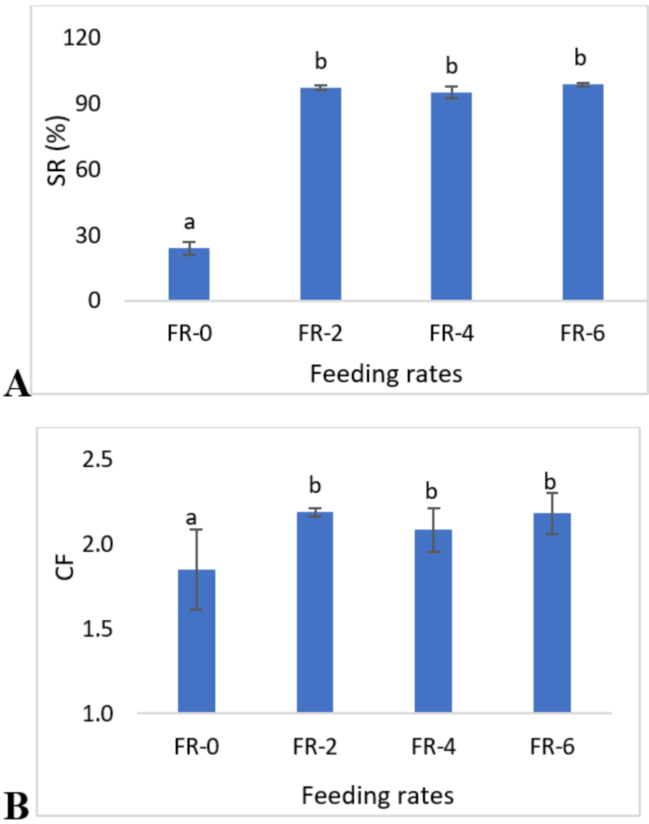

Figure 3: (A) Survival rate (SR) and B) condition factor (CF) of A. testudineus fed diets with different feeding rates in the biofloc tank system (means ± SD in the same column with different superscripts are significantly different ($p < 0.05$); in SR, LR = 0.844 (*) and QR = 0.959 (*), and in CF, LR = 0.685 (*) and QR = 0.782 (*); NS, no significance; *$p < 0.001$).

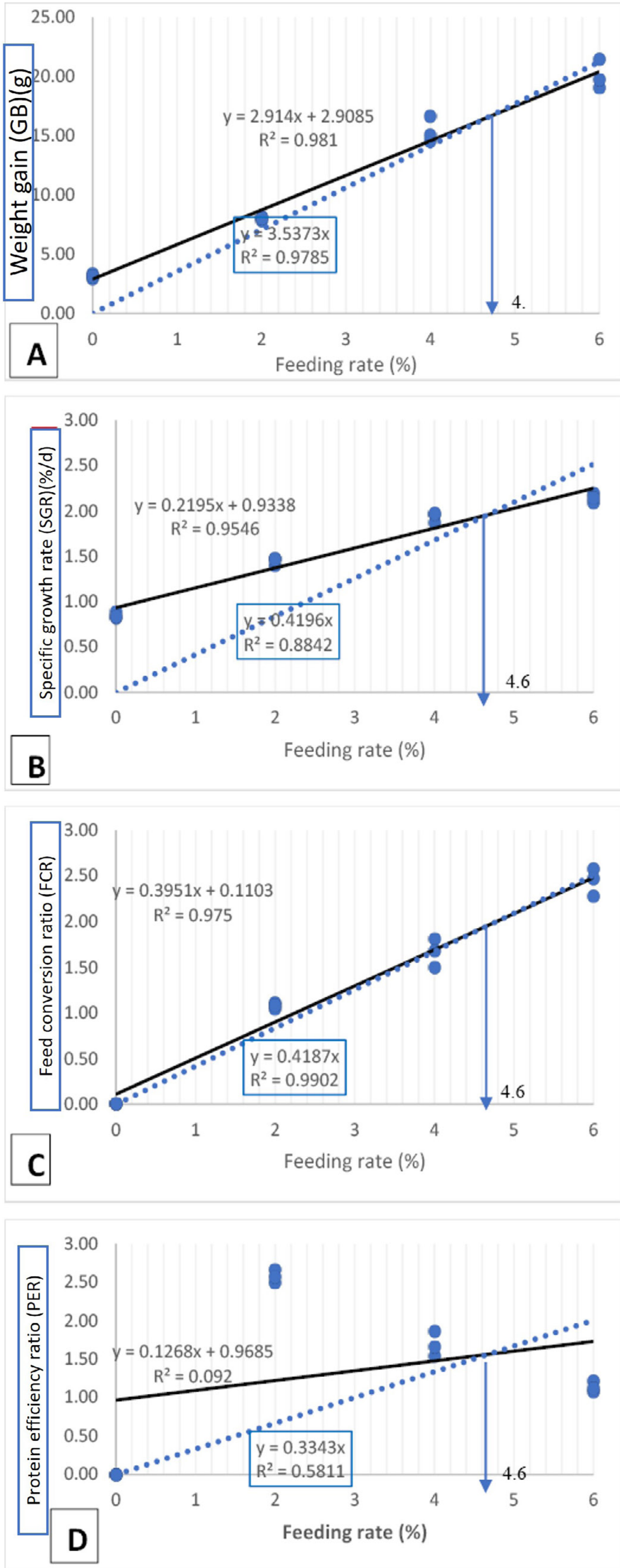

Figure 4: Broken-line regression of A) weight gain (WG) B) specific growth rate (SGR) C) feed conversion ratio (FCR), and D) protein efficiency ratio (PER) of A. testudineus fed diets with different feeding rates.

Optimum feeding rate estimation

The broken-line regression analysis (Figure 4) identified the optimal feeding rates based on mean WG and SGR to be approximately 4.75% and 4.65% of BW/day (Figure 4A, B), respectively. For feed utilization, the optimal FCR and PER were also estimated at feeding rates close to 4.65% (Figure 4C, D).

DISCUSSION

All key water parameters recorded in the rearing tanks at different feeding rates, except for floc volume in FR-0, remained within the optimal ranges for biofloc-based aquaculture systems throughout the rearing period, as reported by Mahanand and Pandey (2022) and Emerenciano *et al.* (2017). The optimal ranges are as follows: temperature (28-32 °C), pH (6.8-8.0), dissolved oxygen (DO) (4-7 mg/L), total ammonia nitrogen (TAN) (<1 mg/L), total dissolved solids (TDS) (<500 mg/L), and floc volume (30-50 ml/L). Water temperature, DO, and TDS were not significantly influenced ($p > 0.05$) by feeding rates across all treatments. However, pH values showed a slight reduction in the fed groups (FR-2-6), likely due to increased microbial activity and CO_2 production. TAN concentrations were significantly lower in the fed groups compared to the control (FR-0), with the lowest TAN recorded in FR-4, affirming the biofloc system's efficacy in maintaining nitrogen balance. These observations validate the functional premise of (BFT): heterotrophic bacterial communities can assimilate inorganic nitrogen into biomass, thus mitigating TAN accumulation (Avnimelech, 2009; Emerenciano *et al.*, 2017). However, the slightly elevated TAN in FR-6 suggests that microbial assimilation capacity may approach saturation under high feed inputs, particularly if carbon dosing or aeration becomes limiting. This emphasizes the need for careful balancing of feed input, carbon source, and system capacity, especially in commercial-scale operations.

Floc volume significantly increased ($p < 0.05$) in all fed groups (FR-2-6) compared to the non-fed group (FR-0). Floc accumulation in the non-fed group was below the recommended concentration of 30 mg/L. This under-accumulation of floc (<30 mg/L) in the feeding tanks may have disrupted the C:N ratio, leading to inefficient microbial biomass synthesis (Emerenciano *et al.*, 2017; Crab *et al.*, 2012). Since microbial biomass served as the sole dietary source in the non-fed tanks, its limited availability likely increased physiological stress, reduced immune competence, and compromised overall health. Consequently, the non-fed group exhibited a significantly lower survival rate (below 25%) and condition factor ($p < 0.05$), a key index of health and body robustness. This finding strongly suggests that biofloc alone could not supplement the nutrition, health, and survival of the fish. Biofloc is reported to be a rich source of diverse nutritional value, which has potential as a protein-sparing ingredient or as a partial feed supplement. However, complete replacement of commercially formulated feed with biofloc is not practical due to the fact that biofloc composition is highly variable with imbalanced nutrient balance, inadequate biomass, and its digestion and absorption inefficiency in fish (Crab *et al.*, 2012; Emerenciano *et al.*, 2017). The results align with observations that biofloc meal or in situ biofloc biomass is suitable as a partial replacement strategy rather than a total replacement, while providing BFT provides environmental, social, and economic benefits. In contrast, the significantly higher survival and condition factor observed in the fed groups can be attributed to reduced stress from adequate feed availability and improved immune responses due to biofloc supplementation with commercial feed. It has been well documented that biofloc supplements nutrition, enhances phagocytic activity, and contributes to nonspecific immune responses (Habib *et al.*, 2022; Cho *et al.*, 2024; Debnath *et al.*, 2025).

The growth parameters, including final weight (FW), weight gain (WG), percentage weight gain (WG%), specific growth rate (SGR), absolute growth rate (GRabs), and total yield (TY), all increased significantly ($p < 0.05$) with ascending feeding rates (FR-0 to FR-6). However, the rate of increase was lower in FR-6 compared to FR-4 and FR-2. Both linear and quadratic regression analyses revealed strong relationships ($p < 0.001$) between feeding rate and growth indices ($R^2 > 0.95$). The observed improvements in final weight, weight gain, and specific growth rate at higher feeding levels observed in this study are consistent with earlier reports from other BFT-based trials aimed at optimizing feeding regimes for various aquaculture species, including tilapia (Silva *et al.*, 2019; Mohammady *et al.*, 2023), African catfish (Zaidy *et al.*, 2022), olive barb (Thoufeek *et al.*, 2025), trout (Barani *et al.*, 2019; Liu *et al.*, 2023), Indian major carp (Abidi and Khan, 2014), and koi (Duan *et al.*, 2025).

Despite the clear benefits of increasing feeding rates, the broken-line regression in this study estimated optimal feeding rates for maximum WG and SGR to be approximately 4.75% and 4.65% of BW/day, respectively. This suggests that while growth performance improves with higher feeding rates up to an optimal point, biological efficiency in weight gain does not increase linearly beyond this point. Feeding rates above this plateau may not enhance metabolic utilization due to limitations in digestion, absorption, or metabolic turnover (Baloi *et al.*, 2017; Lee *et al.*, 2015). Several authors, including Villes *et al.* (2024), Thoufeek *et al.* (2025), and Liu *et al.* (2023), have discussed the concept of a feeding plateau, affirming that biological optima do not always align with maximum yield, particularly in systems that prioritize sustainable resource use. These authors noted that fish often exhibit declining feed assimilation efficiency once their nutritional requirements are met or exceeded.

In this study, the feed conversion ratio (FCR) was calculated only for the commercial feed provided at different rates (FR-2 to FR-6). Since no feed was provided for the fish reared in the FR-0 group, it was not possible to calculate the FCR for this group. Consequently, a zero (0) FCR for the FR-0 group was not considered for comparison. We used this feeding rate group to assess whether the floc generated in the system alone could support growth and survival as effectively as the fed groups, which received a combination of floc and commercial feed. Unfortunately, it was found that the floc in this group could not support three-fourths of the total stocked fish (survival rate <25%), and the surviving fish exhibited very poor growth. In fed groups, the FCR significantly increased ($p < 0.05$) with higher feeding rates, while the protein efficiency ratio (PER) peaked at FR-2 and then declined significantly ($p < 0.05$) at the highest feeding level (FR-6). This indicates reduced protein assimilation efficiency at overfeeding levels. Broken line regression analysis revealed a significant quadratic relationship ($p < 0.001$) between feeding rate and both FCR and PER, with optimal feed efficiency occurring at approximately 4.65% of BW/day. Silva *et al.* (2019) observed in Nile tilapia that while higher feeding levels improved growth, the FCR tended to increase alongside decreasing PER values, indicating wastage and over-satiation with lower protein assimilation.

The overall nutritional composition of biofloc produced in the different rearing tanks in this study was comparable to the floc compositions reported by several researchers who used molasses as a carbon source (Zafar *et al.*, 2021; Debnath *et al.*, 2025). The lipid, ash, and moisture contents of the floc remained unaffected ($p > 0.05$) by feeding rates. However, higher feeding rates were associated with increased protein content in the biofloc. This is likely due to greater nutrient influx and microbial activity, as elevated organic inputs enrich biofloc with microbial protein through stimulated microbial proliferation, provided an appropriate C:N ratio is maintained (Crab *et al.*, 2012). The consistent supply of waste from fish and feed, along with the addition of molasses and FCO in the fed groups, likely maintained a congenial environment with an appropriate C:N ratio (15:1) for microbes to assimilate nitrogenous waste (TAN) into microbial biomass as floc. In contrast, the non-fed group may have disrupted flocculation due to regular mortality in the tanks.

Body protein content of the fish significantly increased in the fed groups compared to the unfed group, with the highest value recorded in FR-4, followed by FR-6. This suggests efficient protein assimilation under moderate feeding conditions. Several studies have found that fish reared in BFT exhibit increased body protein content, particularly when dietary protein is supplemented through floc consumption. However, the reduction in the protein efficiency ratio (PER) at FR-6 in our study, despite high body protein content, indicates a possible inefficiency in converting dietary protein into growth. In this experiment, all water quality parameters, including total ammonia nitrogen (TAN), remained within suitable ranges for fish culture, although FR-6 exhibited higher TAN levels than FR-4. The elevated TAN levels in FR-6 could have stressed the fish, disrupted their metabolism, and limited their ability to utilize protein effectively (Avnimelech, 2009; Azim and Little, 2008). In other words, excess feeding may lead to overfeeding and increased nitrogen excretion, resulting in metabolic wastage and poor water quality, which likely combine to impair protein utilization at an excessive feeding rate (FR-6).

The microbial floc generated in the biofloc system has been reported to contain proteins, lipids, minerals, vitamins, bioactive compounds, and digestive enzymes (Ogello *et al.*, 2021; Mahanand and Pandey, 2022). These nutrients underscore the potential for using floc in various dietary applications for fish, offering numerous benefits for improving zootechnical performance, feed utilization, survival, health, and immunity (Azim and Little, 2008; Zablon *et al.*, 2022). The biofloc environment likely enhances the nutritional quality of the water and supplements feed intake through microbial biomass, contributing to elevated growth at higher feeding rates. Feeding below optimal levels in BFT systems may compromise growth and survival, while excessive feed input can lead to surplus nitrogen and organic matter that may not be fully assimilated by the microbial community. This can result in increased waste accumulation, water fouling, potential stress, and reduced feed efficiency, ultimately leading to decreased growth, feed efficiency, and economic profit. For instance, Thoufeek *et al.* (2025) reported improved growth performance and feed utilization efficiency in olive barb (*Systomus sarana*) cultured in BFT when fed at 1-3% of BW/day, with performance declining above these feeding rates potentially due to feed and nutrient wastage. Similarly, a lower feeding level of 2-4% BW/day in Nile tilapia (*Oreochromis niloticus*) resulted in higher growth performance, potentially due to reduced oxidative stress from the phenolic compounds present in the biofloc, as observed by Villes *et al.* (2024).

CONCLUSION

The findings of this study indicate that fingerlings of climbing perch fed at increasing rates up to 6% of BW/

day exhibited improved growth metrics. However, while higher feeding rates promoted growth, excessive feeding at these levels resulted in reduced feed utilization efficiency. The optimal feeding rate for maximizing biological performance without compromising growth and feed efficiency was determined to be between 4.65% and 4.75% of BW/day, according to the broken line regression analysis. The non-fed group (FR-0) supported the maintenance of less than 25% of the total fish with negligible growth, indicating that floc produced in the BFT system alone could not sustain comparable growth. Further evaluation of long-term impacts and economic analysis is recommended for the species at extended feeding levels. These findings provide practical recommendations for optimizing feeding strategies in *A. testudineus* culture and offer insights for aqua entrepreneurs aiming to advance biofloc-based aquaculture, particularly in water-scarce and resource-limited urban areas.

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NOVELTY STATEMENT

Limited research exists on the BFT protocol for climbing perch, although several studies have optimized BFT protocols, including feeding rates for various fish species. Previous research on the development of culture protocols for climbing perch in biofloc tanks has primarily focused on optimizing stocking density and evaluating system effectiveness, while the appropriate feeding rate and amount remain largely unexplored. This study is the first to determine an optimized feeding rate for climbing perch in biofloc tanks, offering valuable insights into the development of the sustainable BFT protocol for this species.

AUTHOR'S CONTRIBUTION

Sharmin Aktar: Data analysis, funding, original draft preparation; Abdus Samad: Execution of experiment and laboratory analysis; Nusrat Jahan: Data interpretation and statistical analyses, laboratory analysis; Sanjida Yeasmen Urmi: Methodology, original draft preparation; Md. Shahin Parvez: Supervision, funding, review and editing of draft manuscript; and Md. Nazmul Ahsan: Funding, resources, and review & editing of reviewed manuscript

Ethical statement

The study was carried out according to the guidelines of animal ethical procedures of the Khulna University.

Generative AI and AI-assisted technology statement

All the authors of this article declare that generative AI technologies, including large language models (e.g., ChatGPT, Copilot, etc.) and text-to-image generators, were not employed in the preparation, writing, or editing of this manuscript

Conflict of interest

The authors declare that they have no known competing financial or non-financial, professional, or personal conflicts or relationships that could have appeared to influence the work reported in this manuscript.

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