

Effects of carbon-to-nitrogen ratios on sex-reversed climbing perch (*Anabas testudineus*) in biofloc systems

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Abstract. Biofloc technology is a sustainable aquaculture strategy that enhances nutrient recycling and water quality. However, optimizing the carbon-to-nitrogen (C/N) ratio is essential for maximizing production efficiency. This study evaluated the effects of four C/N ratios (10:1, 15:1, 20:1, and 25:1) on growth performance, feed utilization, carcass traits, whole-body proximate composition, water quality, biofloc characteristics, and economic efficiency of sex-reversed climbing perch (*Anabas testudineus*) cultured in biofloc systems. Fish were reared for 8 weeks in a completely randomized design with 4 treatments and 3 replicates per treatment. The C/N ratio significantly affected growth performance, feed utilization, carcass traits, biofloc characteristics, several water quality parameters, and production cost ($p < 0.05$). Fish cultured at C/N 20:1 achieved the highest final weight (15.94 ± 0.77 g) and the lowest feed conversion ratio (1.82 ± 0.02), together with favorable water quality and the lowest total feed and carbon cost (1.41 ± 0.04 USD kg^{-1} of weight gain). Although C/N 25:1 produced the highest floc volume and total suspended solids, C/N 20:1 resulted in the most favorable overall growth and feed utilization performance among the tested treatments. Overall, among the tested C/N ratios, 20:1 provided the most favorable balance between microbial activity, nutrient utilization, water quality, fish performance, and economic efficiency, supporting sustainable biofloc-based culture of sex-reversed climbing perch.

Keywords: biofloc technology, carbon-to-nitrogen ratio, growth performance, water quality.

Introduction. Freshwater aquaculture is one of the fastest-growing food production sectors and plays a vital role in global food security, income generation, and rural development, particularly in Southeast Asia where the demand for freshwater fish continues to increase. Among indigenous species, climbing perch (*Anabas testudineus*) has attracted considerable interest because of its high market value, consumer acceptance, air-breathing ability, and tolerance to adverse environmental conditions, including low dissolved oxygen (Hanafie et al., 2023). The application of sex-reversal technology has further enhanced production efficiency by improving growth uniformity and biomass yield, making *A. testudineus* a promising species for intensive aquaculture (Pengseesang et al., 2024). However, intensive production systems often result in the accumulation of organic matter and toxic nitrogenous compounds, particularly ammonia and nitrite, which impair water quality, growth performance, feed utilization, and fish health (Avnimelech, 1999; Crab et al., 2007). Biofloc technology (BFT) is a sustainable aquaculture strategy that improves water quality and nutrient recycling by stimulating heterotrophic microorganisms to convert inorganic nitrogen into microbial biomass, which serves as an additional source of nutrients for cultured fish (Dheeran et al., 2025; Emerenciano et al., 2017; Zablon et al., 2022). The efficiency of this process largely depends on maintaining an appropriate carbon-to-nitrogen (C/N) ratio, which regulates microbial activity, nitrogen assimilation, biofloc development, and overall system stability

(Avnimelech, 2012; Liu et al., 2018; Saha et al., 2022; Sarker et al., 2026). Previous studies have demonstrated that optimized C/N ratios improve growth performance, feed utilization, body composition, and water quality in a range of aquaculture species cultured in biofloc systems (Huang et al., 2022; Zahra & Mateen, 2025). Conversely, excessive carbon addition may increase suspended solids, alter microbial dynamics, and reduce production efficiency, indicating that the optimal C/N ratio is species- and culture-specific (Khanjani & Sharifinia, 2024; Muthoka et al., 2026).

Although BFT has been increasingly applied in the culture of *A. testudineus*, studies have primarily focused on stocking density, salinity, or general biofloc management. Information regarding the optimal C/N ratio for improving growth performance, feed utilization, carcass traits, whole-body composition, water quality, and economic efficiency in sex-reversed *A. testudineus* remains limited (Das et al., 2025; Fitriani et al., 2025; Hanafie et al., 2023; Rahman et al., 2024). Therefore, this study aimed to evaluate the effects of different C/N ratios on the performance of sex-reversed *A. testudineus* cultured in biofloc systems. The findings provide practical guidance for optimizing C/N management to improve productivity, water quality, and economic sustainability in biofloc-based freshwater aquaculture.

Material and Method

Experimental site and duration. The experiment was conducted at the Aquaculture Research Laboratory, Department of Agricultural Technology, Faculty of Liberal Arts and Science, Sisaket Rajabhat University, Thailand in March 2026. Prior to the experiment, the fish were obtained from the Department of Fisheries, Thailand, and acclimated for 7 days in a 2,500 L fiberglass tank at a stocking density of 500 fish per tank. During acclimation, the fish were fed a commercial diet containing not less than 32% crude protein at 5% of body weight per day, divided into 2 meals. Continuous aeration was provided 24 h per day. After acclimation, fish of similar size were selected and randomly allocated to the experimental tanks at 30 fish per tank. The study included a 14-day biofloc preparation period to establish microbial communities and stabilize culture conditions, followed by an 8-week feeding trial. Growth performance, feed utilization, biofloc characteristics, water quality, carcass traits, whole-body proximate composition, and economic efficiency were evaluated throughout the experimental period.

Experimental fish. Sex-reversed *A. testudineus*, approximately 2 months old, were obtained from the Department of Fisheries, Thailand. According to the supplier, the fish had undergone feminization using 17 β -estradiol (E2) during the early gonadal differentiation stage. The supplier reported that the protocol was based on immersion of approximately 7-day-old fry in E2 at 2.0 mg L⁻¹ for 2 h, followed by rearing on a hormone-free diet until approximately 2 months of age, consistent with previously reported procedures for *A. testudineus* (Nguyen et al., 2024). The sex-reversed status of the supplied fish was established by the supplier prior to transfer to the experimental facility. Because the feminization treatment was completed before acquisition, the present study did not independently evaluate the efficacy of sex reversal or repeat the gonadal sex-verification procedure. The fish were therefore treated as a pre-existing sex-reversed experimental stock. The objective of the present study was to evaluate the effects of C/N ratios on growth performance, feed utilization, carcass traits, whole-body proximate composition, water quality, biofloc characteristics, and economic efficiency of sex-reversed *A. testudineus*, rather than to assess the efficacy of the feminization treatment.

Experimental feed and carbon source. A commercial floating diet containing 32% crude protein was used as the basal feed throughout the experiment. Molasses was applied as an external carbon source to adjust and maintain the target C/N ratios. The amount of molasses required was calculated based on feed nitrogen input following Avnimelech (1999), assuming that 75% of dietary nitrogen was released into the water

and molasses contained approximately 37% carbon. Carbon supplementation was adjusted according to daily feed input to maintain the designated C/N ratios.

Biofloc system preparation. The experiment was conducted in glass aquaria (45×90×46 cm, width×length×height) with a water depth of about 30 cm. Twelve aquaria were used as experimental units. Continuous aeration was provided using air stones connected to an air blower to maintain dissolved oxygen levels and support biofloc development. Before fish stocking, biofloc systems were activated for 14 days using molasses and microbial starter to stimulate microbial biomass formation and nitrogen assimilation. After activation, C/N ratios were adjusted to the experimental levels before fish stocking.

Experimental design and fish stocking. The experiment was conducted using a completely randomized design (CRD) with four C/N ratio treatments and three replicates per treatment: T1 (10:1), T2 (15:1), T3 (20:1), and T4 (25:1). A total of 360 fish were randomly allocated to 12 glass aquaria at a stocking density of 30 fish per aquarium, with each treatment assigned three independent experimental units.

Carbon-to-nitrogen ratio management. The target C/N ratios (10:1, 15:1, 20:1, and 25:1) were maintained by adding molasses as an external carbon source. The nitrogen input from feed was calculated from daily feed intake, dietary crude protein content (32%), and the nitrogen fraction of protein (6.25). Assuming that 75% of dietary nitrogen was released into the culture water, the daily nitrogen release and corresponding carbon requirement were calculated as follows:

$$\begin{aligned}N_{\text{feed}} (\text{g day}^{-1}) &= \text{Feed intake} (\text{g day}^{-1}) \times 0.32 / 6.25 \\N_{\text{released}} (\text{g day}^{-1}) &= N_{\text{feed}} \times 0.75 \\C_{\text{required}} (\text{g day}^{-1}) &= N_{\text{released}} \times \text{Target C/N ratio}\end{aligned}$$

The liquid molasses contained 80% dry matter (DM), according to the product label, and 37% carbon on a dry-weight basis. The required amount of molasses dry matter was calculated as:

$$M_{\text{DM}} (\text{g day}^{-1}) = C_{\text{required}} / 0.37$$

The corresponding amount of liquid molasses was calculated as:

$$M_{\text{liquid}} (\text{g day}^{-1}) = M_{\text{DM}} / 0.80 = C_{\text{required}} (0.37 \times 0.80)$$

The calculated amount of molasses was pre-dissolved in culture water and evenly distributed into the respective aquaria daily after feeding. The molasses dose was adjusted daily according to the actual feed intake in each aquarium to maintain the designated C/N ratio throughout the experimental period.

Feeding management. Fish were fed a commercial floating diet containing 32% crude protein twice daily at 08:00 and 17:00 h. The initial feeding rate was 5% of biomass per day and was adjusted every 2 weeks based on estimated fish biomass. Daily feed intake was recorded for feed utilization calculations.

Growth performance and feed utilization analysis. Fish were randomly sampled from each aquarium at 2-week intervals to estimate mean body weight and adjust the feeding rate. Approximately 30% of the fish in each aquarium (approximately 9 fish) were gently captured using a soft-mesh net, weighed, and immediately returned to the same aquarium. Sampling was performed at the tank level, and individual fish were not intended to be tracked longitudinally. Therefore, the sampled fish represented the population within each experimental unit at each sampling event rather than individually monitored fish. Handling time was minimized during capture, weighing, and transfer to reduce disturbance and potential handling stress. The same sampling procedure was

consistently applied to all tanks throughout the experiment. Growth performance and feed utilization were evaluated using the following equations:

$$\begin{aligned}\text{WG (g)} &= \text{Final weight (g)} - \text{Initial weight (g)} \\ \text{PWG (\%)} &= [(\text{Final weight} - \text{Initial weight}) / \text{Initial weight}] \times 100 \\ \text{SGR (\% day}^{-1}\text{)} &= [(\ln \text{ Final weight} - \ln \text{ Initial weight}) / \text{Culture period (days)}] \times 100 \\ \text{ADG (g fish}^{-1} \text{ day}^{-1}\text{)} &= (\text{Final weight} - \text{Initial weight}) / \text{Culture period (days)} \\ \text{SR (\%)} &= (\text{Final number of fish} / \text{Initial number of fish}) \times 100 \\ \text{FCR} &= \text{Total feed intake (g)} / \text{Weight gain (g)} \\ \text{FE (\%)} &= (1 / \text{FCR}) \times 100\end{aligned}$$

Where:

WG - weight gain;
PWG - percentage weight gain;
SGR - specific growth rate;
ADG - average daily gain;
SR - survival rate;
FCR - feed conversion ratio;
FE - feed efficiency.

Water quality analysis. Water quality parameters were monitored throughout the experimental period. Temperature, dissolved oxygen (DO), and pH were measured daily, whereas TAN and nitrite-N were analyzed twice weekly. Nitrate-N, alkalinity, total suspended solids (TSS), and floc volume were measured weekly.

Biofloc characterization. Biofloc characteristics were evaluated weekly by measuring floc volume (FV) and (TSS). Floc volume was determined using an Imhoff cone, where water samples collected from each aquarium were allowed to settle for 15–20 min before recording the settled biofloc volume (mL L⁻¹). Total suspended solids were analyzed to estimate the concentration of suspended microbial biomass and biofloc particles in each culture system.

Carcass trait analysis. At the end of the experiment, five fish per replicate were randomly selected for carcass evaluation. Fish were dissected to determine fillet, skeleton, liver, and visceral weights. The following indices were calculated:

$$\begin{aligned}\text{Fillet yield (\%)} &= (\text{Fillet weight} / \text{Body weight}) \times 100 \\ \text{Skeleton proportion (\%)} &= (\text{Skeleton weight} / \text{Body weight}) \times 100 \\ \text{Hepatosomatic index (HSI, \%)} &= (\text{Liver weight} / \text{Body weight}) \times 100 \\ \text{Viscerosomatic index (VSI, \%)} &= (\text{Visceral weight} / \text{Body weight}) \times 100\end{aligned}$$

Whole-body proximate composition analysis. At the beginning and end of the experiment, five fish per tank were randomly sampled for whole-body proximate composition analysis. Each tank contained 30 fish, and samples from the five fish collected from each tank were pooled to represent one experimental replicate (n=3 replicates per treatment). Samples were analyzed according to AOAC (2019) procedures to determine moisture, crude protein, crude lipid, and ash contents. All results were expressed on a wet-weight basis.

Economic analysis. Economic evaluation was conducted based on the costs of feed and molasses used during the experimental period. Feed cost and total production cost per gained kilogram (weight gain) were calculated as follows:

$$\begin{aligned}\text{Feed cost (USD kg}^{-1} \text{ gain)} &= \text{Total feed cost (USD)} / \text{Weight gain (kg)} \\ \text{Feed and carbon cost (USD kg}^{-1} \text{ gain)} &= \text{Total feed and molasses costs (USD)} / \text{Weight gain (kg)}\end{aligned}$$

The economic comparison was based on the feed and carbon cost per kilogram of weight gain among treatments.

Statistical analysis. All data are presented as mean±standard deviation (SD). Data were assessed for normality using the Shapiro–Wilk test and for homogeneity of variance using Levene’s test before statistical analysis. The effects of C/N ratios were analyzed using one-way analysis of variance (ANOVA) under CRD. When significant differences were detected, Duncan’s multiple range test (DMRT) was used to compare treatment means at $p<0.05$. All statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Results

Growth performance and feed utilization. The growth performance and feed utilization of sex-reversed *A. testudineus* cultured under different carbon-to-nitrogen (C/N) ratios in biofloc systems for 8 weeks are presented in Table 1 and Figure 1.

Table 1
Growth performance and feed utilization of sex-reversed *Anabas testudineus* cultured under different C/N ratios for 8 weeks

Parameter	C/N 10:1	C/N 15:1	C/N 20:1	C/N 25:1
Initial weight (g)	2.70±0.10	2.69±0.11	2.71±0.09	2.70±0.10
Final weight (g)	13.33±0.44 ^a	15.16±0.67 ^b	15.94±0.77 ^c	13.54±0.69 ^a
Weight gain (g)	10.63±0.44 ^a	12.46±0.67 ^b	13.24±0.77 ^c	10.84±0.69 ^a
PWG (%)	393.62±16.44 ^a	461.56±24.77 ^b	490.41±28.49 ^c	401.36±25.59 ^a
SGR (% day ⁻¹)	2.85±0.07 ^a	3.08±0.10 ^b	3.17±0.10 ^c	2.88±0.11 ^a
ADG (g fish ⁻¹ day ⁻¹)	0.188±0.008 ^a	0.222±0.012 ^b	0.237±0.014 ^c	0.192±0.011 ^a
ADFI (g day ⁻¹)	0.402±0.012 ^b	0.424±0.019 ^c	0.430±0.022 ^c	0.378±0.019 ^a
FCR	2.11±0.02 ^c	1.91±0.02 ^b	1.82±0.02 ^a	1.96±0.03 ^b
FE (%)	47.46±0.40 ^a	52.40±0.51 ^c	54.91±0.54 ^d	51.03±0.65 ^b
Survival (%)	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00

Values are presented as mean±SD (n=3 tanks per treatment). Different superscript letters within the same row indicate significant differences among treatments ($p<0.05$).

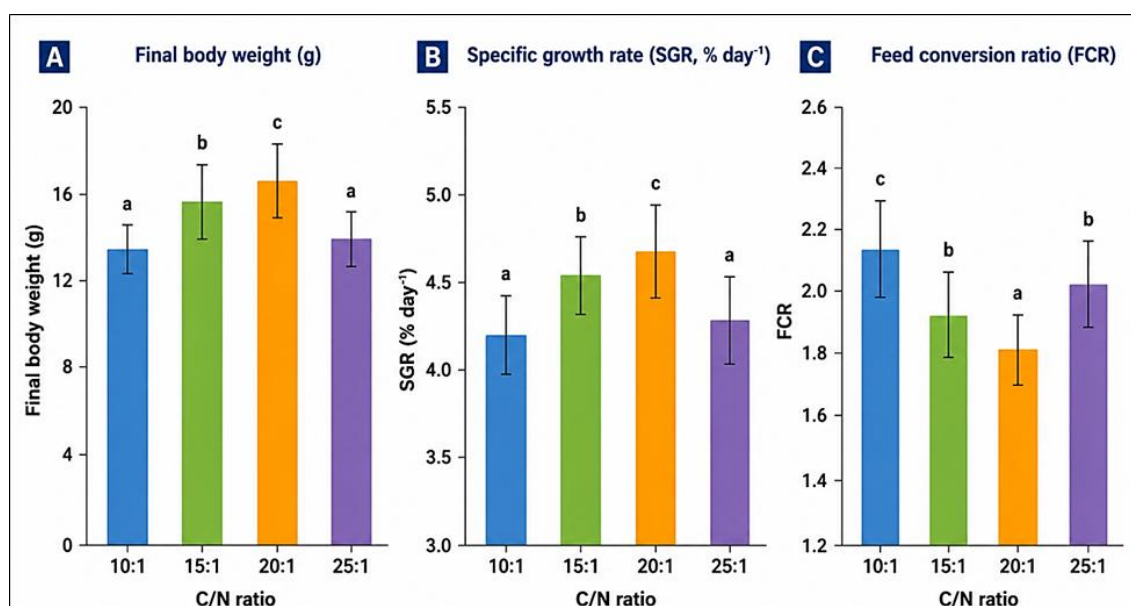


Figure 1. Effects of different carbon-to-nitrogen ratios on (A) final body weight, (B) specific growth rate (SGR), and (C) feed conversion ratio (FCR) of sex-reversed *Anabas testudineus* cultured in biofloc systems for 8 weeks. Bars represent mean±SD (n=3 tanks per treatment). Different lowercase letters indicate significant differences among treatments ($p<0.05$).

The initial body weight did not differ significantly among treatments ($p>0.05$), ranging from 2.69 to 2.71 g, with a coefficient of variation (CV) below 5%, indicating uniform fish distribution. C/N ratios significantly affected final weight, weight gain (WG), percentage weight gain (PWG), specific growth rate (SGR), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and feed efficiency (FE) ($p<0.05$). Fish cultured at C/N 20:1 exhibited the highest final weight (15.94 ± 0.77 g), WG (13.24 ± 0.77 g), PWG ($490.41\pm28.49\%$), SGR ($3.17\pm0.10\%$ day⁻¹), and ADG (0.237 ± 0.014 g fish⁻¹ day⁻¹), whereas the lowest growth performance was observed in the C/N 10:1 and 25:1 treatments. Feed utilization was significantly improved at C/N 20:1, which showed the lowest FCR (1.82 ± 0.02) and highest FE ($54.91\pm0.54\%$). In contrast, the C/N 10:1 treatment resulted in the highest FCR (2.11 ± 0.02) and lowest FE ($47.46\pm0.40\%$). Survival was 100% in all treatments throughout the experimental period.

Carcass traits and whole-body proximate composition. Carcass traits and whole-body proximate composition of climbing perch cultured under different C/N ratios are presented in Table 2. The C/N ratio significantly affected all measured parameters ($p<0.05$). Fillet yield was highest at C/N 20:1 ($36.91\pm0.47\%$), whereas skeleton proportion was lowest at the same treatment ($55.47\pm0.20\%$). HSI was significantly higher at C/N 15:1–25:1 than at 10:1, while VSI was significantly lower at C/N 20:1 and 25:1. For whole-body composition, crude protein increased progressively with C/N ratio up to 20:1, reaching the highest value of $17.36\pm0.61\%$, whereas lipid and ash contents were highest at C/N 25:1 ($6.35\pm0.31\%$ and $3.26\pm0.16\%$, respectively).

Table 2
Carcass traits and whole-body proximate composition of sex-reversed *Anabas testudineus* cultured under different C/N ratios

Parameter	10:1	15:1	20:1	25:1
Fillet yield (%)	34.69 ± 0.41^a	35.23 ± 0.55^b	36.91 ± 0.47^c	35.49 ± 0.30^b
Skeleton (%)	56.72 ± 0.34^c	56.24 ± 0.38^b	55.47 ± 0.20^a	56.64 ± 0.30^b
HSI (%)	1.73 ± 0.25^a	2.01 ± 0.21^b	2.03 ± 0.12^b	2.07 ± 0.05^b
VSI (%)	8.59 ± 0.53^b	8.53 ± 0.56^b	7.62 ± 0.46^a	7.87 ± 0.20^a
Protein (%)	15.82 ± 0.45^a	16.74 ± 0.52^b	17.36 ± 0.61^c	16.81 ± 0.57^b
Lipid (%)	5.42 ± 0.21^a	5.76 ± 0.25^b	6.12 ± 0.28^c	6.35 ± 0.31^c
Ash (%)	3.01 ± 0.12^a	3.15 ± 0.14^b	3.20 ± 0.15^b	3.26 ± 0.16^c

Values are presented as mean $\pm$ SD (n=3 tanks per treatment; five fish per replicate were analyzed). Different superscript letters indicate significant differences ($p<0.05$).

Biofloc characteristics and water quality parameters. Biofloc characteristics and water quality parameters under different C/N ratios are presented in Table 3.

Table 3
Biofloc characteristics and water quality parameters under different C/N ratios

Parameter	10:1	15:1	20:1	25:1
Floc volume (mL L ⁻¹)	12.4 ± 1.2^a	18.6 ± 1.5^b	24.8 ± 2.1^c	27.5 ± 2.4^c
TSS (mg L ⁻¹)	180 ± 15^a	245 ± 20^b	320 ± 25^c	360 ± 30^d
TAN (mg L ⁻¹)	0.42 ± 0.05^c	0.31 ± 0.04^b	0.18 ± 0.02^a	0.25 ± 0.03^b
Nitrite-N (mg L ⁻¹)	0.18 ± 0.03^c	0.12 ± 0.02^b	0.08 ± 0.01^a	0.11 ± 0.02^b
Nitrate-N (mg L ⁻¹)	18.5 ± 1.8^a	22.6 ± 2.1^b	25.8 ± 2.5^c	31.4 ± 3.0^d
Alkalinity (mg L ⁻¹ CaCO ₃)	110 ± 8^a	125 ± 10^b	145 ± 12^c	160 ± 15^d
DO (mg L ⁻¹)	6.23 ± 0.45	6.45 ± 0.51	7.40 ± 0.62	7.57 ± 0.71
pH	7.12 ± 0.15	7.33 ± 0.18	7.44 ± 0.12	7.17 ± 0.20

Values are presented as mean $\pm$ SD (n=3 tanks per treatment). Measurements collected at each sampling time were averaged for each tank over the experimental period before statistical analysis. Each tank was considered an experimental unit. Different superscript letters within the same row indicate significant differences among treatments ($p<0.05$).

Significant differences were observed among treatments for floc volume, total suspended solids (TSS), total ammonia nitrogen (TAN), nitrite-N, nitrate-N, and alkalinity ($p < 0.05$), whereas pH and dissolved oxygen (DO) did not differ significantly among treatments ($p > 0.05$). Floc volume and TSS increased progressively with increasing C/N ratios, with the highest values recorded in the C/N 25:1 treatment ($27.5 \pm 2.4 \text{ mL L}^{-1}$ and $360 \pm 30 \text{ mg L}^{-1}$, respectively). The lowest TAN and nitrite-N concentrations were observed in the C/N 20:1 treatment (0.18 ± 0.02 and $0.08 \pm 0.01 \text{ mg L}^{-1}$, respectively). DO concentrations ranged from 6.23 to 7.57 mg L^{-1} , while pH values ranged from 7.12 to 7.44. Neither DO nor pH differed significantly among treatments ($p > 0.05$).

Economic efficiency of climbing perch production under different C/N ratios is presented in Table 4. The lowest total feed and carbon cost per kilogram of weight gain was observed in the C/N 20:1 treatment ($1.41 \pm 0.04 \text{ USD kg}^{-1}$), whereas the highest cost was recorded in the C/N 10:1 treatment ($1.53 \pm 0.05 \text{ USD kg}^{-1}$).

Table 4

Economic efficiency of *Anabas testudineus* cultured under different C/N ratios

Parameter	10:1	15:1	20:1	25:1
Feed cost (USD kg^{-1})	1.50 ± 0.05^c	1.42 ± 0.04^b	1.34 ± 0.03^a	1.39 ± 0.05^b
Molasses cost (USD kg^{-1})	0.034 ± 0.003^a	0.051 ± 0.006^b	0.069 ± 0.006^c	0.089 ± 0.009^d
Total cost (USD kg^{-1})	1.53 ± 0.05^c	1.47 ± 0.05^b	1.41 ± 0.04^a	1.48 ± 0.05^b

Values are presented as mean $\pm$ SD ($n = 3$ tanks per treatment). Different superscript letters within the same row indicate significant differences among treatments ($p < 0.05$).

Discussion. The present study demonstrated that C/N ratio significantly influenced growth performance and feed utilization of sex-reversed *A. testudineus* cultured in a biofloc system. The best overall response among the tested treatments was observed at a C/N ratio of 20:1. Fish cultured under this condition showed the highest final weight, weight gain, SGR, ADG, and feed efficiency, together with the lowest FCR, indicating improved nutrient utilization and growth efficiency. Among the tested treatments, SGR increased from C/N 10:1 to 20:1 and was highest at C/N 20:1, whereas increasing the ratio to 25:1 did not provide additional growth benefits. The superior performance at C/N 20:1 was likely related to enhanced heterotrophic microbial activity. Also, efficient nitrogen recycling, whereby adequate carbon availability, may have been associated with favorable biofloc development. Although microbial activity was not directly measured in this study, adequate carbon availability in biofloc systems is generally considered to promote heterotrophic bacterial assimilation of inorganic nitrogen into microbial biomass, which can serve as a supplementary nutrient source for fish (Avnimelech, 2012; Emerenciano et al., 2017; Zablon et al., 2022). Similar improvements in growth and feed utilization under optimized C/N conditions have been reported in biofloc systems, where balanced carbon supply enhances microbial protein production and nutrient recycling efficiency (Saha et al., 2022; Sarker et al., 2026). The improved FCR and FE at C/N 20:1 further suggest that biofloc particles contributed additional nutrients, including bacterial biomass and extracellular polymeric substances, which enhanced protein utilization and reduced dependence on formulated feed nutrients (Emerenciano et al., 2017; Zablon et al., 2022). Previous studies on *A. testudineus* cultured in biofloc systems also reported improved growth and survival, supporting the suitability of biofloc technology for this species (Hanafie et al., 2023; Das et al., 2025; Rahman et al., 2024). The lower performance at C/N 10:1 may have resulted from insufficient carbon availability for effective microbial assimilation of nitrogenous waste, limiting biofloc formation and nutrient recycling efficiency (Avnimelech, 1999; Liu et al., 2018). In contrast, despite higher biofloc development at C/N 25:1, excessive carbon supplementation did not improve growth, suggesting that increased microbial biomass does not necessarily enhance nutrient availability. Overall, maintaining a C/N ratio of approximately 20:1 provides an effective balance between microbial nutrient production, nitrogen management, and feed utilization efficiency, thereby improving growth performance of sex-reversed *A. testudineus* in biofloc-based culture systems.

Although increasing the C/N ratio from 10:1 to 25:1 enhanced biofloc development, the highest C/N ratio (25:1) did not further improve growth performance or feed utilization of sex-reversed *A. testudineus*. This finding suggests that excessive carbon availability may exceed the optimal level required for microbial assimilation and nutrient utilization. In biofloc systems, carbon supplementation must be carefully balanced because both insufficient and excessive carbon inputs can alter microbial community dynamics and nutrient availability (Avnimelech, 2012; Khanjani & Sharifinia, 2024). The lower growth response at C/N 25:1 may be associated with excessive microbial biomass accumulation, as indicated by the highest floc volume and TSS values. Although bioflocs provide additional microbial nutrients, excessive suspended solids can increase microbial oxygen demand and disrupt the balance of microbial communities, potentially increasing energy allocation for maintenance rather than growth (Khanjani et al., 2024; Muthoka et al., 2026). Furthermore, excessive carbon input may reduce nutrient transfer efficiency from microbial biomass to fish production by increasing microbial competition for dissolved nutrients and oxygen (Iber et al., 2025; Khanjani & Sharifinia, 2024). Similar findings have shown that increased carbon supplementation can enhance biofloc formation without proportional improvements in fish growth, indicating the presence of species-specific optimal C/N ranges (Huang et al., 2022; Zahra & Mateen, 2025). In the present study, C/N 25:1 produced the highest floc volume and TSS but did not outperform C/N 20:1 in growth or FCR, indicating that biofloc quantity alone is not the primary determinant of production efficiency. Instead, the balance among microbial biomass production, nutrient accessibility, water quality stability, and fish metabolic requirements is critical. Therefore, C/N 20:1 provided a more favorable balance between carbon availability, microbial activity, nitrogen recycling, and nutrient utilization, whereas further increasing the C/N ratio increased biofloc quantity without additional growth benefits.

The present study demonstrated that C/N manipulation significantly affected several water quality parameters in the biofloc system, particularly floc development, TSS, TAN, nitrite-N, nitrate-N, and alkalinity, whereas pH and dissolved oxygen remained statistically comparable among treatments. The lowest TAN and nitrite-N concentrations were observed at C/N 20:1, indicating enhanced microbial nitrogen assimilation and improved environmental stability. An appropriate carbon supply promotes heterotrophic bacterial uptake of dissolved inorganic nitrogen into microbial biomass, thereby reducing toxic nitrogen accumulation and improving nutrient recycling (Avnimelech, 1999; Avnimelech, 2012). The improved nitrogen control at C/N 20:1 was likely associated with a balanced carbon supply that supported microbial conversion of ammonia into microbial protein, reducing ammonia toxicity and enhancing nitrogen cycling (Emerenciano et al., 2017; Liu et al., 2018). Similar effects of C/N optimization on TAN and nitrite reduction have been reported in various biofloc-based aquaculture systems, highlighting the importance of carbon management in microbial nitrogen transformation (Huang et al., 2022; Saha et al., 2022; Sarker et al., 2026). Although C/N 25:1 resulted in the highest floc volume and TSS, it did not provide superior nitrogen control, suggesting that increased biofloc quantity does not necessarily improve nitrogen removal efficiency. Excess carbon input may alter microbial community dynamics, increase organic matter accumulation, and intensify oxygen and nutrient competition among microorganisms (Khanjani et al., 2024; Khanjani & Sharifinia, 2024). The increases in nitrate-N and alkalinity with higher C/N ratios indicated changes in nitrogen dynamics and buffering capacity, respectively, rather than being direct evidence of enhanced microbial activity. Higher nitrate-N may reflect greater nitrification, whereas alkalinity reflects the buffering capacity of the culture water (Alkhamis et al., 2023; Avnimelech, 2012; Khanjani et al., 2024). In contrast, pH values remained within a relatively narrow range (7.12–7.44) and did not differ significantly among treatments ($p>0.05$). In particular, the pH at C/N 25:1 was 7.17 ± 0.20 , indicating that no marked pH decline occurred under this treatment. Therefore, pH depression was unlikely to have been a major factor contributing to the lower growth performance observed at C/N 25:1. Dissolved oxygen remained within an appropriate range throughout the experiment, indicating that aeration and biofloc management were sufficient to support microbial processes and fish metabolism.

(Dheeran et al., 2025; Iber et al., 2025). Overall, C/N 20:1 provided the most favorable balance between microbial activity, nitrogen removal, and water quality stability, demonstrating that optimized carbon management is more important than maximizing biofloc biomass for sustainable climbing perch production.

The present study demonstrated that C/N optimization in biofloc systems influenced carcass traits and whole-body nutrient composition of sex-reversed *A. testudineus*. Fish cultured at C/N 20:1 showed the highest fillet yield, lowest skeleton proportion, and greatest whole-body crude protein content, suggesting improved nutrient deposition and utilization. This improvement was likely associated with increased availability of microbial-derived nutrients from heterotrophic bacterial activity, as bioflocs contain bacterial protein, amino acids, and other bioactive compounds that can supplement dietary nutrients and improve protein utilization (Emerenciano et al., 2017; Zablon et al., 2022). Similar improvements in body composition and nutrient retention under optimized biofloc conditions have been reported in other aquaculture species (Saha et al., 2022; Zahra & Mateen, 2025). The increased lipid and ash contents at higher C/N ratios, particularly C/N 25:1, may indicate altered nutrient partitioning due to excessive carbon availability. Although increased carbon supply promotes microbial biomass production, excessive carbon input may favor energy storage rather than protein deposition when nutrient utilization becomes limited (Avnimelech, 2012; Khanjani & Sharifinia, 2024). The higher lipid content and lower growth performance observed at C/N 25:1 suggest that excessive carbon supplementation may shift nutrient allocation away from muscle growth. The lower VSI values at C/N 20:1 and 25:1 further indicate improved metabolic efficiency and reduced visceral nutrient deposition, which is associated with greater allocation of nutrients toward somatic growth (Durigon et al., 2023; Martínez-Montaña et al., 2024). In addition, the higher fillet yield at C/N 20:1 highlights the potential of optimized biofloc management to enhance the commercial value and nutritional quality of cultured fish through improved microbial nutrient recycling (Hariharan et al., 2025; Zablon et al., 2022). Overall, C/N 20:1 provided the most favorable condition for nutrient deposition, improving protein accretion, carcass quality, and production value of sex-reversed *A. testudineus* in biofloc-based aquaculture systems.

The economic evaluation demonstrated that C/N optimization directly affected production efficiency and operational costs in biofloc-based climbing perch culture. The lowest production cost per kilogram weight gain was obtained at C/N 20:1, corresponding with the highest growth performance, lowest FCR, and highest feed efficiency. This economic advantage was likely related to improved nutrient recycling, whereby microbial assimilation of dissolved nitrogen into protein-rich biomass provided additional nutrients for fish and reduced feed-related costs (Avnimelech, 2012; Emerenciano et al., 2017). Previous studies have shown that biofloc systems can enhance feed utilization and reduce production costs through improved nutrient retention and partial contribution of microbial biomass to fish nutrition (Islam et al., 2023; Zablon et al., 2022). Although C/N 25:1 promoted greater biofloc formation and TSS, it did not improve economic efficiency because increased carbon input and molasses costs were not compensated by enhanced growth or feed utilization. Excessive carbon supplementation may therefore reduce profitability when additional input costs exceed the nutritional benefits derived from biofloc production (Khanjani & Sharifinia, 2024; Muthoka et al., 2026). Similar findings indicate that appropriate carbon management improves economic returns by balancing feed costs, microbial productivity, and water quality management in intensive aquaculture systems (Ramiro et al., 2024; Swain et al., 2025). Therefore, maintaining a C/N ratio of 20:1 represents an economically optimal strategy for sex-reversed *A. testudineus* production by maximizing biological performance while minimizing production costs, supporting sustainable biofloc-based freshwater aquaculture.

The findings of this study highlight the practical importance of C/N optimization for biofloc-based production of sex-reversed *A. testudineus*. Maintaining a C/N ratio of 20:1 effectively balanced microbial activity, water quality stability, growth performance, feed utilization, carcass quality, and production cost, indicating that optimal carbon management is more important than maximizing biofloc biomass. From a practical perspective, a C/N ratio of 20:1 can enhance microbial nitrogen assimilation, reduce toxic

nitrogen accumulation, and improve nutrient recovery, supporting sustainable aquaculture through reduced waste discharge and improved resource utilization (Dheeran et al., 2025; Emerenciano et al., 2017). The successful performance of *A. testudineus* under biofloc conditions further supports its potential for intensive and environmentally sustainable freshwater aquaculture (Das et al., 2025; Hanafie et al., 2023). However, commercial application requires careful control of carbon input, solids accumulation, aeration, and microbial balance, as excessive biofloc development may increase suspended solids and oxygen demand, reducing system stability (Khanjani et al., 2024; Minaz et al., 2024). Future studies should investigate the long-term effects of C/N optimization on immune responses, intestinal microbiota, disease resistance, and reproductive performance, as well as evaluate integration with alternative carbon sources, probiotics, and circular aquaculture approaches to enhance sustainability (Iber et al., 2025; Muthoka et al., 2026). Commercial-scale validation is also required to confirm the economic feasibility and scalability of optimized C/N management. Overall, precise C/N optimization represents a practical strategy for improving the sustainability, profitability, and resource efficiency of climbing perch production in biofloc-based aquaculture systems.

A limitation of the present study is that only four C/N ratios were evaluated. Therefore, the identification of C/N 20:1 as the most favorable treatment should be interpreted within the range of ratios tested and should not be considered a statistically defined optimum for all culture conditions. Future studies should evaluate additional intermediate C/N ratios, such as 18:1 and 22:1, to further refine and validate the optimal C/N ratio for biofloc culture of sex-reversed *A. testudineus*.

Conclusions. This study demonstrated that C/N ratio optimization significantly influenced growth performance, feed utilization, carcass traits, biofloc development, water quality, and economic efficiency of sex-reversed *A. testudineus* cultured in a biofloc system. A C/N ratio of 20:1 provided the most favorable overall performance among the tested treatments, resulting in superior growth, improved feed utilization, enhanced protein deposition, higher fillet yield, better nitrogen control, and the lowest production cost per kilogram of weight gain. Although C/N 25:1 increased biofloc biomass, it did not further improve fish performance or economic efficiency, indicating that maximizing biofloc quantity is less important than maintaining an appropriate balance between microbial activity, nutrient availability, and fish requirements. Therefore, under the conditions of the present study, C/N 20:1 is recommended as the most favorable treatment among those tested for improving productivity, resource utilization efficiency, and sustainability of biofloc-based climbing perch aquaculture.

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Authors Contributions. Conceptualization: RP, YS, and NP; Methodology: RP, YS, and NP; Formal analysis: RP and PP; Investigation: RP and YS; Data curation: RP; Writing – original draft: RP; Writing – review & editing: RP, YS, PP, and NP; Supervision: NP. All authors read and approved the final manuscript.

Conflict of Interest. The authors declare that there is no conflict of interest.

Ethical Statement. The animal study protocol was approved by the Institutional Animal Care and Use Committee of Sakon Nakhon Rajabhat University, Thailand (IACUC-SNRU-22/2025; approved on 28 November 2025). All experimental procedures were conducted in accordance with institutional guidelines and internationally accepted standards for the ethical care and use of animals in research.

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