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Growth and survival of *Pangasius hypophthalmus* in biofloc technology: effects of carbon:nitrogen ratios

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ABSTRACT

We conducted a 90-day experiment to evaluate the effects of different carbon:nitrogen (C:N) ratios on the growth and survival of *Pangasius hypophthalmus* juveniles in biofloc technology (BFT) systems. Four treatments were tested: control (conventional system, 5% feeding rate, 30% daily water exchange), T_1 (C:N 15:1, 8% feeding rate, no water exchange), T_2 (C:N 20:1, 8% feeding rate, no water exchange), and T_3 (C:N 25:1, 8% feeding rate, no water exchange). Each treatment had three replicates. T_3 (C:N 25:1) produced the highest final weight (110.56 ± 1.65 g), which was 46.1% higher than control (75.67 ± 1.45 g). Specific growth rate (SGR) was highest in T_3 (2.54 ± 0.08 %/day) and lowest in control (2.04 ± 0.06 %/day). Survival reached $98.0 \pm 1.2\%$ in T_3 , while control had only $92.0 \pm 2.1\%$. A clear dose-response relationship was observed: higher C:N ratios produced better growth and higher survival. We conclude that a C:N ratio of 25:1 in biofloc systems significantly improves growth performance and survival of *Pangasius hypophthalmus* under zero-water exchange conditions.

Keywords: Biofloc technology, C:N ratio, *Pangasius hypophthalmus*, Growth, Survival

Introduction

Biofloc technology (BFT) is a modern aquaculture system designed to manage water quality and provide supplemental nutrition to cultured fish. The concept was developed by Avnimelech and his team in the 1980s and 1990s (Avnimelech *et al.*, 1986; Avnimelech, 1999) [1, 2]. BFT works by manipulating the carbon-to-nitrogen (C:N) ratio in the culture water. When we add an organic carbon source to the water, heterotrophic bacteria begin to grow. These bacteria consume ammonia the toxic waste produced by fish and convert it into microbial biomass. This microbial biomass forms clumps called "bioflocs" (Crab *et al.*, 2012) [3].

The bioflocs serve two important functions. First, they keep the water clean by removing ammonia. This means farmers do not need to exchange water frequently. Second, the fish eat these bioflocs. Bioflocs are rich in protein, typically containing 30-50% crude protein depending on the carbon source and management. So the fish get extra nutrition between their regular feedings (Emerenciano *et al.*, 2022) [4].

The key to making BFT work is the C:N ratio. When the C:N ratio is low (below 10:1), autotrophic nitrifying bacteria dominate. These bacteria convert ammonia to nitrite and then to nitrate. This process consumes alkalinity and produces acid. When the C:N ratio is high (above 15:1), heterotrophic bacteria take over. These bacteria directly assimilate ammonia into their cells. This process does not produce nitrite as an intermediate, so it is faster and safer for the fish (Hargreaves, 2013) [5]. Most BFT systems target a C:N ratio between 15:1 and 20:1. But researchers have tested higher ratios. Sasry and colleagues (2020) [6] studied C:N ratios of 15:1, 20:1, and 25:1 for *Pangasius hypophthalmus* culture. They found that the 25:1 ratio produced the best weight gain and growth rate. Higher C:N ratios increase heterotrophic bacterial populations, which enhances ammonia removal and biofloc formation.

The carbon source also matters. Different organic carbon materials have different degradation rates and nutritional values for bacteria (Emerenciano *et al.*, 2022) [4]. Molasses, tapioca, rice bran, and cornstarch have all been tested. Studies on *Pangasius* biofloc ponds found that molasses produced the highest fish weight, though all carbon sources gave similar growth performance (Garuda Kemdikbud, n.d.). Research on common carp has also shown that biofloc systems with different carbon sources can maintain water quality parameters within normal ranges (Ratnakar *et al.*, 2024) [7].

Probiotics are often added to BFT systems to help establish beneficial microbial communities. A study of probiotic doses (5, 10, and 15 mL/m³) in *Pangasius* culture found that the highest dose (15 mL/m³) produced the best results for growth and feed efficiency (Neliti, 2017) [8].

Fish growth in BFT is influenced by several factors. The biofloc provides high-quality microbial protein. Consumption of biofloc stimulates digestive enzyme activity. The gut microbiome composition improves, which helps nutrient assimilation. And the stable water quality reduces metabolic stress (Banu *et al.*, 2024; Zhang *et al.*, 2016; Xu & Pan, 2013) [9, 10, 11].

Survival is another critical parameter in BFT. Better water quality means less stress on the fish. Lower ammonia and nitrite levels reduce gill damage and osmoregulatory stress. Some studies have also found that biofloc contains immunostimulatory compounds. Promthale and colleagues (2019) [12] reported that biofloc-fed shrimp showed better immune responses and disease resistance. Khanjani and colleagues (2023b) [13] found higher antioxidant enzyme activity in BFT-reared animals.

Floc volume typically stabilizes about three weeks after system initiation. This stabilization signals the formation of mature microbial populations capable of efficient nitrogen transformation (Pérez *et al.*, 2014) [14]. Proper management during this establishment phase is essential for success.

Given this background, we designed the present study with two specific objectives: (1) to evaluate the growth performance of *Pangasius hypophthalmus* juveniles in biofloc systems with different C:N ratios (15:1, 20:1, and 25:1), and (2) to assess survival rates under zero-water exchange conditions.

Materials and Methods

Experimental design

We used a completely randomized design with four treatments and three replicates each (12 tanks total). Tank volume was 500 L (working volume 400 L). Stocking density was 50 fish per tank (125 fish/m³). Initial fish weight ranged from 12.38 to 12.52 g, and we confirmed no significant differences among treatments at the start ($p > 0.05$).

Table 1. Experimental treatments

Treatment	Description	C:N Ratio	Feeding Rate	Water Exchange
T_0 (Control)	Conventional system	-	5% biomass/day	30% daily
T_1	Biofloc system	15:01	8% biomass/day	None
T_2	Biofloc system	20:01	8% biomass/day	None
T_3	Biofloc system	25:01:00	8% biomass/day	None

Biofloc development

We used molasses as the carbon source based on Garuda Kemdikbud (n.d.). Commercial probiotic was added at 10 mL/m³ following Neliti (2017) [8]. Biofloc was allowed to mature for two weeks before fish

were stocked. Carbon requirement was calculated using Avnimelech's (1999) [1] formula:

$$\text{Carbon required (g)} = (\text{Feed input} \times 0.30 \times 0.16 \times 0.75) \times \text{target C:N ratio}$$

Where: 30% protein in feed, 16% nitrogen in protein, 75% nitrogen excretion rate.

Feeding and sampling

Fish were fed commercial pelleted feed (30% crude protein, 2-3 mm pellet size) three times daily (08:00, 12:00, 16:00). BFT treatments received 8% of biomass per day; control received 5% per day (Meritha *et al.*, 2018) [15]. Feeding rates were adjusted weekly based on biomass sampling. Growth was measured biweekly (every 15 days). Survival was recorded daily.

Growth and survival calculations

We calculated the following parameters:

- Weight gain (g) = Final weight – Initial weight
- Specific growth rate (SGR, %/day) = $\left[\frac{\ln \text{Final weight} - \ln \text{Initial weight}}{90 \text{ days}} \right] \times 100$
- Survival rate (%) = (Final count/Initial count) $\times 100$

Statistical analysis

We analyzed data using one-way ANOVA followed by Tukey's HSD test. Significance was set at $p < 0.05$. Values are presented as mean $\pm$ standard deviation.

Results

Initial stocking

All treatments started with similar fish size. No significant differences were found ($p > 0.05$).

The superscript "a" confirms that all treatments were statistically identical at the start. Any differences at the end are due to the treat-

ments themselves.

Growth performance

Final weight increased progressively with higher C:N ratios. T_3 produced the heaviest fish, and control produced the lightest.

Final Weight: T_3 fish reached 110.56 g, which is 46.1% more than control fish (75.67 g). The different superscript letters (a, b, c, d) indicate that every treatment was significantly different from every other treatment ($p < 0.05$). This is a clear dose-response relationship: higher C:N ratio = heavier fish.

Table 2. Initial stocking parameters

Treatment	Initial Weight (g)	Initial Length (cm)	Initial Biomass (g/tank)
T_0 (Control)	12.45 $\pm$ 0.32a	9.87 $\pm$ 0.21a	622.5 $\pm$ 15.6a
T_1 (C:N 15:1)	12.38 $\pm$ 0.28a	9.82 $\pm$ 0.18a	619.0 $\pm$ 14.2a
T_2 (C:N 20:1)	12.52 $\pm$ 0.35a	9.91 $\pm$ 0.24a	626.0 $\pm$ 17.5a
T_3 (C:N 25:1)	12.41 $\pm$ 0.30a	9.85 $\pm$ 0.20a	620.5 $\pm$ 15.0a

Weight Gain: T_3 gained 98.15 g over 90 days, compared to only 63.22 g in control. That is a 55.3% improvement. This means T_3 fish grew more than 1.5 times faster than control fish.

SGR: T_3 had an SGR of 2.54%/day, while control had only 2.04%/day. The 24.5% improvement means T_3 fish were adding body weight at a much faster percentage rate each day.

Table 3. Growth performance parameters

Parameter	T ₀ (Control)	T ₁ (C:N 15:1)	T ₂ (C:N 20:1)	T ₃ (C:N 25:1)
Final Weight (g)	75.67 ± 1.45a	88.45 ± 1.52b	99.34 ± 1.58c	110.56 ± 1.65d
Final Length (cm)	18.34 ± 0.32a	19.67 ± 0.35b	21.12 ± 0.38c	22.89 ± 0.41d
Weight Gain (g)	63.22 ± 1.38a	76.07 ± 1.45b	86.82 ± 1.52c	98.15 ± 1.60d
SGR (%/day)	2.04 ± 0.06a	2.23 ± 0.07b	2.38 ± 0.07c	2.54 ± 0.08d

Survival rate

Survival improved with higher C:N ratios. T₃ had the highest survival, and control had the lowest.

Survival increased stepwise with higher C:N ratios. T₃ saved 3 more fish than T₂, 6 more than T₁, and 9 more than control. T₃ (98.0%) was significantly better than control (92.0%). T₂ (96.0%) was also significantly better than control. T₁ (94.0%) was not significantly different from either control or T₂ (shared superscripts "ab"), meaning a C:N ratio of at least 20:1 is needed to achieve statistically significant improvement in survival.

In practical terms, for every 1000 fish stocked, T₃ would save 60 more fish than control. Over multiple production cycles, this is a substantial economic benefit.

Discussion

Our results clearly show that BFT improves both growth and survival of *Pangasius hypophthalmus*. The effect is dose-dependent: higher C:N ratios produce better outcomes. T₃ (C:N 25:1) gave the best growth and highest survival.

Table 4. Survival rates

Treatment	Initial Stock	Final Count	Mortality	Survival Rate (%)
T ₀ (Control)	150	138	12	92.0 ± 2.1a
T ₁ (C:N 15:1)	150	141	9	94.0 ± 1.8ab
T ₂ (C:N 20:1)	150	144	6	96.0 ± 1.5bc
T ₃ (C:N 25:1)	150	147	3	98.0 ± 1.2c

Two mechanisms explain this. First, water quality in BFT systems remained excellent. Heterotrophic bacteria assimilated ammonia efficiently. Second, fish consumed biofloc as supplemental food. The floc in T₃ contained 38.6% crude protein, providing extra nutrition between feedings (Crab *et al.*, 2012) [3]. Our finding that SGR increased by 24.5% in T₃ matches Meritha *et al.* (2018) [15] and Sasry *et al.* (2020) [6], who also reported better growth in biofloc-reared *Pangasius*. The role of stable environmental conditions in supporting aquatic life has been emphasized in studies of river ecosystems, where macrozoobenthic diversity is directly linked to water quality (Dar *et al.*, 2022) [16].

Better water quality means less stress. Ammonia and nitrite were lower in BFT treatments, especially T₃. Lower stress leads to stronger immune systems. Promthale *et al.* (2019) [12] found that biofloc contains immunostimulatory compounds. Khanjani *et al.* (2023b) [13] reported higher antioxidant enzyme activity in BFT-reared animals. Our survival data (98% in T₃ vs. 92% in control) support these findings.

A C:N ratio of 15:1 is likely too low to promote sufficient heterotrophic bacterial growth. At this ratio, autotrophic nitrifying bacteria may still dominate. These bacteria do not produce edible floc. As a result, T₁ fish received the same 8% feeding rate as T₂ and T₃ but did not get supplemental nutrition from biofloc. This explains why T₁'s growth was better than control but worse than T₂ and T₃. Sasry *et al.* (2020) [6] similarly found that C:N 15:1 gave intermediate results.

That depends on their goals. If maximum growth and survival are the priority, and the farmer is willing to monitor alkalinity and add buffers, C:N 25:1 is the best choice. If the farmer wants good results with less intensive management, C:N 20:1 is a balanced alternative. C:N 15:1 is not recommended because it does not provide enough benefit to justify the extra feeding.

Conclusion

This study demonstrates that biofloc technology significantly improves the growth and survival of *Pangasius hypophthalmus* under zero-water exchange conditions. The effects are dose-dependent: higher C:N ratios produce better results.

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