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Research Article

Stimulating C: N Ratio in Biofloc culture and their Effect on Floc Formation

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Abstract

The present study demonstrates that manipulating the carbon-to-nitrogen ratio significantly affects various parameters of common carp growth and also influences the culture system. The composition and nutritive value of the biofloc system can be significantly influenced by the carbon-to-nitrogen (C: N) ratios. Higher C/N ratios generally promote faster nitrogen removal since heterotrophic bacteria regenerate more quickly compared to photoautotrophic microalgae. This may result in increased biofloc production. A C:N ratio of 20:1 was found to be effective in maintaining water quality and the biofloc culture system compared to ratios of 15:1 and 10:1. Furthermore, the 20:1 C:N ratio was beneficial for weight gain and length gain of common carp during different observation periods. Proximate composition and floc volume measurements were also maximized in treatment group T3 compared to T2 and T1. Consequently, treatment T3 was determined to be the optimal C:N ratio for the biofloc system, ensuring better growth conditions and system maintenance.

Keywords: Bacteria; Biofloc; Floc; Growth

Introduction

Aquaculture is recognized as one of the most promising industry in the global food production sector. India holds the position of being the second-largest producer of marine food globally, contributing to approximately 7.56 percent of the total global production. The necessity to increase aquaculture production has been triggered by the increasing demand per capita in parallel to the increase of global population. However, the development of a sustainable aquaculture industry is particularly challenged by the limited availability of natural resources as well as the impact of the industry on the environment [6;13]. To develop a sustainable aquaculture industry ecosystem, a variety of environment-friendly technologies are used along with modern innovative technologies in the production of fish [18].

In this regard, inventive technical approaches have been developed to successfully deal with such challenges. Among these, in the biofloc system, the carbon to nitrogen (C/N) ratio is changed to encourage the growth of naturally occurring heterotrophic bacteria [5;15]. This ratio is crucial as it regulates the growth of heterotrophic bacteria, which rely on organic carbon as a food source [12]. Biofloc technology is mainly based on the principle of waste nutrients recycling, in particular nitrogen, into microbial biomass that can be used *in situ* by the cultured animals or be harvested and processed into feed ingredients [4;11]. Bioflocs may contribute to the supply of essential nutrients and digestive enzymes either through the stimulation of endogenous production or microbial secretion [16;1], and the enhancement of nutrient bioavailability that facilitates higher nutrient assimilation. By stimulating the C:N ratio, we can promote the proliferation of heterotrophic bacteria, allowing them to effectively consume hazardous nitrogenous wastes from the water and convert them into protein-rich microbial biomass. This C:N ratio manipulation is crucial for the growth of common carp and also for the water quality maintenance.

Materials and Methods

Fishes were acclimatization for around fifteen days, healthy fingerlings were selected and distributed into twelve rearing circular water tanks having 100-litre capacity. The experiment was divided into four different groups, control, Treatment (T_1), Treatment (T_2), and Treatment (T_3).

The fishes were fed with commercially available pelleted feed at the rate of 5 percent of their body weight two times a day.

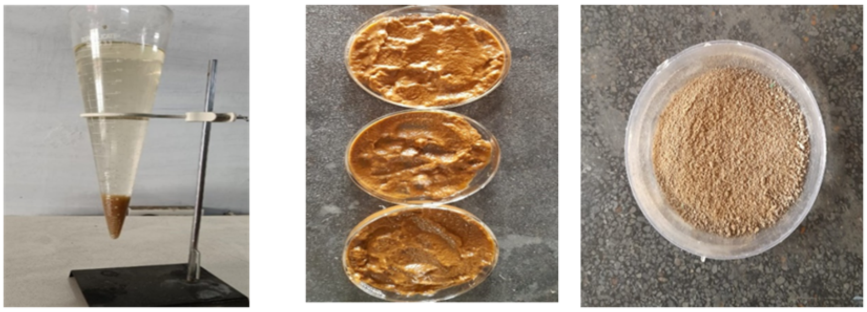
Experiment design	Treatment (T_1) (C: N ratio 10:1) (3 Replicate)	Treatment (T_2) (C: N ratio 15:1) (3 Replicate)	Treatment (T_3) (C: N ratio 20:1)	Control (3 Replicate)	Total
No. of tanks per treatment	3	3	3	3	
Total no. of fish per treatment	30	30	30	30	120

Table 1: Experimental design for the whole experiment.

Biofloc development was achieved following [3] and [10] with slight modifications. 0.05 g of each commercial probiotic was added to 100 L of water (ammonia-containing water from acclimated tanks) and 5-7 ml of jaggery, salt, and calcium carbonate. Jaggery is used as a carbon source for stimulating C:N ratio.

Growth parameter

To determine the growth parameters, the experimental fish weight (g) and length (cm) were measured using an electric weighing balance and scale, respectively.



Floc measured via Imhoff cone   Collected wet floc   Powdered form of floc

Figure a

Proximate composition of collected floc

The nutritional properties of the floc collected from different treatments were analysed based on five factors. The crude protein amount in the floc was determined by following the Kjeldhal method. A fat extraction method was employed to determine the crude fat content in the floc. However, crude fiber content was determined using the following AOAC method [2]. Similarly, moisture and ash content ware determined by using standard methods.

Floc volume measurement

Floc volume was measured by collecting a 1000 ml sample of culture water into an Imphoff cone, following the method described by Eaton., *et al.* [9] on a fortnightly basis up-to 90 days.

Statistical analysis

The data was analysed using SPSS software. One-way ANOVA was employed to determine the proximate composition, while growth parameters and floc volume were measured through two-way ANOVA. Two-way ANOVA was used to account for two factors, with one being floc volume and the other being the observation period. ANOVA was followed by post-hoc analysis to determine significant differences among the treatment groups.

Results and Discussion

Weight gain

The initial body weight in different treatment groups and control was similar, whereas final weight gain showed a significant difference ($p < 0.01$). Among the different treatment groups, T_3 (47.58 ± 0.11 g) showed significantly higher weight gain followed by T_1 (42.71 ± 0.31g) and T_2 (42.33 ± 0.06g). Among the different observation periods, weight gain continuously increased as we moved from 0 days to 90 days.

Length gain

At initial stocking, mean length did not significantly differ across the various treatment groups. Length gain among the different observation periods increased gradually during the whole duration of the experiment. Among the different treatment groups, T_3 (14.80 ± 0.05) has shown significantly higher length gain followed by T_1 (14.70 ± 0.05) and T_2 (14.60 ± 0.05).

Observation days	Weight (g) of *Cyprinus carpio* fingerlings in biofloc culture water			
	Treatment 1	Treatment 2	Treatment 3	Control
0	11.04 ± 0.01^{a}	11.11 ± 0.06^{a}	11.09 ± 0.05^{a}	11.06 ± 0.00^{a}
15	13.17 ± 0.07^{b}	13.25 ± 0.02^{b}	13.14 ± 0.07^{a}	13.06 ± 0.01^{b}
30	16.29 ± 0.52^{c}	15.60 ± 0.30^{c}	16.98 ± 0.00^{b}	16.24 ± 0.02^{c}
45	22.39 ± 0.05^{d}	22.85 ± 0.37^{d}	24.25 ± 0.43^{c}	22.52 ± 0.03^{d}
60	28.70 ± 0.21^{e}	29.31 ± 0.08^{e}	31.88 ± 0.80^{d}	28.23 ± 0.33^{e}
75	35.64 ± 0.15^{f}	35.31 ± 0.47^{f}	39.77 ± 0.58^{e}	33.52 ± 0.37^{f}
90	42.71 ± 0.31^{g}	42.33 ± 0.06^{g}	47.58 ± 0.11^{f}	40.51 ± 0.07^{g}

Table 2: Effect of different treatments on the weight of *Cyprinus carpio* culture at different durations.

Values in the same column with different superscripts differ significantly ($p < 0.05$) (Tukey post hoc analysis <5%)

Observation days	Length (cm) of *Cyprinus carpio* fingerlings in biofloc culture water			
	Treatment 1	Treatment 2	Treatment 3	Control
0	9.30 ± 0.05^{a}	9.33 ± 0.08^{a}	9.33 ± 0.08^{a}	9.40 ± 0.05^{a}
15	9.53 ± 0.03^{a}	9.53 ± 0.03^{a}	9.56 ± 0.08^{a}	9.50 ± 0.05^{ab}
30	10.56 ± 0.08^{b}	10.26 ± 0.18^{b}	10.63 ± 0.08^{b}	9.83 ± 0.06^{b}
45	12.30 ± 0.11^{c}	12.20 ± 0.05^{c}	12.66 ± 0.08^{c}	11.33 ± 0.17^{c}
60	13.66 ± 0.03^{d}	13.60 ± 0.05^{d}	13.76 ± 0.08^{d}	12.70 ± 0.05^{d}
75	13.86 ± 0.03^{d}	13.86 ± 0.03^{d}	14.00 ± 0.04^{d}	12.80 ± 0.05^{d}
90	14.70 ± 0.05^{e}	14.60 ± 0.05^{e}	14.80 ± 0.05^{e}	13.66 ± 0.08^{e}

Table 3: Effect of different treatments on the weight of *Cyprinus carpio* culture at different durations.

Values in the same column with different superscripts differ significantly ($p < 0.05$) (Tukey post hoc analysis <5%).

The results of an ANOVA clearly show that the combined effects of each of the treatments and the days have a significant effect ($p < 0.01$) on the length gain in common carp (Table 3).

Proximate composition of floc

The proximate composition of floc collected from the different treatments is presented in Table 4. Crude protein, crude lipid, and fiber content were found to be maximum in the treatment T_3 (25.22 ± 0.02%, 4.34 ± 0.02%, and 2.45 ± 0.01%) followed by T_1 (23.09 ± 0.06%, 2.04 ± 0.03% and 2.02 ± 0.00%) and T_2 (21.04 ± 0.32%, 2.01 ± 0.04% and 1.20 ± 0.00%), respectively. However, crude fiber and ash content among the treatment group did not differ significantly ($p > 0.05$). Moreover, moisture and ash content in the floc was found to be maximum in the treatment T_1 (10.89 ± 0.14%, 20.04 ± 0.29%) followed by T_3 (10.24 ± 0.02%, 19.69 ± 0.34%) and T_2 (10.90 ± 0.02%, 19.21 ± 0.07%), respectively.

Proximate analysis	Treatment 1	Treatment 2	Treatment 3
Crude Protein (%)	23.09 ± 0.06^{b}	21.04 ± 0.32^{b}	25.22 ± 0.02^{a}
Crude Lipid (%)	2.04 ± 0.03^{b}	2.01 ± 0.04^{b}	4.34 ± 0.02^{a}
Crude Fiber (%)	2.02 ± 0.00^{a}	1.20 ± 0.00^{a}	2.45 ± 0.01^{a}
Moisture (%)	10.89 ± 0.14^{b}	10.90 ± 0.02^{b}	10.24 ± 0.02^{c}
Ash (%)	20.04 ± 0.29^{a}	19.21 ± 0.07^{a}	19.69 ± 0.34^{a}

Table 4: Proximate composition of floc collected from each treatment during the experimental period.

Values in the same row with different superscripts differ significantly (Tukey post hoc analysis $p < 0.05$).

Floc volume measurement

It was observed that the initial slow growth of the floc in the first few days can be attributed to the clean surfaces of the tanks, which may have hindered the development of the floc. However, as time progressed, floc formation started on the 21st day and gradually increased at the end of the culture period. The maintenance of a stable amount of floc after the 21 days suggests that the system has reached a state of equilibrium, where the floc formation and degradation processes are balanced (Figure 1). Among the different treatments T_3 (17.00 ml/l) was found to maximum floc volume followed by T_1 (13.94 ml/l) and T_3 (12.72 ml/l). Considering the whole experiment, there was a significant difference in floc volume between the treatment groups as $p > 0.05$. This indicates that the biofloc system established a stable and self-sustaining floc population.

Proximate analysis	Treatment 1	Treatment 2	Treatment 3
Crude Protein (%)	23.09 ± 0.06^b	21.04 ± 0.32^b	25.22 ± 0.02^a
Crude Lipid (%)	2.04 ± 0.03^b	2.01 ± 0.04^b	4.34 ± 0.02^a
Crude Fiber (%)	2.02 ± 0.00^a	1.20 ± 0.00^a	2.45 ± 0.01^a
Moisture (%)	10.89 ± 0.14^b	10.90 ± 0.02^b	10.24 ± 0.02^c
Ash (%)	20.04 ± 0.29^a	19.21 ± 0.07^a	19.69 ± 0.34^a

Table 4: Proximate composition of floc collected from each treatment during the experimental period.

Values in the same row with different superscripts differ significantly (Tukey post hoc analysis $p < 0.05$).

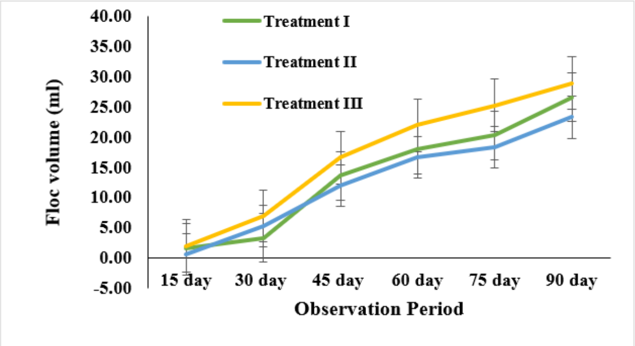


Figure 1: Shows floc volume variations in different treatment groups.

In the present study, treatment T_3 (47.58 ± 0.11g) had a statistically significant weight gain followed by T_1 (42.71 ± 0.31g), T_2 (42.33 ± 0.06g), and the control group. The findings of Dash., *et al.* [7] are consistent with the current study, as they also reported enhanced growth performance and improved feed utilization in tilapia raised under biofloc conditions. Moreover, higher growth was observed in C/N 20:1 and other treatments compared to control.

The results from the proximate composition of floc revealed that treatment T_3 was found to have maximum protein, lipid, and fiber content. Values of crude protein, crude lipid, and fiber content were found to be maximum in the treatment T_3 (25.22 ± 0.02%, 4.34 ± 0.02% and 2.45 ± 0.01%) followed by T_1 (23.09 ± 0.06%, 2.04 ± 0.03% and 2.02 ± 0.00%) and T_2 (21.04 ± 0.32%, 2.01 ± 0.04% and 1.20 ± 0.00%), respectively.

This composition proved that C:N ratio of 20:1 was most suitable for the culture conditions in the biofloc system because the floc formed from this treatment was found to be highly nutritious when compared with the other treatment groups. However, C:N ratio of 15:1 was found to be a less efficient system when considering the same fact. The C:N ratio is an added factor affecting the density of heterotrophic bacteria and other microorganisms in biofloc systems. In intensive aquaculture systems, high-protein diets with low carbon-to-nitrogen ratios below 10:1 are commonly used, which may not help efficient breakdown of inorganic nitrogen by bacteria. However, another study suggests that a higher C:N ratio stimulates the growth of heterotrophic bacteria, resulting in significant improvement in water quality and biofloc composition [8]. Some researcher [14] detected enhanced growth performance and immune activation in biofloc-raised carp when a higher carbon-nitrogen ratio of 20:1 or 25:1 was sustained.

Floc volume was considered as another factor for the determination of C:N ratio for a better biofloc system. In this study, the floc volume of Treatment T_3 was found to be maximum (17 ml/l) which signifies the highest bacterial growth for culture maintenance when compared with treatment T_2 and treatment T_1. According to another study [14], the use of higher C/N ratios, specifically 19 and 23, proved to be more efficient in regulating nitrogenous waste levels during the cultivation of different carp species when compared to lower ratios of 11 and 15. This improved performance was attributed to the increased production of bioflocs and a more diverse composition of live feeds, resulting in enhanced carp production.

Conclusion

This study indicates that a C:N ratio of 20:1 was found to be optimal for fish growth and for maintaining better water quality. The floc formed in different treatment groups served as an additional feed source for the fish and was also found to be at its maximum in the treatment with the highest C: N ratio.

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