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Characteristics and quality of biofloc in vannamei shrimp culture with different carbon sources

Karakteristik dan kualitas bioflok dalam pemeliharaan udang vanname dengan sumber karbon berbeda

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ABSTRACT

The different carbohydrate complexities are thought to affect the bioavailability of carbon sources for heterotrophic bacteria in the biofloc formation process and produce different biofloc characteristics. Based on these, the purpose of this study was to evaluate the effect of using carbon sources with different levels of complexity on the characteristics and quality of biofloc in pacific white shrimp (*Penaeus vannamei*) rearing. The carbon sources used in this study were dextrose (DX), potato starch (ST), and α-cellulose (CL). The experimental tests were the TAN (total ammonia nitrogen) reduction test which was carried out for 16 hours and the shrimp rearing test which was carried out for 30 days. Parameters measured to determine the characteristics of biofloc in shrimp rearing test were bifloc volume, biofloc particle size, biofloc nutrient composition, and water quality. The DX treatment produced the lowest TAN in the TAN reduction test. The shrimp rearing test showed the biofloc had different nutrient quality between the treatments. Meanwhile, the water quality between the treatments had relatively close values, but the CL treatment was able to suppress TAN and NO_2 lower than the DX and ST treatments during the rearing period. In addition, it is feared that the biofloc volume of the DX treatment which is higher than the ST and CL treatments is feared to be the main cause of stress in shrimp. The conclusion of this study is that complex carbohydrates, especially cellulose, have more potential in improving the performance of the biofloc system.

Keywords: biofloc volume, carbohydrate complexity, nutrient composition, particle size

ABSTRAK

Kompleksitas karbohidrat diduga dapat memengaruhi bioavaibilitas sumber karbon bagi bakteri heterotrof dalam proses pembentukan bioflok, sehingga kompleksitas karbohidrat yang berbeda diduga dapat menghasilkan karakteristik bioflok yang berbeda. Berdasarkan hal tersebut maka tujuan dari penelitian ini adalah untuk mengevaluasi pengaruh penggunaan sumber karbon dengan tingkat kompleksitas yang berbeda terhadap karakteristik dan kualitas bioflok pada pemeliharaan udang vaname (*Penaeus vannamei*). Sumber karbon yang digunakan dalam penelitian ini adalah dekstrosa (DX), tepung kentang (ST), dan α-selulosa (CL) dan rancangan percobaan yang digunakan adalah RAL (rancangan acak lengkap). Penelitian ini memiliki dua jenis pengujian yang dilakukan secara paralel, yaitu uji penurunan TAN (total ammonia nitrogen) yang dilakukan selama 16 jam dan uji pemeliharaan udang yang dilakukan selama 30 hari. Parameter yang diukur untuk mengetahui karakteristik bioflok pada pemeliharaan udang adalah volume biflok, ukuran partikel bioflok, komposisi nutrien bioflok, dan kualitas air pemeliharaan. Perlakuan DX menghasilkan TAN paling rendah pada uji penurunan TAN. Bioflok yang dihasilkan dari uji pemeliharaan udang menunjukkan kualitas nutrien bioflok yang berbeda-beda antar perlakuanya. Sementara itu kualitas air antar perlakuan memiliki nilai yang relatif berdekatan, tetapi perlakuan CL mampu menekan TAN dan NO_2 lebih rendah dari perlakuan DX dan ST selama masa pemeliharran. Selain itu, volume bioflok akhir perlakuan perlakuan DX yang lebih tinggi dari perlakuan ST dan CL dikhawatirkan dapat menjadi penyebab utama stres pada udang. Kesimpulan dari penelitian ini adalah karbohidrat kompleks khususnya selulosa lebih berpotensi dalam meningkatkan performa sistem bioflok.

Kata kunci: kompleksitas karbohidrat, komposisi nutrisi, ukuran partikel, volume bioflok

INTRODUCTION

The biofloc system is characterized by minimal or no water exchange (Cardona *et al*., 2016). The addition of organic carbon to the biofloc system aims to stimulate the growth of heterotrophic bacteria that utilize inorganic nitrogen (ammonia) in the aquaculture medium. This is crucial as high ammonia levels, as indicated by Qiu *et al*. (2018), can compromise the immune response of shrimp and increase their risk to pathogens. Within the biofloc system, heterotrophic bacteria utilize ammonia and organic carbon through biological assimilation to synthesize new cells (Yang *et al*., 2019) and produce microbial proteins (Jamal *et al*., 2020).

By maintaining a balanced C:N ratio in the aquaculture medium, the biofloc system fosters a dense bacterial community, leading to a system dominated by bacteria rather than algae. Consequently, this promises a robust maintenance system and a preventive disease management approach (Nisar *et al*., 2022). Biofloc, in this context, refers to the aggregation of abundant heterotrophic bacteria mixed with aquatic microorganisms, colloids, organic polymers, cations, and dead cells. These components serve as a feed source for aquaculture organisms, and the nutrient waste can be efficiently recycled to form biofloc (Ekasari *et al*., 2010).

Currently, the biofloc system continues to exhibit potential for integration into aquaculture practices. The management processes and production outcomes in aquaculture utilizing the biofloc system can be influenced by the characteristics of biofloc, which can be categorized into three main aspects: physical (volume and particle size of biofloc), chemical (nutrient quality of biofloc), and biological (biofloc microbiome). Elevated quality in each of these categories has the potential to enhance the efficiency of biofloc functions, which traditionally focused on water quality improvement but can also serve additional functions, such as providing supplementary feed. However, the establishment of favorable biofloc characteristics necessitates precise control measures.

Generally, the carbon source utilized by the biofloc system consists of carbohydrates, encompassing both simple and complex carbohydrates (Abakari *et al*., 2020). The complexity of carbohydrates is presumed to influence the bioavailability of the carbon source for heterotrophic bacteria during the biofloc formation process, leading to the development of distinct biofloc characteristics. In previous research, Wei *et al*. (2016), reported that the protein content in biofloc produced from glucose was significantly greater than that from starch ($P<0.05$), and Deng *et al*. (2018), observed that plant cellulose yielded a higher biofloc volume compared to tapioca flour. Therefore, the objective of this study is to evaluate the impact of carbon source complexity on the characteristics, quality, and functions of biofloc in relation to the performance of the biofloc system.

MATERIALS AND METHOD

Ethical statement

The experimental procedures conducted with Pacific white shrimp have received approval from the local Indonesian authorities, specifically the Animal Care and Use Committee at Bogor Agricultural University, Indonesia. Additionally, the shrimp used in the study have obtained accreditation as per the standards set by the National Standardization Agency of Indonesia (BSN, 2014).

Research design

This study was conducted from September 2021 to May 2022 at the Fisheries Pond of the Vocational School, IPB University. The study employed three types of carbon sources as treatments, namely dextrose (DX), potato starch (ST), and α-cellulose (CL). Cellulose exhibits a higher level of complexity compared to starch due to the presence of β-glucan bonds, and its simplification process can only be carried out by bacteria producing cellulase enzymes (Shendurse & Khedkar, 2016) before becoming available as a carbon source for heterotrophic bacteria. The use of pure carbohydrates is anticipated to enhance the precision of research outcomes. The experimental design employed was a completely randomized design with three treatments and three replications.

Shrimp maintenance

The test organism employed in this study were Pacific white shrimp (*Penaeus vannamei*) sourced from PT Suri Tani Pemuka Unit Carita, Banten, Indonesia. Pacific white shrimp was chosen due to the successful implementation of biofloc technology in shrimp aquaculture (Ahmad *et al*., 2017), and it is also a significant high-value economic commodity in Indonesia.

The shrimp were maintained for a period of 30 days at a density of 20 individuals per aquarium, with an initial shrimp weight of 1.07 ± 0.01 g. The aquariums utilized in the experiment had dimensions of 100×50×50 cm³ with a water volume of 187.5 L. The seawater used maintained a salinity level of 25 mg/L.

Feeding was administered four times a day at 07:00, 11:00, 15:00, and 19:00 WIB, employing the restricted feeding method with an initial feeding rate set at 6% of the shrimp biomass per aquarium. The feeding rate was utilized to determine the daily feed quantity, which was subsequently divided into four portions based on the feeding schedule. The feeding rate increased in tandem with the shrimp biomass, which was obtained through shrimp sampling conducted every seven days. This sampling involved selecting 10 shrimp for weighing, determining their average weight, and multiplying it by the total number of shrimp in each aquarium. The feeding rate could also be adjusted, either increased or decreased, based on the remaining feed to prevent an accumulation of excess feed in the aquaculture water.

Organic carbon source addition

The addition of carbon sources is carried out every day, specifically one hour after the first feeding (07:00 WIB). The determination of the amount of carbon sources added to the maintenance water is calculated based on Avnimelech (1999).

$$\Delta CH = \frac{\{\text{feed} \times \% \times \%N \times \%N\} \times \text{C/N ratio}}{\%C}$$

Note:

ΔCH	= Amount of carbon addition
Pakan	= Daily feed intake
% protein	= Feed protein percentage
%N pakan	= N feed content percentage
%N ekskresi	= Percentage of N excreted by shrimp
Rasio C/N	= C/N ratio used
%C	= C percentage in the organic carbon source

The calculation of the amount of carbon sources uses a 16% N content in the feed, an 80% excreted N percentage (Bucking, 2017), a C/N ratio of 15 (Xu *et al.*, 2016), and a 50% carbon percentage in carbohydrates (Abakari *et al.*, 2020).

TAN decreasing test

The experiment utilized 10 L of seawater with a salinity of 25 g/L in containers, to which biofloc inoculum and various nutrient sources were added, including $(NH_4)_2SO_4$ at 95.54 mg/L, KH_2PO_4 at 31 mg/L, and Na_2HPO_4 at 63.7 mg/L (Ekasari, 2008; De Schryver & Verstraete, 2009). Each container received only one carbohydrate treatment, administered at the beginning of the experiment. The testing period spanned 16 hours, with water samples collected every two hours. The parameters assessed included total ammonia nitrogen (TAN), temperature, and pH. TAN measurements were conducted at the Aquaculture Environmental Laboratory, Bogor Agricultural University, using the phenate method based on APHA (2012), guidelines, while temperature and pH were measured using a pH meter.

Biofloc physical characteristics

Observations of biofloc volume were conducted daily, one hour prior to the second feeding, utilizing an Imhoff cone. Particle measurements of biofloc were carried out at the Fish Seed Production Laboratory, Bogor Agricultural University, by capturing images of biofloc particles through a microscope with a 10 ×magnification. Subsequently, these images were analyzed using IndomicroView software, measuring the two-dimensional features in terms of horizontal diameter, vertical diameter, area, and perimeter.

Biofloc nutrient composition

The observed nutrient composition of biofloc included protein, fat, crude fiber, non-nitrogen extractable substances (BETN), carbohydrates, and ash, analyzed through proximate analysis following standard procedures (BSN, 2006). The carbohydrate content was determined by summing the levels of crude fiber and BETN. Proximate analysis of biofloc was conducted at the Fish Nutrition Laboratory, Bogor Agricultural University. The biofloc samples for proximate analysis were obtained from the filtered aquaculture water using a plankton net. The collected biofloc was placed in a 50 mL centrifuge tube and stored in a freezer at -20°C to maintain its stability, as proximate analysis procedures could not be completed within a single day.

Water quality

Water quality parameters measured included temperature, pH, dissolved oxygen (DO), total ammonia nitrogen (TAN), nitrite, nitrate, and alkalinity. Temperature, DO, pH, and settleable solid biofloc were measured each morning. TAN, nitrite, nitrate, and alkalinity concentrations were measured weekly using Tetra test kits (Tetra®, Blaksburg US). Meanwhile, TAN, nitrite, nitrate, and alkalinity concentrations at the end of the cultivation period were measured in the laboratory using the methods outlined by the American Public Health Association (APHA, 2012).

Data analysis

The acquired data were tabulated utilizing Microsoft Excel 2019 software. The tabulated data underwent analysis through analysis of variance (ANOVA) with a confidence interval of 95%. In the event of significant differences, further testing was conducted using Duncan's test with a confidence interval of 95%. Both ANOVA and Duncan's test were executed utilizing R Studio software version 4.1.3.

RESULT AND DISCUSSION

Result

The final biofloc volume produced in the DX treatment is significantly higher than the ST and CL treatments ($P<0.05$) (Table 1). The particle size of biofloc in the DX treatment is not significantly different from the ST treatment, except for the horizontal diameter parameter ($P<0.05$). Meanwhile, the CL treatment shows the smallest values for all biofloc particle size parameters compared to the DX and ST treatments ($P<0.05$). Microscopic images of biofloc particles also demonstrate that the CL treatment's biofloc particles are smaller than those in the DX and ST treatments (Figure 1). Additionally, the CL treatment's biofloc appears to have the densest matrix.

The protein content of biofloc in the DX treatment is higher than that of biofloc in the ST and CL treatments, but the carbohydrate and fat content in the biofloc of the DX treatment is lower than that of the ST and CL treatments ($P<0.05$) (Table 2). Additionally, the ash content of biofloc in the DX treatment is smaller than in the other treatments ($P<0.05$). The CL treatment shows a high fat content ($P<0.05$), while the ST treatment is high in carbohydrate content, although not significantly different from the CL treatment.

The water quality profile over the 30-day shrimp rearing period remains relatively consistent for parameters such as DO, temperature, pH, and NO_3 (Table 3). However, there are differences in alkalinity, TAN, and NO2 concentrations among treatments (Figures 2, 3, 4). In the TAN reduction test, the DX treatment is capable of suppressing TAN concentrations lower than the ST, CL, and control treatments (Figure 5). The DX treatment also achieves the lowest TAN concentration at the end of the shrimp rearing period (Figure 3).

Table 1. Volume and size of biofloc at the end of shrimp aquaculture using the biofloc system with different carbon sources.

Parameter	Treatment		
	DX	ST	CL
Volume (mL/L)	24.67 ± 2.33^{a}	9.50 ± 1.76^{b}	13.33 ± 0.67^{b}
Horizontal diameter (mm)	1.50 ± 0.26^{a}	0.77 ± 0.06^{b}	0.34 ± 0.04^{b}
Vertikal diameter (mm)	0.80 ± 0.07^{a}	0.96 ± 0.03^{a}	0.35 ± 0.03^{b}
Surface area (mm^2)	0.97 ± 0.23^{a}	0.58 ± 0.05^{a}	0.10 ± 0.02^{b}
Circumference (mm)	3.78 ± 0.58^{a}	2.73 ± 0.11^{a}	1.09 ± 0.11^{b}

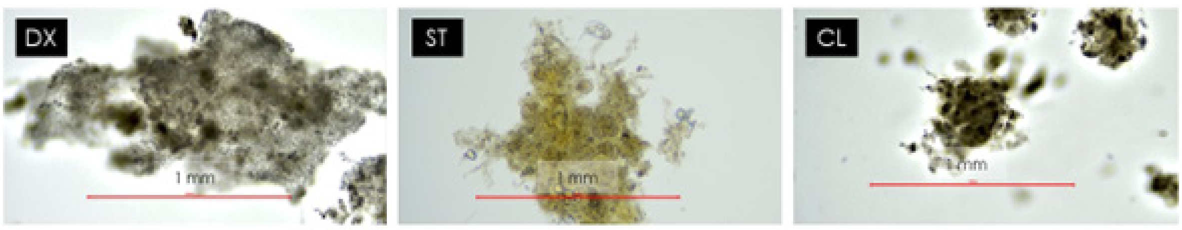

Figure 1. Biofloc particles in shrimp aquaculture photographed using a microscope with a magnification of 10×.

Table 2. Proximate composition of biofloc (on a dry weight basis) in shrimp aquaculture water using the biofloc system with different carbon sources.

Parameter	Treatment		
	DX	ST	CL
Protein (%)	59.76 ± 0.84[a]	34.06 ± 2.10[b]	37.94 ± 1.89[b]
Lipid (%)	4.00 ± 0.04[c]	6.70 ± 0.14[b]	9.29 ± 0.20[a]
Fibre (%)	9.84 ± 0.07[b]	20.68 ± 0.23[a]	22.18 ± 1.00[a]
BETN (%)	5.37 ± 0.87[a]	10.68 ± 1.37[a]	5.61 ± 2.22[a]
Carbohydrate (%)	15.21 ± 0.91[b]	31.37 ± 1.58[a]	27.79 ± 1.74[a]
Ash (%)	21.03 ± 0.70[c]	27.87 ± 0.39[a]	24.97 ± 0.23[b]

The data represents the mean values ± standard error (n=3); the same uppercase letters in the same row indicate no significant difference at the 5% level of significance (tested using Duncan's multiple range test). DX = Dextrose, ST = Potato starch, CL = α-cellulose.

Table 3. The minimum, maximum, and average values of water quality calculated based on daily, weekly, and end-of-rearing water quality data.

Parameter	Treatment					
	DX		ST		CL	
	Distribution	Mean	Distribution	Mean	Distribution	Mean
DO (mg/L)	5.5–6.9	6.4	5.5–6.9	6.4	5.2–69	6.3
Temperature (°C)	27.1–28.4	27.9	26.9–28.4	27.8	27.0–28.5	27.9
pH	6.8–8.0	7.8	6.8–7.9	7.6	6.8–7.8	7.6
Alkalinity ($CaCO_3$ mg/L)	122–180	146	112–139	120	111–130	119
TAN (mg/L)	0.00–0.08	0.03	0.00–0.16	0.07	0.00–0.25	0.05
NO_2^- (mg/L)	0.01–1.33	0.45	0.01–0.80	0.54	0.01–0.47	0.38
NO_3^- (mg/L)	2.66–83.33	40.53	2.92–83.33	57.25	3.18–83.33	48.97

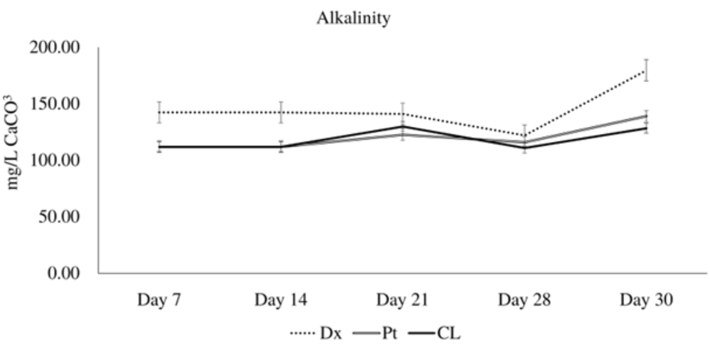

Figure 2. Fluctuations in the alkalinity concentration in the biofloc system aquaculture water over 30 days with different carbon sources.

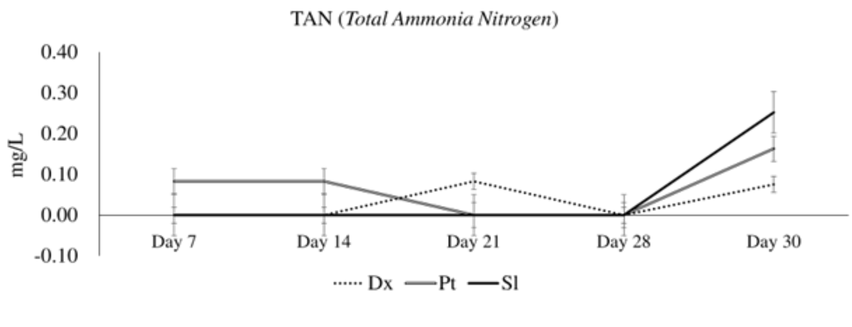

Figure 3. Fluctuations in the TAN (Total Ammoniacal Nitrogen) concentration in the biofloc system aquaculture water over 30 days with different carbon sources.

Discussion

Okomoda *et al*. (2022), reported that an initial increase in biofloc volume during aquaculture resulted in a decrease in total ammonia nitrogen (TAN) concentration in the shrimp cultivation water. This finding aligns with the results indicating a reduction in TAN concentrations for each treatment after the introduction of a carbon source. However, in shrimp cultivation, the DX treatment with a high biofloc volume yielded the lowest decrease in TAN concentration at the end of the cultivation period. Nevertheless, the outcomes of this study indicate that simple carbohydrates are more readily and rapidly utilized by heterotrophic bacteria. The water quality in the shrimp cultivation varied slightly among treatments, with differences observed primarily in alkalinity, total ammonia nitrogen (TAN), and NO2 concentrations.

The average concentration of alkalinity in the shrimp cultivation water for the DX treatment was higher than that for the ST and CL treatments. One possible explanation is that the process of complex carbohydrate simplification took too long, leading to autotrophic NH3 conversion by autotrophic bacteria utilizing CO_2 from alkalinity. Denitrification without organic material is considered to result in nitrate reduction and slower bacterial growth kinetics compared to heterotrophic denitrification with organic material, due to the low solubility of hydrogen and the hydrogenotrophic bacteria's need to assimilate inorganic carbon for growth (Albina *et al*., 2019). Bacterial species responsible for reducing TAN and NO_2 concentrations in shrimp cultivation water could also contribute to the lower alkalinity observed in the CL and ST treatments. Qiao *et al*. (2020), reported that Amonium removal is primarily performed by *Arthrobacter, Pseudomonas, Rhodococcus, Bacillus, Massilia*, and *Rhizobium* through complete heterotrophic aerobic nitrification, while Chryseobacterium and other incomplete denitrification species can only reduce nitrification products (NOX) through aerobic denitrification.

In the absence of dissolved CO_2, $CaCO_3$ will dissolve, resulting in hardness concentrations and alkalinity less than 10 mg/L as $CaCO_3$ (Boyd *et al*., 2016). Not only alkalinity, but the average pH values for the ST and CL treatments were also lower than those for the DX treatment. The lower average pH values in the ST and CL treatments compared to DX are presumed to be due to the inability of water alkalinity to support pH stability. The reaction of CO_2 with water, forming H_2CO_3, is one of the causes of a decrease in water

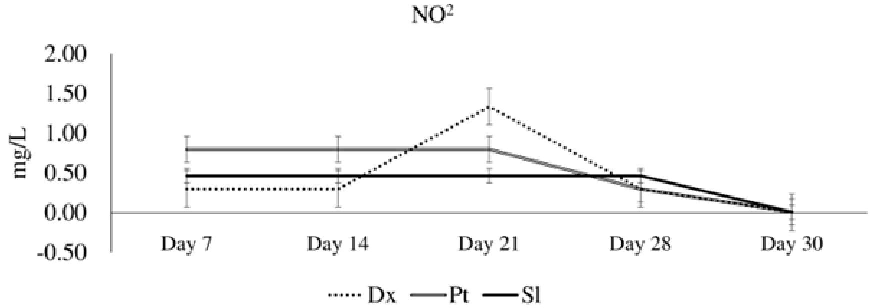

Figure 4. Fluctuations in the NO_2 (Nitrite) concentration in the biofloc system aquaculture water over 30 days with different carbon sources.

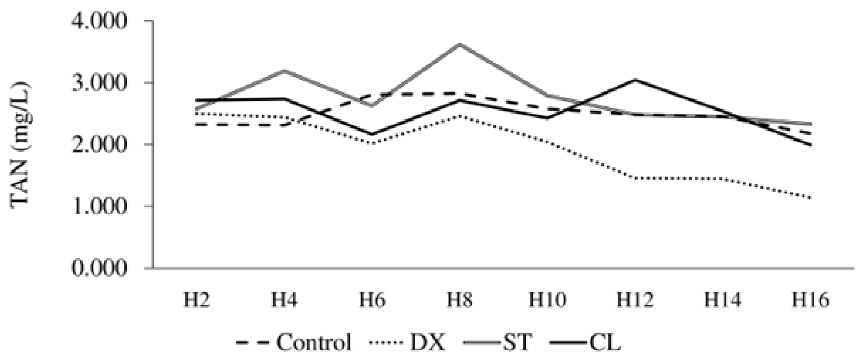

Figure 5. Comparison of TAN reduction rates among different carbon sources over 16 hours.

pH (Boyd *et al*., 2016). Biofloc also serves as an additional feed with implications for nutrient quality and biofloc particle size.

Krummenauer *et al*. (2020) reported that during the shrimp grow-out phase, biofloc can contribute 63-100% of carbon (C) and 35-86% of nitrogen (N) to shrimp tissue composition. With sequential protein content in DX, PT, and CL bioflocs being 59.76%, 34.06%, and 37.94%, respectively, DX biofloc emerges as the biofloc with the greatest nutrient contribution and highest shrimp yield. However, in the current study, the CL and PT treatments resulted in the highest final shrimp biomass (data reported in another publication). Ekasari *et al*. (2014) disclosed that shrimp exhibit a nibbling feeding habit, rendering differences in biofloc particle size inconsequential for shrimp utilization.

Similar findings are evident in Nile tilapia, where smaller particle-sized biofloc leads to superior growth performance and minimal impact on the gills of Nile tilapia (Lyu *et al*., 2023). These observations suggest that high nutrient quality in biofloc is not the sole factor supporting shrimp growth performance. The average final shrimp biomass reveals that shrimp in the PT and CL treatments are larger than those in the DX treatment ($P<0.05$) (data reported in another publication). Shrimp that extensively utilize biofloc may experience faster satiety, leading to a reduced appetite for feed.

This can result in lower shrimp growth performance, as commercial feed is proven to meet the nutritional needs of shrimp. This also indicates that the availability of biofloc in the DX treatment, which has larger particles compared to the PT and SL treatments, fills the shrimp intestines more quickly. As a consequence, the shrimp may deplete energy more rapidly due to nibbling processes involved in biofloc utilization, causing a lack of appetite for consuming feed. Consequently, this results in a significantly lower average final shrimp biomass in the DX treatment compared to the PT and SL treatments ($P<0.05$).

On the other hand, a lower final shrimp biomass can also be attributed to excessively dense biofloc volume. Okomoda *et al*. (2022), reported that an increase in biofloc volume during the early stages of cultivation led to a reduction in total ammonia nitrogen (TAN) concentrations in shrimp cultivation water. While an increase in biofloc volume is beneficial, excessively dense biofloc in the cultivation water is concerning as it may induce stress in aquaculture organisms. Assan *et al*. (2021), reported that stressed fish exhibit reduced feed consumption and slower growth performance compared to non-stressed fish.

Moreover, stress conditions are closely associated with the intensification of systems and fluctuations in the environmental water quality, which can lead to vibriosis outbreaks (Chen *et al*., 2018; Long *et al*., 2019). The biofloc volume generated by the DX treatment is higher than that in the ST and CL treatments. The use of simple carbohydrates as a carbon source in the biofloc system is a concern as it may potentially compromise shrimp health and growth performance. Conversely, the use of complex carbohydrates as a carbon source in the biofloc system has a greater potential to enhance shrimp health and growth performance.

CONCLUSION

The conclusion drawn from this study is that each type of carbon source is capable of improving water quality; however, the characteristics generated by complex carbohydrates, particularly cellulose, have a greater potential to enhance the performance of the biofloc system.

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