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The effect of molasses, tapioca and sago flour on biofloc system and volume of growth performance in whiteleg shrimp *Litopenaeus vannamei*

Tamrin Tamrin[1,*], Muhammad Aris[1], Waisya Ade Muntahar[1], Taufiq Abdullah[2]

[1]*Department of Aquaculture, Faculty of Fisheries and Marine Science, Khairun University, Ternate, Indonesia.*
[2]*Department of Aquaculture, Faculty of Marine and Fisheries Technology, State University of Gorontalo, Gorontalo, Indonesia.*

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ABSTRACT

Despite the high commercial success the whiteleg shrimp industry has achieved, challenges related to water quality and disease remain major issues. One approach to address these problems is Biofloc Technology (BFT). Carbon sources have been one of the factors influencing the characteristics of BFT. Some organic carbon sources used include molasses, tapioca flour, and sago flour. This study aimed to evaluate and compare the use of molasses, tapioca flour, and sago flour as organic carbon sources in BFT. It focused on examining, their impact on floc density and the growth performance of whiteleg shrimp. The study was conducted from November to December 2023 using a completely randomized design with treatments including a control, molasses, tapioca flour, and sago flour, each with three replicates. Parameters measured included biofloc volume, weight gain, average daily growth, survival rate, and feed conversion ratio. The results showed that BFV treatments with molasses, tapioca flour, and sago flour had significant differences compared to the control group. Sago flour provided a significant increase in BFV. The growth performance of whiteleg shrimp in treatments with molasses, tapioca flour, and sago flour improved and showed significant differences compared to the control. The application of BFT using molasses, tapioca flour, and sago flour as organic carbon sources significantly enhanced the biofloc volume, growth performance, feed conversion ratio, and survival rate of whiteleg shrimp. Among the tested carbon sources, sago flour demonstrated the highest biofloc volume and the most significant improvement in shrimp growth and feed efficiency.

Introduction

Whiteleg shrimp *Litopenaeus vannamei* is the most economically valuable global aquaculture commodity. In 2022, whiteleg shrimp contributed the most to global fisheries production, with 6.8 million tonnes, making it the top species produced (FAO, 2024). The potential for the development of whiteleg shrimp culture remains vast, especially due to the large target market (Purwanto *et al.*, 2023). Despite its high commercial success, the industry faces challenges related to water quality management and disease control, which directly affect the survival and growth of shrimp (Quyen *et al.*, 2020; Pardede *et al.*, 2024; Akbar & Fazli, 2023).

One approach that has gained increasing attention in recent years is the application of Biofloc Technology (BFT) (Deng *et al.*, 2018; Yu *et al.*, 2024). Bioflocs are aggregates composed of bacteria, microalgae, fungi, zooplankton, and other organic materials that can be utilized by cultured organisms (Ogello *et al.*, 2021; Khanjani *et al.*, 2022). BFT offers numerous benefits, including reduced water usage, improved nutrient recycling within the water, and suppression of pathogens in the culture environment (Kumar *et al.*, 2018; Zafar & Rana, 2022). Several studies have demonstrated the positive effects of BFT applications, such as enhanced survival and biomass, reduced cannibalism at high stocking densities, and suppression of disease-causing bacteria (Gustilatov *et al.*, 2022; Huang *et al.*, 2023; Izel-Silva *et al.*, 2024).

* Corresponding author.
Email address: tham.ibrahim@unkhair.ac.id

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Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Volume 14, Number 1, Page 98-103

The fundamental principle of the BFT is to recycle waste nutrients, particularly nitrogen, into microbial biomass (Ekasari *et al.,* 2014; McCusker *et al.,* 2023). One of the factors influencing the characteristics of BFT is the carbon source used. Selecting the appropriate organic carbon source is crucial for optimizing the BFT (Wei *et al.,* 2016; Ekasari *et al.,* 2024). Carbon sources such as molasses and tapioca flour have been shown to suppress *Vibrio* sp. populations, increase floc density, and improve the growth performance of whiteleg shrimp (Rahim *et al.,* 2023). Another carbon source with significant potential for use is sago flour.

Sago flour is derived from the sago palm *Metroxylon sagu*, which is widely found in Southeast Asia and the Pacific regions (Roslan *et al.,* 2020; Sidiq *et al.,* 2021). Sago flour can be utilized as an organic carbon source in BFT due to its high carbohydrate content, ranging from 81% to 96% (Uthumporn *et al.,* 2014; Liestianty *et al.,* 2016; Gunawan *et al.,* 2018). Sago flour also supports the growth of heterotrophic microbes (Thompson *et al.,* 2023). In addition, the application of sago flour as a carbohydrate source in tilapia feed has been proven to enhance growth and feed efficiency (Senawi *et al.,* 2020). Therefore, sago flour holds great potential for use as an organic carbon source in BFT.

Although various carbon sources in BFT have been studied, there has been no direct comparison of the effects of molasses, tapioca flour, and sago flour on floc density and the growth performance of whiteleg shrimp. This research offers novelty in its comprehensive evaluation of these three organic carbon sources within BFT, specifically focusing on their relative effectiveness in promoting optimal floc density and shrimp growth. It aimed to evaluate and compare the use of molasses, tapioca flour, and sago flour as organic carbon sources in BFT, assessing their impact on floc density and the growth performance of whiteleg shrimp to determine the most effective carbon source for enhancing shrimp aquaculture.

Materials and Methods

Location of research

This study was conducted for two months, from November to December 2023, at the Wet Laboratory of the Faculty of Fisheries and Marine Science, Khairun University, Ternate.

Container preparation

The containers for rearing the test shrimp were 12 round tanks, each with a diameter of 40 cm and a height of 22 cm. Before use, the tanks were thoroughly cleaned and then dried. Each tank was filled with 20 liters of seawater and equipped with aeration. The seawater had been previously filtered using a filtration system consisting of layers of charcoal, coral, sand, and gravel.

Biofloc preparation

The floc used for rearing was first fermented for 24 hours. The molasses-based carbon source floc was prepared by mixing 2 tablespoons of yeast, 5 ml of probiotics, 1 ml of molasses, and 1 liter of distilled water, followed by fermentation processes. Next, the tapioca flour-based carbon source floc was prepared by mixing 2 tablespoons of yeast, 5 ml of probiotics, 50 g of tapioca flour, and 1 liter of distilled water, followed by fermentation. Then, the sago flour-based carbon source floc was prepared by mixing 2 tablespoons of yeast, 5 ml of probiotics, 50 g of sago flour, and 1 liter of distilled water, followed by fermentation. After 24 hours, each fermentation product was cultured in a fiberglass tank with a diameter of 40 cm and a height of 22 cm, and aerated for seven days, with the addition of each carbon source molasses, tapioca flour, and sago flour-estimating the C/N ratio according to Ekasari *et al.* (2014), which is 15. The amount of carbon source added to the rearing water was calculated based on Avnimelech (1999). Once the floc suspension was formed, it was added to the rearing tanks at a volume of 10% of the total rearing container volume (2 liters for each 20-liter tank).

Animal testing

The juveniles of whiteleg shrimp at PL-16 stage, with an average weight of 0.02±0.00 g, were obtained from PT. Esaputlii Prakarsa Utama (Benur Kita), located in Barru Regency, South Sulawesi Province. The whiteleg shrimp from the hatchery have been declared Specific Pathogen Free (SPF) by Sarmayani *et al.* (2021). The whiteleg shrimp used for the treatments were stocked at a density of 20 shrimp per tank. During the rearing period, no water exchange was performed except for the control treatment, where 50% water was exchanged every 3 days, and aeration was provided continuously.

Experimental Design and Observation Parameters

This study employed a completely randomized design with treatments including a control (without BFT), molasses, tapioca flour, and sago flour, each consisting of three replicates. Feeding trials were conducted to satiation four times daily (at 7 a.m., 11 a.m., 3 p.m., and 10 p.m. Eastern Indonesia Time) for

Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Volume 14, Number 1, Page 98-103

60 days using commercial feed. The proximate composition of the commercial feed was 38% protein, 7% fat, 3% fiber, 13% ash, and 10% moisture content. Different parameters of the experiment were estimated using standard procedures reported by earlier researchers. These include biofloc volume (BFV) (Deng *et al.,* 2018) and growth performance, including weight gain (ΔW), average daily growth (ADG), specific growth rate (SGR), survival rate (SR), feed efficiency (FE) survival rate (SR), and feed conversion ratio (FCR) (Zokaeifar *et al.,* 2012; Lugert *et al.,* 2018; Rostika *et al.,* 2020). The calculation of these parameters was performed using the following formulas:

BFV = Volume of Floc in 1 L of water in the Imhoff cone
ΔW = Final weight (W_t) / Initial weight (W_0)
ADG = $(W_t - W_0)$/Days
SGR = ([ln Wt - ln W_0]/Days) × 100
SR = (Final numbers - Initial numbers) × 100
FE = ΔW / Total Feed Given
FCR = Total Feed Given / ΔW

Data analysis

Data were analyzed using Microsoft Excel 2010 and tested with analysis of variance (ANOVA). If, there was a significant effect, Tukey's follow-up test was applied. On the other hand, clinical signs parameters were analyzed descriptively.

Results

Biofloc volume (BFV)

The BFV observed in treatments with molasses, tapioca flour, and sago flour as carbon sources is presented in Figure 1. There were significant differences ($P < 0.05$) in BFV between the treatments and the control group. The BFV in the molasses treatment was 230.00 ± 16.52 mL L^{-1}, in the tapioca flour treatment it was 258.67 ± 12.06 mL L^{-1}, and in the sago flour treatment, it reached 341.67 ± 9.29 mL L^{-1}. However the control treatment without BFT did not show any floc suspension.

Growth performances

The growth performance of whiteleg shrimp in treatments with molasses, tapioca flour, and sago flour as carbon sources is presented in Table 1. The results indicate that these treatments were effective in enhancing the growth of whiteleg shrimp compared to the control group. This was evidenced by significant differences ($P < 0.05$) in the parameters of Wt, ΔW, ADG, and SGR between the treatments and the control. The treatments with molasses, tapioca flour, and sago flour as carbon sources also significantly improved the SR of whiteleg shrimp ($P < 0.05$) compared to the control. The SR observed were 82.00 ± 2.00% for molasses, 84.33 ± 1.53% for tapioca flour, and 85.33 ± 1.53% for sago flour. However, the control group without BFT showed a lower SR of 73.67 ± 4.16%.

The treatments with molasses, tapioca flour, and sago flour as carbon sources also significantly ($P < 0.05$) increased FE compared to the control. The FE values were 0.80 ± 0.00 for the control, 0.90 ± 0.00 for molasses, 0.93 ± 0.06 for tapioca flour, and 0.97 ± 0.06 for sago flour. Moreover, the carbon source treatments significantly ($P < 0.05$) reduced the FCR compared to the control. The FCR values were 1.12 ± 0.04 for molasses, 1.08 ± 0.03 for tapioca flour, and 1.03 ± 0.03 for sago flour. Meanwhile, the control group without BFT recorded a higher FCR of 1.25 ± 0.05.

Discussion

The application of BFT has become an alternative and attractive solution due to its role in promoting the success of sustainable aquaculture. BFT has been applied to many prominent aquaculture species such as catfish *Clarias gariepinus* (Fauji *et al.,* 2018; Chen *et al.* 2020), tilapia *Oreochromis niloticus* (David *et al.,* 2021; Khanjani *et al.,* 2022), milkfish *Chanos chanos* (Sontakke *et al.,* 2021), striped catfish *Pangasianodon hypophthalmus* (Singh *et al.,* 2024), and tiger shrimp *Penaeus semisulcatus* (Kaya *et al.,* 2023).

Whiteleg shrimp farming systems are also popular with the application of BFT (Rangka and Gunarto, 2012; Ferreira *et al.,* 2021). Positive effects have been found in BFT-based whiteleg shrimp farming systems, including improved growth performance, enhanced digestibility, improved health, increased gut microflora which leads to better digestibility, reduced water usage, improved nutrient recycling within the water, and the suppression of pathogenic bacteria causing diseases such as *Vibrio* sp (Kumar *et al.,* 2018; Gustilatov *et al.,* 2022; Huang *et al.,* 2023; Raza *et al.,* 2024).

One of the factors influencing the characteristics of BFT is the carbon source used. Selecting the appropriate organic carbon source is crucial for optimizing the BFT (Wei *et al.,* 2016; Ekasari *et al.,* 2024). Our study found that different carbon sources significantly ($P < 0.05$) affected the BFV. This is consistent with a finding by Deng *et al.* (2018), who also found that different carbon sources significantly impact BFV in whiteleg shrimp farming systems. Our findings also revealed an interesting observation regarding the use of plant-based carbon sources, particularly sago flour, which was able to increase BFV more than molasses and tapioca flour. This can be attributed to the fact that sago flour can be utilized as an organic carbon source in BFT due to its high carbohydrate content, ranging from 81% to 96% (Uthumporn *et al.,* 2014; Liestianty *et al.,* 2016; Gunawan *et al.,* 2018). Sago flour also supports the growth of heterotrophic microbes (Thompson *et al.,* 2023), making it a viable alternative carbon source alongside molasses and tapioca flour.

The fundamental principle of BFT is to recycle waste nutrients, particularly nitrogen, into microbial biomass, which can then be utilized by cultured organisms (Ekasari *et al.,* 2014; McCusker *et al.,* 2023). This study found that BFT with carbon sources from molasses, tapioca flour, and sago flour significantly ($P < 0.05$) influenced the growth of whiteleg shrimp. This aligns with the findings of Chethurajupalli and Tambireddy (2021), who found that BFT can enhance the growth of whiteleg shrimp. The role of BFT in supporting growth also positively impacts feed efficiency (Oliveira *et al.,* 2021). This study found that BFT with carbon sources from molasses, tapioca flour, and sago flour significantly ($P < 0.05$) affected the FCR. This is in line with the research by Suleman *et al.* (2024), which found that the application of BFT in catfish can reduce FCR more effectively compared to non-BFT systems. In addition to its positive impact on growth and feed efficiency, BFT also enhances SR. The results of this study indicate that BFT with carbon sources from molasses, tapioca flour, and sago flour significantly ($P < 0.05$) influenced the SR of whiteleg shrimp. This is similar to the findings of Panigrahi *et al.* (2018), who found that the application of BFT can improve SR.

BFT can enhance growth, feed efficiency, and SR because the flocs in the medium serve as supplementary feed that supports growth performance (Castro *et al.,* 2021). Furthermore, bioflocs contain amino acids, fatty acids, vitamins, and minerals that are beneficial for shrimp growth (Toledo *et al.,* 2016; Ballester *et al.,* 2018; Castro *et al.,* 2021). Additionally, bioflocs are aggregates composed of bacteria, microalgae, fungi, zooplankton, and other organic materials that can be utilized by cultured organisms (Ogello *et al.,* 2021; Khanjani *et al.,* 2022). Thus, the growth, FCR, and SR in treatments of molasses, tapioca flour, and sago flour in BFT are higher compared to the control one.

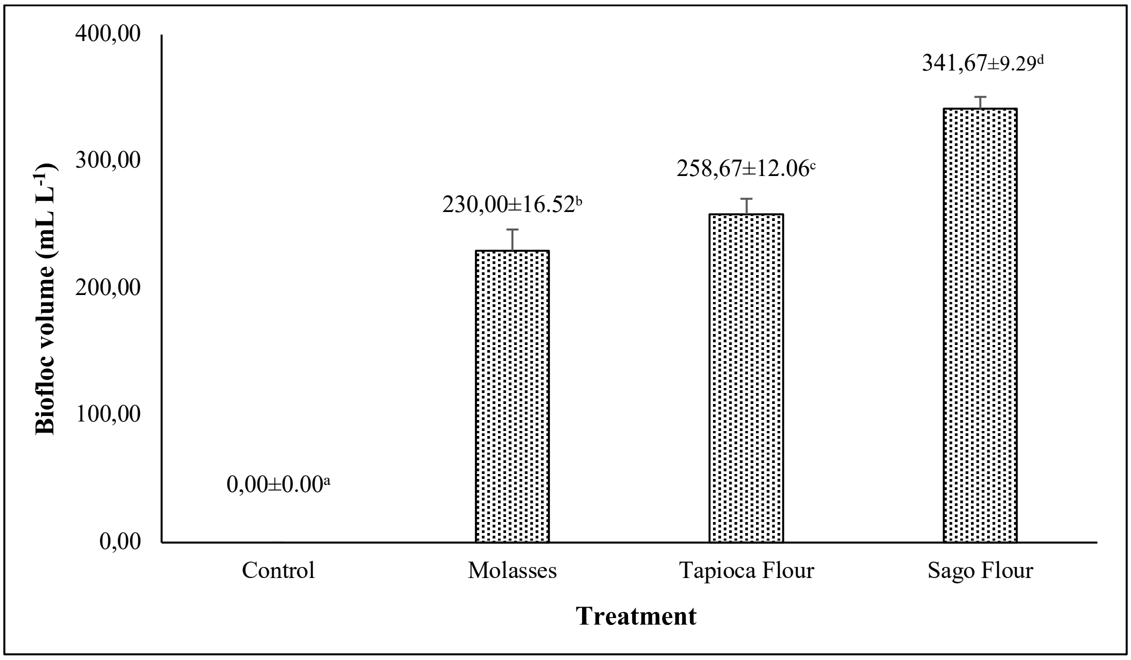

Figure 1. Biofloc volume in treatment. Data (mean ± SD) followed by different letters indicate significant different ($P<0.05$) at the 5% level (Tukey test).

Depik Jurnal Ilmu-Ilmu Perairan, Pesisir dan Perikanan
Volume 14, Number 1, Page 98-103

Table 2. The initial weight (W_0), final weight (W_t), weight gain (ΔW), average daily growth (ADG), specific growth rate (SGR), survival rate (SR), feed efficiency (FE) and feed conversion ratio (FCR) of whiteleg shrimp in different treatment.

Parameter	Treatment			
	Control	Molasses	Tapioca Flour	Sago Flour
W_0 (g)	0.02±0.01[a]	0.01±0.01[a]	0.02±0.01[a]	0.03±0.01[a]
W_t (g)	2.29±0.20[a]	2.95±0.23[b]	3.17±0.34[b]	3.32±0.07[b]
ΔW (g)	2.28±0.20[a]	2.93±0.24[b]	3.15±0.34[b]	3.29±0.07[b]
ADG (g day-1)	0.04±0.01[a]	0.05±0.00[b]	0.05±0.01[b]	0.05±0.01[b]
SGR (%)	0.80±0.03[a]	0.89±0.03[b]	0.92±0.03[bc]	0.97±0.03[c]
SR (%)	73.67±4.16[a]	82.00±2.00[b]	84.33±1.53[b]	85.33±1.53[b]
FE	0.80±0.00[a]	0.90±0.00[ab]	0.93±0.06[b]	0.97±0.06[b]
FCR	1.25±0.05[b]	1.12±0.04[a]	1.08±0.03[a]	1.03±0.03[a]

Description: Data (mean ± SD) different superscripts in the same line with different letters are significantly different (P<0.05) at the 5% level (Tukey test).

Conclusion

The application of BFT using molasses, tapioca, and sago flour as organic carbon sources significantly enhanced the BFV and growth performance of whiteleg shrimp *L. vannamei*. Among the tested carbon sources, sago flour demonstrated the highest BFV and the most significant improvement in shrimp growth and feed efficiency. The findings suggest that sago flour is a promising alternative carbon source in BFT systems for whiteleg shrimp aquaculture, contributing to more sustainable and efficient shrimp production.

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