



Effects of difference C/N ratio on water quality of white leg shrimp (*Litopenaeus vannamei*) cultivation

Gamal Mustik Samadan*, Fatma Muchdar, Ismawati Ridwan

¹Study Program of Aquaculture, Fisheries and Marine Science Faculty, Universitas Khairun, Ternate, Indonesia.

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ABSTRACT

Biotechnology is used to apply technology to the C/N ratio by stimulating the activities of heterotrophic microorganisms, which derive food from carbon and nitrogen substrates in a certain ratio. The goal of this research was to see how the optimal carbon-nitrogen ratio affected water quality and floc volume in white leg shrimp (*Litopenaeus vannamei*) farming. This study took place in the UPT Integrated Laboratory Unkhair in Ternate City in July and August 2021. A fully randomized design (CRD) with four treatments and three replications was used in this investigation. Different C/N ratios had an effect on ammonia, nitrite, nitrate, floc volume, and feed conversion ratio, according to the research. The water quality parameters are still suitable for white leg shrimp cultivation. In white leg shrimp culture, the addition of C-organic molasses (different C/N ratios) was able to reduce the concentrations of (NH₃), (NO₂), and (NO₃), and also greatly increase the flock volume and streamline the feed conversion ratio.

Introduction

White leg shrimp is a brackish water commodity in high demand because to its disease resistance, relatively fast growth, and high maintenance survival rate (Panigrahi *et al.*, 2018). White leg shrimp culture is currently carried out at a high density. In an effort to increase production, high stocking densities are used during cultivation without environmentally suitable waste management, resulting in a decrease in aquaculture water quality (Samadan *et al.*, 2018; Akbar and Fazli, 2023). Aquaculture waste with a high nutrient content has the potential to harm the aquaculture ecosystem. The usage of a biofloc system in shrimp and fish farming is one of the approaches for handling aquaculture waste. (Avnimelech, 1999; Crab *et al.*, 2007; Ekasari, 2009). Biofloc is a composite of autotrophic and heterotrophic organisms as well as waste that combines in easily with the water. Biofloc method involves adding organic carbohydrates to the maintenance medium to improve the C/N ratio and encourage the development of heterotrophic bacteria

transforming inorganic nitrogen into bacterial biomass (Crab *et al.*, 2007).

According to De Schryver *et al.*, (2008), heterotroph bacteria will be using N, both inorganic and inorganic forms, for the creation of biomass in the condition of a balanced C/N ratio in development medium, decreasing the concentration of N in water. In the biofloc system, the comparison of carbon (C) and nitrogen (N) materials (ratio C/N) is critical for bacteria to reproduce effectively, which impacts the structure of floc production (Maulina, 2009). Biofloc required a carbon-to-nitrogen ratio of at least 1:12 to function properly (Suryaningrum, 2012).

The C/N ratio is one technique to improve the intense cultivation system and the use of low-cost, practical technologies in aquaculture waste management (Avnimelech *et al.*, 1981). Biotechnology is the application of technology to the C/N ratio because it initiates the work of heterotrophic microorganisms (Ekasari *et al.*, 2015). The C/N ratio and the operating mechanism of bacteria were related as bacteria take nutrition from carbon and nitrogen

* Corresponding author.

Email address: gmsamadan@unckhair.ac.id

substrates in a certain ratio (Miao et al., 2017). As a result, bacteria would also function at their optimum to convert hazardous inorganic nitrogen into non-toxic organic nitrogen, ensuring that water quality is preserved and bacterial biomass is important as a source of protein for fish and shrimp. This mechanism contributes to the high feed efficiency. In general, a C/N ratio of higher than 15 is often recommended in aquatic environment (Avnimelech, 1999). The objective of this research would be that the carbon-to-nitrogen ratio affects water quality and floc volume in a laboratory-scale white leg shrimp culture.

Materials and Methods

Time and site

This research was conducted in the Integrated Wet Laboratory of Khairun University in Ternate City, North Maluku Province, over 60 days (July-August 2021).

Materials and Tools

Tools and materials used during the research, such as aquarium, aerator, analytical balance, tissue, stationery, white leg shrimp fry (PL10), molasses, bran, yeast and probiotic EM4.

Experimental Design

This study was carried out utilizing an experimental approach using a fully randomized design (CRD) with four treatments and three replications. Control treatment (A): no biofloc; treatment B: C/N ratio 10; treatment C: C/N ratio 12; and treatment D: C/N ratio 15 were the therapies that were attempted.

Parameters Measured

Water quality includes salinity, temperature, pH, dissolved oxygen (DO), ammonia (NH₃), nitrate (NO₃), and nitrite (NO₂) (APHA, 2005). Growth performance (Budidardi, 2008) such as specific growth rate (SGR), absolute growth, survival (SR), flock volume (Suprpto and Samtafsir, 2013), and feed conversion rate (FCR) (Efendie, 2003).

Data Analysis

Prior to analysis of variance, all data were subjected to normality and homogeneity tests. The least significant difference test (LSD) was employed if the treatments were different, and analysis of variance was done to determine the impact of treatment at the 95% confidence level (Zar, 2010). StatCal software version 1.1 was used to analyze the data. The water quality was descriptively assessed and presented in the form of a table.

Results

Ammonia (NH₃)

Figure 1 shows the results of the ammonia (NH₃) measurements taken during the experiment.

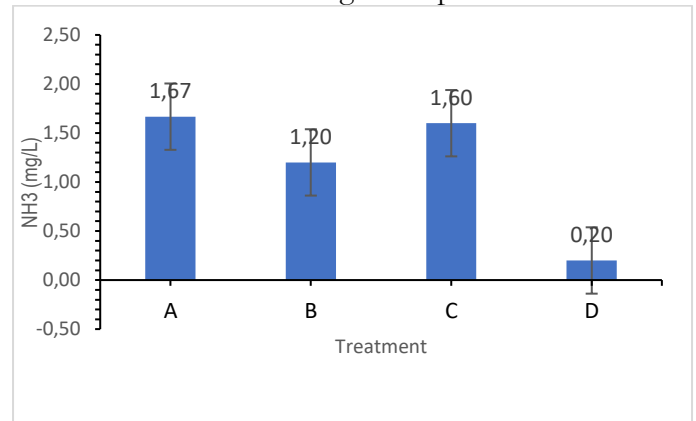


Figure 1. Ammonia concentration (NH₃) for each treatment

Table 1 shows the results of data analysis of variance of the impact of the C/N ratio on the ammonia (NH₃) parameter.

Table 1. The result of analysis of variance of ammonia concentration (NH₃)

Variable	db		F Statistic	P Value
	db nom	denom		
NH ₃	3	8	103**	0

**significance different

Table 1 shows that various C/N ratios have an effects on the ammonia (NH₃) parameter, according to the findings of the analysis of variance. Treatment D was the treatment that considerably lowered the ammonia levels, according to the test findings (LSD).

Nitrate (NO₃)

Figure 2 shows the results of nitrate (NO₃) measurement data during the experiment.

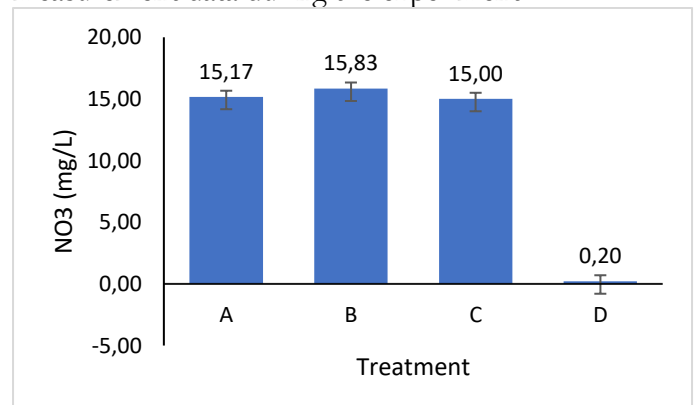


Figure 2. Nitrate (NO₃) concentrations for each treatment

Table 2 shows the results of the analysis of variance of the effect of the C/N ratio on the ammonia (NO₃) parameter.

Table 2. The result of a nitrate concentration analysis of variance

Variable	db nomin	db denomin	F Stat	P Value
NO ₃	3	8	52,17**	0,00

**significance different

The analysis of variance data in Table 2 show that various C/N ratios have had an effect on nitrate (NO₃) concentrations. Treatment D was found to be the treatment that considerably decreased the nitrate content in the test results (LSD).

Nitrite (NO₂)

Figure 3 shows the results of nitrite (NO₂) measurement data during the experiment.

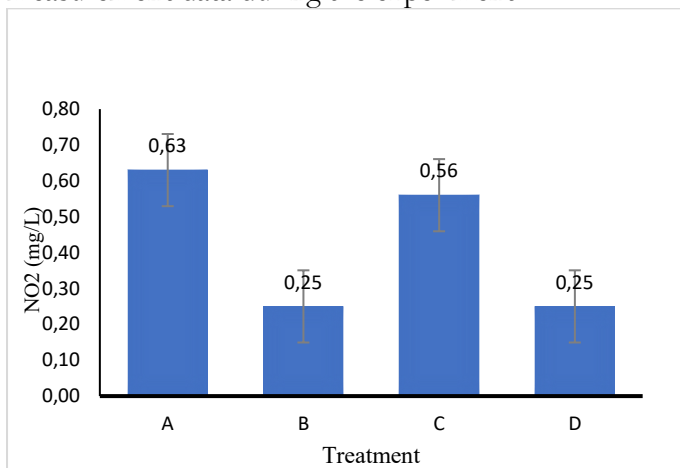


Figure 3. Concentration of nitrite (NO₂) during the study

Table 3 shows the results of the analysis of variance of the effect of the C/N ratio on the ammonia (NO₂) parameter.

Table 3. The result of a nitrite concentration analysis of variance

Variable	db nomin	db denom	F Stat	P-Value
NO ₂	3	8	265,40**	0,00

**significance different

The analysis of variance results in Table 3 show that various C/N ratios have had an effect on the nitrite (NO₂) parameter. Treatment D significantly lowered the levels of nitrite, according to the test results (LSD).

Floc Volume

Figure 4 shows the result of measuring the volume of floc during the experiment.

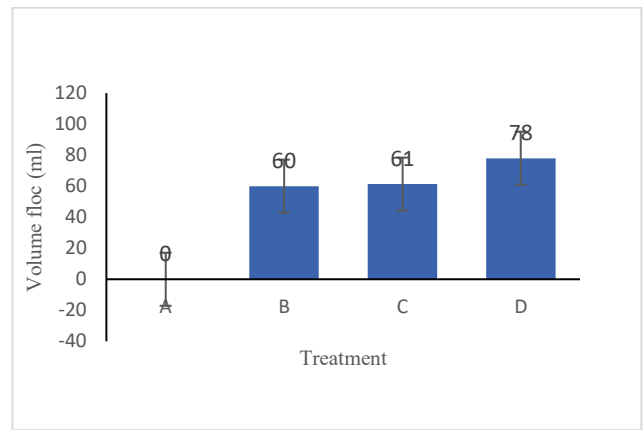


Figure 4. Floc volume in each treatment

Table 4 shows the results of the analysis of variance of the effect of the C/N ratio on the volume of the floc.

Table 4. The results of the analysis of the variance of the floc volume

Variable	db nomin	db denom	F Stat	P-Value
Volume floc	2	6	6,84**	0,03

**significance different

The analysis of variance results in Table 4 show that various C/N ratios have an impact on the volume of the floc. Treatment D was tested for significance in the follow the test (LSD).

Absolute Weight Growth

Figure 5 shows the results of measuring absolute weight gain all through the experiment.

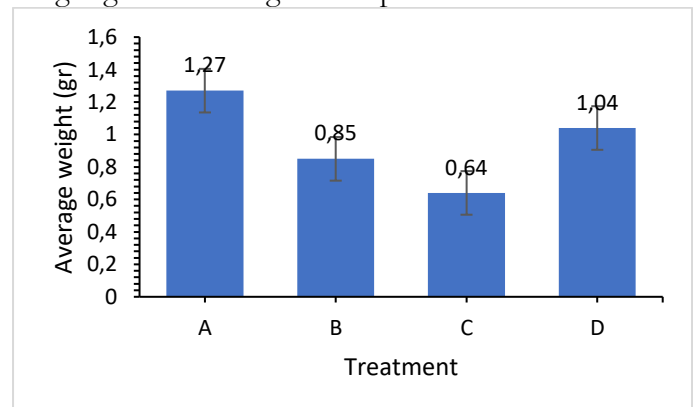


Figure 5. Absolute weight growth in each treatment

Table 5. Result of analysis of variance of absolute weight growth (gr)

Variable	db nomin	db denom	F Stat	P-Value
Weight gain	3	8	2,21*	0,16

*nonsignificance

The analysis of variance results in Table 5 show that the application of different C/N has no effects on the absolute weight growth parameters.

Specific Growth Rate (SGR)

Figure 6 shows the results of the shrimp weight measurements during the experiment.

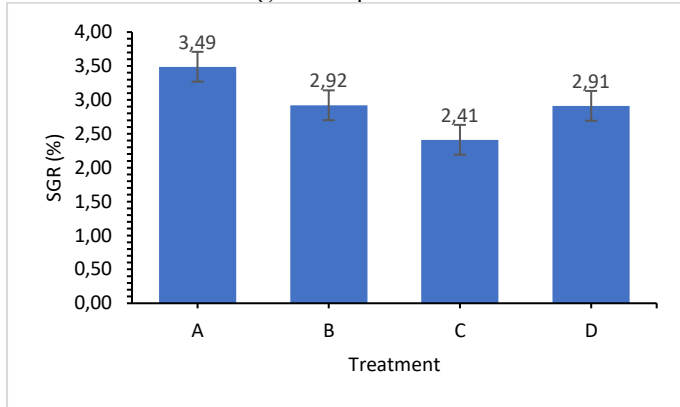


Figure 6. Specific growth rate (SGR) for each treatment

Table 6 shows the results of the analysis of variance of the C/N ratio's effect on the specific growth rate. The variance result showed that the C/N ratio has really no effect on the particular growth rate.

Table 6. Result of analysis of variance of specific growth rate (SGR %)

Variable	db nomin	db denom	F Stat	P Value
SGR	3	8	0,552*	0,6604

*nonsignificance

Survival Rate (SR)

Figure 7 shows the survival of white leg shrimp during the experiment.

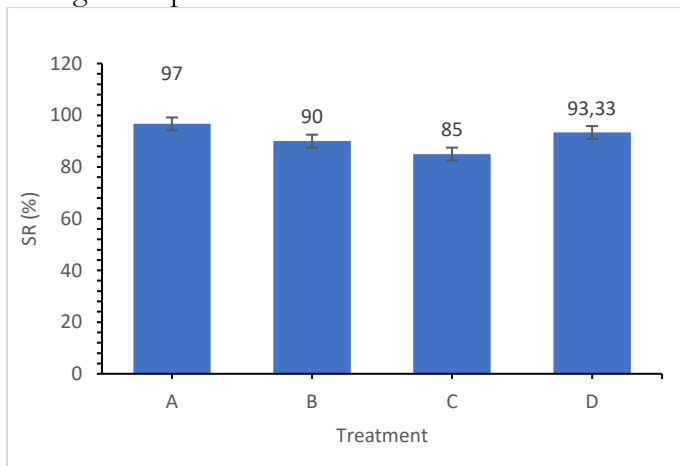


Figure 7. Average survival rate (SR) of each treatment during the study

Table 7 shows the results of the analysis of variance of the effect of the C/N ratio on survival.

Table 7. The results of the survival rate variance analysis (SR %)

Variable	db nominator	db denominator	F Stat	P Value
SR	3	8	412,39**	0

**significant different

The analysis of variance results in Table 7 show that various C/N ratios have an effect on survival, particularly treatment B being a significant treatment.

Feed Conversion Ratio (FCR)

Figure 8 shows the result of the feed conversion ratio calculation performed during the experiment.

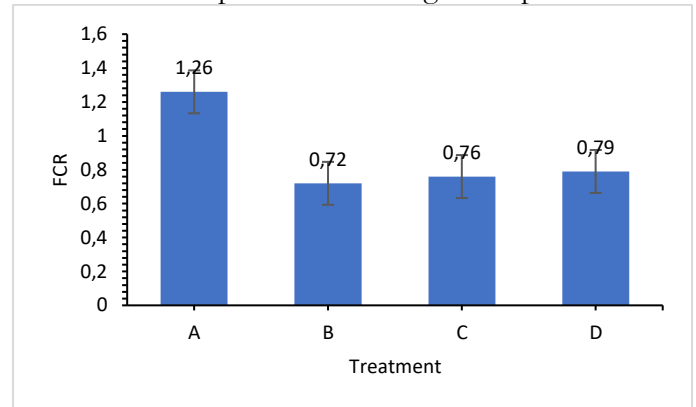


Figure 8. Average of feed conversion ratio (FCR) for each treatment

Table 8 shows the results of the analysis of variance of the effect of the C/N ratio on the feed conversion ratio.

Table 8. The result of analysis of variance of feed conversion ratio (FCR)

Variable	db nomin	db denom	F Stat	P-Value
FCR	3	8	25,26**	0,00

**significance different

Table 8 shows the results of the analysis of variance of the effect of the C/N ratio on the feed conversion ratio.

Water Qualities

Table 9 shows the study's water quality metrics, such as temperature, salinity, pH, and DO.

Table 9. The results of the water quality samples collected during the research

Parameter	Treatment			
	A	B	C	D
Temperature (°C)	25,3-25,4	25,2-25,3	25,7-25,6	28-29
Salinity (ppt)	11,9-10,11	12,0-11,5	11,9-12,4	11,2-11,0
pH	7-8	8	8	7-8
DO (mg/L)	26,7	26,6	27,8	27,3

Discussion

Based on the results of the analysis in Table 1, treatment D appears to have a substantial effect in lowering ammonia concentration when compared to the other treatments. Figure 1 illustrates this as well. The optimum C/N ratio for the development of bacteria that use nitrogen in the biofloc environment is shown in this condition. According to Nootong et al., (2011), when heterotrophic bacteria and molasses as a carbon source are added to the culture media, bacteria use the nitrogen and create flocs, lowering the quantity of ammonia. The biomass of heterotrophic bacteria included in the floc converted both organic and inorganic nitrogen present in the rearing water, in addition to phytoplankton and nitrifying bacteria, lowering the quantity of ammonia (NH₃) in the growth media (Avnimelech et al., 1981; Azhar, 2013).

The addition of C/N affected the concentrations of nitrite (NO₂) and nitrate (NO₃) in the same way as ammonia did, with treatment D being significantly different from the other treatments (Tables 2 and 3). According to Anand et al., (2013), adding carbon to the raising container reduced the levels of nitrate and nitrite. Similarly, Samocha et al. (2012) found that adding carbon to a system with a higher C/N ratio affects the nitrate level. The addition of molasses content will be easily utilized by microorganisms, resulting in faster water quality improvement (Tinh et al., 2021).

The addition of the C/N ratio affected the formation of floc capacity in the medium in Table 4, where treatment D was highly important, according to the results of the floc volume analysis. This is related to the occurrence of floc-forming bacteria, which will decrease organic materials in water (proteins, carbohydrates, fats, etc.) (Suprpto dan Samtafsir 2013). Meanwhile, Purnomo (2012) on the other hand, indicated that the increase in bacteria happened when carbon was added to the maintenance medium. The potential of bacteria to create biofloc is described by the high value of floc volume (Suwoyo dan Tampangallo, 2015; Salamah, 2018).

The effects of adding the C/N ratio on vaname shrimp growth performance, including as absolute growth and specific growth rate, were not significant in any of the treatments (Tables 5 and 6). While survival is useful (Table 7). Regardless of the fact that the four treatments were not significant in the study, the growth performance of vaname shrimp was nevertheless good (Figures 5 and 6), due to effective feeding (Table 8). It was also found that adding carbon (C/N ratio) improved shrimp survival significantly. This demonstrates that shrimp make reasonable utilization of feed (Avnimelech, 1999; Pantjara, 2008). During the

research, the water quality parameters (Table 9) were still effective in the development of white leg shrimp (Pan et al., 2007; Samadan et al., 2018).

Conclusion

Based on the result of the research and the discussion that describes, it can be shown that addition C-organic molasses (C/N 15) towards the white leg shrimp culture system biofloc can reduce ammonia (NH₃), nitrite (NO₂), and nitrate (NO₃) concentrations while also significantly increasing flock volume and improving efficiency the feed conversion ratio in white leg shrimp culture..

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