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Research Article

Utilization of Sugarcane Bagasse (*Saccharum officinarum* Linn.) as a Carbon Source in Biofloc System of Vaname Shrimp *Litopenaeus vannamei*

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Abstract

Background and Objective: Vaname shrimp (*Litopenaeus vannamei*) is one of the main economic commodities in aquaculture in the world. Biofloc is a cultivation technology that effectively improves the growth and health status of vaname shrimp. This research aimed to analyze the use of bagasse as a carbon source in the biofloc system for white shrimp cultivation. **Materials and Methods:** The shrimp used were 18 g/individual shrimp obtained from the Bone Marine and Fisheries Polytechnic Pond. Sugarcane bagasse processed from sugar factory waste was dried in an oven at 60°C and ground using a flouring machine. The research treatments included biofloc application where sugarcane bagasse played a role as a carbon source (L), biofloc application where wheat flour's role was as a carbon source (T) and control or no biofloc application (K). **Results:** This research showed that sugarcane bagasse could be used as a carbon source for white shrimp biofloc cultivation where the growth value tended to be the same as wheat flour. Total hemolytic count (THC) and shrimp survival in sugarcane bagasse biofloc were as good as wheat flour biofloc. Sugarcane bagasse biofloc had the same ability as wheat flour biofloc in reducing ammonia levels in the rearing media. Sugarcane bagasse biofloc had the same ability as wheat flour biofloc in reducing ammonia levels in the rearing media. The application of bagasse had no effect on temperature, pH, dissolved oxygen and salinity of the rearing media because this treatment was in the optimal range for the growth of vaname shrimp. **Conclusion:** Sugarcane bagasse has the potential to be a carbon source in biofloc systems because it could improve growth, health status, survival and water quality.

Key words: Aquaculture, biofloc system, carbon source, shrimp cultivation, sugarcane bagasse, water quality

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The white shrimp (*Litopenaeus vannamei*) stands as a prominent aquaculture commodity globally. Key producing nations for Vannamei shrimp include Japan, Taiwan, China, the Philippines, Malaysia and Indonesia¹. The global demand for vaname shrimp in the market is projected to improve annually, creating a significant shortfall in supply within the industry². One of the technologies currently being developed is biofloc. Biofloc is an effective cultivation technology used to improve the growth and health status of vaname shrimp^{3,4}. The core concept of biofloc is to repurpose nutrients produced by shrimp excretion and residual feed into bacterial biomass through the activity of heterotrophic bacteria, creating a resource that shrimp can utilize. Biofloc itself consists of various microbial communities such as protozoa, bacteria and zooplankton which contain vitamins, the amino acid methionine, enzymes and minerals that act as food supplements that help shrimp digestion, so biofloc has the potential to save the use of shrimp feed^{5,6}. Probiotics in biofloc play a role in increasing the nutritional value of feed and the performance of the immune response to disease, improving the quality of the cultivation environment⁷, increasing the feed conversion ratio and inhibiting pathogenic bacteria⁸⁻¹⁰. Growing probiotics in biofloc media require a balance between the ratio of carbon (C) and nitrogen (N).

Generally, carbon sources are obtained from molasses and flour¹¹, while nitrogen sources are obtained from animal organic materials such as fish meal or pelleted feed itself. The use of nitrogen sources from leftover feed and chicken manure has been studied in tilapia. The research results show optimal outcomes^{12,13}. The use of various types of fertilizer and NH₄Cl on vaname shrimp can also be a good source of nitrogen for the growth of biofloc microbes^{14,15}. One material containing carbon and nitrogen that has potential in biofloc cultivation is sugarcane bagasse (*Saccharum officinarum*L.)¹⁶. Sugarcane is a plantation commodity used as raw material for making sugar. Sugarcane bagasse is an organic waste often produced in sugarcane processing factories in Indonesia¹⁷. This by-product is available in abundance in Bone Regency, South Sulawesi¹⁸.

Several studies have been carried out to utilize sugarcane bagasse in cultivation activities. Research by Krishnani *et al.*¹⁹ shows that sugarcane bagasse can be used as an ammonia bioremediation agent in shrimp cultivation. Similar results were shown by research by Hassan *et al.*²⁰ that the use of bagasse as a carbon source in vaname shrimp larvae has the potential to express genes related to growth and immunity. These findings recommend that the addition of bagasse as a

carbon source in cultivation without water changes and artificial feed can improve the zootechnics and immunity of vaname shrimp. The use of sugarcane bagasse as biofloc has also been researched. The results of this research show the potential of sugarcane bagasse as a carbon source. The research results of Yassien *et al.*²¹ show that sugarcane bagasse can effectively increase the activity of nitrifying bacteria so that this material reduces inorganic nitrogen levels and improves the water quality of vaname shrimp biofloc. Sugarcane bagasse contains 1.5% protein, 1.5% lipid, 7.6% ash content, 65% fiber, 24.4% carbohydrates and 39.45% organic carbon. The flour generally used in biofloc contains 12.2% protein, 1.2% lipid, 4.1% ash content, 1.3% fiber, 81.2% carbohydrates and 41% organic carbon²². Sugarcane bagasse can be used as a carbon source because it can support ammonia elimination and the production of higher biofloc volumes. Sugarcane bagasse applied to biofloc can also increase shrimp growth, feed utilization, total heterotrophic bacteria and water quality. Based on this description, an analysis of the use of sugarcane bagasse as a carbon source in the biofloc system needs to be carried out for growing vaname shrimp.

MATERIALS AND METHODS

The study was conducted between March and August, 2023 in Teaching Factory, Polytechnic of Marine and Fisheries, Bone Regency, South Sulawesi.

Preparation of animal test: Vannamei shrimp (*L. vannamei*) measuring 18 g/individual came from the Pond of Polytechnic of Marine and Fisheries, Bone. The shrimp were adapted one week before being given aquarium treatment. During adaptation, shrimp were given commercial 35% protein feed with a biomass feeding rate (FR) of 5% with a feeding frequency of 4 times/day following the Robertson *et al.*²³ method. The shrimp were put into a research container with a volume of 120 L with a density of 20 fish/120 L of water which referred to the intensive density of SNI 8008-2014 which ranged from 80 to 120 fish/m³²⁴. The container was sterilized using chlorine before use. The feeding procedure was the same as the feeding procedure during adaptation.

Preparation of sugarcane bagasse: Sugarcane bagasse was obtained from sugar factory waste in Arasoe Village, Bone Regency, South Sulawesi, Indonesia. Preparation referred to the method of Mansour *et al.*²² where the bagasse was cleaned and dried using an oven at a temperature of 60°C.

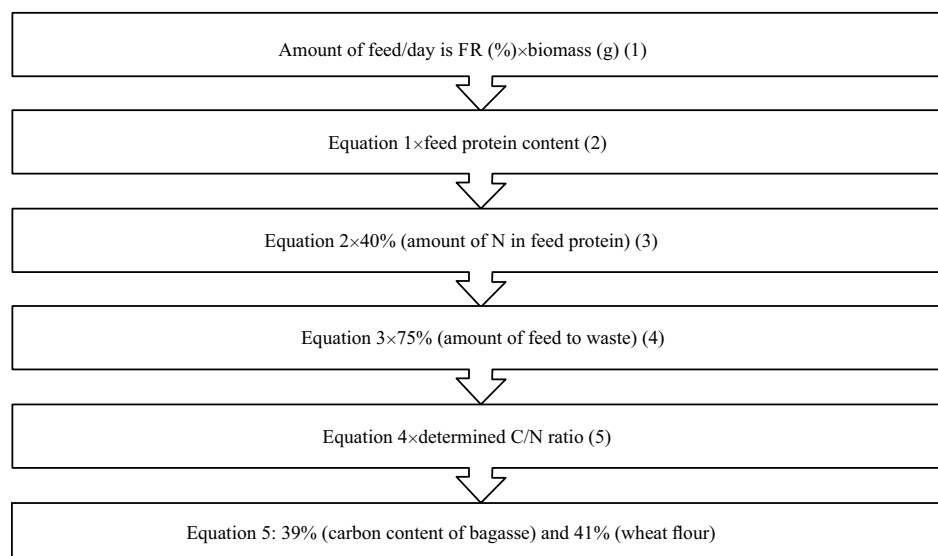


Fig. 1: Calculation of the requirement of carbon sources with a C:N ratio of 16

Table 1: Results of proximate test of sugarcane bagasse

Sample	Parameter (%)				
	Ash content	Water content	Lipid	Protein	Crude fiber
A	41.06	3.50	0	1.92	27.79
B	44.31	2.95	0	2.08	35.46

The bagasse was ground using a flouring machine until it became powdered flour and then it was sieved using a sieve. The bagasse flour was then tested proximately to determine its carbon content. The proximate results of bagasse can be seen in Table 1.

Preparation of biofloc: Sea water, as a maintenance medium, was obtained from the waters around Waetuo Village, Bone Regency, Indonesia. It was sterilized using 15 ppm chlorine. The water was allowed to aerate for 10 days before use. Biofloc was grown by gradually adding material to the maintenance medium for 14 days. The prepared wheat flour and bagasse were then used as a carbon source. Feed was used as a nitrogen source to form a floc with a C:N ratio of 16:1 according to the recommendations of Husain *et al.*²⁵. Probiotic bacteria obtained commercially were *Bacillus* sp. *Lactobacillus* sp. *Nitrosomonas* sp. and *Nitrobacter*. Material application refers to the method of Gustilatov *et al.*⁴. The amount of carbon added used the carbon calculation scheme by de Schryver *et al.*²⁶, namely 21.5 g of sugarcane bagasse and 20.5 g of wheat flour. As a source of nitrogen, shrimp were given feed 10 g/day with a protein content of 35%. The calculation method can be seen in Fig. 1.

Maintenance of shrimp: A total of 20 shrimp were put into the rearing medium. Maintenance was carried out for 30 days. Shrimp were given commercial pellet feed containing 35% protein with a frequency of 4 times/day and FR of 5%. During maintenance, monitoring of growth and flock volume was carried out at the beginning (0 days), middle (15 days) and end (30 days). Floc volume was measured using an Imhoff cone and controlled through microscope observation. Water quality measurements included observations of temperature, marine oxygen, salinity and pH which were carried out every day, namely morning and evening. Temperature and dissolved oxygen measurements used YSI Pro20I; salinity measurement utilized a refractometer and pH measurement used a pH meter. Ammonia (NH₃) and Nitrate (NO₃) measurements were carried out at the end of maintenance using the Ammonia and Nitrate Test Kit.

Experimental design: The research used a Completely Randomized Design (CRD) with 3 treatments and 3 replications. Each unit was placed randomly. The research treatments included biofloc application where bagasse was used as a carbon source (L), biofloc application where wheat flour was used as a carbon source (T) and control or no biofloc application (K). Observations made during the study were growth, blood picture (total hemocyte count), floc volume and survival. Shrimp weight was measured at the beginning, middle and end of rearing as Average Body Weight (ABW, g/ind). Absolute growth (AG, g/ind) was measured by referring to the Araneda *et al.*²⁷ equation, namely the

difference between final weight and initial weight. Total hemocyte count (THC, cell/mL) is a parameter measure of shrimp immunity which refers to the method of Liu and Chen²⁸, namely the number of cells counted $\times 1/\text{large box volume} \times \text{dilution factor}$. The growth parameter was measured following the method described by Begenal²⁹. Survival rate (SR, %) was calculated according to the formula³⁰:

$$SR = \frac{\text{Remaining shrimp number}}{\text{Stocked shrimp number}} \times 100$$

Ethical consideration: All procedures were approved by the Center for Research and Community Service of Polytechnic of Marine and Fisheries, Bone with number B.432/POLTEK.BN/RSDM.430/II/2024.

Statistical analysis: All the data collected for the parameters were analyzed using the IBM SPSS Statistic for Windows, version 16.00 (IBM Corp., Armonk, New York, USA) at a significance level of 0.05. Significant differences between treatments were determined using a *post hoc* Duncan's test.

RESULTS

The results showed that there was no real difference in ABW from all treatments at the beginning (0 days of maintenance) and the middle of the study (15 days of maintenance). At the final observation (30 days of rearing), the ABW of the T treatment was significantly higher, namely 26.3 ± 4.47 g/ind, compared to other treatments and controls as in Fig. 2. The highest absolute growth of shrimp occurred in the T treatment, namely 10.01 ± 4.54 g/ind as in Fig. 3.

The results of floc volume measurements showed that the floc volume was not significantly different at the beginning of the observation, but the results were different at the middle and end of the study. In the middle of the study, the floc volume in treatment L became higher, namely 12.66 ± 4.16 mL/L. At the end of the study, the volume of the T treatment flock was higher, namely 31.01 ± 3.61 mL/L as in Fig. 4. Total hemocyte count (THC) increased at the end of the study compared to the beginning of the observation. The THC of treatment L and treatment T was higher than the control. The THC values in treatment L, treatment T and control,

Table 2: Water quality was measured every day during the study

Treatment	Measuring time	Temperature (°C)	pH	Salinity (g/L)	Dissolved oxygen (ppm)
L	Morning	25.10-29.10	7.10-8.60	20.00-25.00	4.20-6.20
	Afternoon	28.60-29.70	7.10-8.20	20.00-26.00	2.30-6.60
T	Morning	27.10-28.80	6.94-8.60	22.00-26.00	4.34-6.95
	Afternoon	28.40-29.90	6.91-8.01	21.00-21.25	3.70-6.11
K	Morning	25.80-29.90	6.82-8.45	20.00-26.00	5.18-8.29
	Afternoon	27.10-29.80	6.80-7.99	18.00-26.00	5.36-8.81
Standard ²⁴		28-30	7.5-8.5	26-32	>4

L: Sugarcane bagasse, T: Wheat flour and K: Control

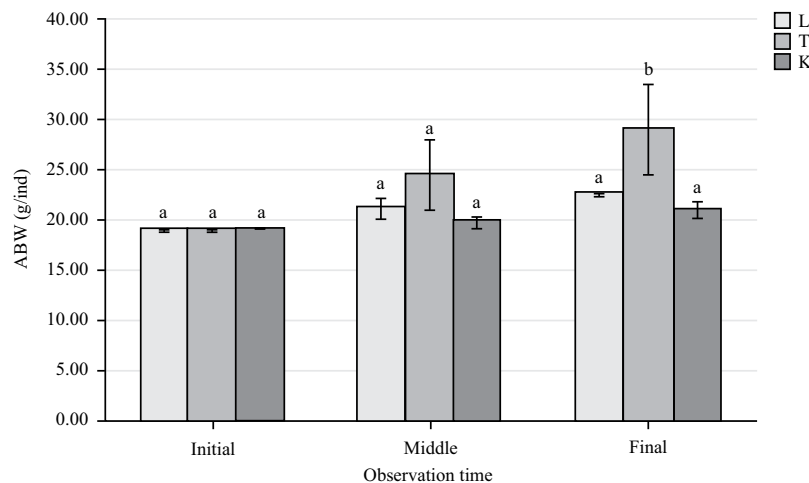


Fig. 2: Average body weight of vaname shrimp

Initial observation was 0 days after stocking, the middle was 15 days after stocking and the final was 30 days after stocking, L: Sugarcane bagasse, T: Wheat flour and K: Control. Different letter notations at the same observation time (Mean $\pm$ SD) indicate significantly different results (Duncan's $p < 0.05$)

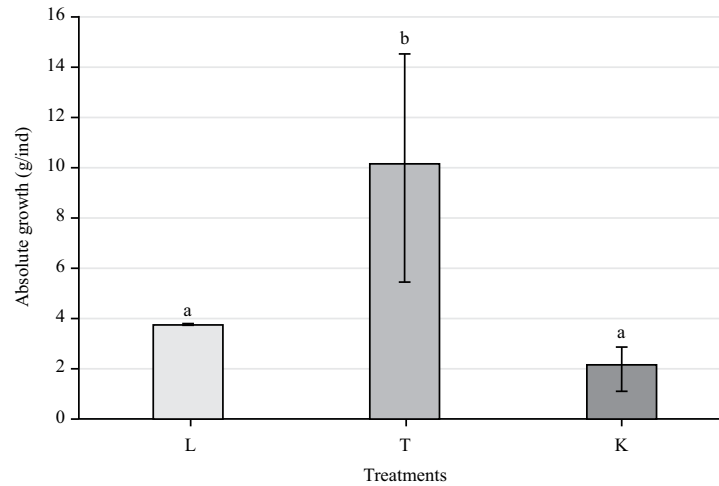


Fig. 3: Effect of various treatment on absolute growth of vaname shrimp after 30 days of rearing

L: Sugarcane bagasse, T: Wheat flour and K: Control, Different letter notations at the same observation time (Mean $\pm$ SD) indicate significantly different results (Duncan's $p < 0.05$)

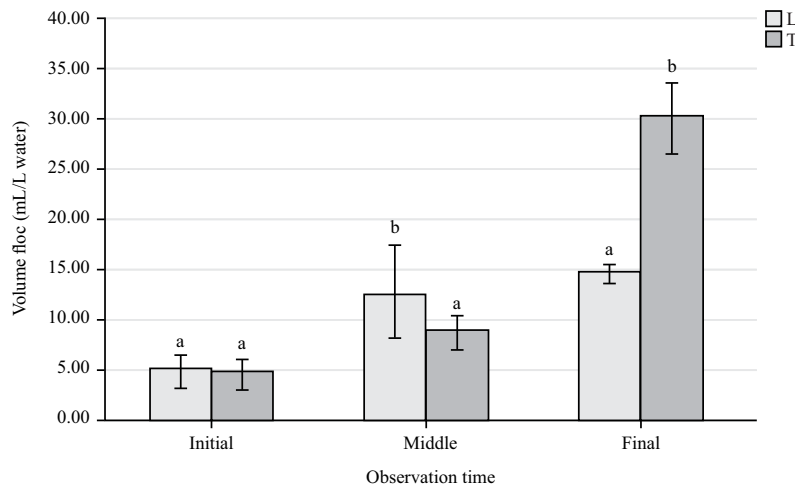


Fig. 4: Effect of floc volume on observation time

Initial observation was 0 days after stocking, the middle was 15 days after stocking and the final was 30 days after stocking, L: Sugarcane bagasse, T: Wheat flour and K: Control. Different letter notations at the same observation time (Mean $\pm$ SD) indicate significantly different results (Duncan's $p < 0.05$)

Table 3: Water quality was measured at the end of the study

Treatment	Ammonia (NH ₃) (ppm)	Nitrate (NO ₃) (ppm)
L	0.83 $\pm$ 0.05 ^b	5.62 $\pm$ 0.03 ^a
T	0.30 $\pm$ 0.29 ^b	6.33 $\pm$ 1.27 ^a
K	1.46 $\pm$ 0.31 ^a	5.56 $\pm$ 0.05 ^a
Standard ²⁴	<0.1	<0.5

L: Sugarcane bagasse, T: Wheat flour and K: Control, Different letter notations (Mean $\pm$ SD) indicate significantly different results (Duncan's $p < 0.05$)

respectively were 3.41×10^3 , 3.34×10^3 and 3.05×10^3 cells/mL as in Fig. 5.

The highest survival in all treatments, namely treatment L, treatment T and control, respectively were as follows, 88.33 ± 2.89 , 87.66 ± 5.77 and $66.33 \pm 2.89\%$ as in

Fig. 6. Results of water quality measurements in the form of temperature, salinity, pH, DO and nitrate showed the same range in the biofloc and control treatments (Table 2). The quantity of ammonia in all biofloc treatments was lower than the control.

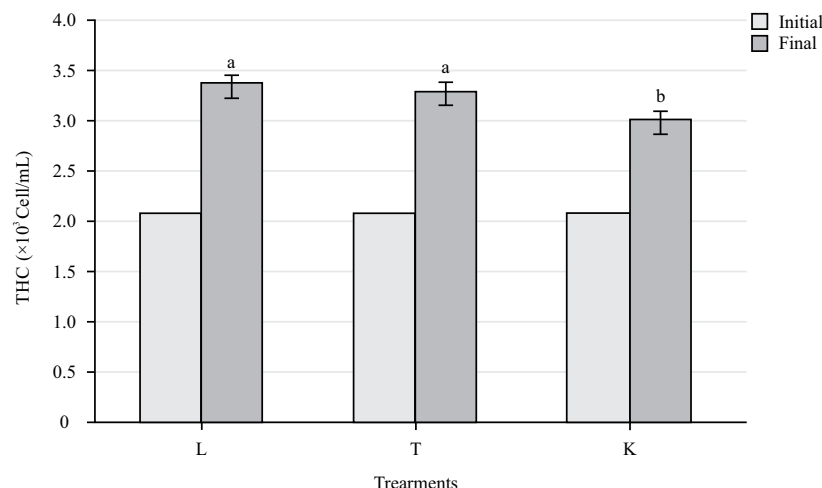


Fig. 5: Effect of various treatment on total hemocyte count

L: Sugarcane bagasse, T: Wheat flour and K: Control. Different letter notations at the sea observation time (Mean $\pm$ SD) indicate significantly different results (Duncan's $p < 0.05$)

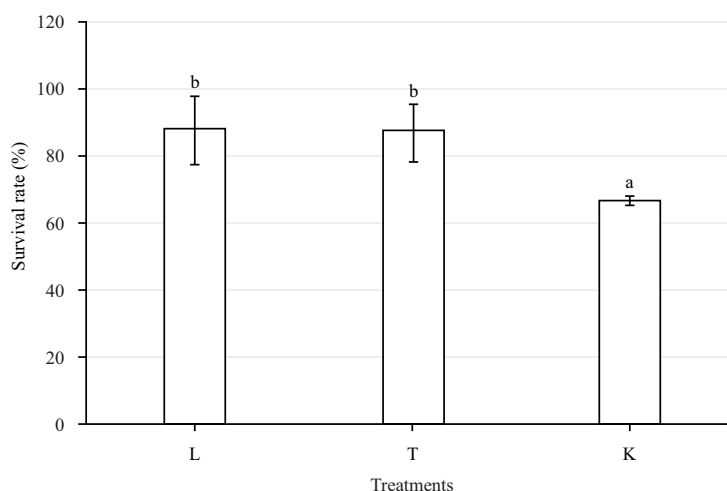


Fig. 6: Effect of various treatment on shrimp survival rate

L: Sugarcane bagasse, T: Wheat flour and K: Control. Different letter notations at the same observation time (Mean $\pm$ SD) indicate significantly different results (Duncan's $p < 0.05$)

DISCUSSION

The results of this research showed that bagasse used as a carbon source was able to increase growth and tended to be better than the control. This revealed that sugarcane bagasse had the potential to be a carbon source in rearing vaname shrimp using a biofloc system. Mansour *et al.*²² found the same thing in the use of sugarcane bagasse as a carbon source for vaname shrimp biofloc. The ABW of vaname shrimp raised using sugar cane bagasse was better than without biofloc. Hassan *et al.*²⁰ stated that bagasse has the potential to be a carbon source in vaname shrimp biofloc because of several

advantages, namely having a fairly high carbon content and acting as a substrate for heterotrophic bacteria to grow in the rearing medium. Shrimp reared in a biofloc system using sugarcane bagasse as a carbon source can increase the transcription of shrimp growth genes up to 5 times compared to controls²⁰. Biofloc is a collection of various types of microorganisms (floc-forming bacteria, filamentous bacteria, fungi), suspended particles, various colloids and organic polymers, various cations and dead cells²⁶. The principle of biofloc technology is to grow microorganisms in water with the help of activator bacteria. These bacteria will grow rapidly if they are given a carbon source³¹. The carbon source in

biofloc technology is very necessary to support the growth of microbes, especially floc-forming bacteria in the form of prebiotics (as a food source)³². Several carbon sources from various materials have been tested in previous research, namely molasses, glycerol, wheat flour and sugars such as dextrose and bran³³.

It was further explained that the growth of vaname shrimp on the application of wheat flour as a carbon source showed better results than sugar cane bagasse. Chakrapani *et al.*³⁴ reported that the application of wheat flour and rice flour as a carbon source was able to increase the growth of vaname shrimp in the biofloc system compared to other carbon sources such as bran. According to Yassien *et al.*²¹, wheat flour has a carbohydrate content of 81.2% (higher than bagasse flour which contains carbohydrates of 24.4%). Organic carbon from wheat flour is 41% and sugar cane bagasse is 39.45%. Apart from having a higher carbon source, wheat flour also dissolves easily in water so it is easily used as a substrate by bacteria. Sugarcane bagasse is high in fiber and has low solubility, limiting its use as a substrate by biofloc bacteria²¹. Therefore, wheat flour is superior as a carbon source in biofloc technology in supporting shrimp growth. The success of wheat flour in increasing the growth of vaname shrimp in a biofloc system has been previously reported by Citria *et al.*³⁵ and Erlangga *et al.*³⁶. In general, the application of biofloc in this study showed better results in increasing growth. Almuqaramah *et al.*³⁷ reported that the biofloc system in the rearing container was able to produce feed protein in it so that the floc formed could be used by shrimp as additional nutrition.

The success of the floc in providing a source of nutrients for shrimp was identified from the measured floc volume. Floc formation involves probiotic bacteria and other microorganisms through the formation of biofilms, namely extracellular compounds that initiate biofloc formation³⁸. This mechanism is naturally carried out by microorganisms to maintain their viability. Leftover feed and other organic particles are incorporated by microorganisms in the cultivation media⁵. The fiber contained in bagasse can be a good medium for the growth of microorganisms, so that biofloc formation becomes faster¹⁹. This is what causes the floc volume on day 15 (middle) of shrimp rearing to be higher than wheat flour. The increase in floc volume did not occur significantly until the 30th day because sugarcane bagasse fiber was difficult to decompose, so its use as a food source for floc-forming microorganisms was less than optimal. The floc volume increased gradually from the beginning to the end of

the observation. The maximum floc volume observed in the bagasse treatment was 15 mL/L, while the maximum floc volume in the wheat flour treatment was 35 mL/L. The floc volume observed in all treatments was still within the optimal range for white shrimp growth. According to Avnimelech³⁹, the maximum floc volume for shrimp growth is 50 mL/L. If the floc volume is greater than 50 mL/L, then the phenomenon that occurs is depletion of dissolved oxygen which interferes with shrimp growth.

Biofloc application can stimulate the immune system and improve the health status of shrimp. The non-specific shrimp immune system is composed of cellular and humoral. Hemocytes play a role in both cellular and humoral immune responses^{40,41}. Changes in the number of hemocytes (THC) are an indicator of shrimp health. Biofloc consists of probiotic bacteria containing lipopolysaccharide or peptidoglycan, fungi containing β -glucan and other microbes stimulating hemocytes and other immune parameters through pathogen-associated molecular pattern signals⁴²⁻⁴⁴. The increased THC in the shrimp's body can protect the shrimp from infection by pathogens and foreign objects. This shows that sugarcane bagasse biofloc has the potential to improve the health status of shrimp. Several studies related to the use of biofloc provide a protective effect against infection with the bacteria *Vibrio parahaemolyticus* which causes Acute Hepatopancreatic Necrosis Disease (AHPND) which has been previously reported^{4,45,46}. The tendency for higher THC in the bagasse treatment shows its potential as a good carbon source for bacterial growth and an inducer that increases THC in shrimp. Hassan *et al.*²⁰ reported that the application of sugarcane bagasse can increase the expression of the immunity genes β BGP (β -1,3-glucan binding protein) and L-GBP which is higher than the control. The β BGP and L-GBP are components of the immune response to infection that activate the coagulation cascade of the prophenoloxidase system and antibacterial effectors.

The survival of vaname shrimp is influenced by various factors including the immune system, quality, water and feed availability. The biofloc system is able to increase immunity, improve water quality and provide the food source that shrimp need, so that their survival is better. One indicator of increased shrimp immunity in this study was the THC value which increased higher than the control. Biofloc can be used as an additional food source so that it has a positive influence on the survival of vaname shrimp cultivation^{47,48}. Shrimp survival in the sugarcane bagasse treatment was not significantly different from the wheat flour treatment. This showed the potential of sugarcane bagasse as a carbon

source. The fiber in sugarcane bagasse could encourage bacterial growth because it acted as a substrate. Apart from that, the fiber in bagasse also increases the viability of bacteria considering its role as a good attachment point for water¹⁹. Probiotic bacteria provide various benefits for growth, pathogen protection and shrimp survival. The results of the study by Kurniaji *et al.*⁴⁹ show that differences in probiotics in biofloc can influence the growth performance and survival of shrimp by up to 88.9%. Kumar *et al.*³ reported that probiotics combined with biofloc application can control bacterial infections and provide antimicrobial agents.

Ammonia (NH₃) levels in biofloc are lower than controls (without biofloc). This shows that biofloc can reduce ammonia levels in waters. Hostins *et al.*⁵⁰ reported that the application of biofloc combined with probiotics in vaname shrimp cultivation can reduce TAN and speed up the nitrification and denitrification processes. This research also shows that the application of bagasse as a carbon source does not harm water quality, especially ammonia. According to Yassien *et al.*²¹ the use of bagasse in biofloc does not harm water quality. Utilizing sugarcane bagasse improves water quality by reducing TSS and reducing ammonia levels. High levels of ammonia and nitrite in water can be toxic to shrimp that are reared through nitrogen assimilation by heterotrophic bacteria⁵¹. Measurements of temperature, salinity and pH showed the same range in the biofloc treatments and control. The application of biofloc technology could help maintain water quality so that water quality remains in the optimal range. The DO measurement results showed different DO values in the treatment and control. This indicated that there was higher dissolved oxygen utilization in biofloc compared to the control. Dissolved oxygen in biofloc media was lower than without biofloc. This might be caused by the high activity of heterotrophic bacteria in utilizing oxygen for the breakdown of organic material. The rate of nitrification and respiration of microorganisms increased carbon dioxide levels in the floc and a decrease in dissolved oxygen levels.

CONCLUSION

The use of sugarcane bagasse as a carbon source in biofloc systems can enhance the growth, immune performance and survival of vannamei shrimp. Additionally, the use of sugarcane bagasse can aid in the formation of bioflocs within 15 days after stocking and improve water quality. Sugarcane bagasse can be used as a biofloc carbon source to replace commercial carbon sources with a maintenance period of 30 days. The use of sugarcane bagasse in vaname shrimp biofloc needs to be evaluated for a longer period of time.

SIGNIFICANCE STATEMENT

This research discovered the ability of sugarcane bagasse to be a carbon source for cultivating vaname shrimp using a biofloc system. Sugarcane bagasse was able to provide a carbon source to improve the growth and health status of vaname shrimp. This research provides information on managing sugarcane bagasse from factory waste as an alternative to reduce the costs of providing a large carbon source in the vaname shrimp biofloc system. Communities can apply this technology to optimize vaname shrimp production.

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