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Short Communication

Analysis of White Feces Disease (WFD) caused by *Vibrio* sp. and Dinoflagellata in Vannamei Shrimp (*Litopenaeus vannamei*) in Brackishwater Culture Pond

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Abstract

Shrimp disease that currently causes economic loss to shrimp farmers is White Feces Disease (WFD). This disease appeared due to several factors, such as poor pond management, unhealthy shrimp seed, and poor water quality which resulted in the appearance of *Vibrio* sp. bacteria and Dinoflagellata. This study aimed to analyze the cause of WFD outbreak in vannamei shrimp pond. The study method through direct experiment was applied in shrimp pond. Sampling was performed three times in each feeding tray to collect ten shrimps. Overall, sampling was performed twice a week. The Sample Survey Method was used to collect sample in this study. Result of study showed that clinical symptom was observed through changes in pattern and behavior of vannamei shrimp during culture. However, this observation resulted in insignificant data. Vannamei shrimp infected WFD tended to swim slower and often rose to the surface with body color turned red. Moreover, total organic matter (TOM) increased on week-7 along with the increasing growth of plankton, particularly from the Dinoflagellata group. However, bacterial growth of *Vibrio* sp. on week-7 was insignificant, yet many shrimps were found dead with white feces during that period. The result of analysis indicated that white feces disease was caused by Dinoflagellata besides the main trigger, namely, *Vibrio* sp.

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1. Introduction

Shrimp, including vannamei shrimp, is a superior commodity of aquaculture, particularly for export purposes. Vannamei shrimp (*Litopenaues vannamei*) is known as a fast growing shrimp that can be cultured in high stocking density (Aldama-Cano *et al*., 2018), has high export value and disease resistance (Nur'aini et al., 2019). However, the problem faced by shrimp farmers is mass mortality due to disease attack in shrimp pond (Sun *et al*., 2013). Various diseases are successfully identified and resulted in great loss, either caused by bacteria (Li *et al*., 2016) or viruses (Patil *et al*., 2021), including the White Feces Disease (WFD) in 2012 (Fatmala *et al*., 2019).

White Feces Disease (WFD) is caused by the attack of Vibrio sp. (Longyant *et al*., 2008). Clinical symptoms shown by shrimp infected with WFD include decreased appetite (Sanguanrut *et al*., 2018), intestine which turns white or even empty due to lack of food intake (Wang *et al*., 2020), white feces floating on the water surface (Apitanyasai *et al*., 2016), and abnormal growth (Tang *et al*., 2015). Moreover, the disease is also caused by poor aquaculture management (Velmurugan *et al*., 2015), unhealthy shrimp seed (Aranguren *et al*., 2017), poor water quality (Mello *et al*., 2011), and the limited knowledge of shrimp farmers concerning disease that attacks shrimp thus results in slow disease treatment (Rakasiwi & Albastomi, 2017). Shrimp farmers should increase their knowledge about shrimp disease to appropriately prevent and treat the disease (Wahyudi & Fadlil, 2013). WFD is rapidly distributed through water, particularly in ponds with high-stocking density, thus quick treatment is necessary (Rusadi *et al*., 2019).

However, in several cases, WFD occurs together with the existence of several types of toxic plankton (Ning *et al*., 2019), such as Dinoflagellata which causes Paralytic Shellfish Poisoning (PSP) disease in shrimp and fish (Adnan, 2014). At high concentrations, Harmful Algal Bloom (HAB) or blooming of toxic phytoplankton may occur (Choirun *et al*., 2015). Dinoflagellata is identified as toxic species which produces toxins that will enter the food chain of shrimp (Yuliana *et al*., 2020). In other words, shrimp that consumes the plankton will be dead. The appearance of the disease which possibly attacks vannamei shrimp and the lack of experts who are able to diagnose shrimp disease according to the clinical symptom requires appropriate solution, hence the type of diseases that attack shrimp could be identified effectively and quickly by observing the symptom appears (Adam *et al*., 2019).

This condition underlined the study on the occurrence of WFD in vannamei shrimp pond. This study aimed to analyze the appearance of WFD caused by the abundance of *Vibrio* sp. bacteria and Dinoflagellata plankton.

2. Materials and Methods

The study was conducted from December 2020 – March 2021 in the pilot brackish water Pond of PT. Matahari Sakti, Mangunharjo, Central Java. Parameters observed in this study included Total Vibrio Count (TVC), clinical symptom, plankton abundance, and water quality. This study survey method wasthrough purposive sampling. Sampling location was purposely determined by certain reasons and considerations to collect samples that could represent the area or sample group, allowing researchers to obtain a comprehensive description of the research location. Sampling was done three times in each feeding tray to collect ten shrimps from the pond. Sampling was performed twice a week.

2.1 Total Vibrio Count

Bacterial counting was conducted at the end of sampling. Shrimp intestine contents were collected and dissolved in 9 ml of NaCl solvent to be further mixed using vortex until homogenous. Later, 1 ml of sample was cultured on TCBS agar and incubated for 24 hours at 31.4°C (Jayadi *et al*., 2016).

2.2 Clinical Symptom

Observation of clinical symptom was performed by checking abnormal change that occurred in experimental shrimp. Clinical symptom of shrimp was observed by looking for any changes in symptom that might occur after diet treatment was given, such as appetite, change in body color, change in intestine color, and shrimp behavior. The data obtained were given a score by referring to (Alfiansah & Gardes, 2019). Normal or healthy shrimp is visually active with bright white body color, filled intestinal tract, and non-porous carapace.

2.3 Plankton Abundance

The method applied to count total plankton was the swept area method (Tait, 1972). The calculation was done under the microscope at 10x10 magnification and repeated for three times. Plankton abundance was determined using the formula (APHA, 1998).

$$N = Z\ x\ \frac{X}{Y}\ x\ \frac{1}{V}$$

Description:

N = Individual abundance of phytoplankton (individual/liter)

Z = Individual number of phytoplankton
X = Volume of filtered water sample (40 ml)
Y = Volume of 1 drop of water (0.06 ml)
V = Volume of filtered water (100 l)

2.4 Water Quality

Parameter of water quality observed during the study included temperature, pH, salinity, alkalinity, NO_2, NO_3, NH_4, TOM, PO_4, and N/P ratio. Measurement of water quality was done for 8 weeks of culture.

3. Results and Discussion

3.1 Total Vibrio Count (TVC)

The result of the study showed a fluctuating increase in bacterial count on the third week, however it could be controlled and remained stable since the fourth week. Total bacterial count experienced another increase on the eighth week, but total Vibrio count remained stable (Figure 1). Total Vibrio count of 440 CFU/ml of the total bacterial count (239.875 CFU/ml) (Figure 2).

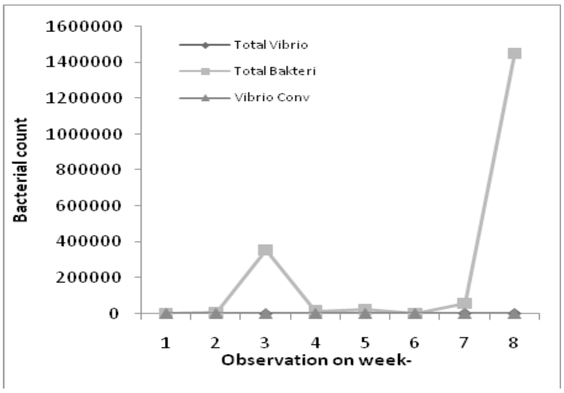

Figure 1. Daily bacterial count in vannamei shrimp culture.

Vibrio bacteria are normally found in waters, yet they are not dangerous if their number is still below the safety limit. Maximum bacterial count threshold for *Vibrio* sp. in water is 10^4 CFU/ml, while it is 10^6 CFU/ml in open waters. If bacterial count exceeds this threshold (Sanguanrut *et al*., 2018), mass mortality of cultured shrimp in the pond may occur (Hatmanti, 2003; Lestari *et al*., 2018; Roza & Zafran, 1998).

3.2 Clinical Symptoms

The clinical symptom was observed through changes invannamei shrimp pattern and behavior during culture.Measurement of clinical symptom was done to identifyand examine the changes that occurred. Observationconducted on vannamei shrimp infected with WFD resulted in insignificant data. Vannamei shrimp attacked by WFD in pond tended to swim slower and often rose to the surface with body color that turned red. In 24 hours after the symptom appeared, many shrimps were found dead.

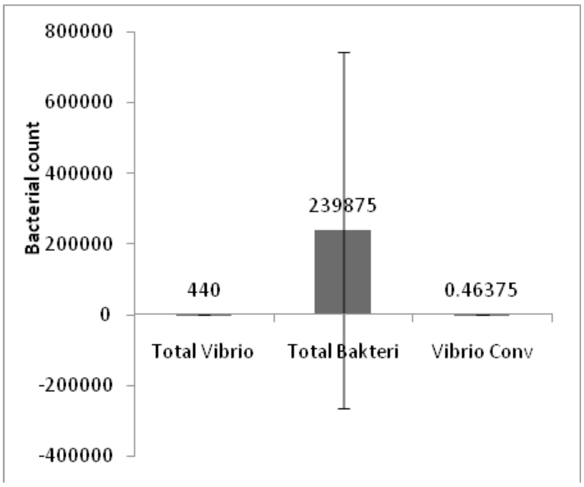

Figure 2. Comparison of bacterial count in vannamei shrimp culture.

3.3 Identification and Plankton Abundance

The result of identification showed plankton diversity in waters which included green algae, blue green algae, Diatom, Euglena, Dinoflagellata, and Protozoa (Figure 4). The study result found three dominant group of plankton, namely Green Algae, Blue Green Algae, and Diatom. However, Green Algae were found to have the highest abundance and diversity (Figure 3).

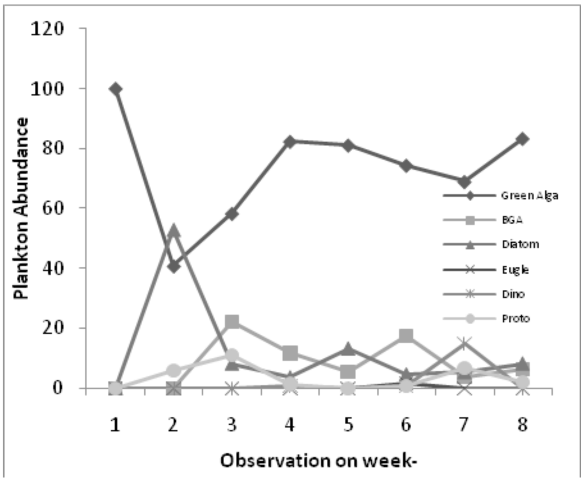

Figure 3. Daily plankton abundance in vannamei shrimp culture.

Green Algae (Chlorophyta) are the main producer in aquatic ecosystem since most (single cell and motile) phytoplankton are member of chlorophyta group which have chlorophyll pigment to effectively perform photosynthesis (Fauziah & Laily, 2015). This result of Green Algae domination showed that the water have excellent light intensity. Chlorophyta contains higher pigment of chlorophyll a and chlorophyll b compared to carotene and xanthophyll; is a cosmopolitan species; particularly lives in waters with adequate light such as in pond, lake, puddle, and flowing water like river and drain (Siregar & Hermana, 2012). Chlorophyta is also found in semi aquatic environment, such as rocks, damp soil, and damp tree banks (Ariana *et al*., 2014).

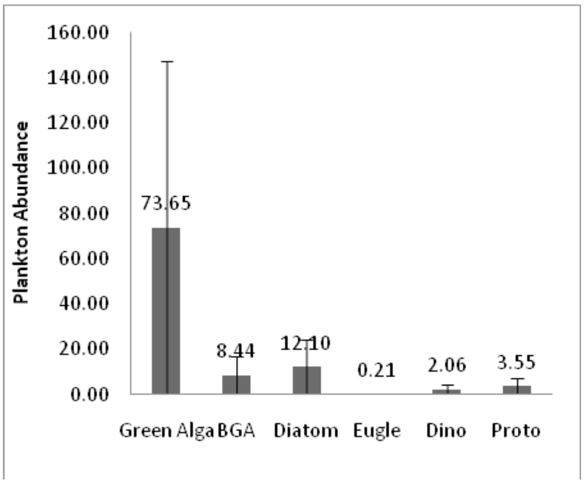

Figure 4. Comparison of plankton abundance in vannamei shrimp culture

3.4 Water Quality

In an aquaculture system, physical and chemical parameters highly affect both organic and inorganic nutrients. The additional treatment applied in the form of lime and fermented probiotics also play important role. The number of plankton increased along with the culture period. Observation of water quality during shrimp culture reflected weekly increase in alkalinity concentration, yet the value was still within the standard for shrimp culture of 100-150 mg/l (Supito *et al*., 2017). This finding showed that alkalinity is pH buffer in waters. Moreover, (Suwarsih *et al*., 2016) stated that the increase in alkalinity also affects the nutrient availability which functions as foods for phytoplankton, where increasing alkalinity will result in phosphorus release (Adam & Maftuch, 2015) and increase the availability of carbon for photosynthesis process of phytoplankton**.**

Result of the study showed an increase in total organic meter (TOM) on week-7 along with the growth of plankton, particularly Dinoflagellata. Moreover, bacterial growth of *Vibrio* sp. on week-7 was found to be insignificant. However, vannamei shrimps were found dead with feces during week-7. This situation indicated that the white feces observed was caused by dinoflagellates. According to Alfiansah and Gardes (2019), population of phytoplankton will increase along with nutrient intake in the pond and duration of

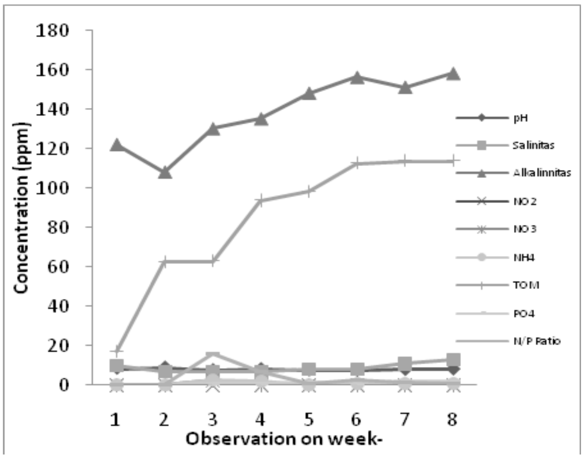

Figure 5. Daily water quality in vannamei shrimp culture.

shrimp culture. Concentration of ammonium and nitrite may cause vannamei shrimp mortality (Sun *et al*., 2013). *Alteromonas*, *Photobacterium*, *Pseudoalteromonas*, and *Vibrio* are bacteria dominantly found when shrimp disease occurs (Maftuch *et al*., 2016), either in sample of pond water or in the infected shrimp. Moreover, (Marbun *et al*., 2019) stated that clinical symptom of shrimp attacked by white feces disease is indicated by change in intestine, such as intestine damage and unfilled intestine content (Aldama-Cano *et al*., 2018). Furthermore, shrimp are also found to eat less with white pale body color and hepatopancreas damage (Adam *et al*., 2019). Later, (Nur'aini *et al*., 2019) said that the pathology of vannamei shrimp infected with WFD indicates the existence of bacterial mass in tubular lumen of hepatopancreas and nodular formation in tubular lumen hepatopancreas caused by high stocking density (Tang *et al*., 2015). Hence, the growth of bacteria and plankton is related to the increase of total organic matter.

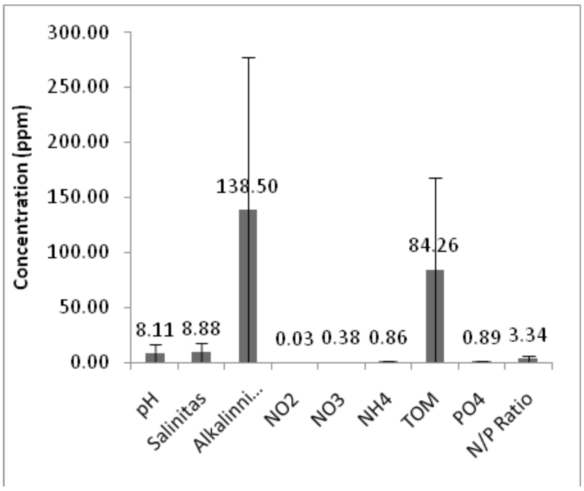

Figure 6. Comparison of water quality in vannamei shrimp culture.

4. Conclusion

Result of analysis indicated that white feces appeared along with the appearance of dinoflagellates which resulted in the disease besides its main trigger which is *Vibrio* spp.

The appearance of the disease which possibly attacks vannamei shrimp and the lack of experts who are able to diagnose shrimp disease according to the clinical symptom requires appropriate solution, hence the type of diseases that attack shrimp could be identified effectively and quickly by observing the symptom appears.

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Authors' Contributions

All authors have contributed to the final manuscript. The contribution of each author as follows, Moh. Awaludin Adam and Ach. Khumaidi; collected the data, drafted and designed the figures. Ira Mei Widiastuti, Ernawati, Achmad Yani, Era Invisitawati, Yuliana, and Rini Fitriasari Pakaya conceptual ideas and critical revision of the article. All authors discussed the results and contributed to the final manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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