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# CORRELATION BETWEEN TOTAL ORGANIC MATTER AND ABUNDANCE OF *Vibrio* sp. BACTERIA IN INTENSIVE VANNAMEI SHRIMP (*Litopenaeus vannamei*) PONDS

## Hubungan Bahan Organik Dengan Kelimpahan Bakteri *Vibrio* sp. Pada Tambak Udang Vaname (*Litopenaeus vannamei*) Skala Intensif

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**ABSTRACT**

The cultivation of vannamei shrimp (*Litopenaeus vannamei*) requires attention to the quality of the rearing water to prevent the occurrence of *Vibrio* sp. bacterial infections. This study aims to analyze the abundance of *Vibrio* sp., Total Organic Matter (TOM), and the relationship between organic matter and the abundance of *Vibrio* sp. in the cultivation media. The research stages include sample preparation, KMnO4 standardization, TOM measurement, equipment sterilization, TCBS media preparation, bacterial inoculation, and the enumeration of *Vibrio* sp. The data obtained were analyzed for correlation using the SPSS26 application. The results showed that TOM levels in the water samples increased as the cultivation period progressed, with the lowest TOM level being 105 ppm at the early DOC and the highest 223 ppm at the late DOC. The abundance of *Vibrio* sp. fluctuated, with the lowest being $2{,}0\times10^2$ CFU/mL at DOC 54 and the highest at DOC 89, reaching $3.0\times10^3$ CFU/mL. The correlation value between organic matter and *Vibrio* sp. abundance was 0.486, indicating a moderate relationship and showing a positive linear correlation, meaning the relationship was directly proportional. This study shows that TOM positively correlates with the abundance of *Vibrio* sp. bacteria in intensive vannamei shrimp farming.

Keywords: abundance, water quality, correlation, direct relationship

**ABSTRAK**

Kegiatan budidaya udang vaname (*Litopenaeus vannamei*) memerlukan perhatian terhadap kondisi kualitas air media pemeliharaan untuk mencegah peluang timbulnya infeksi bakteri *Vibrio sp.* Penelitian ini bertujuan menganalisis jumlah kelimpahan *Vibrio sp*, *Total Organic Matter* (TOM) dan hubungan bahan organik dengan kelimpahan *Vibrio sp* pada media budidaya. Tahapan penelitian meliputi preparasi sampel, standarisasi KMnO4, pengukuran TOM, sterilisasi alat, pembuatan media TCBS, inokulasi bakteri dan penghitungan jumlah bakteri *Vibrio sp.* Data yang diperoleh dianalisis korelaisinya menggunakan aplikasi SPSS26. Hasil penelitian menunjukkan nilai TOM pada sampel air mengalami peningkatan seiring bertambahnya masa budidaya dengan nilai kadar TOM terendah yaitu 105 ppm pada DOC awal dan tertinggi 223 ppm pada DOC akhir. Jumlah kelimpahan *Vibrio sp* mengalami fluktuasi.

Jumlah *Vibrio sp* terendah yaitu $2{,}0\times10^{2}$CFU/mL pada DOC 54 dan tertinggi pada DOC 89 yaitu $3.0\times10^{3}$ CFU/mL. Nilai korelasi antara bahan organik dengan kelimpahan *Vibrio sp* yaitu 0,486 yang diartikan terdapat hubungan yang moderat atau sedang dan menunjukan garis korelasi yang linear positif yaitu hubungan yang searah. Penelitian ini menunjukkan bahwa TOM berkorelasi positif terhadap kelimpahan bakteri *Vibrio* sp. pada budidaya udang vaname sistem intensif.

Kata Kunci: kelimpahan, kualitas air, korelasi, searah

## INTODUCTION

Vannamei shrimp (*Litopenaeus vannamei*) is one of Indonesia's fishery commodities with high economic value. This species has a number of advantages, such as resistance to disease, efficient use of space, rapid growth, and tolerance to environmental changes (Utami et al., 2016). The high market demand encourages farmers to choose vannamei shrimp as a superior commodity thereby increasing the intensification of shrimp cultivation (Samocha et al., 2013). One of the obstacles in the vannamei shrimp cultivation process is disease attacks caused by pathogenic infections (Anderson et al., 2019). The increasingly widespread practice of intensive vannamei shrimp cultivation with improper procedures has the potential to arise various problems such as the accumulation of organic waste is getting higher (Kurniaji et al., 2023). This organic waste is increasing along with the increase in material inputs in the cultivation system (Wafi et al., 2021).

Organic matter in pond waters is a collection of complex organic compounds that have been or are undergoing decomposition, both in the form of humus and mineralization (Farraosi et al., 2022). Artificial feeding to meet the nutritional needs of shrimp indirectly increases the content of organic matter in the pond. The main source of this organic matter comes from uneaten feed and biota feces that are preserved. According to Putra et al., (2014) dissolved organic matter is less than 0.5 µm in size, while insoluble organic matter is more than 0.5 µm in size. In addition to causing environmental problems, the existence of organic waste in waters also triggers the growth of various types of diseases caused by bacteria (Ariadi & Mujtahidah, 2021). Bacteria are microscopic organisms that can interact favorably or adversely with other organisms (Amrullah & Mar'iyah, 2023). In vannamei shrimp farming, it is important to manage the bacterial community so that it cannot cause disease. Bacterial diseases caused by pathogenic bacteria have been reported to significantly affect shrimp farming worldwide (Fosyal & Lisa, 2018). One of the diseases that often appears is vibriosis caused by bacteria from the genus *Vibrio* sp. This bacteria attacks the shrimp farming industry a lot and causes many losses (Sutiknowati, 2014).

*Vibrio* sp. is an opportunistic pathogen that is commonly found in brackish waters such as ponds, seas, rivers, and estuaries (Ihsan, 2017). In shrimp farming, the bacteria *Vibrio* sp. being the main pathogen that causes vibriosis (Sarida & Harpeni, 2010). This disease can reduce shrimp survival by up to 80% in a few days at the hatchery and rearing stage due to its virulent nature (Rusadi et al., 2019). According to Idami & Nasution, (2020) *Vibrio* sp. considered pathogenic if the amount reaches $8.35\times10^{4}$ CFU/mL or more. If their growth is not prevented, the presence of these bacteria can cause mass death in cultivated organisms (Kurniawan, 2012). Therefore, early detection of the abundance of *Vibrio* sp. It is important to prevent the spread of disease. Pond water quality also plays an important role in maintaining the health and performance of shrimp (Gao et al., 2016). Fluctuations in water quality parameters, such as those affected by aquaculture inputs and waste, can cause stress in shrimp, which increases the risk of vibriosis disease (Ariadi et al., 2021). This study aims to analyze the relationship between organic matter and the abundance of *Vibrio* sp. In the water of the vannamei shrimp pond.

## METHODS

### Place and Time

This research was carried out from January to March 2024. The research location is in the VIP Laboratory of PT. Central Proteina Prima Tbk (CP. Probolinggo, East Java. The laboratories used consist of the Water Quality Lab and the Microbiology Lab.

### Tools and Material

The tools used in the research include dry and wet sterilizers (ovens, autoclaves), bacterial incubators, showcases, glass equipment (erlenmeyer, petri dishes, test tubes, measuring cups), hot plate stirrers, magnetic stirrers, micropipettes, tube racks and other lab equipment. The ingredients used include aquaades, $CO_2$ free equiades, 96% entanol, alcohol, thiosulfate citrate bile salt sucrose agar (TCBS), alumnium foil, physiological NaCl, $H_2SO_4$ (sulfuric acid) $KMnO_4$ (potassium permanganate), oxalid acid (oxalic acid).

### Procedure

Water sampling for Total Organic Matter (TOM) measurement is carried out every week at 06.00 WIB from vannamei shrimp ponds (*Litopenaeus vannamei*) at PT. Viva Vaname Intinusa Prima. Samples are taken from several pond points to obtain optimal water quality representation and then placed in sterile bottles. Each sample is separated into containers according to the parameters to be tested, including for TOM measurements. The first step in standardization is to mix 50 mL of aquiade with 5 mL of $H_2SO_4$ 6 N, then heat it to a temperature of about 70°C on a hot plate. After reaching the desired temperature, 10 mL of 0.01 N Oxalic acid is added which reacts with KMnO4 during titration. Titration was carried out until the first pink color appeared, which indicates that the entire oxalic acid has reacted with $KMnO_4$. The volume of $KMnO_4$ used in titration is recorded and averaged to obtain precise results. This standardization process is carried out in a duplex.

The tools are sterilized by the dry method using an oven for petri dishes and an autoclave for other materials. 96% ethanol is used to wash certain tools before further sterilization. The preparation of bacterial growth media was carried out by preparing a 0.85% physiological NaCl solution and TCBS agar media. TCBS media was prepared by mixing 71.3 g of TCBS with 8 g of NaCl in 800 mL of aquaades. Once the media has been heated and cooled to 50°C, the media is poured into a sterile petri dish of 15-20 mL per cup. Bacterial inoculation is carried out using the spread plate method where bacterial samples from dilution are pipetted into petri dishes containing media. Petri dishes were incubated at 30°C for 24 hours, and the number of bacterial colonies of *Vibrio* sp. that grow is calculated using the Total Plate Count (TPC) method, with the colonies counted in the range of 30 to 300 colonies per petri dish (Madigan, 2003)

### Research Parameters

The equation used in calculating TOM and *Vibrio* sp. bacteria as follows:

$$\text{TOM (ppm)} = (\text{x} - \text{y}) \frac{NKMnO4\ x\ BEKMNO4\ x\ 1000}{\text{Sample Volume}}$$

Information:

| | |
|---|---|
| x | = 0.01 N $KMnO_4$ volume required for sample water titration |
| y | = 0.01 N $KMnO_4$ volume required for blank titration |
| $NKMnO_4$ | = Normality of $KMnO_4$ |
| $BEKMNO_4$ | = 31,6 |
| 1000 | = Conversion from mL to L |

$$\text{Number of bacterial colonies (CFU/mL)} = \Sigma C \times \frac{1}{Fp} \times \frac{1}{S}$$

Information:
ΣC = Number of colonies on a dish
Fp = Dilution factor
S = Inoculation volume (mL)

Table 1. Correlation coefficient criteria

| No | R value | Interpretation |
|---|---|---|
| 1. | 0.00-0.199 | Very low |
| 2. | 0.20-0.399 | Low |
| 3. | 0.40-0.599 | Medium |
| 4. | 0.60-0.799 | Strong |
| 5. | 0.80-1000 | Very Strong |

**Data Analysis**

The data obtained were in the form of TOM levels and the total population of *Vibrio* sp. The correlation relationship was analyzed using the IBM SPSS 26 application. The correlation coefficient value is used according to the coefficient criteria (Table 1).

## RESULT

**Total Organic Matter (TOM) Levels**

The results of the Total Organic Matter (TOM) level measurement that has been carried out can be seen in Figure 1. The results showed that TOM increased during the cultivation period. The increase began to occur in DOC 54 to DOC 89. From a TOM level of 105 ppm to 223 ppm.

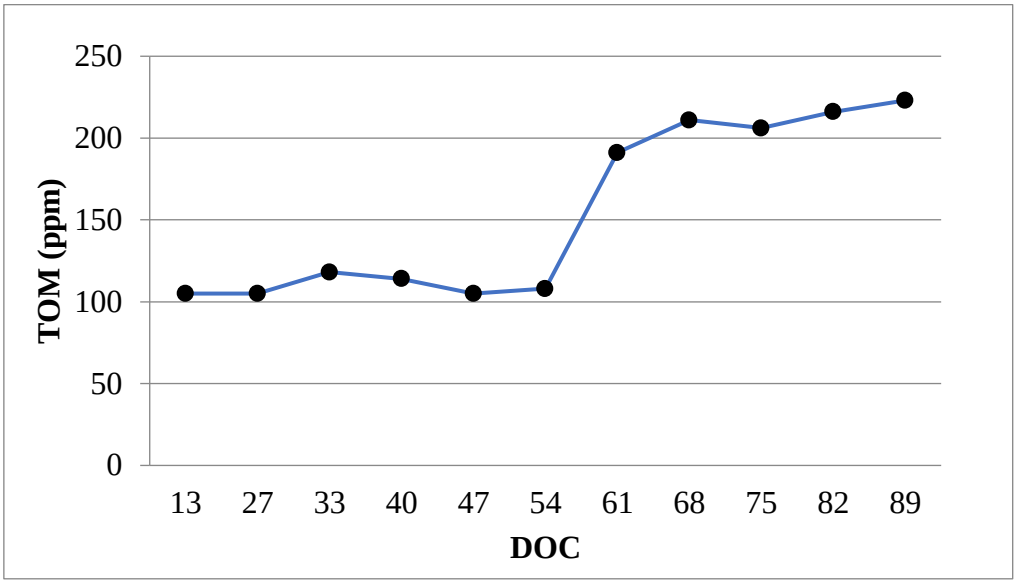


Fig 1. TOM levels in vannamei shrimp cultivation media

***Vibrio* sp. Bacteria Population**

The results of the total observation of *Vibrio* sp. can be seen in Figure 2. The study showed that there was a fluctuation in the total abundance of *vibrio* sp bacteria during vannamei shrimp (*Litopenaeus vannamei*) cultivation activities. Total bacteria increased from 2.0×102

CFU/mL to 3.0×103 CFU/mL. The increase began to occur in DOC 27 and then rebounded in DOC 33. Furthermore, in DOC 61, it increased again until DOC 89.

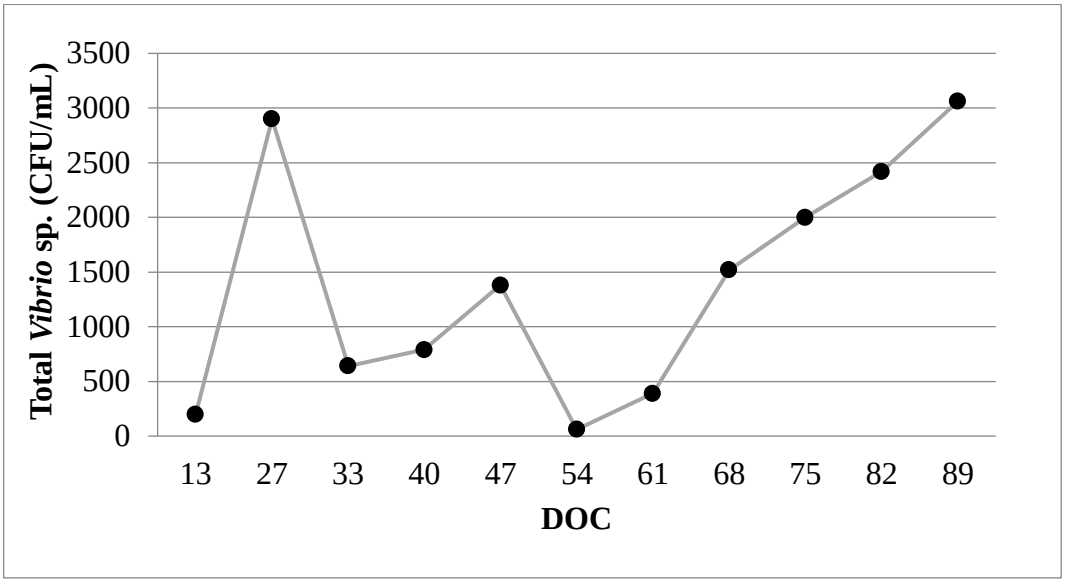


Fig 2. The total population of *Vibrio* sp. On Vannamei shrimp cultivation media

**Correlation of TOM and Total *Vibrio* Bacteria sp.**

The results of the correlation analysis of TOM and *Vibrio* sp. presented in Figure 3. The results of the research on the relationship between total organic matter and the abundance of *Vibrio* sp show a positive linear correlation line direction, which is a unidirectional relationship, so it can be interpreted that if the amount of organic matter increases, the number of *Vibrio* sp bacteria will also be larger. From the correlation graph, the correlation value (r) can also be known by looking at the linear r2 value, which is 0.237 which if squared the result is 0.486 (correlation value).

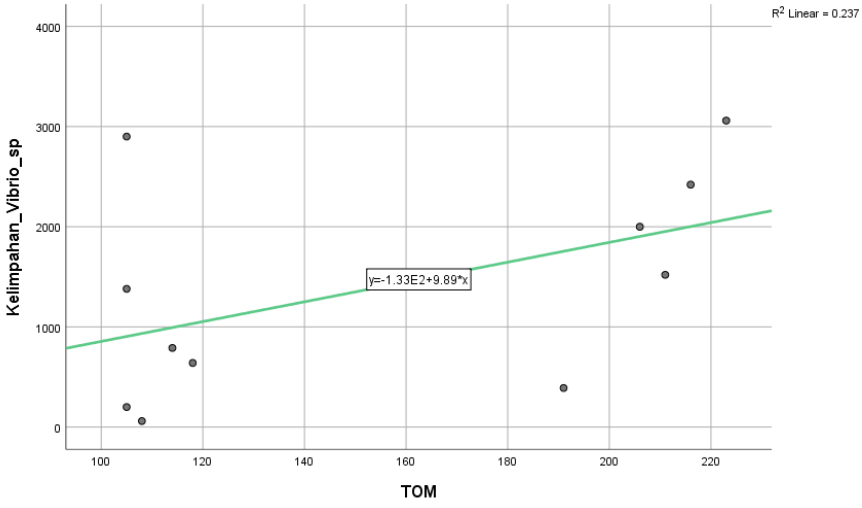


Fig 3. Correlation of TOM and *Vibrio* sp bacteria

## DISCUSSION

**Total Organic Matter (TOM) Levels)**

TOM is a total of organic matter derived from a collection of various complex organic compounds that are undergoing or have undergone a decomposition process (Manengkey,

2010). Organic matter in sediments comes from deposition that is affected by the conditions during which the sedimentation process occurs. High stocking density with 100% feed in the form of pellets will produce high organic matter during the cultivation process. This can have a bad impact on shrimp, where waste from feces and feed residues will degrade water quality to the minimum acceptable limit for shrimp (Halim et al., 2023).

From the results of measuring TOM levels, it is known that TOM levels increase as cultivation activities progress. Arfiati et al., (2019) stated that the increase in total organic matter is in line with the length of cultivation time. In DOC 13, the TOM level was recorded at 105 ppm, exceeding the optimal threshold of <80 ppm. The TOM level increased drastically in DOC 61 to 191 ppm, and in DOC 68 it reached 211 ppm. This increase is suspected to be due to the death of microorganisms such as plankton, the death of shrimp, and the presence of klekap that is not immediately lifted from the surface of the pond and experiences decay at the bottom of the pond. Gunarto & Mansyur, (2016) also stated that the abundance of klekap increases organic matter and decreases oxygen content due to the degradation process of organic matter. In addition, as the cultivation period increases, feeding increases with the growth of shrimp's lifespan and biomass, which contributes to an increase in organic matter from metabolic waste, feces, and uneaten feed residues (Kamal et al., 2017). Renitasari et al., (2021) also mentioned that TOM increases due to the accumulation of feces, feed residues, and dead plankton. The decrease in organic matter levels occurred in DOC 75, which was 206 ppm, allegedly caused by the activities of folliction, water change, and reduction in the amount of feed due to shrimp deaths. Feed reduction is one of the efforts to reduce the metabolic rate and accumulation of feed residues (Tahe, 2008). The highest TOM level was recorded at DOC 89 at 223 ppm, which is the end of the cultivation period.

**_Vibrio_ sp Bacteria Population.**

The total fluctuation of bacteria is affected by the water condition of the aquaculture medium during the rearing of vannamei shrimp. The abundance of *Vibrio* sp bacteria in pond waters fluctuates due to the dynamics of dynamic pond aquatic ecosystems (Madusari et al., 2022). There are water quality factors such as total organic matter, temperature, salinity, and nutrient content, as well as interactions between organisms in the pond ecosystem. In addition, there are also external factors such as weather and the administration of probiotics in ponds that can affect the population of *Vibrio* sp. In DOC 13, the total abundance of *Vibrio* is still 2×102 CFU/mL, which means that it is still within the tolerance limit, which is <104 CFU/mL (Anjasmara et al., 2018). In DOC 27, there was an increase in the *Vibrio* population to 2.9×103CFU/mL so that treatment was needed as an effort to avoid the occurrence of vibriosis disease in shrimp by reducing the abundance of bacteria in the cultivation medium. Population increase *Vibrio* sp. This was influenced by the increase in TOM levels in water at DOC 27, which was 105 ppm. In addition, temperature is also one of the factors in the high number of *Vibrio* sp. bacteria. in a pond, because the increase in temperature is able to provide a chance for the generation of *Vibrio* sp.

There was a decrease in the abundance of *Vibrio* sp. in DOC 33 and 40, which is 6.4-7.9×102CFU/mL which is suspected to be influenced by the activity of discharging and administering probiotics. The administration of probiotics in the form of *Lactobacillus* sp. which functions as a floc-forming coagulant for the attachment of other bacterial communities so that the process of decomposing organic matter in the pond becomes more optimal. A combination of *Lactobacillus* sp. with *Bacillus subtillis* is able to produce more effective bacteriocin to inhibit the growth of pathogenic bacteria (*Vibrio* sp.) (Kuebutornye et al., 2020). This has led to a decrease in the number of *Vibrio* sp. on Vannamei shrimp pond media after probiotic administration. There was a drastic decrease in DOC 54 which was the lowest number of *Vibrio* sp. is suspected to be caused by water change and the administration of probiotics, as

explained by Puspitasari et al., (2020) that water that already contains shrimp residues and feed residues replaced with new clean water can reduce the population of *Vibrio* sp.

*Vibrio* sp. in DOC 61 is caused by the increasing amount of organic matter in the water medium, characterized by a TOM level of 191 ppm and continues to increase following the period of vannamei shrimp cultivation activities that take place. The increase in bacteria is also influenced by weather factors that trigger eutrophication in the water medium and also the shift of plankton so that algae blooming occurs (Krismono & Yayuk, 2007). Poor water quality can be the cause of the abundance of *Vibrio* sp. in the water of vannamei shrimp rearing (Kharisma & Manan, 2012). Total bacteria *Vibrio* sp. the highest is at DOC 89, which is 3.0×103CFU/mL as with the period of cultivation activities that have been ongoing where there is also an increase in organic matter, water change is not carried out immediately so that it can trigger the development of *Vibrio* sp. in shrimp ponds (Mangampa, 2015).

**Correlation of TOM and Total *Vibrio* Bacteria sp.**

The results showed a positive linear correlation, namely the increase in the amount of organic matter followed by an increase in the number of *Vibrio* sp. bacteria. Widigdo et al., (2020) stated that the high content of organic matter in vannamei shrimp ponds can cause a high population of *Vibrio* sp. Likewise, according to Jayadi, (2016) that high organic levels in water will increase the population of bacteria including *Vibrio* sp. Because organic matter will be used by bacteria as a food source for growth and development. In addition to energy sources, organic compounds also contain nutrients such as carbon, nitrogrn, phosphorus and other minerals that are utilized by *Vibrio* sp. bacteria. for their growth. Organic matter will continue to increase as the cultivation inputs provided increase. A high amount of organic matter is known to be able to support an increase in the abundance of pathogenic bacteria in ponds (Alfiansah et al., 2018). Pathogenic bacteria in the form of *Vibrio* sp. will grow abundantly in pond waters that have an accumulation of organic matter levels of >90 mg/L (Ariadei et al, 2020,) and also according to Kharisma & Manan, (2012) that poor water physico-chemical quality factors are the main trigger for the abundance of *Vibrio* in intensive ponds.

The amount of organic matter derived from feed waste and feces cumulatively will have a negative impact on the aquaculture pond aquatic ecosystem (Liu et al., 2021). The increase in TOM levels and the *Vibrio* sp. in pond waters can have an impact on the decline and stability of water quality, where an increase in TOM levels can trigger an increase in the level of eutrophication in pond waters because too many nutrients enter the water so that it can cause excessive algae growth. From excessive algae growth, it will reduce the dissolved oxygen content in the water in addition to being used in the decomposition process, as according to Zavala & Espino, (2000) that the low oxygen content in the waters is caused by the high content of organic matter and the rate of decomposition, so that as long as it is collected in a certain area, organic matter is decomposed by microorganisms and causes the dissolved oxygen content to decrease which triggers stress in farmed shrimp. Population increase *Vibrio* sp. caused by an increase in TOM levels can increase the risk of vibriosis in shrimp, thereby reducing the productivity of aquaculture businesses. In addition to the provision of probiotics, it is still necessary to monitor water quality periodically to monitor changes in the water quality of aquaculture media. Spraying and changing water can also be done as an effort to reduce the amount of organic matter which can also be followed by a decrease in *Vibrio* sp.

## CONSLUSSION

Based on the results of the study, the abundance of *Vibrio* sp. in vannamei shrimp ponds showed significant fluctuations, with the highest value reaching 3,060 CFU/mL. In addition, the Total Organic Matter (TOM) level also varied with the highest increase at the end of the cultivation period, which was 216 ppm. The results of the correlation test between TOM and

the abundance of *Vibrio* sp. showed a moderate relationship with a correlation value of 0.486, which means that there was a unidirectional relationship between the two. The higher the TOM level, the greater the number of *Vibrio* sp. bacteria. in ponds, which shows that the increase in organic matter also contributes to the increase in the population of *Vibrio* sp.

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