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Monitoring of population density of *Vibrio* sp. and health condition of hepatopancreas Pacific white shrimp *(Litopenaeus vannamei)* cultivated with intensive systems in Bulukumba Regency, South Sulawesi, Indonesia

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ABSTRACT

Pacific white shrimp (*Litopenaeus vannamei*) has advantages in terms of growth and rapid adaptation process to its environment. Shrimp are also very risky once exposed to pathogenic bacteria. This study aims to monitor the population density of *Vibrio* sp. and the health status of hepatopancreas in pacific white shrimp. This research was conducted for 80 days using 58 Pacific white shrimp. There are seven ponds which are intensive cultivation ponds with days of culture (DOC) from 10-80. Measurement of bacterial populations using the spread platting method. The hepatopancreas obtained was then observed the level of necrosis, the presence of gregarine, and the percentage of hepatopancreas depletion. The resulting data were analyzed descriptively qualitatively. The results showed the highest *Vibrio* sp. population came from DOC 70 which were sequentially found in P2 pond at $5.8x10^3$ CFU/gr. The highest percentage of hepatopancreas depletion occurred at DOC 30, 50, and 80 which was 90%. The presence of necrosis can be confirmed by the appearance of lesions in the hepatopancreas. Gregarine was also found in this study after sneaking into pacific white shrimp hepatopancreas.

Key word : *Monitoring, Vibrio, Hepatopancreas, Shrimp, Pathogen*

Introduction

Pacific white shrimp *(Litopenaeus vannamei)* is a commercial commodity that is often cultivated especially in Indonesia. Growth and its ability to adapt to disease infections are the advantages of pacific white shrimp as a cultivation material (Cuzon *et al*., 2004). Lately, intensive shrimp culture has increasingly gained worldwide attention as a potential vehicle for increasing production, survival, growth, and high stocking densities (Wasielesky Jr *et al*., 2013).

Bulukumba Regency, South Sulawesi is one area that has the potential in developing products in the field of marine and fisheries, especially for the cultivation of pacific white shrimp (Sipahutar *et al*.,

2019). The increase in production can occur due to intensive cultivation patterns carried out by farmers in several areas in the Regency of Bulukumba (Sipahutar *et al*., 2019).

The high stocking density of white shrimp culture can cause pathogenic threats, such as vibriosis caused by *Vibrio harveyi*, *Vibrio alginolyticus*, *Vibrio cholera*, and *Vibrio parahaemolyticus* (Park *et al*., 2018). Bacteriosis disease is already endemic so that it can result in large losses due to mass death in the cultivation of pacific white shrimp (Taslihan, 2017).

The use of antibiotics is not entirely effective for preventing vibriosis infection. Therefore, several efforts in activities such as periodic monitoring, biosecurity, administration of probiotics, and prebiotics as well as immunostimulants have been carried out to improve the defense mechanism of shrimp body so that production results can increase rapidly (Dash *et al*., 2017; Widanarni *et al*., 2014; Taslihan, 2017; Wiradana *et al*., 2019).

Based on this problem, this study aims to monitor the population density of *Vibrio* sp. and the impact of its presence on the hepatopancreas organ so that it can provide further information for early prevention efforts on white shrimp in Bulukumba Regency, South Sulawesi.

Materials and Methods

Research period and location

This research was conducted on the intensive pacific white shrimp farms in Bulukumba Regency, South Sulawesi and carried out for 80 days starting from Days of Culture (DOC) 10-80 on pacific white shrimp.

Study area and culture details

Seven intensive cultivation ponds of 2,600-4,700 m^2 were used in this study. Intensive ponds are equipped with aerators to supply oxygen and molasses. Stocking density of shrimp seedlings in each pond of white shrimp ranges from 189-236 (pcs/m^2).

Monitoring of population density of *Vibrio* sp.

Calculation of bacterial populations from pacific white shrimp samples in each pond was carried out using the *spread platting* method (Abraham and Sasmal, 2009). Dilution of hepatopancreas and intestinal samples of pacific white shrimp using 50% sterile sea water. A total of 0.1 mL of diluted sample is then distributed to Petri dishes which contain Thiosulphate-citrate-bile-salts-sucrose (TCBS) agar media. A total of 8.9 g TCBS media was heated to boiling point. After that, the media was cooled to a temperature of 50°C and poured into a sterile Petridish and allowed to medium harden. Petridish containing the sample was then incubated in an upside down position at 37°C for 20-24 hours. After the incubation period ends, the colonies of *Vibrio* sp. were counted with their population densities (Abraham and Sasmal, 2009; Marwiyah *et al*., 2019).

Monitoring the health status of hepatopancreas in pacific white shrimp

Pacific white shrimp was collected from each pond (P1-P7) starting from DOC 10-80 by using nets in each aquaculture pond so as to obtain a total of 58 shrimp. The collected shrimps were then examined for their hepatopancreas to observe necrosis severity, gregarine presence, tubulus and lipid (Lightner, 1993; George *et al*., 2016).

Data analysis

Observation results of *Vibrio* sp. processed using Microsoft Excel and displayed in graphical form. The Monitoring results for pacific white shrimp hepatopancreas health status were analyzed descriptively.

Results and Discussion

Monitoring of population density of *Vibrio* sp.

The monitoring results are shown in Figure 1. This result showed that the highest number of *Vibrio* sp. populations was found in pond P2, which was $5.8x10^3$ CFU/g from DOC 70. Otherwise, the lowest average population was found in pond P7 from DOC 10 at $1.7x10^2$ CFU/g. The high population of *Vibrio* sp. in pond P2 is caused by increasing organic matter content in aquaculture ponds to support bacteria growth. In addition, *Vibrio* sp. has the potential to be an opportunistic pathogen in pacific white shrimp (Heenatigala and Fernando, 2016; Rungrassamee *et al*., 2016). According to Zhou *et al*. (2012), particulate matter in aquaculture ponds is an important part that must be considered. This is related to increasing in *Vibrio* sp. number and the virulence of these bacteria due to dramatic changes in environmental quality.

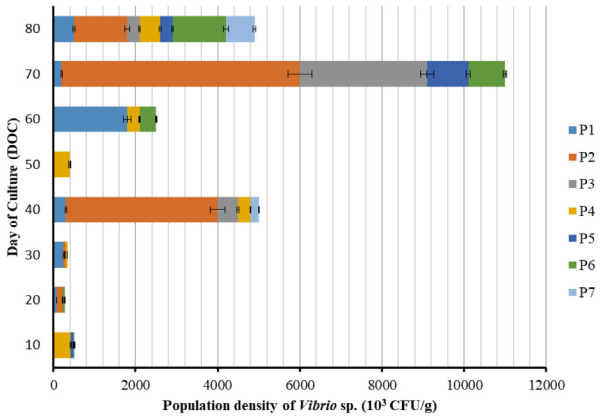

Fig. 1. Population density of *Vibrio* sp. on the TCBS medium.

Taslihan (2017) also reported that the total *Vibrio* sp. analysis conducted on shrimp ponds in Jepara Regency ranged from 2.7×10^4-3.1×10^4 and all samples showed the number of white shrimps infected by *Vibrio parahaemolyticus*. Meanwhile, this bacterium often grows naturally in estuaries and oceans and is a major cause of infectious diseases through seafood intermediaries in several countries especially Asia (Oh *et al*., 2011; Haendiges *et al*., 2014; Elmahdi *et al*., 2016). In addition, early mortality syndrome (EMS) that occurs in shrimp species *P. vannamei*, *P. monodon* and *P. chinensis* is referred to as acute hepatopancreatic necrosis disease (AHPND) (NACA, 2012; FAO, 2013).

Healthy pacific white shrimp has an abundance of *Vibrio* sp. with a total of $2.5 \pm 0.5\text{x}10^4$ CFU/g. This condition shows that the presence of *Vibrio* sp. in healthy shrimp will not be pathogenic (Supono *et al*., 2019). Furthermore, Taslihan *et al*. (2015) stated that the abundance of *Vibrio* sp. exceeding 10^4 CFU/gr has a susceptibility to vibriosis infection resulting in mass mortality in cultured shrimp. The existence of *Vibrio* sp. naturally can be derived maternally from shrimp broodstock into eggs, larvae and post-larvae. In addition, an increase in organic content derived from the remainder of the feed and feces can trigger the acceleration of the development of *Vibrio* sp. to be an opportunistic pathogen (Hameed *et al*., 2003).

Health status of pacific white shrimp hepatopancreas

Percentage of hepatopancreas organs depletion is shown in Figure 2. This study indicate that the highest percentage of depletion was obtained in pond P7 with an average of 90% in DOC 30, 70 and 80. Depreciation of hepatopancreas cells can be caused by atrophy whereas the state of decreasing cells number in tissue or abnormalities in cells and it makes hepatopancreas tubules will look dilated (Otta and Karunasagar, 2001). Cell depletion is also caused by decreasing in shrimp appetite.

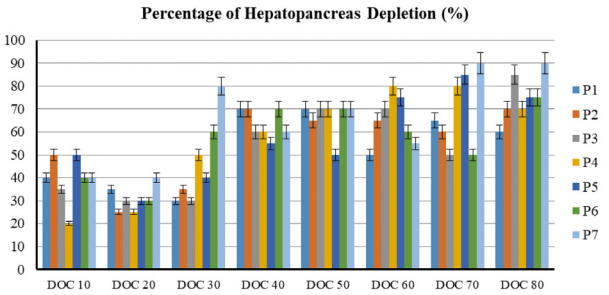

Fig. 2. Percentage of pacific white shrimp hepatopancreas depletion (%). Note: 0%: no depletion; < 25%: few depletion; 26-50%: high depletion; > 50%: very high depletion (George *et al*., 2016).

Water quality measurement and infectious diseases identification in pacific white shrimp must often be done in order to prevent an increase in the percentage of depletion that occurs in hepatopancreas. These precautions can be like observing white stools number due to intestine filled with agregated transformed microvilli (ATM) particles so that the shrimp must be immediately separated from other shrimp for prevention before prolonged stress (Sriurairatana *et al*., 2014). Moreover, white dung is also a damage result from aggregated white shrimp hepatopancreas and finally released through the digestive tract of shrimp (Taslihan, 2017).

One of indicator in health status of pacific white shrimp hepatopancreas is the number of necrosis, the results of which can be seen in Figure 3. The qualitative observations showed that pacific white shrimp pancreas has experienced necrosis with the highest number found in DOC 50 and DOC 80 and marked by the occurrence of tubular tissue damage.

The presence of necrosis in hepatopancreas is pacific white shrimp indicator with infections such as WSSV, IMNV, and AHPND. Moreover, necrosis can occur due to hepatopancreatic tubular epithelial cell decay and infiltration of hemocytosis. Hepatopancreas is a large gland that has functions such as liver and pancreas in shrimp and is often used as a bioindicator related to physiological health condi-

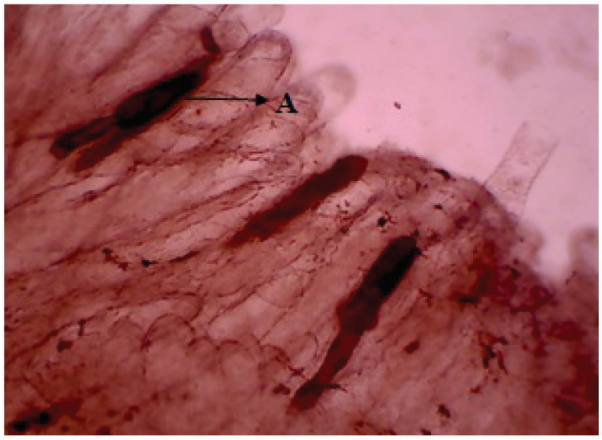

Fig. 3. Necrosis in pacific white shrimp hepatopancreas (label A) in intensive system ponds.

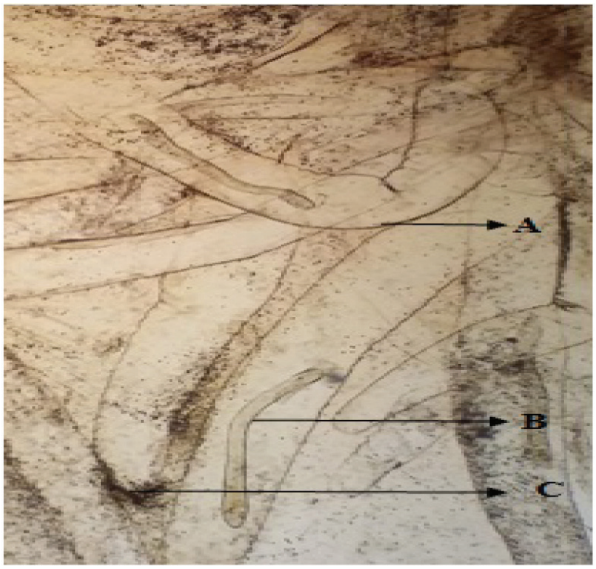

Fig. 4. Hepatopancreas condition of pacific white shrimp in intensive system ponds. Notes: **A** (Tubular); **B** (Gregarine protozoa); **C** (Lipid).

tions (Manan *et al*., 2015).

Infection greatly influences the texture of hepatopancreas to become less dense, slimy, and has a whitish blue color which causes the shrimp to have no appetite. In this study, there were shrimp hepatopancreas that experienced necrosis due to EMS infection. The existence of gregarine could be seen in Figure 4. In general, the presence of gregarine is abundant in DOC 80 in all aquaculture ponds. The existence of gregarine from *Nematopsis* sp. and microsporidia from the Enterocytozoon group are the cause of white feces disease (WFD) (Taslihan *et al*., 2015; Anjaini *et al*., 2018).

Conclusion

This study showed that the highest population of *Vibrio* sp. was found in P2 pool, which was $5.8x10^3$ CFU/g from DOC 70. The lowest average population was found in pool P7 from DOC 10 at $1.7x10^2$ CFU/g. Shrimp health indicators such as necrosis and the presence of gregarine were found in pacific white shrimp which indicate that it is susceptible to infectious diseases. The results of this study must be further developed using other indicators such as hepatopancreas histology and using molecular approaches to further clarify the existence of the *Vibrio* sp. However, this research can be a pioneer in research process regarding the health care of shrimp culture.

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