Change of hepatopancreas conditions in intensive shrimp aquaculture (*Litopenaeus vannamei*) at Mayangan Village, Legon Kulon District West Java

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**Abstract.** The study of hepatopancreas purpose to monitor changes in the health condition of shrimp, which is hepatopancreas is a vital organ of metabolism and digestive system. This study was conducted in intensive shrimp ponds in Mayangan Village, Legon Kulon for 82 days. Sampling was carried out every 10 days. Hepatopancreas preparation was conducted by taking 30-50 mg with adding 0.1-0.2 ml of immersion liquid and observing used a microscope at 40x magnification. The parameters of hepatopancreas observed such as microphili, lipids, wrinkles, and necrosis. After monitoring the microphili in the Day of Culture (DOC) 1-40, the average score range shows that the culture shrimp is 90-100% healthy. in DOC 43 until the last sampling has decreased by 40-0% which is assumed to be the effect of a White Feces Disease (WFD). The lipid and wrinkle in DOC 1-40 sampling showed that the average range shrimp culture was 90-100% healthy. While in DOC 43 sampling until the last of lipid and wrinkle sampling decreased by 60-0%. Hepatopancreas necrosis in DOC 1-31 sampling does not exceed from 20%, while DOC 40 sampling until the last of necrosis sampling continues to increase to 100% assumed by WFD and WSSV (White Spot Syndrome Virus).

1. **Introduction**

Mayangan Legon Kulon Village, West Java is an area that is a center for shrimp producers in Indonesia. One type of shrimp commodity produced is shrimp from the panaiidae family, vannamei (*Litopenaeus vannamei*). Shrimp production produced from 2015 to 2017 continues to decline. The shrimp export volume in 2015 was 4,230 tons, in 2016 there were 4,110 tons, and in 2017 it was 3,800 tons [4].

Decrease in average production results due to poor waste management and pond construction using mulsa (low density polyethylene) material [3]. Health observations are needed to determine the morphology of shrimp bodies, the presence of parasites and pathogens, and assess the health condition of shrimp bodies (Scoring Health Larvae) to be able to increase shrimp production again [16]. Hepatopancreas is a vital organ of shrimp that is used as a digestive and metabolic system. So it is possible to observe the condition of hepatopancreas can be a reference in monitoring the sick and healthy shrimp.

2. **Materials and methods**

This study was carried out for 84 days from March to May 2019, located in the Vannamei Shrimp Farm, Mayangan Village, Legon Kulon Sub-District, Subang Regency, West Java with descriptive method, which is a method that describes the facts or characteristics of a particular population in an
actual and meticulous way to look for elements, characteristics, traits or problems that exist [7, 13, 14]. The area of the pond plot is 3,000 m² with a population density of 140/m². The tools used include light microscopy, pH meters, and digital scales. Materials used include nitrite test kits, ammonium test kits, glass objects, cover glass, alcohol 70% -90%, shrimp test WSSV, and aquades.

2.1. Shrimp Sampling
Shrimp sampling is done based on age / Day of Culture (DOC) on shrimp. At DOC 1-30 days sampling is done once every 10 days at DOC 10, 20 and 30. Samples were taken from anco (bridge sampling place) in pond ponds as many as 3 animals/ponds from 3 different anco points. At DOC 30 until harvest sampling is done every 7 days.

Shrimp DOC 30 and above sampling is done by using a net with an estimated take of 1.5-2 kg/pond and select 3 shrimp as the sample. The net used in the sampling was immersed in a PK solution of 10 ppm. Shrimp from the sampling results are hung on a jarring and separated based on normal carapace or kropos and shrimp which are under-sized.

2.2. Hepatopancreas Preparation
Hepatopancreas preparation is done by taking 50-60 mg of organs and then placed on glass objects. Immersion is added 1-2 drops and covered with glass cover. On observing the hepatopancreas organs can be observed wrinkles (wrinkles) and necrosis. The assessment indicators can be seen in Figures 1 and 2 below.

![Figure 1. Wrinkle Indicator in Shrimp Hepatopancreas](Source: CV Andulang Laboratory (2019))

![Figure 2. Indicators of Necrosis in Shrimp Hepatopancreas](Source: CV Andulang Laboratory (2019))
Further observation of lipids and microphili of shrimp is the lipids contained in microphili. The full lipid is given the value "+", if the half lipid is given the value "medium" and the blank is given the value "-". Whereas the microphili assessment is "+" if the tip is thick and looks double, the medium for the microphili tip is thickened and "-" if the tip is 1 layer and thin.

2.3. ADG (average daily growth) and MBW (mean body weight)
ADG and MBW were measured every shrimp sampling. Shrimp are counted and weighed. The ADG and ABW formulas are as follows Hidayat et al. [6].

1) MBW
Is the mean body weight of shrimp from sampling

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MBW = \frac{weight \ of \ entire \ sampling}{number \ of \ samples}
\]

2) ADG
It is the average daily growth calculated for each sampling

\[
ADG = \frac{ABW \ end - ABW \ start}{long \ maintenance}
\]

3) Shimples Test WSSV
Shrimples WSSV tests are carried out at the time of H-1 harvest. Shrimples tests use the WSSV Test Kit by taking 5-7 shrimp samples. Then cut 40-50 mg of gills and uropods then extracted using a pastel test kit. The extraction results are taken 1 ml using a pipette test kit and put into a well. Waiting for the indicator process to run 3-5 minutes. If the stripe 2 appears positive the sample while the stripe 1 sample is negative.

2.4. Water quality monitoring
a. Water Sampling
Sampling is done 2 times a day at 7:00 and 15:00. Water samples taken water must be evenly distributed from the surface to the bottom using a bottle tied to a paralon / wooden stick. Water samples taken are tested with a maximum time limit of 1 hour after sampling.
b. Water Quality Measurement
Water quality test is carried out with several parameters observed. The water quality testing schedule can be seen in Table 1 below:

| Parameters  | Time  |
|-------------|-------|
| pH          | 07.00 |
| Nitrite     | 15.00 |
| Ammonia     | 15.00 |

The water quality parameter test standard is compared with SNI 8037.1.2014 [9]. The reference for water quality test can be seen in Table 2 below.

| No | Parameter  | Unit   | Score |
|----|------------|--------|-------|
| 1  | pH         | -      | 7.5-8.5 |
| 2  | Nitrite    | Mg/l   | <0.1  |
| 3  | Ammonia    | Mg/l   | <0.1  |

Source: SNI 8037.1.2014 [9]
1. pH
pH is measured twice a day using a pH-pen with SNI 06-6989. 11 [1]. Water samples that have been taken are moved in glasses with a diameter of 4.5-5 cm and filled with water with a height of 5 cm. The pH pen is inserted and stirred slowly until the indicator stops.

2. Nitrite
Nitrite was measured using the Merck kit 1.14408 SNI 06-6989.30 [2]. The water sample is taken 6 ml in a test tube and reagent gave as much as 1 measuring spoon. Samples were homogenized and allowed to stand for 3 minutes. Then observed and matched with the indicators on the test kit.

3. Ammonia (NH₃)
Ammonia was measured using the Merck kit No. 1.14423 SNI 06-6989.30 [2]. Water samples were taken 5 ml in a test tube and 0.4 ml of reagent 1 were given using a split. Reagent 2 is given as much as 1 measuring spoon then homogenized and allowed to stand for 5 minutes. Reagent 3 inserted as much as 4 drops, homogenized then allowed to stand for 7 minutes. Samples were observed and matched with the indicators in the test kit.

3. Results and discussion
3.1. Microphili and lipids
Microphili is a membrane that wraps lipid fluid in the hepatopancreas, observing microphiles can be used as an indicator of immunity as well as the absorption capacity of shrimp to feed nutrition. Lipid observations were carried out as an indicator of shrimp feed nutrition that came in. The results of microphili monitoring and lipid percentage of healthy shrimp based on microphili observations can be seen in the following graph 3.

![Monitoring of Microphili and Lipid Percentage](image)

**Figure 3.** Microphili and Lipid Monitoring Graph

Figure 3 showed microphilis in the 1st to 6th sampling averaging that shrimp farming is healthy. While the 7th sampling until the end of the microphili sampling showed a decrease which is the effect of the WFD (White Feces Disease) attack on DOC 49. The microphilis of shrimp that were attacked by WFD experienced erosion on the wall. So that the shrimp in the 9th and 10th sampling, the average microphili observed was thin.

Lipids in the 1st to 7th sampling showed that the average shrimp was healthy. This is in accordance with FAO [5] which states that the presence of relatively large hepatopancreas with large amounts of vacuoles lipid is considered a sign of good health. Whereas the 8th sampling until the end of the microphilic sampling showed a decrease which is the effect of a WFD (White Feces Disease) attack on
DOC 49 making the shrimp malnourished and reduced the size of the hepatopancreas. The differences in the microphilic and lipid images of healthy and sick shrimp can be seen in Figure 4 below.

![Microphilic and healthy shrimp lipids (left), microphilic and WFD shrimp lipids (right)](image)

**Figure 4.** Microphilic and healthy shrimp lipids (left), microphilic and WFD shrimp lipids (right)

### 3.2. Necrosis
Necrosis is a fleck/spots caused by dead cells and the result of inflammation of the wound in the hepatopancreas. The necrosis value in the results of weekly shrimp sampling can be seen in the following graphs 5.

![Necrosis Percentage](image)

**Figure 5.** Monitoring Necrosis

Figures 5 show hepatopancreas necrosis in the 1st to 5th sampling does not exceed 20% and shrimp can be said to be healthy. This is consistent with the Pelatihan Pengawas Budidaya Perikanan [8] which states that necrosis in hepatopancreas causes shrimp to be less active and pale in color. Increased necrosis is triggered by a WFD attack that goes from DOC 40 to harvest. Necrosis caused by WFD cannot be recovered properly. The 11th sampling showed 2 samples had 100% necrosis which was an indicator of weak shrimp immunity and was proven by WSSV attack on cultured shrimp. The reduction in necrosis is a sign that shrimp can recover from the body so that the cultivation process. White Muscle Disease attack on the 11th sampling causes total necrosis of the hepatopancreas and cannot be recovered by shrimp. The picture of necrosis in hepatopancreas can be seen in the following Figure 6.
3.3. Wrinkle (wrinkle)
Wrinkle is the predominance of microphiles in the hepatopancreas. If wrinkle has a high value, we can be sure that shrimp growth will be good and optimal and has high immunity. The wrinkle value in the results of weekly shrimp sampling can be seen in the following Figure 7.

![Figure 6. Monitoring Necrosis in Hepatopancreas](image)

Figure 7 showed wrinkles begin to decrease in score at the 6th sampling caused by EHP and WFD attacks. EHP attacks cause shrimp hepatopancreas to contract and experience degradation of the wasting. In advanced infections, WFD attacks shrimp, making absorption of food nutrients reduced and not optimal. Causing the lipid/wrinkle strands to decrease or shrink. This is in accordance with FAO [8] which states that a large number of cilia (wrinkles) in the hepatopancreas is an indicator of nutrient absorption in shrimp.

3.4. Parameters support
1. pH
Measured daily pH fluctuations in ponds range from 7.7 to 9.1, while for daytime monitoring ranges from 8.2 - 9.2. This is not in accordance with SNI 8037.1.2014 [9] which states that the optimal pH range is 7.5 - 8.5. An illustration of average weekly pH fluctuations in one production cycle as shown in Figures 8.
2. Nitrite (NO₂)
Nitrite results from ammonia oxidation in the nitrification process by the autotrophic bacterium *Nitrosomonas*, which uses ammonia as an energy source. High levels of nitrites are due to the slow change from nitrates to *nitrobacteria*. The nitrite measurement results can be seen in Figure 9 below.

The range of nitrite fluctuations in the test was 0-0.7 ppm. This is not in accordance with SNI 3087.1.2014 [9] which states that the ammonium content in aquaculture should not exceed 0.1 ppm. On the 8th week, the nitrite level has died and is not in accordance with the quality standard of aquaculture. The toxicity of nitrites is very dependent on the internal and external conditions of the fish such as species, age of the fish, and water quality. Nitrite ions enter the fish with the help of gill chloride cells [15]. In the blood, nitrite will unite with hemoglobin, which results in an increase in methemoglobin [15]. This will reduce the ability to transport oxygen in the blood.

3. Ammonia (NH₃)
The results of checking ammonia in a vaname shrimp enlargement pond can be seen in the following Figure 10.
Figure 10 showed that the ammonia content fluctuation is higher according to the standard. Ammonia fluctuation range in testing is 0-0.7 ppm. This is not in accordance with SNI 3087.1.2014 which states that the ammonium content in aquaculture should not exceed 0.1 ppm. Ammonia levels in weeks 7 to 8 have increased due to high mortality and triggers ammonia levels. If ammonia is high the thing to do is to give probiotics that contain Nitrobacteria and Nitrosomonas bacteria. Ammonia will soon be overhauled and decomposed well if the nitrification process goes well.

4. ADG (Average Body Weight) and MBW (Mean Body Growth)
ADG and MBW from the sampling results can be seen in the following Figure 11 and 12.

![Monitoring Average Daily Growth](image1)
![Monitoring Mean Body Weight](image2)

Figure 11. ADG monitoring  
Figure 12. Monitoring of MBW

Figure 11 showed the ADG in shrimp ranging from -0.003-0.59. ADG did not experience even negative growth in DOC 57 when shrimp were infected with WFD. The 8th DOC 68 shrimp sampling did not experience ADG increase because the hepatopancreas and shrimp intestines were unable to absorb nutrients optimally which caused the shrimp not to gain weight.

Figure 12 showed the MBW of the results of the sampling conducted. Sampling on DOC 60 and 68 does not show the weight gain of shrimp due to WFD attacks. Shrimp are harvested at DOC 74 with a weight of 11.43 grams.

4. Conclusion
Shrimp cultivation with mulsa construction in the main village of legon kulon which was studied by observing changes in hepatopancreas showed lipid parameters, microphilic wrinkles decreased and necrosis increased. This is supported by data on water quality, ADG and MBW of shrimp affected by WFD on DOC 43 and WSSV on DOC 72.

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