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Histopathological in Gill, Hepatopancreas and Gut of White Shrimp (*Litopenaeus vannamei*) Infected White Feces Disease (WFD)

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KEYWORDS

White Feces Disease
Gregarine
Histopatology

Abstract White feces disease which is the presence of white feces floating on the surface of the water. Physical disruption of white feces disease attacked by exocytes becoming soft. the cause of white feces disease (WFD) is a decrease in environmental quality such as salinity, dissolved oxygen and ammonia. White feces disease is associated with gregarine protozoa in the Apicomplexa phylum. Gregarine parasitic infection from the type of *nematopsis* that was interacted with *Vibrio* and *Vibrio* bacteria found in white shrimp (*Litopenaeus vannamei*) which was attacked by white impurities was *V. harveyi*. This study aims to analyze the presence of gregarine protozoa in the gills, intestines and hepatopancreas of white shrimp infected with white feces disease. The method used in this research is descriptive method. Histopathological results showed that there were protozoa (gregarine) in the intestine, hepatopancreas and gills of white shrimp infected with WFS in 3 different locations and found Aggregated Transformed Microvilli (ATM) that resembled gregarine.

Introduction

Direktorat Jenderal Perikanan Budidaya (2016) reported that the production of white shrimp was 488,019 tons from the initial target of 484,002 tons that it reached a percentage of 100.83%. This shows that white shrimp production is on target and rose by 19.86% from 2015. The national shrimp production target in 2018 according to the Kementerian Kelautan dan Perikanan Indonesia (2018), aims to reach 800 thousand tons of shrimp that requires white shrimp and tiger shrimp. The increase in national shrimp production can be done through intensive cultivation even though the technology still faces many obstacles so that the productivity of white shrimp *(L. vannamei)* is not sufficient for the demand (Garno, 2004).

White shrimp *(Litopenaeus vannamei)* is an alternative shrimp besides tiger shrimp *(Penaeus monodon)* which can be cultivated intensively. white shrimp has the advantage of being able to grow as fast as tiger shrimp (3 gram/week), can be cultivated in a wide range of salinity (0.5-45 ppt), lower protein requirements (20-35%) than tiger shrimp, able to convert feed better (FCR 1.2-1.6) and can be stocked with high density up to more than 150 tails/m2 (Budiardi, *et al*., 2005).

White shrimp production decreases caused by white feces disease. Beginning in 2014 this disease began to enter Indonesia and attacked intensive white shrimp *(L. vannamei)* ponds in Sumatra, Java, Sulawesi, Bali and West Nusa Tenggara (Taslihan *et al*., 2015). Limsuwan (2010), an indication of white feces disease, namely the presence of white dirt floating on the surface of the water. Physical disruption of white feces disease attacked by exocytes becoming soft. Durai *et al*. (2015) one of the causes of white feces disease (WFD) is decreasing environmental quality such as salinity, dissolved oxygen and

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ammonia. Ammonia arises due to poor feed management. Tangtopiros (2010), white feces disease is associated with gregarine protozoa in the Apicomplexa phylum. The spread of white feces syndrome (WFS) is when shrimp consume invertebrates that have gregarine in them. It is strongly suspected that this disease is caused by a combination of Gregarine parasitic infection from the type of *nematopsis* that is interacted with *Vibrio* and *Vibrio* type bacteria found in white shrimp which is attacked by white feces disease is *V. harveyi* (Chaweepack, *et al*., 2015).

Identification of protozoa gregarine in shrimp body organs is very necessary to provide further information about gregarine as one of the agents causing white feces disease (WFD). One way that can be done to identify gregarine is to use tissue histopathology studies. Histopathology is a tissue study that is used to study disease in organisms. Histopathological analysis can be used as a biomarker to determine health conditions through structural changes that occur in organs that are the main target of pollutants such as gills, liver, kidneys and so on (Setyowati, *et al*., 2010). According to Asniatih *et al*. (2013), histopathological examination of organisms can provide a picture of changes in tissue infected with the disease.

Based on the above problems, it is necessary to further analyze the presence of gregarine protozoa in the gills, intestines and hepatopancreas of white shrimp infected with white feces disease. Identification of gregarine in the gills, intestine and hepatopancreas is done by observing tissue or histopathology in the laboratory. Through identification of gregarine in the gills, intestines and hepatopancreas of shrimp, it is expected to provide information about one of the causes of white feces disease, namely gregarine protozoa.

Materials and methods

The method used in this research was descriptive method. Suryana (2010), descriptive method is a method used to look for elements, characteristics, characteristics of a phenomenon. This method starts with collecting data analyzing data, and interpreting the data. Data is collected by careful observation, including descriptions in the context that approaches and analyzes documents and records (Hamdi and Bahrudin, 2014).

The process of data collection in this study was carried out by direct and indirect observation. Hasanah (2016), defines observation as the activity of recording a symptom with the help of instruments and recording it with scientific goals or other purposes. The observation technique in this study is direct observation techniques and indirect observation techniques. Direct observation technique is a data collection technique in which the researcher makes direct or no observations of the symptoms of the subject being investigated both observations made in the actual situation and carried out in artificial situations, specifically held. The indirect observation technique is a data collection technique in which the researcher observes the symptoms of the subject he is researching through the means of a tool (Diantha 2016)

Samples of white shrimp *(L. vannamei)* taken from 3 farms infected with white feces disease, (A) Tasikharjo Village, Jenu Subdistrict, Tuban District, (B) Kranji Village, Paciran Subdistrict, Lamongan District and (C) Peleyan Village, Panarukan Subdistrict, Situbondo District. This research was conducted from September to November 2018.

Histopathology Procedure

1. Process of cutting the tissue.
   Soaking with Davidson's solution for 7 hours. Furthermore, the tissue is chosen the best according to the location to be studied. The tissue is cut more or less with a thickness of 2-3 millimeters. The tissue is put on tapes and coded according to the gross code of the researcher. The tissue is processed by the Automatic Tissue Tek Processor for 90

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minutes. Waiting until the Alarm Tech Tissue goes off the sign has finished.

2. Blocked and Cutting Process of Tissue.
   The treated tissue is blocked with paraffin which has been melted with a hot plate and adjusted to the network code. The network that has been blocked with paraffin is waiting until hardens. Next the tissue is cut with the microtome with a thickness of 3-5 microns.
3. Depression process.
   Enter the incision into the waterbath with a temperature of 40°C. The best incision results are selected and prepared by glass objects (for preparation of HE coloring) which previously had to be smeared with polysin adhesive. Next the sample was dried in an oven for 30 minutes at a temperature of 70-80°C. The tissue inserted into 2 tubes of Xylol solution for 20 minutes each, it is added to 4 alcohol tubes. Dyeing on each alcohol tube was carried out for 3 minutes (Hydration), and the last one was rinsed with running water for 15 minutes.
4. Coloring process.
   Tissue was immersed in hematoxillin lead for 10-15 minutes. Then wash with running water for 15 minutes. The next step is immersion in 1% alcoholic acid solution as much as 2-5 times dyeing. The last coloring step is by immersing the tissue using Eosin dye for 10-15 minutes.
5. Dehydration.
   After the coloring process is complete, the tissue is immersed in a multilevel alcohol, 70% Alcohol for 3 minutes. Then immersion in Alcohol 80% for 3 minutes, Alcohol 96% for 3 minutes and the last one with Absolute Alcohol for 3 minutes.
6. Purification.
   Purification was done by inserting the results of tissue incision into Xylol 1 solution for 15 minutes and Xylol 2 for 15 minutes.
7. Finishing.
   The slide or object is covered with a glass cover and glued with Micromouth Mounting medium and left to slide until it is dry at room temperature. After the dry slide the slide is ready to be observed with a microscope.

Results and discussion

White feces disease is a disease that is often found in white shrimp. Shrimp infected with white feces tend to be smaller and darker in color. In the hepatopancreas and shrimp intestines infected with white feces disease, the effects are quite serious. These organs tend to be white and paler. An indication of the initial disease appears in the feed container and on the surface of the water, where many white feces are found. Shrimp affected by this disease show a softened exoskeleton and the presence of gregarine protozoa causes dark colors in the gills (Mastan, 2015). Gregarine is protozoa in the digestive tract and tissues of various invertebrate animals. They attack the digestive tract of shrimp and are most often observed during the trophozoite stage or sometimes gametocyst. The condition of poor water quality, gregarine-type protozoa together with infection with *Vibrio* bacteria in the digestive tract of shrimp causes a change in the tissue structure of the hepatopancreas to microvilli tissue in the intestine. There are fears that severe infections caused by gregarine can potentially cause economic losses through the death of cultured organisms. In addition, it is suspected that the growth of the organism will also decrease and cause mass mortality (Kua *et al*., 2013)

White Shrimp Hepatopancreas Histopathology

The histopathological results of shrimp hepatopancreas infected with white feces disease showed that the three white shrimp samples had hepatopancreas tissue which appeared irregular (Figure 2). This irregular shape indicates a lysis structure in the white shrimp hepatopancreas tissue. Histological tissue of normal shrimp

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hepatopancreas has a good tubular and distal lumen and has no changes (Figure 1) (Nazaruddin *et al*., 2014). Damage to hepatopancreas tissue is due to the presence of gregarine protozoa found to infect these organs. Histopathological results showed the presence of gregarine protozoa characterized by dark circles on the tissue indicated by arrows (Figure 2).

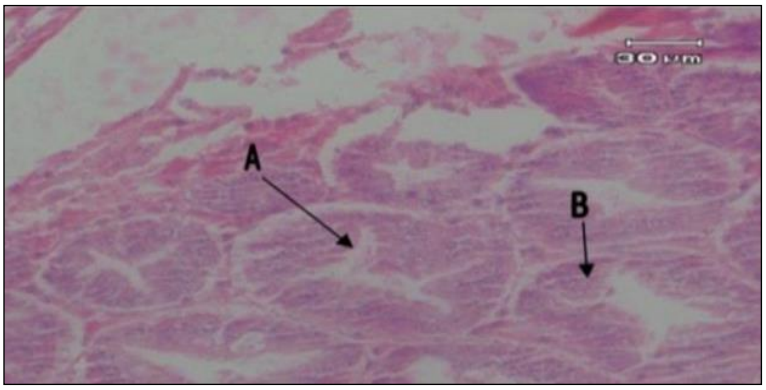

Figure 1. Normal white shrimp tissue histology (A = lumen, B = distal tubule; HE, 400X) (Nazaruddin *et al*., 2014).

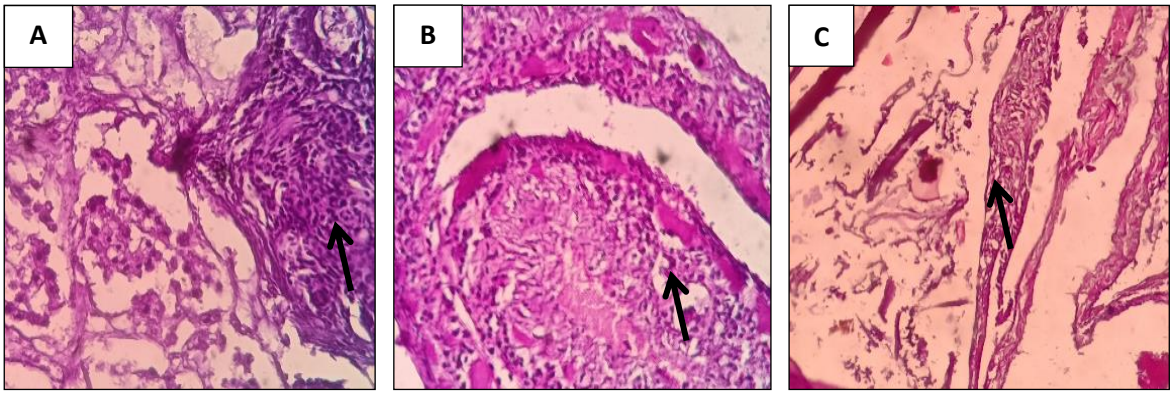

Figure 2. Histopatology of white shrimp hepatopancreas infected by WFD from (A) Lamongan, (B) Situbondo, (C) Tuban (H & E, 100X).

Besides severe tissue degradation or damage, it can be seen also that the hepatopancreas tissue of shrimp infected with WFD experiences tissue atrophy. Tissue atrophy is characterized by the condition of the hepatopancreas that looks empty. According to Jayadi (2016), tissue atrophy is a narrowing of the tissue accompanied by a widening of the hepatopancreatic lumen. The thickened lumen of the hepatopancreas indicates that the hepatopancreas lumen is empty because the shrimp do not eat for days.

Another thing that can be seen from the *L. vannamei* hepatopancreas which is infected with WFS is the exfoliation of hepatopancreas microvilli. The results of the exfoliated microvilli will be aggregated and accumulated which will then form the Aggregated Transformed Microvilli (ATM). Mastan (2015) explained that, white bowel disease in shrimp arises from the transformation, exfoliation and aggregation of hepatopancreas microvilli which then leads to the intestine. The exfoliating microvilli forms Aggregated Transformed Microvilli (ATM) which resembles gregarine. Jones *et al*. (1994) suggested that abnormal microvilli tissue could affect the body's metabolic system so that it would have an adverse growth effect.

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White Shrimp Intestinal Histopathology

In addition to the hepatopancreas, an organ that can be used as a parameter to diagnose White Feces Disease in white shrimp is the intestine. In white shrimp intestine infected with WFD, there was found a gregarine protozoa infection which was seen in (Figure 3), that the WFD-infected hepatopancreas tissue had protozoa gregarine which was shown with a black arrow. Gregarines are "primitive" apricomplex parasitic groups that have a single large single cell that infects the intestine and other extracellular spaces of lower invertebrates and vertebrates (especially arthropods, mollusks and annelids), which are abundant in natural water sources (Apicomplex phylum; Gregarinomorphea class) (Ryan *et al*., 2016). Ramadan (2016), bacteria from the genus *Vibrio* were found that infect the intestines and hepatopancreas of white shrimp infected with white feces disease.

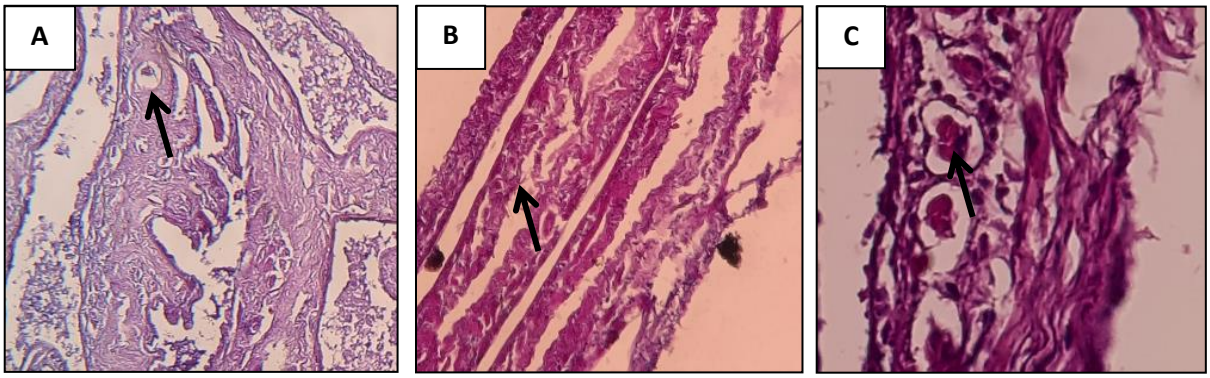

Figure 3. Histopathology of white shrimp intestine infected with WFD from (A) Lamongan, (B) Situbondo, (C) Tuban (H & E, 100X)

The histopathological results of healthy white shrimp intestine revealed that intestinal villi tissue looked good. However, it is suspected that damage to the intestinal villi layer is an indication of an infection from the bacteria that attack the white shrimp intestine. Kurniawan and Susianingsih (2014), exposing damaged intestinal villi is a sign of antigen attachment carried out by bacteria. Previous research has suggested that food absorption or intestinal blockage by protozoa can have a detrimental effect. White shrimp intestine which was infected by White Feces Disease showed higher damage compared to white shrimp intestine that was not infected with white feces disease.

Maskur *et al*. (2014), vibriosis in shrimp is generally a secondary infection when the shrimp is stressed and weak. This bacterial infection is usually associated with stress conditions due to high density, malnutrition, poor handling, parasitic infections, high organic matter, low oxygen, poor water quality, extreme water temperature fluctuations. Ramadhan (2016), found bacteria from the genus *Vibrio* which infects the intestine and the hepatopancreas in white shrimp infected with white feces syndrome.

White Shrimp Gill Histopathology

The histopathological results of shrimp gills infected with WFD can be seen that the gill tissue appears to be lysis and disjointed and messy. This messy tissue indicates the level of degradation in the white shrimp gills is high and the gill function is not able to work normally.

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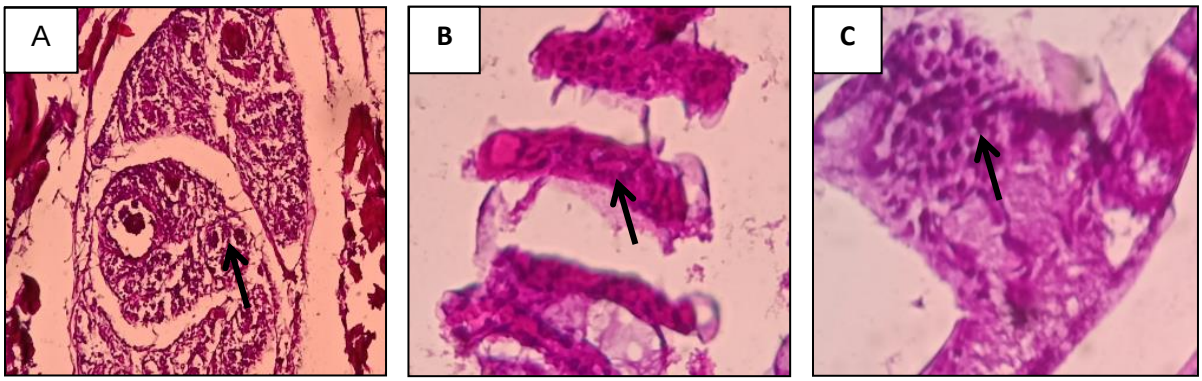

Figure 4. Histopathology of white shrimp gills infected with WFD from (A) Lamongan, (B) Situbondo, (C) Tuban (H & E, 100X).

Histology results of gills seen with the aid of a microscope showed little change in network structure that was not very clear. Normal epithelial cells do not show a striking structure or size. However, in the research of Suppamataya, *et al*. (1998) suggested gregarine protozoa found in gill tissue. Gills infected with gregarine protozoa have different tissue structures with normal gill tissue structures. Gill tissue infected with gregarine protozoa experiences necrosis or tissue damage in the part of the lamella. Damaged tissue structure will result in organ function not going well (Irina and Svetlana, 2010).

Aggregated Transformed Microvilli (ATM)

The histopathological analysis of gill, intestines and hepatopancreas of white shrimp infected with WFD found that there was gregarine. In addition to Gregarine, it was found that ATM resembles gregarine protozoa. In the study of tissue histopathology, gregarine is characterized by the presence of cell organelles, such as the presence of nuclei, mitochondria, ribosomes or other cell organelles while ATMs do not have these cell organelles. ATMs are formed from collections of membranes that experience constriction so that the lumen tubules widen. The membranes were subsequently degraded and aggregated to form ATM. ATM is the result of damaged and transformed hepatopancreatic tubular cells resembling the shape of gregarine (Sriurairatama *et al*., 2014). The exfoliated hepatopancreas microvilli will then be aggregated and collected in the intestine (Figure 5).

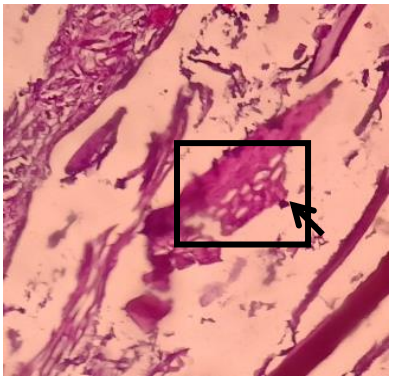

Figure 5. ATMs in white shrimp intestines infected with WFS are marked with arrows (H & E, 100X).

Jayadi (2016), the histopathological diagnosis of the digestive organs namely hepatopancreas and intestine is known that there is a picture like a worm that was once thought to be gregarine which was later called Aggregated Transformed Microvilli (ATM). ATM itself has a form resembling gregarine or a type of protozoa. According to Rajendran *et al*. (2016), white feces syndrome arises because of the transformation, decay and aggregation of the microvilli hepatopancreas tissue into the intestine. The identification results showed that *Vibrio* bacteria and six species of fungi had been isolated from shrimp infected with white feces syndrome. In several studies, it was suggested

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that microvilli tissue undergoing changes related to WFS infections had not been fully identified. However, it has been reported that an increase in ATM prevalence coincides with an increased prevalence of acute hepatopancreatic necrosis. Therefore, the outline can be taken that white impurities can be caused by hepatopancreatic dysfunction and pathological results that experience necrosis caused by one of these pathogens or a combination of biotic and abiotic factors.

Water Quality Observation

The results of measurements of water quality. The purpose of measuring water quality is to find out whether white feces disease is driven by poor water quality. *Vibrio* sp. bacteria. at a density of 104 CFU/ml pathogenic in shrimp, attacking hepatopancreas, especially coupled with poor water quality with increased organic matter in ponds (Taslihan *et al*, 2015). This measurement has been carried out at the Hydrology Laboratory of the Division of Aquatic Biotechnology and Environment, Faculty of Fisheries and Marine Sciences, Brawijaya University, Malang. Measuring water quality parameters carried out on white shrimp ponds in three different places obtained results as found in Table 1.

Table 1. Measurement Results Parameters of Water Quality of Shrimp Ponds infected with white feces disease.

No	Water Quality Parameters	Tasikharjo Village, Jenu District, Tuban Regency	Kranji Village, Paciran District, Lamongan Regency	Peleyan Village, Panarukan District, Situbondo Regency	Standard Parameters
1	Temperature (°C)	30.7	29.8	27.3	28.5-31.5°C (SNI. 2006)
2	pH	7.1	6.9	6.6	7.5-8.5 (SNI. 2006)
3	DO (mg/L)	6.2	5.8	7.2	>3.5 mg/L (SNI. 2006)
4	Salinitas (ppt)	27.6	27.7	29.3	15 – 25 ppt (SNI. 2006)
5	Amonia (mg/L)	0.154	0.184	0.188	<0.1 mg/L (Effendi. 2003)
6	Nitrat (mg/L)	0.158	0.118	0.160	<0.01 mg /L (SNI. 2006)
7	Nitrit (mg/L)	0.003	0.007	0.006	0.11-0.54 mg/L (SNI. 2006)

1. Temperature

   From the results of field temperature measurements, the results were not much different between the white shrimp farms that were healthy and infected with White Feces Syndrome. When the temperature is measured, the weather around the pond area is bright and there is no cloud cover covering the area around it.

   According to the results issued by the Badan Standardisasi Nasional (2006), with SNI 01-7246-2006 number, it was explained that the requirements for temperature values for white shrimp cultivation were in the range of 28.5-31.5°C. According to Hadi (2006), there are several factors governing the balance of temperature, namely rainfall, evaporation, humidity, air temperature, wind speed and solar radiation.

2. Acidity (pH)

   The results of pH measurements on ponds were obtained below the pH requirements used for white shrimp farming. According to the results issued by the Badan Standardisasi Nasional (2006), with SNI 01-7246-2006, it was explained that the requirements for the pH value for white shrimp cultivation were in the range of 7.5-8.5. The degree of acidity (pH) has a very important role for the life of organisms in the waters. PH is closely related to chemical

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reactions in a water. As suggested by Suwarsih *et al*. (2016), the acidity (pH) of water shows the levels of hydrogen ions or protons contained in a water. pH also influences the process and speed of chemical reactions in the media and biochemical reactions in the shrimp's body. In additions pH also influences the toxicity of a compound, the survival and growth of shrimp.

3. Dissolved Oxygen (DO)

   As we know, shrimp is an organism with high oxygen demand. In the pond, the system implements super intensive cultivation with very high stocking densities and high feeding. The oxygen in the white shrimp pond remained at optimal value because it was assisted by a water mill. Waterwheel is very necessary in shrimp farming especially super intensive cultivation considering the supply of oxygen for shrimp is important and must always be considered.

   The results of DO measurements obtained indicate that the DO value is categorized as very fulfilling the requirements set by the Badan Standardisasi Nasional regarding the maintenance of white shrimp in ponds. According to the Badan Standardisasi Nasional (2006), with SNI 01-7246-2006, it was explained that the requirement for DO values for white shrimp cultivation was > 3.5 mg / L.

4. Salinity

   From the three water quality parameter data obtained it was found that the salinity value for farms infected with WFS exceeded the standard salinity values issued by the Indonesian Badan Standardisasi Nasional. According to the Badan Standardisasi Nasional (2006), with SNI 01-7246-2006, it was explained that the requirements for salinity values for white shrimp cultivation were in the range of 15-25. According to Buwono (1993), the optimum range of pond salinity was between 15-25 ppt. Too high salinity can inhibit the occurrence of shrimp moulting. Conversely, salinity between 5-10 ppt can accelerate moulting, but shrimp are sensitive to disease.

   According to Haliman and Adijaya (2004), salinity is one aspect of water quality which has an important role in influencing shrimp growth. High salinity will affect the growth of shrimp. Shrimp growth is slow because the osmoregulation process is disrupted. Osmoregulation is the process of regulating and balancing osmotic pressure between the inside and outside of the shrimp body. If salinity increases, then the growth of shrimp will slow down because more energy is absorbed for the osmoregulation process than for growth. With the condition of a bad aquatic environment can affect the health conditions of shrimp that are being cultivated.

5. Amonia (NH_3)

   The results of the measurement of Ammonia (NH3) showed that the ammonia value in the white shrimp ponds exceeded the optimum level. Effendi (2003) suggests that ammonia levels that exceed the optimum limit can affect the growth of aquatic organisms. If the ammonia value continues to increase, it will be dangerous for the survival of cultivated white shrimp. Ammonia levels in water should not exceed 0.1 mg/L. This is because it can interfere with the growth of the organism. According to Kilawati and Maimunah (2015), high ammonia levels in the waters can also increase the concentration of ammonia in the blood thereby reducing blood activity (hemocyanin) in binding to oxygen. In addition, high levels of ammonia can also increase shrimp susceptibility to disease.

6. Nitrit (NO_2)

   From the results of nitrite measurements, the nitrite content of the two ponds is still within the range that can be tolerated by the organism so that it does not affect the survival of the organism. According

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to the Badan Standardisasi Nasional (2006), with SNI 01-7246-2006, it was explained that the requirements for the salinity value for white shrimp farming ranged from < 0.01 mg/l.

7. Nitrat (NO_3)

Nitrates in a water can determine the productivity of phytoplankton. The nitrate value of the two ponds is still in the optimal range for the pond waters themselves. According to Boyd and Clay, (2002), nitrates are not toxic to shrimp at concentrations below 5 ppm

Nitrate content that is too high can cause eutrophication (nutrient enrichment) which is characterized by the occurrence of phytoplankton blooms and can cause the death of biota. The main source of phosphate and nitrate nutrients comes from the waters themselves, namely through the processes of decomposition of weathering or decomposition of plants and the remains of dead organisms. Apart from that, it also depends on the surrounding conditions including donations from the land through the river flow consisting of various industrial wastes containing organic compounds (Patty *et al*., 2015).

Conclusions and suggestion

The results of this study concluded that histopathological analysis showed that there were protozoa (Gregarine) in the intestine, hepatopancreas and gills of white shrimp *(L. vannamei)* infected with WFS in 3 different locations and found Aggregated Transformed Microvili (ATM) that resembled gregarine. In measuring water quality there are two parameters that exceed the threshold, namely ammonia and nitrate. This is thought to be the trigger for the cause of white feces disease. Suggestions need to be identified for the type of gregarine that attacks white shrimp which is infected with white feces disease.

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