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ANALYSIS OF THE IMPACT OF pH FLUCTUATIONS ON THE PATHOLOGICAL CONDITION OF THE HEPATOPANCREAS IN WHITELEG SHRIMP (*Litopenaeus vannamei*)

Analisis Dampak Fluktuasi pH Terhadap Kondisi Patologi Hepatopankreas Udang Vaname (*Litopenaeus vannamei*)

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ABSTRACT

Whiteleg shrimp (Litopenaeus vannamei) aquaculture faces serious challenges from disease, often triggered by environmental stressors. This research aims to analyze the correlation between weekly water pH variations and qualitative histopathological damage in the shrimp hepatopancreas. Sampling and pH measurements were conducted weekly in an intensive pond from 20 to 41 days of culture (DOC). Data analysis was performed descriptively by comparing pH values with the results of hepatopancreas wet mount observations. The results showed extreme pH fluctuations of up to 0.7 and a lowest pH value of 7.4, which is outside the ideal range for aquaculture. This condition created environmental stress that triggered detrimental physiological effects, including a decrease in immune activity. Shrimp at DOC 41 showed significant hepatopancreas damage in the form of tissue necrosis, tubule atrophy, and cellular disorganization. This damage was exacerbated by the finding of Enterocytozoon hepatopenaei (EHP) spores and a strong suspicion of Vibrio spp. infection. The extreme pH fluctuations acted as a triggering factor that weakened the shrimp's immune system, allowing opportunistic pathogens to thrive. The resulting hepatopancreas damage can threaten feed efficiency and the sustainability of aquaculture.

Keywords: pH Fluctuation, Hepatopancreas, Litopenaeus vannamei

ABSTRAK

Budidaya udang vaname (*Litopenaeus vannamei*) menghadapi tantangan serius dari penyakit, yang sering dipicu oleh stresor lingkungan. Penelitian ini bertujuan menganalisis korelasi antara variasi nilai pH air mingguan dengan kerusakan histopatologis kualitatif pada hepatopankreas udang. Pengambilan sampel dan pengukuran pH dilakukan per minggu di tambak intensif dari DOC 20–41. Analisis data dilakukan secara deskriptif dengan membandingkan nilai pH dengan hasil pengamatan wet mount hepatopankreas. Hasil menunjukkan bahwa terjadi fluktuasi pH yang ekstrem hingga 0,7 dan nilai pH terendah 7,4,

yang berada di luar batas ideal budidaya. Kondisi ini menciptakan stres lingkungan yang memicu dampak fisiologis merugikan, termasuk penurunan aktivitas imun. Udang DOC 41 menunjukkan kerusakan hepatopankreas signifikan berupa nekrosis jaringan, atropi tubulus, dan disorganisasi seluler. Kerusakan ini diperkuat dengan temuan spora *Enterocytozoon hepatopenaei* (EHP) serta dugaan kuat infeksi *Vibrio* spp. Fluktuasi pH yang ekstrem berperan sebagai faktor pemicu yang melemahkan sistem imun udang, memungkinkan patogen oportunistik untuk berkembang. Kerusakan hepatopankreas yang diakibatkan dapat mengancam efisiensi pakan dan keberlanjutan budidaya.

Kata Kunci: Fluktuasi pH, Hepatopankreas, *Litopenaeus vannamei*

INTRODUCTION

Whiteleg shrimp (Litopenaeus vannamei) is a leading aquaculture commodity that plays a crucial role in supporting the national economy. This commodity's contribution to Indonesia's fisheries export value is significant (Ministry of Maritime Affairs and Fisheries, 2024). However, the rapid intensification of cultivation implemented to achieve maximum production also presents significant challenges. Among the various obstacles faced, disease remains a major threat, potentially causing high mortality rates and substantial economic losses for farmers (Asrido et al., 2024). Precise and optimal water quality management is a key factor in maintaining stable shrimp homeostasis and is a fundamental strategy for suppressing and controlling the risk of disease outbreaks.

Among various water quality parameters, pH is the most dynamic chemical factor and the most sensitive to environmental stressors in aquatic organisms. Extreme pH fluctuations outside the optimal range can immediately trigger a stress response (Yu et al., 2020). Physiologically, large pH fluctuations disrupt the body's acid-base balance, forcing shrimp to divert energy for osmoregulation and homeostasis restoration (Pusporini, 2018). Environmental stress resulting from pH changes has significant pathological implications, particularly for the hepatopancreas. Damage to the hepatopancreas directly impacts immunity and growth. Prolonged pH stress has been shown to cause cellular damage and tissue degeneration in the hepatopancreas, manifested as necrosis and inflammation (Kholifah, 2016; Pusporini, 2018). Environmental pH imbalance acts as a physiological stressor that negatively impacts shrimp cellular immunity. This stress leads to decreased hemocyte activity and limits the efficacy of innate defense mechanisms, such as phagocytosis and melanization, thus collectively increasing the risk of infection by opportunistic pathogens (Liu et al., 2012).

The detrimental impact of pH fluctuations on the health of shrimp internal organs has been demonstrated in several studies, where this stressor consistently causes serious pathological changes in the hepatopancreas. Transcriptomic analysis indicates that low pH causes cellular damage mediated by oxidative stress (Hou et al., 2025), which is manifested as cellular degeneration/necrosis and inflammatory infiltration in the hepatopancreatic tubules. Another study by Tao et al. (2016) showed that pH stress causes rupture of the basement membrane of hepatopancreatic tubules, followed by an increase in the size and quantity of vacuoles within the tubules, and a reduction in the hepatocyte population. In the context of intensive aquaculture, where daily pH fluctuations are difficult to avoid, research focusing on the pathological conditions of shrimp is crucial. This analysis goes beyond clinical observation and provides qualitative data on internal organ changes in response to pH stress.

This study was conducted considering the critical role of pH as a stress factor triggering tissue damage and the limited qualitative data on the microscopic pathological conditions of the whiteleg shrimp hepatopancreas in real-world aquaculture environments. To address these data limitations, this study offers a novel approach by qualitatively analyzing histopathological damage in the whiteleg shrimp hepatopancreas, directly correlating it with weekly pH

variations in real-world aquaculture environments. The main objective of this study was to analyze the correlation between weekly variations in water pH values and the qualitative histopathological characteristics of damage in the hepatopancreas of whiteleg shrimp through microscopic observation. The results of this study are expected to provide in-depth qualitative information as a scientific basis for formulating water quality management strategies, especially in controlling pH fluctuations, to reduce the risk of pathology and increase the success of intensive whiteleg shrimp cultivation.

RESEARCH METHODS

This research was conducted over a one-month period (January – February 2025). Data and sample collection was conducted at an intensive aquaculture pond located in Wringi Putih Village, Muncar District, Banyuwangi Regency, East Java. The tools used included a microscope, glass objects, cover glasses, glass pipettes, and petri dishes. The materials used were distilled water, physiological NaCl, and glycerol. Whiteleg shrimp samples were taken randomly from one of the aquaculture plots monitored at weekly intervals. pH data collection was also conducted in the same pond to ensure valid temporal correlation.

pH Measurement

pH measurements are conducted weekly to monitor trends in aquatic environmental conditions. Data collection is conducted twice daily, in the morning and evening. Measurements are conducted in situ using a portable pH meter (Tosani, 2023). To ensure data validity and implement biosecurity principles, after measurement, the electrodes are washed with distilled water and dried with sterile tissue to remove contamination and prevent potential pathogen transmission between pond units.

Hepatopancreas Sampling and Analysis (Wet Mount)

Whiteleg shrimp samples were randomly taken from the monitored cultivation ponds at weekly intervals, with 5 shrimp taken at the beginning of each week, starting at Day 20 and ending at Day 41. The shrimp were uniform in size and clinically showed normal activity. The shrimp were immediately placed in a refrigerated container and transported to the laboratory for observation. Observations of the pathological condition of the shrimp focused on the hepatopancreas. The specimens were prepared using the wet mount method. The shrimp samples were dissected using sterile surgical instruments, then the hepatopancreas was removed aseptically and placed on a cleaned glass slide. Next, one drop of physiological NaCl was dripped onto the hepatopancreas and covered with a cover glass (Sriurairatana et al., 2014). Observations were made using a microscope at 40x magnification to identify any changes or abnormalities in the structure of the hepatopancreas.

Data Analysis

Data analysis was conducted using a descriptive method (Sugiyono, 2013) by directly comparing pH values with qualitative evaluation results from microscopic observations. This was done to specifically identify indications of cellular damage and tissue degeneration in the hepatopancreas, thereby manifesting and documenting the shrimp's pathological response to pH stress exposure.

RESULT

The results of pH parameter measurements showed significant variations between morning and afternoon. In the morning, pH values were recorded ranging from 7.4 to 7.6. This value then increased in the afternoon, with a higher range of 7.7 to 8.4. This daily variation resulted in significant pH fluctuations within a single day, with the range of

changes recorded ranging from 0.2 to 0.7 (Figure 1). These data clearly indicate that the sampled ponds experienced pH instability that exceeded the ideal daily limits for vannamei shrimp cultivation.

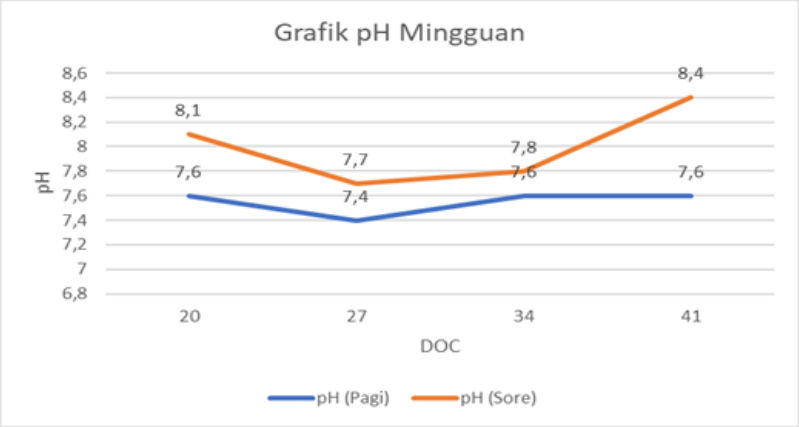

Figure 1. Morning and Evening pH Measurement Graph

Qualitative microscopic observations were performed on the hepatopancreas to assess the impact of environmental conditions. Microscopic observations revealed that although most shrimp samples exhibited normal hepatopancreas, significant pathological abnormalities were observed in DOC 41 shrimp samples (Figure 2).

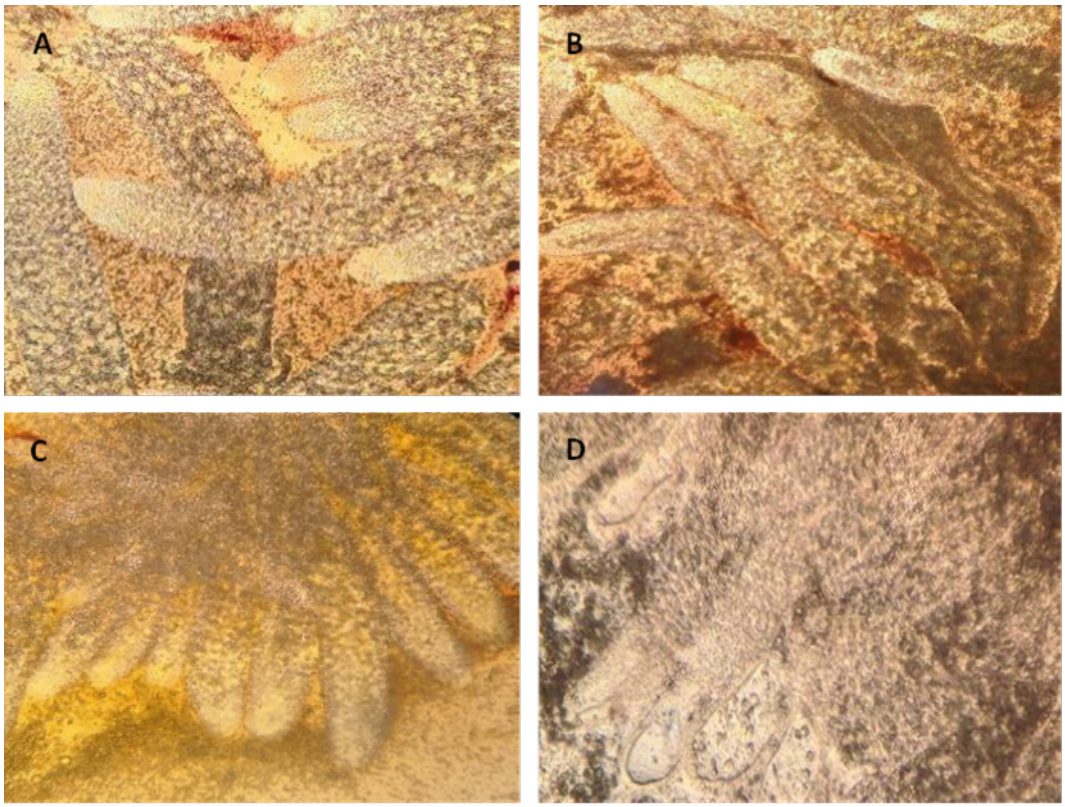

Figure 2. Wet Mount preparations, A) DOC 20; B) DOC 27; C) DOC 34; D) DOC 41

The organ damage observed in DOC 41 samples was specifically diagnosed as hepatopancreatic necrosis, characterized by changes in cellular morphology and structure. Atrophy of the hepatopancreatic tubules was also observed, accompanied by disorganization of the surrounding cellular structure.

DISCUSSION

Water quality measurements in the research ponds indicate that pH conditions tend to be suboptimal for sustainable whiteleg shrimp cultivation. The lowest pH value recorded in the morning reached 7.4, which is below the optimal minimum limit of 7.5 recommended in the Indonesian National Standard (SNI) for whiteleg shrimp production requirements in intensive-technology ponds (Standar Nasional Indonesia, 2006). This low pH value is causally related to biological processes occurring overnight, particularly the intensive respiration activity of all aquatic organisms. Respiration produces carbon dioxide (CO2), which, when dissolved in water, forms carbonic acid. This increased CO2 concentration leads to an increase in hydrogen ions (H+), ultimately causing a decrease in water pH in the morning (Mahmudi et al., 2020).

In addition to the suboptimal pH value, the research ponds also exhibited extreme daily pH fluctuations, reaching 0.7. This fluctuation far exceeds the ideal daily pH range of 0.2–0.3, indicating serious environmental instability. These large pH fluctuations trigger detrimental physiological consequences for shrimp. Specifically, extreme pH changes have the potential to disrupt the acid-base balance of the shrimp's body, forcing them to divert metabolic energy that should be used for growth to osmoregulation and homeostasis maintenance. Pusporini (2018) emphasized that significant pH fluctuations can cause cellular damage to vital organs such as the hepatopancreas, including pathological manifestations such as tissue necrosis.

The impact of extreme pH fluctuations extends to the shrimp's systemic functions. pH instability leads to decreased digestive enzyme activity, which impairs nutrient digestion and feed absorption efficiency. The combination of high temperature and low pH places a heavy metabolic burden on shrimp, ultimately triggering increased food intake and a higher osmorespiratory response. Increased energy consumption under stressful conditions does not result in improved or increased shrimp growth (Shirly-lim et al., 2024). Furtado et al. (2015) emphasized that shrimp exposed to pH stress exhibit an imbalance in antioxidant enzyme activity, forcing them to allocate more energy to reduce Reactive Oxygen Species (ROS) production. The consequence of this extra energy allocation is a decrease in growth rates in the culture system. Furthermore, large pH fluctuations and the ingress of pathogens into the body of whiteleg shrimp also result in decreased hemocyte activity (Kadek et al., 2018; Lu-qing et al., 2005), cells that play a crucial role in shrimp immune defense through mechanisms such as phagocytosis. This weakened immune response collectively increases the shrimp's susceptibility to disease transmission. The stressful environment caused by pH fluctuations is a significant triggering condition, increasing the risk of bacterial infections, such as vibriosis, in shrimp populations (Andayani et al., 2024; Pattano & Mittraparp-arthorn, 2025).

The hepatopancreas of DOC 20, 27, and 34 shrimp samples was normal, with no changes, and intact tubules with unaltered or blocked lumens. However, different results were found in DOC 41 shrimp samples, with tissue necrosis, tubule atrophy, and cellular structural disorganization in the hepatopancreas. DOC 41 shrimp samples were likely caused by opportunistic pathogen infection such as Vibrio spp. These bacteria are the main cause of pathological damage to the hepatopancreas and immunity (Asrido et al., 2024; Kumar et al., 2021). Tubular damage also directly disrupts nutrient absorption. Therefore, the observed necrosis indicates significant infectious pressure, which may have been exacerbated by unstable cultivation conditions, as seen in drastic pH fluctuations. The occurrence of necrosis and extreme vacuolation in the hepatopancreas reflects significant damage to the organ. This pathological condition, characterized by severe necrosis and vacuolation, can be a strong indicator of bacterial infection in shrimp (Mei et al., 2020).

Microscopic observations were made on the hepatopancreas. Its function as a detoxification organ makes it highly sensitive to physiological changes and environmental influences. According to Abbas et al. (2024), the hepatopancreas functions as the primary

digestive gland, responsible for producing enzymes for digesting food, as well as absorbing and storing nutrients. Furthermore, the crucial function of the hepatopancreas makes this organ a primary focus of histological analysis to understand the physiological adaptations of crustaceans to their environment (Vogt, 2019).

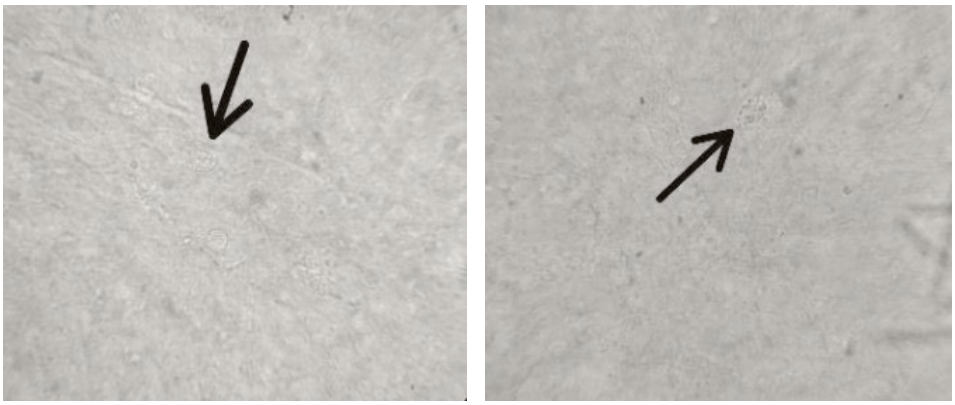

Figure 3. EHP Spores of DOC 41 Shrimp Sample

Histopathological observations of DOC 41 shrimp samples revealed the significant presence of Enterocytozoon hepatopenaei (EHP) spores. Morphologically, the identified EHP spores exhibited a characteristic oval shape and tended to cluster, with a dense, nucleus-like structure within them. The presence of these spores confirmed EHP microspridia infection in shrimp, a shrimp disease that poses a serious threat to the global aquaculture industry (Tang et al., 2016). EHP spores specifically demonstrated the ability to survive and replicate within the shrimp hepatopancreas. This organ serves as the central storage site for various vital nutrients in crustaceans. Therefore, it is also essential as the main center for regulating all metabolic processes in the body (Zhang et al., 2023).

Intensive nutrient absorption in the hepatopancreas provides an abundant energy source, supporting the life cycle and development of EHP. EHP infection occurs when spores successfully penetrate and infect the epithelial cells of the hepatopancreatic tubules. Infected cells become sites for parasite proliferation, causing abnormal enlargement of the cell nucleus and subsequent vacuolar degeneration of the cytoplasm. The increasing number of spores within the cells ultimately triggers massive epithelial cell lysis. This damage manifests as characteristic histopathological lesions, including tubular epithelial necrosis and tubular atrophy (Kumar et al., 2017). This severe tissue damage directly disrupts the vital functions of the hepatopancreas, namely nutrient absorption and storage, which are crucial for understanding the physiological adaptation and growth of infected crustaceans (Vogt, 2019).

The ability of EHP spores to remain active and multiply effectively in the hepatopancreas is facilitated by the organ's stable internal environment. This environment provides optimal physicochemical parameters (temperature, pH, and nutrient concentration). Studies have shown that environmental stress or poor water quality can accelerate the severity of EHP infection and its spread within ponds, making it a disease influenced by environmental management (Rajendran et al., 2016). This allows EHP to thrive, particularly in the tubules adjacent to the intestine. Therefore, the ideal internal conditions of the hepatopancreas for EHP combined with poor environmental management, will collectively exacerbate tissue damage and accelerate the clinical manifestations of the disease in culture.

CONCLUSION

The results of this study confirm a strong relationship between unstable water quality and pathological conditions in the hepatopancreas of whiteleg shrimp

(Litopenaeus vannamei). This environmental instability creates environmental stress that exacerbates the pathological conditions seen in DOC 41 shrimp, which include tissue necrosis, tubular atrophy, and cellular disorganization in the hepatopancreas. This damage is likely a result of EHP infection. Extreme pH fluctuations are a triggering factor that weakens the shrimp's immune system, allowing opportunistic pathogens such as EHP to thrive and cause hepatopancreatic damage, ultimately disrupting feed efficiency and threatening the sustainability of the culture.

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