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Histopathological Alterations of Hepatopancreas and Intestines in the Vaname Shrimp *(Litopenaeus vannamei)* Infected by White Feces Disease (WFD)

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ABSTRACT

The White Feces Disease (WFD) significantly impacts Vannamei shrimp (*Litopenaeus vannamei*), leading to substantial economic losses in aquaculture due to reduced growth rates and increased mortality. This study investigated the histopathological changes in the hepatopancreas and intestines of Vannamei shrimp affected by WFD. A total of 60 shrimp, averaging 10–15 grams, were acclimatized and divided into two groups: healthy and WFD-infected. The WFD infection was confirmed through clinical symptoms and histopathological examination. Histological analysis revealed severe damage in the hepatopancreas of infected shrimp, including tissue lysis, necrosis, and atrophy compared to the normal hepatopancreas structure. The study identified a high prevalence of damage in ponds adjacent to WFD-infected areas, suggesting possible contamination through shared water or equipment. This emphasizes the importance of stringent biosecurity measures to prevent disease spread. In the intestines, significant necrosis and vacuolation were observed, indicating extensive cellular death and tissue degradation. These changes compromise the shrimp's ability to digest and absorb nutrients, leading to deteriorating health and reduced survival rates. The findings highlight the need for effective management strategies, including early detection and improved biosecurity protocols, to mitigate the impact of WFD and to ensure sustainable shrimp aquaculture practices.

INTRODUCTION

White Feces Disease (WFD) has emerged as a critical health issue in shrimp aquaculture, particularly affecting the Pacific white shrimp, (*L. vannamei*), which is a dominant species in global shrimp production. The disease is characterized by the

presence of white fecal strings floating on the water surface, coupled with clinical signs such as anorexia, lethargy, and significant declines in shrimp health **(Sumini & Kusdarwati, 2020; Damora *et al.*, 2022)**. WFD outbreaks have been linked to substantial economic losses due to reduced growth rates, increased feed conversion ratios, and elevated mortality rates among affected shrimp populations **(Adam *et al.*, 2022; Rahardjo, 2023)**. The rapid spread of WFD, particularly in high-density farming conditions, necessitates immediate intervention strategies to mitigate its impact on shrimp health and aquaculture productivity **(Rahardjo, 2023)**.

The etiology of WFD remains incompletely understood; however, research indicates that multiple pathogens, including various *Vibrio* species, play a significant role in its manifestation **(Mastan & Begum, 2016; Sriurairatana *et al.*, 2014)**. Histopathological studies have revealed that the hepatopancreas and intestines of (*L. vannamei*) are particularly susceptible to infections, leading to tissue damage and functional impairments **(Sriurairatana *et al.*, 2014; Damora *et al.*, 2022)**. For instance, the sloughing of hepatopancreatic microvilli and the transformation of intestinal epithelial cells have been observed in WFD-affected shrimp, indicating severe histological alterations that compromise the shrimp's digestive and immune functions **(Sriurairatana *et al.*, 2014; Alfiansah *et al.*, 2020)**. Understanding these histopathological changes is crucial for developing effective management and treatment strategies for WFD, as they provide insights into the pathological processes that underlie the disease **(Damora *et al.*, 2022; Rahardjo, 2023)**.

Despite the significance of WFD in shrimp aquaculture, there is a notable lack of comprehensive studies focusing on the specific histopathological changes occurring in the hepatopancreas and intestines during WFD infection. This gap in knowledge highlights the need for systematic evaluations of these tissues in both diseased and healthy shrimp to elucidate the pathological mechanisms associated with WFD **(Damora *et al.*, 2022; Rahardjo, 2023)**. By comparing the histological features of infected and healthy shrimp, researchers can gain a deeper understanding of the disease's impact on shrimp health and identify potential biomarkers for early diagnosis and intervention **(Adam *et al.*, 2022; Damora *et al.*, 2022)**. Such insights are essential for improving diagnostic and therapeutic approaches, ultimately aiding in the sustainability of shrimp aquaculture practices. WFD poses a significant threat to the shrimp aquaculture industry, necessitating further research into its etiology and the histopathological changes it induces in critical organs such as the hepatopancreas and intestines. By addressing the current knowledge gaps, the aquaculture sector can develop more effective strategies for managing this disease and mitigating its economic impacts.

MATERIALS AND METHODS

Sample collection and maintenance

A total of 60 *Litopenaeus vannamei* shrimp were obtained from shrimp farmers in Bungatan District, Situbondo Regency, East Java. Post-larval stage shrimp were selected to facilitate the examination of the hepatopancreas and intestines. The sample size of 60 shrimp was chosen to ensure sufficient statistical power for detecting significant differences between the healthy and WFD-infected groups (Table 1). Based on previous studies, this number of samples was determined to provide an 80% statistical power with a confidence level of 95%, allowing for a reliable comparison of histopathological changes between groups. The shrimp samples were divided into two groups of 30 each: healthy shrimp and those exhibiting clinical signs of WFD. This allocation allows for robust statistical analysis while accounting for variability within each group.

Table 1. Criteria for selecting healthy and WFD-infected *Litopenaeus vannamei* shrimp based on physical and behavioral observations

Criteria	Healthy shrimp	WFD-infected shrimp
Intestinal Condition	Full and continuous intestine with normal digestive activity.	Empty or fragmented intestine.
Feeding Behavior	Strong feeding activity, rapid response to feed.	Reduced or absent feeding activity.
Carapace Condition	Hard, intact carapace with a consistent white or pinkish body color.	Softened carapace with dull brown or dark discoloration on the body.
Body and Gills Appearance	No visible signs of stress or discoloration on the gills or body.	Discolored body and gills, indicative of stress or advanced disease.
Other Symptoms	No signs of lethargy or abnormal behavior.	Lethargy and high mortality rates.
Presence of White Fecal Strings	Not observed.	White fecal strings floating on the water surface.

Healthy shrimp were characterized by a full intestine, good appetite, a hard carapace, and a white body color. In contrast, shrimp with WFD symptoms exhibited an empty or fragmented intestine, reduced or lost appetite, softening of the carapace, discoloration of the body and gills, and higher mortality rates. After collection, the shrimp were immediately preserved in cool boxes at 4°C to maintain the integrity of their organs. The hepatopancreas and intestines were dissected from the shrimp for further histopathological analysis.

Identification of White Feces Disease (WFD)

Shrimp exhibiting clinical signs of White Feces Disease (WFD), such as floating white fecal strings and reduced feeding activity, were selected for the study. Disease

confirmation was done through microscopic examination of fecal samples, which revealed the presence of abnormal bacterial populations or protozoans.

Histopathological sampling

Ten shrimp (five showing WFD symptoms and five healthy controls) were selected for histopathological analysis. Shrimp were dissected under sterile conditions, and samples of the hepatopancreas and intestines were immediately collected and fixed in 10% buffered formalin for 24–48 hours at room temperature.

Histological processing

Fixed tissue samples were dehydrated through a graded ethanol series (70, 80, 90, and 100%), cleared in xylene, and embedded in paraffin wax. Serial sections (4–5µm thickness) were cut using a rotary microtome and were mounted on glass slides. Sections were stained with hematoxylin and eosin (H&E) for general histological examination.

Microscopic examination

Stained tissue sections were examined under a light microscope at magnifications of 40x and 100x. Histopathological alterations in the hepatopancreas and intestines were assessed, focusing on key indicators such as:

- Hepatopancreas: Tubule necrosis, vacuolization, hemocyte infiltration.
- Intestines: Epithelial erosion, sloughing of intestinal cells, inflammation.

Scoring and statistical analysis

Histopathological changes were graded using a semi-quantitative scoring system, with scores assigned for the severity of lesions (mild, moderate, severe). Data were statistically analyzed using a t-test to compare the WFD-infected and control groups. A *P*-value of <0.05 was considered statistically significant. This methodology allows for the systematic evaluation of the histopathological effects of WFD on the hepatopancreas and intestinal tissues in *L. vannamei*.

RESULTS AND DISCUSSION

Morphological alterations in *Litopenaeus vannamei* infected with White Feces Disease (WFD)

Shrimp infected with White Feces Disease (WFD) exhibit distinct morphological changes, particularly in the hepatopancreas and intestines. The hepatopancreas of affected shrimp often appears shrunken and discolored, taking on a dull brown hue, which is indicative of significant pathological alterations **(Sriurairatana *et al.*, 2014; Damora *et al.*, 2022)**. In the intestines, a characteristic fragmented and white appearance can be observed, often resulting from the shrimp's decreased appetite and subsequent inability to properly digest food **(Adam *et al.*, 2022; Farastuti, 2023)**. One of the diagnostic hallmarks of WFD is the mass exfoliation of the epithelial cells from the hepatopancreatic

tubules, which has been linked to the transformation, shedding, and aggregation of hepatopancreatic microvilli into vermiform bodies resembling gregarine parasites **(Sriurairatana *et al.*, 2014)**.

The presence of floating white fecal strings on the surface of the water is another hallmark of WFD, serving as a visual cue for aquaculture practitioners to identify potential outbreaks **(Adam *et al.*, 2022; Damora *et al.*, 2022)**. Histopathological analyses of the gills, intestines, and hepatopancreas of WFD-infected shrimp reveal significant structural damage. The intestines, in particular, are often empty due to the shrimp's decreased appetite, which disrupts nutrient absorption and impairs growth **(Adam *et al.*, 2022; Farastuti, 2023)**. This condition is exacerbated by the presence of opportunistic pathogens, such as *Vibrio* species, which thrive in the compromised environment created by WFD **(Mastan & Begum, 2016)**.

In recent years, Enterocytozoon hepatopenaei (EHP), a microsporidian parasite, has been frequently associated with WFD outbreaks. EHP has been detected in both the hepatopancreas and feces of shrimp from WFD-affected ponds, indicating its role in the disease's pathology **(Desrina *et al.*, 2020)**. The emergence of WFD is often attributed to several factors, including suboptimal pond management, poor seed quality, and deteriorating water quality, which create favorable conditions for opportunistic pathogens like *Vibrio* spp. **(Mastan & Begum, 2016)**. The morphological differences observed in shrimp infected with WFD are exhibited in Table (2).

Table 2. The morphological differences observed in shrimp infected with WFD

ORGAN	HEALTHY SHRIMP	WFD-INFECTED SHRIMP
HEPATOPANCREAS	Normal size, smooth surface, white to pinkish color	Shrunken, dull brown, mass exfoliation of epithelial cells
INTESTINE	Full, uniform, and continuous	Fragmented, empty, and white-colored
FECES	Normal, brown to greenish	White, floating on water surface
APPETITE	Normal, strong feeding activity	Reduced or absent, resulting in empty intestines

Table (2) highlights the key morphological changes associated with WFD, aiding in the diagnostic process for detecting the disease in *L. vannamei* populations. The morphological changes observed in shrimp infected with WFD, particularly in the hepatopancreas and intestines, are critical for diagnosing and understanding the disease. The combination of histopathological findings, clinical signs, and the involvement of pathogens such as EHP underscores the complexity of WFD and the need for effective management strategies in shrimp aquaculture.

Histopathological analysis of hepatopancreas

Histopathological analysis is a key method used to diagnose diseases and to assess the health condition of shrimp by observing changes in organ structure. For shrimp

diagnosed with White Feces Disease (WFD), histopathological examination of the hepatopancreas shows significant alterations (Figs. 1, 2). The hepatopancreas tissue in WFD-infected shrimp exhibits irregular structures compared to healthy shrimp. These irregularities suggest tissue lysis and severe structural damage, including atrophy, which results from prolonged anorexia in the shrimp **(Anjaini *et al.*, 2018; Damora *et al.*, 2022)**.

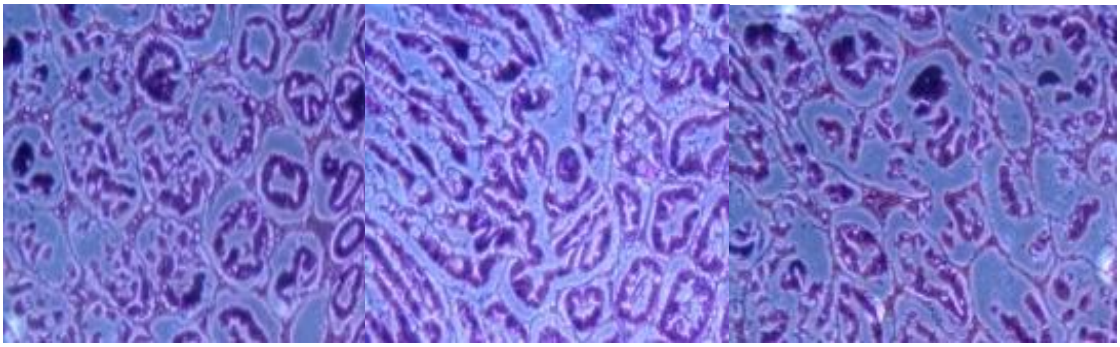

Fig. 1. Normal hepatopancreas histopathology

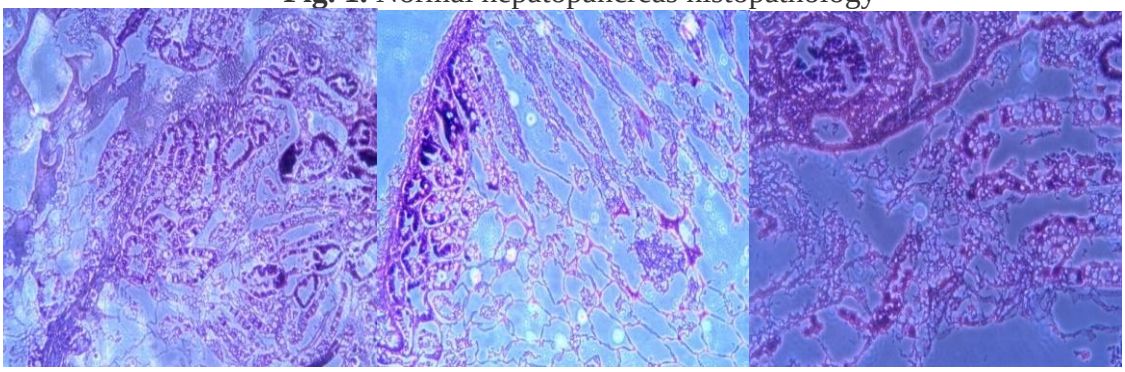

Fig. 2. Histopathology of hepatopancreas diagnosed with WFD

The histopathological examination of the hepatopancreas is indeed a crucial tool for understanding the impact of White Feces Disease (WFD) on *L. vannamei* shrimp. The hepatopancreas, which is responsible for vital functions such as digestion, nutrient absorption, and metabolism, becomes a primary target during WFD infection. Recent studies have highlighted the extensive damage caused by WFD in this organ, with significant observations of necrosis, tissue lysis, and atrophy. In healthy shrimp, the hepatopancreas displays a well-organized tissue structure, characterized by clearly defined tubular cells and no visible signs of degeneration. However, in WFD-infected shrimp, histological analyses have revealed significant structural abnormalities. Tissue lysis, a hallmark of WFD, is evident, indicating cellular disintegration and the breakdown of tissue integrity **(Damora *et al.*, 2022)**. This lysis disrupts nutrient absorption and digestive processes, severely compromising shrimp health **(Damora *et al.*, 2022)**.

Atrophy, or the shrinking of tissue size due to cell death, was prominently observed in the hepatopancreas of WFD-infected shrimp. This phenomenon is typically associated with prolonged anorexia in shrimp, as WFD leads to a drastic reduction in feeding

activity. Without proper nutrient intake, the hepatopancreas depletes its reserves, resulting in a reduction in tissue mass, which further weakens the shrimp's ability to combat the disease **(Damora *et al.*, 2022)**. The findings align with prior research that identified the hepatopancreas as one of the primary organs affected by WFD, emphasizing that damage not only compromises shrimp digestion but also increases susceptibility to secondary infections from bacteria and viruses **(Caro *et al.*, 2021)**. The comparison between normal and WFD-affected ponds underscores the contagious nature of WFD.

Shrimp in ponds adjacent to WFD-infected ones often show moderate organ damage, likely due to cross-contamination through shared water systems or equipment. This finding emphasizes the need for improved biosecurity measures to limit the spread of the disease **(Nguyen *et al.*, 2023)**. In summary, the histopathological examination of the hepatopancreas provides critical insights into the impact of WFD on shrimp health. The extensive damage observed, including necrosis, tissue lysis, and atrophy, highlights the need for effective management strategies to mitigate the effects of this disease in aquaculture settings.

The ANOVA analysis in this study confirmed that WFD significantly affects hepatopancreas structure, with F-values indicating a highly significant difference between normal and WFD-affected shrimp. The results suggest that interventions focused on early detection and the management of WFD can mitigate the impact on shrimp health by preserving the integrity of vital organs like the hepatopancreas. The histopathological analysis demonstrates the devastating effects of WFD on the hepatopancreas, highlighting the importance of managing water quality, monitoring shrimp health, and implementing biosecurity measures to prevent the spread of this debilitating disease.

The data were further analyzed using ANOVA, and the results indicated that there was no significant damage in normal shrimp (F-value < F-table). In contrast, the WFD-affected ponds showed significant organ damage (F-value > F-table). This suggests that WFD plays a major role in the structural deterioration of the hepatopancreas **(Alfiansah *et al.*, 2020)**.

Table 3. Scoring of hepatopancreas damage in normal and WFD-infected shrimp

TREATMENT	POND	DAMAGE LEVEL	DESCRIPTION
S1	Pond 2	Moderate	Neighboring WFD-affected pond, moderate damage likely due to cross-contamination.
S2	Pond 7	Mild	Light damage, not directly affected by WFD.
S3	Pond 8	Mild	Similar to S2, light structural damage.

The scoring in Table (3) indicates that ponds adjacent to WFD-infected areas experience higher levels of hepatopancreas damage, likely due to cross-contamination through shared water systems or equipment. Ponds in closer proximity to infected areas show increased tissue lysis, necrosis, and atrophy, which are key indicators of WFD.

These findings emphasize the need for strict biosecurity measures, such as the isolation of infected ponds, regular disinfection of equipment, and careful monitoring of water flow, to prevent the spread of the disease between ponds.

Implementing these preventive strategies could significantly reduce the transmission risk of WFD and limit the associated economic losses in shrimp farming operations. Additionally, early diagnosis through regular histopathological monitoring could help in timely intervention, reducing the severity of tissue damage and improving shrimp recovery rates.

Histopathological analysis of intestines

In addition to the hepatopancreas, the intestines of shrimp infected with WFD show severe damage, as evidenced by histopathological examinations. In healthy shrimp, the intestinal structure is intact, with a filled lumen, columnar epithelium, and organized muscle layers. In contrast, WFD-infected shrimp show significant tissue necrosis, vacuolation, and edema **(Kurniawinata *et al.*, 2022)**. The presence of necrotic tissues and structural irregularities is a direct consequence of parasitic infection by *Enterocytozoon hepatopenaei* (EHP) and opportunistic bacterial pathogens like *Vibrio* spp. The damage is exacerbated by the shrimp's reduced appetite, leading to white and fragmented intestines **(Farastuti *et al.*, 2023)**.

Table 4. Scoring of intestinal damage in WFD-infected shrimp

POND	DAMAGE (%)	DAMAGE LEVEL	DESCRIPTION
P1	51%	Severe	Necrosis, vacuolation, and edema present in multiple regions.
P2	56%	Severe	Extensive tissue damage, including severe edema and necrosis.
P3	45%	Moderate	Significant necrosis and vacuolation, but less extensive than P1 and P2.

The damage observed in the P1, P2, and P3 ponds (Table 4 & Fig. 3) highlights the severe impact of White Feces Disease (WFD) on the shrimp's intestines. Histopathological analysis revealed significant signs of necrosis and vacuolation, both of which are associated with cellular death and tissue degradation. Necrosis, characterized by abnormal cell death, compromises the integrity of intestinal tissues, while vacuolation, the formation of vacuoles or empty spaces within cells, further indicates a breakdown in cellular structure.

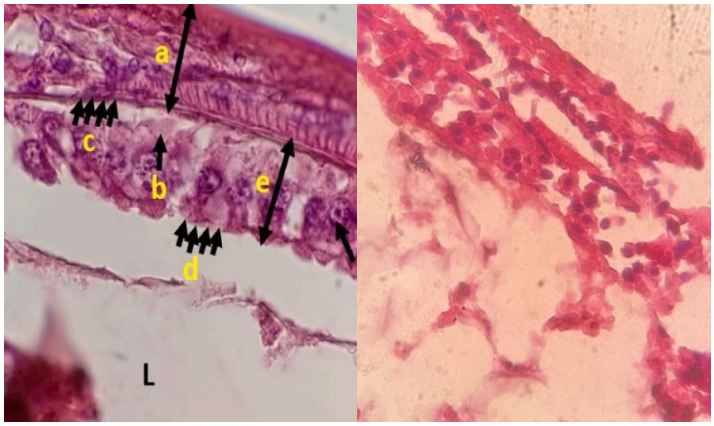

Fig. 3. Histopathology of vaname shrimp (left) **(Kurniawinata *et al.*, 2022)**, vaname shrimp infected with WFD (right)

These pathological changes severely impair the shrimp's ability to properly digest and absorb nutrients, which can lead to malnutrition, stunted growth, and reduced survival rates. The weakened intestinal structure caused by WFD not only disrupts normal physiological functions but also increases the susceptibility of shrimp to secondary infections. Consequently, the cumulative effect of these conditions may result in substantial losses in shrimp populations if the disease is not managed effectively.

Percentage of damage to the intestines of the whiteleg shrimp is depicted in plots P1, P2, and P3 (Table 3). Plot P1 has a percentage of intestinal damage of 51%, indicating that WFD causes damage with a score of 4. Plot P2 has a percentage of intestinal damage of 56%, indicating that WFD causes damage with a score of 3. Plot P3 has a percentage of intestinal damage of 45%, indicating that WFD causes damage with a score of 3.

Damage to the intestines of whiteleg shrimp infected with WFD usually occurs in the form of necrosis, vacuolation, and edema. It is caused by WFD disease agents such as vibrio bacteria. The genus *Vibrio* in shrimp pond waters is often used as a biomarker of disease in shrimp intestines, this is due to the large number of vibrio bacteria in the intestines of shrimp affected by the disease **(Reyes *et al.*, 2022)**. Vibrio bacteria in the shrimp intestines will attack the intestinal tissue, resulting in necrosis. The results of histopathological tests showing necrosis, vacuolation, and edema are shown in Fig. (4).

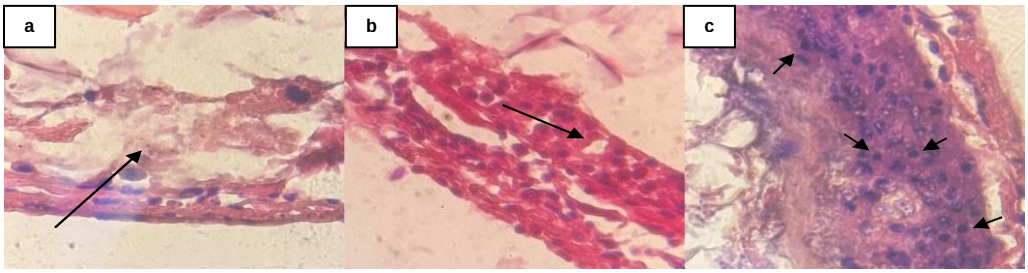

Fig. 4. Intestinal organ damage showing: **(a)** necrosis, **(b)** vacuolation, **(c)** edema as indicated by the arrow

As seen in Fig. 4 (a), necrosis occurred in the histopathology test of White Feces Disease (WFD)-infected vaname shrimp. Necrosis is a condition in which cells or tissues are damaged so that their condition is not normal. Shrimp infected with WFD usually experience necrosis caused by disease agents such as vibrio. *Vibrio* bacteria attack the shrimp's digestive organs, one of which is the intestine. Necrosis is the death of cells or organ tissue where the condition is abnormal and no longer intact. Necrosis can be caused by attacking bacteria, wounds on the body, stress, and also toxic substances in the water **(Piamsomboon *et al.*, 2017)**. Vacuolation is also seen in the histopathology test of White Feces Disease (WFD)-infected vaname shrimp in Fig. (4b). Vacuolation is the widening or narrowing of organ tissue caused by cell loss. The occurrence of vacuolation can be seen from the presence of empty holes in the organ tissue. Vacuolation is a condition of cell or tissue loss, resulting in narrowing and widening of the tissue that indicates changes in organ structure **(Ina-Salwany *et al.*, 2018)**.

Edema is also seen in the histopathology test of vaname shrimp infected with white feces disease (WFD) in Fig. (4c). Edema is cell swelling caused by inflammation due to bacterial attacks, cells experience swelling. In histology tests edema will look like swollen cells filled with fluid like blood. Edema causes erythrocytes to rupture easily until degeneration occurs. This condition can cause cell rupture until necrosis occurs. Edema can be seen in the color of cells that are darker than normal cells **(Salazar-Lugo *et al.*, 2011)**.

To mitigate the progression of WFD, shrimp farms must adopt early detection protocols and focus on maintaining optimal water quality and biosecurity measures. These actions, combined with close monitoring of intestinal health, can help reduce the incidence of WFD and improve overall shrimp farm sustainability. The molecular identification of this disease has been studied by **Kilawati *et al.* (2024)**. Future research could explore the potential of various natural substances for enhancing immunity and promoting wound healing using materials that have been previously investigated **(Islamy *et al.*, 2024a, b, c)**. The provision of natural feed for aquatic animals as an eco-friendly alternative has been suggested from a variety of natural plants that have been listed **(Islamy *et al.*, 2024)**, which should also consider several nutritional factors **(Serdiati *et al.*, 2024)**.

CONCLUSION

The histopathological analysis of Vannamei shrimp infected with White Feces Disease (WFD) reveals significant damage to both the hepatopancreas and intestines. Infected shrimp exhibit severe hepatopancreas damage characterized by tissue lysis, atrophy, and necrosis, which impairs their ability to digest and absorb nutrients. Adjacent ponds also show increased damage due to potential contamination, emphasizing the need for stringent biosecurity measures. In the intestines, necrosis and vacuolation indicate substantial cellular death and tissue degradation, leading to further health deterioration.

Effective management strategies, including early detection, water quality control, and biosecurity, are crucial to mitigating WFD's impact and improving shrimp health and farm productivity.

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