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Evaluation of Garlic Powder as a Control Agent of *Vibrio* sp. in the Digestive Tract of White-leg Shrimp

Afandi Saputra[1*], Suharyadi [1], Erni Marlina[1], Sinar Pagi Sektiana[1], Sri Budiani Samsuharapan[1], and Ade Maria Ulfa[2]

[1]*Department of Aquaculture, Jakarta Technical University of Fisheries, Ministry of Marine Affairs and Fisheries, Republic of Indonesia, Jl. Raya Pasar Minggu, Jati Padang, Jakarta-12520, Indonesia*
[2]*Pharmacy Study Program, Faculty of Health Sciences, Malahayati University, Jl. Pramuka No. 27, Bandar Lampung, Indonesia*
*Corresponding author's Email: afandi.saputra38@gmail.com

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ABSTRACT

Garlic (*Allium sativum*) is a natural plant recognized for its active compounds, such as allicin, which possess antibacterial properties that can combat pathogenic microorganisms. *Vibrio* sp. bacteria can be pathogenic to vannamei shrimp (*Litopenaeus vannamei*), causing mortality marked by necrosis and melanization in the hepatopancreas cells. The present study aimed to examine the chemical compositions of garlic powder and its potential to inhibit the growth of *Vibrio* sp. bacteria in vannamei shrimp. Vannamei shrimp, with a population of 400 shrimp/m^3, were orally administered garlic powder mixed into their feed. The experimental doses were 0.1 mg of garlic powder (Group A), 0.5 mg of garlic powder (Group B), and 1 mg of garlic powder in 1 g of shrimp feed (Group C), along with shrimp that did not receive garlic powder (K, control group). Vannamei shrimp at the post-larva stage were used with a density of 1000 shrimp in each tank. Phytochemical analysis of the garlic powder revealed the presence of compounds such as flavonoids, alkaloids, tannins, phenols, and saponins. Thin-layer chromatography using quercetin as the reference standard yielded a positive result, indicated by a retention factor of 0.66. Notably, there was a significant reduction in total bacterial counts of *Vibrio* sp. in 40 days of treatment. A garlic powder at a dose of 0.5 mg/g was particularly effective in inhibiting the growth of *Vibrio* sp. bacteria. The current results indicated that shrimp not given garlic powder (control group) exhibited significant atrophy and hepatopancreas infiltration.

Keywords: Garlic, Hepatopancreas, Vannamei Shrimp, *Vibrio* sp.

INTRODUCTION

Endemic diseases of vannamei shrimp (*Litopenaeus vannamei)* include white spot syndrome virus (Balakrishnan et al., 2011), taura syndrome virus (Flegel, 2012), early mortality syndrome, and acute hepatopancreatic necrosis disease (AHPND; Saputra et al., 2023a). Somboon et al. (2012) found that vannamei shrimp infected with white feces syndrome (WFS) contained *Vibrio* (*V.*) species of different types, including *V. parahaemolyticus*, *V. cholerae*, and *V. alginolyticus. Vibrio parahaemolyticus*, at a density of 10^4 CFU $ml^{-1,}$ can be pathogenic in vannamei shrimp (Saputra et al., 2023a). *Vibrio parahaemolyticus* exhibits pathogenic effects, including necrosis, hemocyte infiltration, and melanization, in shrimp hepatopancreas cells (Lai et al., 2015). Saputra et al. (2023a) found that a concentration of 10^6 CFU ml^{-1} resulted in 100% mortality in shrimp within 73 hours after challenge. Conversely, Ananda Raja et al. (2017) reported that the lethal dose (LD) of *V. parahaemolyticus* in vannamei shrimp was 2.6×10^4 CFU ml^{-1} under different conditions.

Recently, efforts to prevent shrimp diseases by using herbal ingredients such as *Piper crocatum*, *Citrus limon*, *Zingiber officinale*, and *Borassus flabellifer* as an alternative to antibiotics have been reported by Reverter et al. (2014), Palanikumar et al. (2020), and Saputra et al. (2023b). Antibiotics act selectively but can lead to the development of bacterial resistance (Haifa-Haryani et al., 2022). Antibiotics can harm fish and the environment; therefore, residue-free treatment should be used, incorporating environmentally friendly, natural herbal ingredients. Garlic (*Allium sativum*) is one of the candidates for natural antibacterial ingredients that function as a medicine, reported to have an inhibitory effect of 20 µL on *Erwinia carotovora* and *Xanthomonas campestris* bacteria (Chen et al., 2018; Chirawithayaboon et al., 2020). Chen et al. (2018) stated that allicin is the main component responsible for the onion aroma and is one of the active substances that can eliminate pathogenic bacteria or exhibit antibacterial properties. Vinyl-1,2-dithiacyclopent-3-en-1,2-dithiacyclohex-5-en- and 2-vinyl-4H-1,3-dithin in *Allium sativum* exhibit broad pharmacological effects with low levels of toxicity (Mikaili et al., 2013).

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The application of herbal treatments is most effective in preventing issues in shrimp farming (Saputra et al., 2023b). Monitoring the clinical condition of shrimp during maintenance is crucial for assessing their health before outbreaks of pathogenic diseases, such as AHPND, and potential mortality (Tran et al., 2013). Low cell necrosis levels in the hepatopancreas and intestines of vannamei shrimp are indicators of shrimp health (Li et al., 2018). The present study evaluated the chemical compounds of garlic powder and its ability to prevent the growth of *Vibrio* sp. bacteria in orally administered vannamei shrimp, focusing on measuring the total bacterial count, the level of *Vibrio* sp. in the hepatopancreas, and observing histopathological changes.

MATERIALS AND METHODS

Ethical approval

The present study was conducted in June and August 2020 at the Microbiology and Chemistry Laboratory of the Ahli Usaha Perikanan (AUP) Polytechnic, Serang, Banten, Indonesia. In the present study, the shrimp were raised according to the standard operating procedures for good management practices in vannamei shrimp aquaculture, as outlined by the Ministry of Marine Affairs and Fisheries of the Republic of Indonesia.

Study design

The study employed a completely randomized design with four treatment groups, each having three replications. The treatments involved adding garlic powder to commercial feed at different concentrations, including a control group with no garlic powder (Group K). The second group received 0.1 mg of garlic powder per 1 g of vannamei shrimp feed (Group A). The third group was given 0.5 mg of garlic powder per 1 g of feed (Group B), and the fourth group received 1 mg of garlic powder per 1 g of vannamei shrimp feed (Group C). Vannamei shrimp (*Litopenaeus vannamei*) were used at the post-larva stage with a density of 1000 shrimp in each tank. These shrimps were obtained from the Cultivation Laboratory at Politeknik AUP in Serang, Banten, Indonesia. During the maintenance period, vannamei shrimp were fed at a dose of 3% four times a day (Zainuddin et al., 2019). The commercial feed (Gold Coin Marine Shrimp Feed, Vietnam) has a particle size and nutritional composition that range from 0.5 to 2.2 mm, with crude protein at 36%, crude fat at 5%, crude fiber at 4%, ash at 15%, moisture at 12%, and a metabolic energy content of 3.200 kcal/kg. For the 40-day maintenance of vannamei shrimp, garlic powder was prepared at approximately 500 to 1000 grams using a kinetic method originating from farmers in Banten province, Indonesia (Pathak et al., 2022). Before the experiment, the shrimp were acclimated to a temperature of 28-30°C in a 2.5 m^3 round plastic tub for one month (Zhao et al., 2024).

Container preparation

Vannamei shrimp were housed in a circular container with a diameter of two meters and a height of one meter. A total of 12 circular tanks made of tarpaulin were sterilized using chlorine (Tjiwi-Kimia, Sidoarjo, East Java, Indonesia) at a dose of 0.3 mg/l. The sterilization process for equipment, including containers, aeration devices, and water, required a 24-hour sterilization period. Cleaning was performed with sterile water until the chlorine was gone. Aeration was provided at nine points per tank, each holding one cubic meter, to ensure proper oxygen diffusion during shrimp maintenance (Meidiana et al., 2023).

Garlic phytochemicals

Garlic extraction was carried out by dissolving 50 grams of garlic powder into 250 mL of ethanol 99.9%. The kinetic extraction process was performed for 24 hours with a magnetic stirrer on a hot plate (Sundang et al., 2012). A phytochemical analysis was performed on ethanol extracts of *Allium sativum*, including tests for flavonoids, alkaloids, tannins, terpenoids, phenols, and saponins, using the method described by Ahmad et al. (2015). For thin-layer chromatography (TLC; Sigma-Aldrich, St. Louis, Missouri, USA), the procedure described by Matysik et al. (2016) was employed. The garlic flavonoids were screened via TLC using ethanol as the solvent. The retardation factor (RF) value on silica gel (Sigma-Aldrich, St. Louis, Missouri, USA) helped to identify the quercetin component (Patel et al., 2017). The silica gel used for TLC had a density of approximately 0.5 to 0.6 g/mL, with the silica content reaching up to 99.9% silicon dioxide (Pathak et al., 2022). The ethanol extract of garlic was identified separately on the TLC plate and then developed in the solvent system. The lethal concentration 50 (LC_{50}) of garlic powder given to vannamei shrimp was 1 mg/g in shrimp feed (Syngai et al., 2016).

High-performance thin-layer chromatography analysis

High-performance thin-layer chromatography (HPTLC; CAMAG, Switzerland) analysis was performed using the method described by Patel et al. (2017). The standard solution was prepared by dissolving 2 mg each of quercetin and gallic acid in 10 mL of methanol 99.9%. For application on the TLC plate, 1-6 µL of this standard solution was used. For

the extraction and hydrolysis of the garlic sample, garlic (10 g) was subjected to ethanol extraction for two hours per cycle. The resulting extract was dried using an evaporator, and the residue was re-dissolved in 80 mL of methanol 70%. This solution was then hydrolyzed for two hours with 40 mL of 2 M ammonium chloride (NH_4Cl) and 40 mL of toluene. The hydrolyzed mixture was neutralized with NaOH 5% and refluxed for one hour. The mobile phase from this reaction was subsequently extracted twice with 30 mL of toluene, followed by four extractions with 50 mL of ethyl acetate. The combined ethyl acetate extracts were evaporated to dryness. The resulting residue (10 mg) was dissolved in 5 mL of methanol to create the test sample. A volume of 15 µL of this sample solution was spotted onto the TLC plate. The stages in thin-layer chromatography column (CO-TLC) were performed on silica gel (Sigma-Aldrich, St. Louis, Missouri, USA) using a solvent ratio of 5:4:4 (v/v/v). In the mobile phase, toluene, ethyl acetate, and formic acid solvents were dissolved in a ratio of 5:4:1 (v/v/v). Then, the TLC reading was scanned at a wavelength of 254 nm.

Gastrointestinal sampling and bacterial observation

The collection of hepatopancreatic organs was carried out exclusively from deceased shrimp. Bacteria collection was carried out by inoculating the hepatopancreas using the modified method of Supono et al. (2019). Samples of bacterial abundance and *Vibrio* sp. were collected on days 0, 20, and 40 during post-treatment garlic powder exposure maintenance. Samples of vannamei shrimp hepatopancreas and intestines, each weighing 1.0 g, were collected to test for total bacteria and total *Vibrio* sp. bacteria. At the same time, histology testing of hepatopancreas cells was performed on day 40 of rearing. The total plate count method was used to determine the bacterial count in the shrimp digestive tract (Ananda Raja et al., 2017). The digestive tract, including the hepatopancreas and intestines, was placed into sterile microtubes for weighing. Then, the sample was crushed using a sterile micro-pestle, and 1mL of sterile physiological solution (NaCl 0.9%) was added. The sample solution was then diluted stepwise with sterile physiological solution (Supono et al., 2019).

After incubation for 24 hours, the bacterial colonies were counted using a colony counting device, with the petri dish placed on top. The bacterial colonies were then counted using the following formula.

$$N = C \times \frac{1}{P} \times \frac{1}{V}$$

N means colony count (CFU mL^{-1}), C means the number of bacterial colonies on the petri dish (Colonies mL^{-1}), P means dilution factor, and V means sample volume (mL; Supono et al., 2019).

Histological analysis

The method of Ananda Raja et al. (2017) was used to perform histopathological examination of the hepatopancreas in vannamei shrimp infected with *Vibrio* sp. bacteria. Shrimp health was assessed by visually inspecting the color and structure of the hepatopancreas and intestines. After determining the shrimp's health condition, the hepatopancreas and intestines were collected and stored in Davidson's fixative (Sigma-Aldrich, St. Louis, Missouri, USA) for 24 hours (Ananda Raja et al., 2017). For observation and differentiation of hepatopancreas and intestinal cells, staining was performed using hematoxylin and eosin (H&E; Sigma-Aldrich, St. Louis, Missouri, USA).

Statistical analysis

A logarithmic transformation was used to process the data on bacterial abundance in the digestive tract. The SPSS program was employed to analyze the abundance of *Vibrio* sp. in each treatment. The transformed data were then analyzed using analysis of variance (ANOVA) with Minitab 26 for Windows at a confidence level of 95% ($p < 0.05$). After identifying a significant difference in the current results, the test was conducted using the least significant difference (LSD) method. The RF value on silica gel was used to identify the quercetin component. The data processing outcomes were then displayed in graphs to clearly illustrate the results of each treatment.

RESULTS AND DISCUSSION

Phytochemical screening

The *Allium sativum* phytochemical test results (Table 1) indicated that it contains flavonoids, saponins, tannins, phenols, terpenoids, and alkaloids, as reported by Dalhat et al. (2018). The phytochemical content in *Allium sativum* exhibited diverse secondary metabolite properties, making it a potential treatment for vannamei shrimp farming (Palanikumar et al., 2020). The phenolic compound (quercetin), reported by Rempe et al. (2017), functions as an antibacterial by disrupting the cell membrane (membrane rigidification), intercalating into DNA, inhibiting DNA gyrase, inactivating type III secretion, and inhibiting bacterial zinc (HPFABZ), dehydratase, hydroxyphenyl-phenylalanine ammonia-lyase, and protein kinase. In addition to phenol, other compounds with antibacterial properties were found in tannins. Tannin, a multidentate ligand, can bind to proteins primarily through hydrophobic interactions and hydrogen bonds, which can inhibit bacterial metabolism (Jöbstl et al., 2006). A relationship exists between the activity of tannic

acid against bacteria and its inhibition of receptor binding, which affects bacterial cell wall damage (Theisen et al., 2014). Pandey and Negi (2018) observed that at concentrations below the minimum inhibitory level, tannins possess antivirulence effects, alter the expression of virulence factors, and reduce bacterial pathogenicity, which prevents bacteria from attaching to cell receptors, thereby blocking their entry onto different surfaces.

Table 1. Phytochemical test results of *Allium sativum*

Compound identification	Color indicator parameters	Results
Flavonoids	Fading yellow color	Positive
Alkaloids	Orange deposition	Positive
Tannins	Blackish brown, blackish blue	Positive
Terpenoids	Orange, orange-brown	Positive
Phenol	Blackish brown, blackish blue	Positive
Saponins	Permanent foam	Positive

High-performance thin-layer chromatography analysis

The HPTLC chromatogram of the ethanol extract from *Allium sativum* was confirmed through chromatography and absorption spectrum analysis, with the reference standard scanned at a wavelength of 254 nm (Figure 1). The peak appeared at an Rf value of 0.66, with the peak area calculated as 0.039% (w/w). Patel et al. (2017) reported that the HPTLC screening of *Lagerstroemia indica* extract yielded an Rf value of 0.63 with a peak area of 0.041%. Pure compounds can be isolated when the ratio of solvents and eluents used has the correct polarity (Saputra et al., 2016). Pathak et al. (2022) employed HPTLC screening of quercetin and gallic acid standards using a toluene-ethyl acetate-formic acid (v/v/v) eluent mixture.

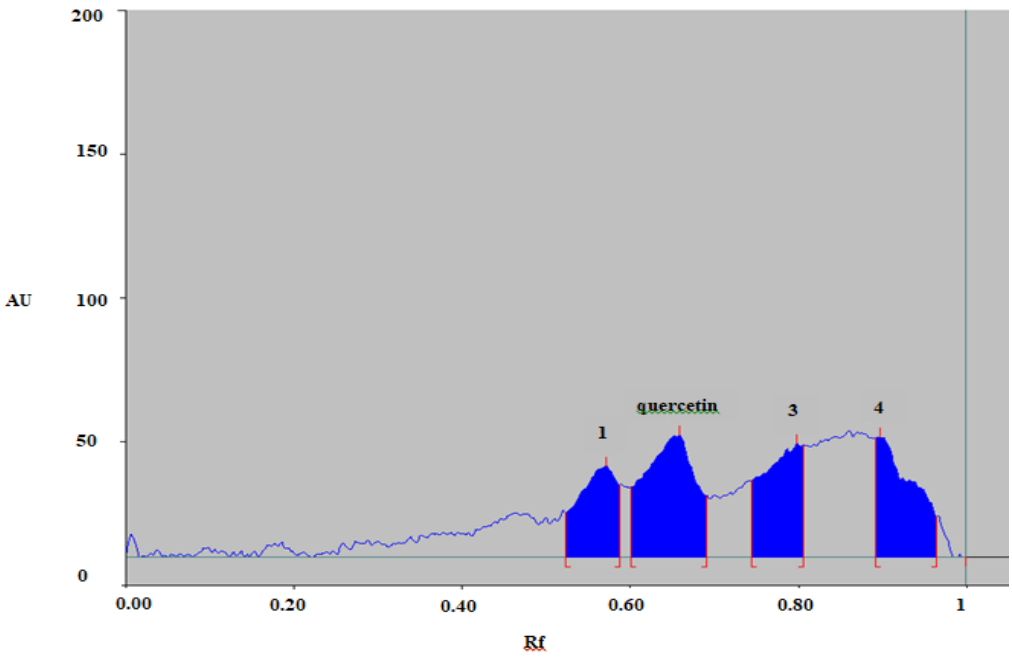

Figure 1. *Allium sativum* ethanol extract using quercetin standard, scanned at 254 nm. Rf: Retardation factor, AU: Absorbance units. The peak appeared at an Rf value of 0.66, and the AU was 55 for the *Allium sativum* ethanol extract.

Total bacterial abundance and total *Vibrio* species

The levels of bacteria and *Vibrio* sp. in the digestive tract of vannamei shrimp fed with *Allium sativum* powder, as well as in the control group, indicated an increasing pattern ($p \leq 0.05$) throughout the shrimps' rearing period. This present study demonstrated a correlation between the administration of *Allium sativum* powder and the proliferation of bacteria, including *Vibrio* sp., indicating that *Allium sativum* powder can inhibit the growth of *Vibrio* sp. bacteria for up to 40 days of culture (DOC). The results of the data analysis indicated that the total bacterial count after applying *Allium sativum* powder in DOC 40 significantly decreased ($p \leq 0.05$) in both the treatment and control groups. The total bacteria in DOC 40 in the digestive tract of vannamei shrimp in the control group had a value of 8.5×10^6 CFU g^{-1}, and in the treatment groups were 2.4×10^6 CFU g^{-1} (Group A), 3.4×10^6 CFU g^{-1} (Group B), and 2.1×10^6 CFU g^{-1} (Group C).

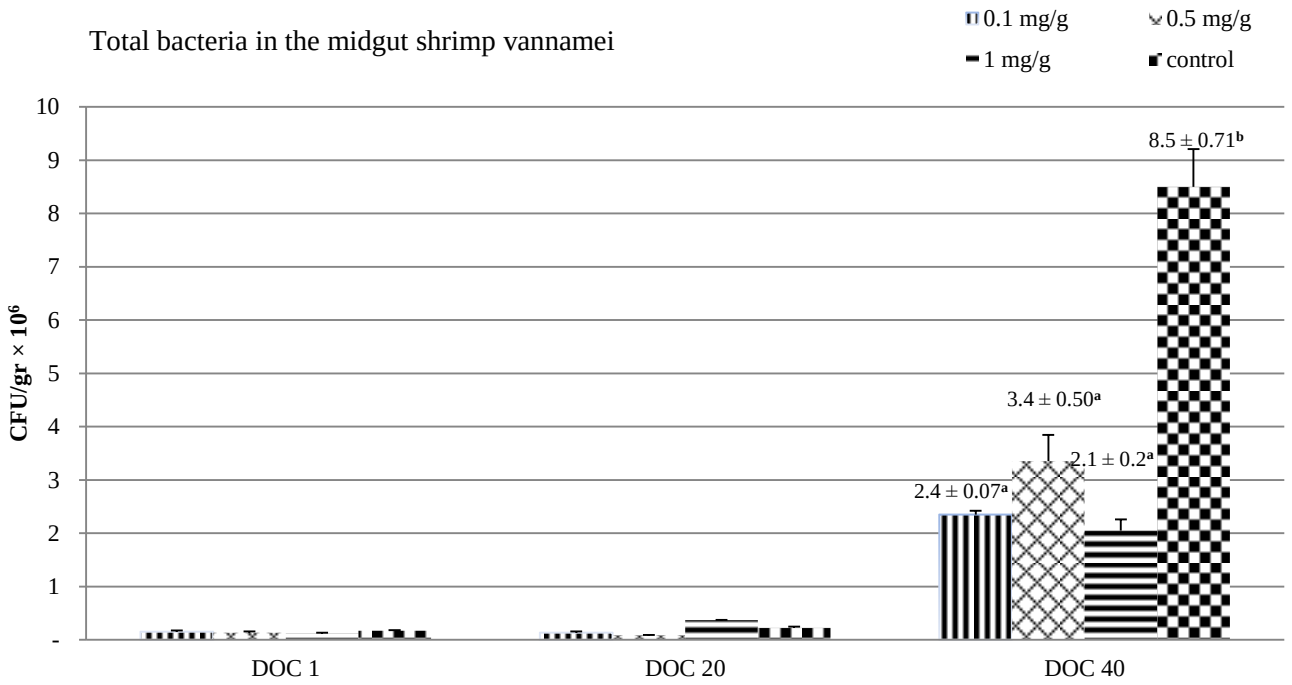

Figure 2. Bacterial abundance in the digestive tract of vannamei shrimp fed with *Allium sativum* powder for 40 days of culture. DOC: Day of culture. [a,b] The difference in letters indicates a significant difference ($p < 0.05$).

The total bacterial population in DOC 40 in the shrimp intestine in the control group, a value of 8.5×10^6 CFU g^{-1} ($p \leq 0.05$), was similar to the total number of bacteria in shrimp digestion found in nature (5×10^5 CFU g^{-1}) as reported by Buntin et al. (2008). The current results indicated that all doses of *Allium sativum* powder had the same effect in suppressing the growth of *Vibrio* sp. bacteria. Administering *Allium sativum* powder has been demonstrated to be effective in maintaining the total bacterial population during the digestive process of vannamei shrimp, with bacterial counts ranging from $2.1 \pm 0.2 \times 10^6$ to $3.4 \pm 0.5 \times 10^6$ CFU g^{-1}, showing statistically significant differences ($p \leq 0.05$). Therefore, this dosage can be considered an acceptable and effective level of application. Kongnum and Hongpattarakere (2012) reported a decrease (5.0 ± 0.14 CFU g^{-1} to 3.34 ± 0.21 CFU g^{-1}; $p \leq 0.05$) in the total bacterial count in vannamei shrimp after giving *Lactobacillus plantarum* during the 6-week treatment. In DOC 40, the bacterial abundance percentages between Gram-negative and Gram-positive bacteria indicated that the control group had a ratio of 49.4% to 50.6%. In comparison to the present study, Group A had a ratio of 12.5% to 87.5%, Group B displayed a ratio of 8.82% to 91.18%, and Group C had a ratio of 14.3% to 85.7% ($p \leq 0.05$). Figure 2 illustrates that administering *Allium sativum* powder to all treatment groups effectively suppressed total bacterial growth in vannamei shrimp maintenance compared to the control group. *Allium sativum* has been reported to function as a secondary metabolite, such as an antibacterial agent, in preventing WFS (Palanikumar et al., 2020) and also acts as an immunostimulant (Breyer et al., 2015).

The current results indicated that the abundance of *Vibrio* sp. after applying *Allium sativum* powder in DOC 40 was significantly different ($p \leq 0.05$) among treatments compared to the control group. Based on Figure 3, the total amount of *Vibrio* sp. in DOC 40 within the digestive tract of vannamei shrimp in the control group reached the highest level at 4.2×10^6 CFU g^{-1} ($p \leq 0.05$). Meanwhile, the treatment groups receiving garlic powder had an abundance of *Vibrio* sp. of 0.290×10^6 CFU g^{-1} (Group A), 0.275×10^6 CFU g^{-1} (Group B), and 0.280×10^6 CFU g^{-1} (Group C).

As shown in Figure 3, the *Vibrio* sp. abundance graph at DOC 40 demonstrated that the control group exhibited a natural increase in *Vibrio* sp. levels within the hepatopancreas of vannamei shrimp. Several factors contributed to these conditions, including the high content of organic matter in the vannamei shrimp farming environment. In the maintenance of vannamei shrimp, the duration, the size of the shrimp, and the condition of the aquaculture environment are increasingly critical, particularly the organic matter content (Ariadi et al., 2019). Organic matter plays a significant role in increasing the abundance of Gram-negative bacteria such as *Vibrio* sp. Sun et al. (2016) stated that synergistic conditions with organic sediments in vannamei shrimp culture indicated that organic sediments could significantly influence the abundance of shrimp gut bacteria. Supono et al. (2019) reported that in the vannamei shrimp farming environment, the abundance of *Vibrio* sp. ranged from 1.3 to 2.2×10^5 CFU $ml^{-1,}$ including *V. parahaemolyticus* and *V. alginolyticus*. Saputra et al. (2023a) added that *V. parahaemolyticus* found in the vannamei shrimp farming environment infected with AHPND in Serang District, Banten Province, Indonesia, had a pathogenic abundance value of 1×10^4 CFU

ml^{-1}. Similar findings were reported by Ananda Raja et al. (2017), indicating that the LD value of *V. parahaemolyticus* infection in vannamei shrimp is 2.6×10^4 CFU ml^{-1}. The total abundance of *Vibrio*, including specific *Vibrio* species, exhibited different pathogenicity. In the case of *V. parahaemolyticus* and *V. harveyi*, as reported by Chen et al. (2017), these species produce toxins that can affect the shrimp's immune system, leading to hepatopancreas damage, mortality, and luminescent vibriosis (Subagiyo et al., 2020).

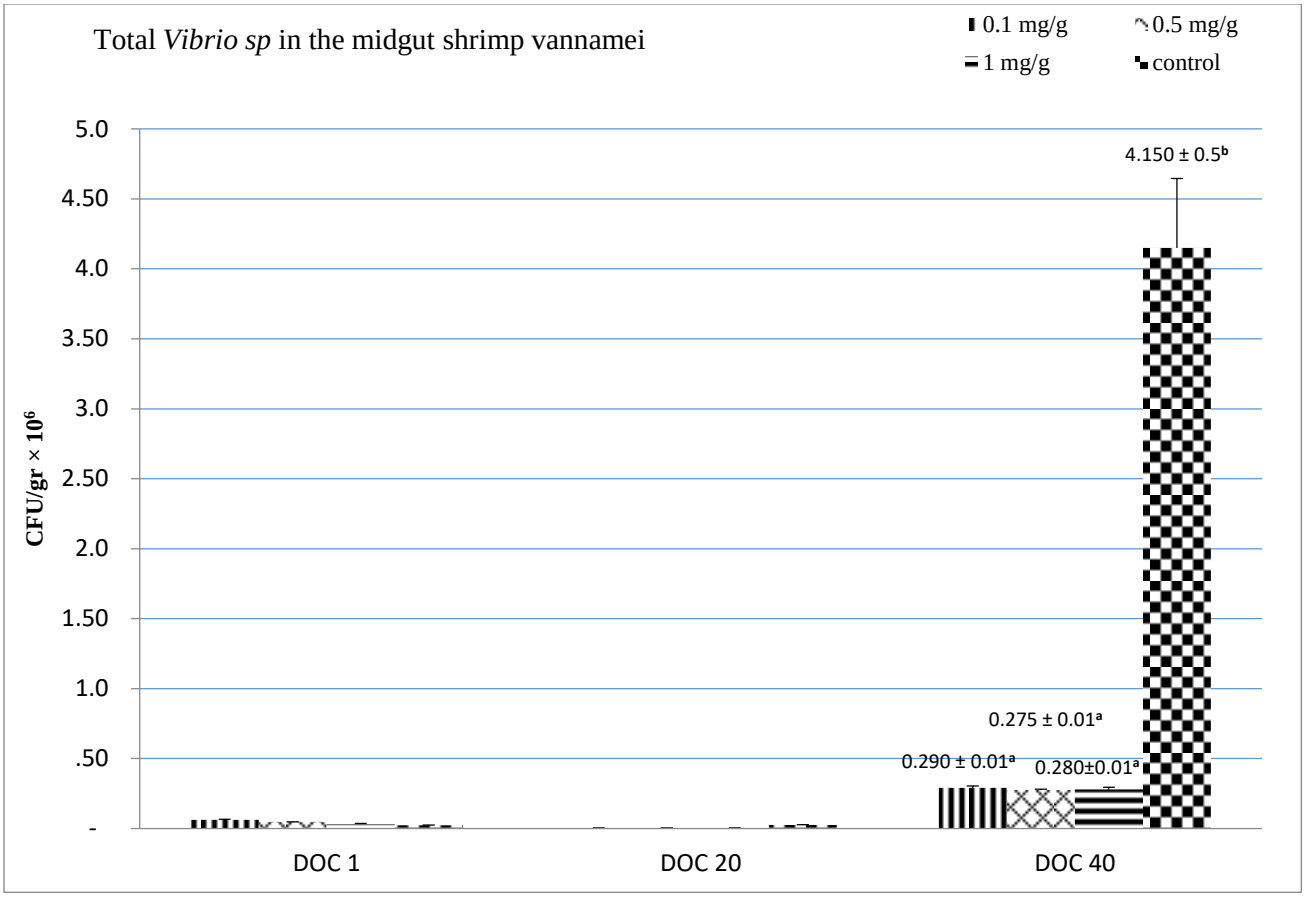

Figure 3. The abundance of *Vibrio* sp. in the digestive tract of vannamei shrimp fed with *Allium sativum* powder for 40 days of culture. DOC: Day of culture. [a,b] The difference in letters indicates a significant difference ($p < 0.05$).

The total abundance of *Vibrio* indicated a high potential for infection caused by *V. parahaemolyticus* (Saputra et al., 2023a). Previous studies have shown that the abundance of *Vibrio* in shrimp farming can affect shrimp health, where high numbers of *Vibrio* colonies can contribute to increased incidence of diseases such as AHPND in shrimp (Gustilatov et al., 2024). In a study conducted by Ananda Raja et al. (2017), it was found that *V. parahaemolyticus* was one of the most common pathogenic species among isolates from shrimp ponds, suggesting that the high abundance of these colonies may increase the risk of disease exposure in the farming environment (Ananda Raja et al., 2017). Amoah et al. (2021) observed that dietary supplementation with black garlic positively affected the immune response and health of *L. vannamei*. The current results exhibited an increase in beneficial bacteria and a decrease in *Vibrio* in the intestines of treated shrimp (groups A, B, and C), supporting the idea that garlic can help maintain the balance of the shrimp gut microbiota, which is essential for overall health. Adding *Allium sativum* powder to shrimp feed helped to keep *Vibrio* bacteria lower in groups A (0.290×10^6 CFU g^{-1}), B (0.275×10^6 CFU g^{-1}), and C (0.280×10^6 CFU g^{-1}) compared to the control group (4.150×10^6 CFU g^{-1}). *Allium sativum*, widely recognized for its antimicrobial properties, has the potential to positively modulate the intestinal microbiota of shrimp, thereby enhancing health and resistance to infections (Chen et al., 2018). Several studies have indicated that dietary garlic can significantly reduce pathogenic bacterial populations while supporting beneficial microbial communities, which are essential in regulating *Vibrio* growth (Amoah et al., 2021). Specifically, a study by Maneesin et al. (2014) reported that shrimp treated with garlic oil exhibited a decrease in total viable count, reinforcing the evidence that garlic is effective in suppressing the growth of harmful microorganisms, including *Vibrio* spp.

Histopathology of hepatopancreas

Histopathological examination of the hepatopancreas in vannamei shrimp treated with garlic powder, compared to the control group, provided insights into how *Vibrio* sp. infection affects the health of the hepatopancreatic tissue. The

hepatopancreas of vannamei shrimp indicated a level of damage that did not differ in groups A, B, and C compared to the control group ($p \leq 0.05$; Table 2). Based on general histology, vannamei shrimp with the control treatment on DOC 40 demonstrated a high abundance of *Vibrio* sp. ($4.2 \pm 0.5 \times 10^6$ CFU g^{-1}) compared to groups A, B, and C ($p \leq 0.05$). The histopathology findings in the control group revealed hemocyte infiltration in the tubular space and atrophy of the tubular epithelium in the hepatopancreas tissue. Figures 4a and 4b depict the hepatopancreas with hemocyte infiltration on DOC 40, while Figures 4c and 4d show atrophy of the tubule epithelium in the control group on the same day.

Table 2. Hepatopancreas damage level of vannamei shrimp by *Vibrio* pathogenicity

Treatments	Average level of damage	Damage (%)	Damage rates
control	1.98 ± 0.15	25.87	Medium
0.1 mg/g	0.29 ± 0.11	5.13	Light
0.5 mg/g	0.18 ± 0.09	3.44	Light
1 mg/g	0.20 ± 0.16	4.17	Light

Notes: Classification of hepatopancreas tissue damage levels; Normal (Grade 0): At this level, the structure of the hepatopancreas tubules appears intact with tightly arranged epithelial cells without any signs of degeneration or necrosis. Light (Grade 1): Mild vacuolization is seen in the epithelial cells, as well as slight changes in the structure of the tubules that are still functioning. Medium (Grade 2): Vacuolization is seen moderately to severely, with epithelial cells beginning to atrophy. Severe (Grade 3): At this level, extensive cell necrosis and total disintegration of the hepatopancreas tubules are evident. Very Severe (Grade 4): Complete damage to the hepatopancreas tissue with an unrecognizable organ structure. Source: Aguilar-Rendón et al. (2020); Saputra et al. (2023a).

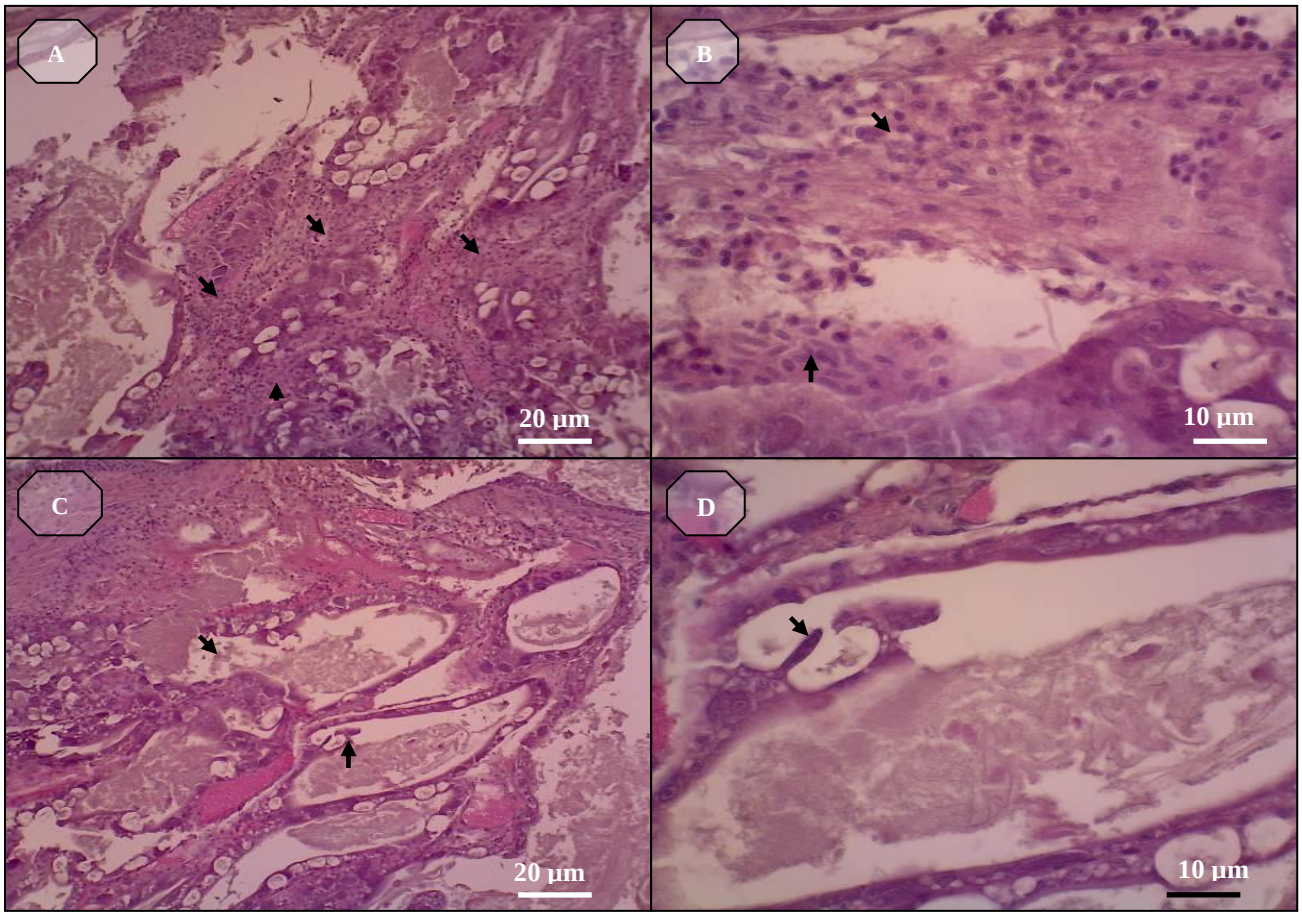

Figure 4. Hepatopancreas of *Litopanaeus vannamei* in the control treatment after 40 days of culture. **A** and **B:** Hemocyte infiltration in the inter-tubular space (arrow), **C** and **D**: Tubule atrophy (arrows). H&E staining, 10 and 20 µm.

Histopathological examination of the shrimp hepatopancreas was performed after 40 days of maintenance. Observations focused on the effects of *Allium sativum* powder administration and the control group. Acute disease occurred in the control group after DOC 40, characterized by hemocyte infiltration between tubules and atrophy. Some peeling cells were observed in the tubular lumen, and hemocytes caused infiltration around the tubules, likely due to the bacterial toxin *Vibrio* sp., as reported by Han et al (2017). *Vibrio* sp. damaged the hepatopancreas by affecting the tubules and causing hemocyte infiltration. Saputra et al. (2023a) noted that pathogens, including *V. parahaemolyticus*, can severely harm the hepatopancreas tissue in vannamei shrimp, leading to the appearance of inflamed tubules, atrophy, and peeling of tubule cells. The current results indicated that as the maintenance time of vannamei shrimp increased,

there was a corresponding rise in the total abundance of *Vibrio* sp. bacteria, both in all treatment groups and the control group. A high abundance of *Vibrio* affected the health of vannamei shrimp. Soto-Rodriguez et al. (2015) and Saputra et al. (2023a) reported a significant correlation between maintenance time and the *Vibrio* sp. virulence.

CONCLUSION

Adding *Allium sativum* powder to vannamei shrimp feed can inhibit the growth of *Vibrio* sp. bacteria at an effective dose of 0.5 mg g^{-1}, with a corresponding abundance of *Vibrio* sp. at $0.275 \pm 0.01 \times 10^6$ CFU g^{-1}. Further studies should focus on optimizing the application of garlic flour for large-scale use in shrimp ponds by farmers.

DECLARATIONS

Availability of data and materials

All data generated during the study are relevant and included in this published article.

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Authors' contributions

Afandi Saputra and Suharyadi conducted the research, administration, conceptualization, and writing. Erni Marlina has done the methodology. Sinar Pagi Sektiana, Sri Budiani Samsuharapan, and Ade Maria Ulfa wrote the draft and prepared the article. All authors checked and approved the last edition of the manuscript for processing and publication in this journal.

Competing interests

All authors affirmed that they have no competing interests.

Ethical considerations

The authors declared that the manuscript is original, has not been published elsewhere, and confirmed the latest version before publication.

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