DOI: http://dx.doi.org/10.15578/iaj.19.2.2024.157-165 *Indonesian Aquaculture Journal, 19 (2), 2024, 157-165*

Available online at: http://ejournal-balitbang.kkp.go.id/index.php/iaj

CONTROL OF VIBRIOSIS BY USING TURMERIC-KALMEGH EXTRACT WITH DIFFERENT PERIOD TREATMENTS IN WHITELEG SHRIMP IN THE FLOATING NET-CAGES

Dinamella Wahjuningrum[*)#], Irzal Effendi[*)], Shavika Miranti[**)], Khoirul Umam[*)], Nida Ulzanah[*)], and Taufiq Abdullah[***)]

[*)] Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor

Kampus IPB Darmaga, Jl. Agatis, Babakan, Kec. Dramaga, Kabupaten Bogor, Jawa Barat 16128, Indonesia

[**)] Department of Aquaculture, Faculty of Marine Science and Fisheries, Raja Ali Haji Maritime University, Tanjungpinang

Jl. Politeknik Senggarang, Tanjung Pinang, Kepulauan Riau 29111, Indonesia

[***)] Department of Aquaculture, Faculty of Marine and Fisheries Technology, State University of Gorontalo, Gorontalo

Dulalowo Tim., Kec. Kota Tengah, Kota Gorontalo, Gorontalo 96138, Indonesia

(Received: March 15, 2024; Final revision: October 22, 2024; Accepted: October 22, 2024)

ABSTRACT

Whiteleg shrimp Penaeus vannamei cultured in marine floating net-cage systems are highly susceptible to stress and diseases caused by Vibrio harveyi. This study aimed to evaluate the effect of turmeric Curcuma longa and kalmegh Andrographis paniculata on the growth performance and immune system of whiteleg shrimp against V. harveyi infection. The study utilized a completely randomized design with treatments including control (C), one week (1W), two weeks (2W), and four weeks (4W) of supplementation. The challenge test consisted of C+, C-, 1W, 2W, and 4W treatments. Parameters observed included growth performance, immune response, and resistance to V. harveyi. Results showed that the combination of turmeric and kalmegh significantly improved final biomass, weight gain, specific growth rate, and feed conversion ratio compared to the control. Survival rates after challenge tests revealed the highest survival in the C- group (93.33 ± 5.77%), followed by treatment 4W (86.67 ± 11.55%), 2W (76.67 ± 20.82%), 1W (46.67 ± 35.12%), and the lowest in C+ (33.33 ± 26.46%) with significant differences ($P < 0.05$) across treatments. Immune responses, including total hemocytes, phagocytosis activity, phenoloxidase activity, and respiratory burst, were also significantly improved in the treatment groups compared to the positive control. In conclusion, the combination of turmeric and kalmegh (2:1 ratio, 6 mL/kg of diet) significantly enhances both growth and immune responses of whiteleg shrimp, offering a potential alternative to antibiotics for controlling vibriosis in shrimp aquaculture.

KEYWORDS: *Andrographis paniculata*; *Curcuma longa*; *Penaeus vannamei*; *Vibrio harveyi*

INTRODUCTION

P. vannamei, also known as white leg shrimp, is a highly valued species in global aquaculture, accounting for 51.7% of global aquaculture production in 2020 (FAO, 2022). Advancements in white leg culture in marine floating net cages have, reduced production costs and improved shrimp quality by eliminating the need for aeration (Zarain-Herzberg *et al.,* 2010; Maicá *et al.,* 2014). However, challenges such as elevated LDL and glucose levels, decreased total hemocyte count and respiratory burst, reduced HDL, indicate stress in shrimp (Effendi, 2016). These conditions may weaken the immune system, increasing susceptibility to diseases (Chen & He, 2019; Kathyayani *et al.,* 2019).

Disease is a major threat to shrimp farming, causing significant economic losses. Between 2009 and 2018, the Asian shrimp industry incurred annual losses of up to US$4 billion due to disease outbreaks (Shinn *et al.,* 2018). Vibriosis, caused by *Vibrio* spp. including *V. harveyi*, is a prominent disease in shrimp farming, resulting in mortality rates of up to 100% in infected shrimp (Manefield *et al.,* 2000).

Current vibriosis control through antibiotics negatively affect aquaculture by promoting antibiotic-resistant bacteria and causing residue accumulation in

[#] Correspondence: Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University, Bogor
E-mail: dinamella@yahoo.com

aquaculture products, potentially endangering human health and aquatic environments (Cabello, 2006; Kathleen *et al.,* 2016). Therefore, natural, safe, and effective alternatives are needed, one of which is the use of medicinal plants, which have been proven to control disease in aquaculture practices (Van Hai, 2015).

Medicinal plants, such as turmeric (*C. longa*) and kalmegh (*A. paniculata*) have emerged as promising solutions, possessing antibacterial properties that can effectively combat pathogens like *V. harveyi* while mitigating the adverse impacts associated with antibiotic use, such as environmental pollution and antibiotic resistance (Van Hai, 2015; Loo *et al.,* 2020). Research has indicated that incorporating turmeric and kalmegh into the diet can enhance shrimp growth and immunity (Moghadam *et al.,* 2021; Yin *et al.,* 2023).

Because the effectiveness of immunostimulants like turmeric and kalmegh may diminish over time, requiring periodic administration and potentially increasing production costs, this study aims to determine the optimal administration period for a turmeric and kalmegh combination to enhance the growth and immune performance of white leg shrimp, with a focus on controlling *V. harveyi* infection in a marine floating net-cage system.

MATERIALS AND METHODS

Study Area

This study consisted of two stages: rearing in floating net cages and challenge tests in the laboratory. The rearing stage was conducted at the Sea Farming Center, Center for Coastal and Marine Resources Studies, Institute for Research and Community Development, IPB University (5^0 43' 12.49" S and 106^0 35' 32.72" E). The challenge tests, on the other hand, were conducted at the Aquatic Organism Health Laboratory, Department of Aquaculture, Faculty of Fisheries and Marine Science, IPB University (6^0 33' 31.36" S and 106^0 43' 21.84" E).

Container Preparation

The rearing stage utilized floating net-cages made of high-density polyethylene (HDPE). Each cage, measuring 1 × 1 × 2.5 m, was constructed with 1 mm mesh nets and weighted with 2 kg at each corner. A total of 12 floating net-cages were used, consisting of 3 for the control treatment and 9 for the feed treatment. The challenge test utilized glass tube containers with a diameter of 14 cm and a height of 30 cm, equipped with aeration. A total of 15 glass tube containers were used, including 3 for the positive control treatment, 3 for the negative control treatment, and 9 for the feed treatment. The water quality for the rearing and challenge tests had a dissolved oxygen concentration of 7.4-7.9 mg L^{-1}, salinity of 29-34 mg L^{-1}, temperature of 29.0-31.3!, pH of 7.25-7.75, and total ammonia nitrogen (TAN) of 0.007-0.017 mg L^{-1}. These water quality conditions comply with SNI 7311:2009.

Whiteleg shrimp Preparation

The whiteleg shrimp used were PL-10 fry with an average length of 0.5 ± 0.00 cm and weight of 0.12 ± 0.03 g, sourced from the hatchery of PT. Suri Tani Pemuka, Karang Suraga, Serang, Banten (60 09' 28.02" S and 1050 51' 14.06" E). The whiteleg shrimp on the hatchery of PT Suri Tani Pemuka have been described as Specific Pathogen Free (SPF) by Kemal *et al*. (2021).

Turmeric *C. longa* and Kalmegh *A. paniculata* Preparation

Turmeric (*C. longa*) and kalmegh (*A. paniculata*) extracts were obtained at the Research Center for Spice and Medicinal Plants (BALITTRO), Bogor, West Java. The part of turmeric used for the study was the rhizome, while the kalmegh part consisted of a mixture of leaves and stems. The extracts obtained were then incorporated into the treatment diet.

Treatment Diets Preparation

Treatment diets used a commercial diet (40% protein) prepared by the re-pelleting method following Munaeni *et al.* (2020). The treatments consisted of a control diet (CD) and a diet containing turmeric *C. longa* and kalmegh *A. paniculata* (CAD). The CD diet in this study received additional vitamin C at a concentration of 1 g kg^{-1} and carboxymethyl cellulose (CMC) at a concentration of 30 g kg^{-1} diet as a binder. The CAD diet was supplemented with a turmeric and kalmegh extract in a 2:1 ratio, based on the preliminary trial results of Miranti (2016). The turmeric and kalmegh extract (2:1) was added to the feed at a dose of 6 mL kg-1 feed, with vitamin C at a concentration of 1 g kg-1, and CMC at a concentration of 30 g kg-1 feed. The dosage of turmeric and kalmegh extract in the CAD diet followed the method used by Miranti (2016).

V. harveyi Preparation

The *V. harveyi* bacteria used is green colonies and fluorescent in a dark room in thiosulfate citrate bile salt sucrose (TCBS) agar media. The *V. harveyi* bacteria used were collected from the BPKIL Serang Laboratory, Umbul Tanjung, Serang, Banten (6^0 14' 23.56" S and 105^0 49' 35.70" E). *V. harveyi* was made rifampi-

cin-resistant at a concentration of 50 μg mL^{-1} as a marker (referred to as *V. harveyi*^{Rf}). The virulence of *V. harveyi*^{Rf} was then increased using Koch's Postulate method following Ilmiah *et al.* (2012). *V. harveyi*^{Rf} bacteria were cultured on TCBS agar media for 18 hours at 28°C, then re-cultured in 25 mL seawater complete (SWC) broth (1 g yeast extract, 3 mL glycerol, 5 g bactopeptone, 250 mL distilled water, and 750 mL seawater) in a 150 rpm water bath shaker for 18 hours at 28°C. The resulting SWC broth culture was then centrifuged at 6000 rpm for 5 min. The separated supernatant was discarded, and the bacterial pellet was resuspended in phosphate-buffered saline solution (PBS: 8 g NaCl, 1.5 g Na_2HPO_4, 0.2 g KCl, 0.2 g KH_2PO_4, 1000 mL distilled water) and homogenized with a vortex (this process was repeated twice). Next, 0.1 ml of the suspension was injected through the ventral sinus of the cephalothorax. Whiteleg shrimp showing clinical symptoms were then isolated and identified. Isolates showing positive *V. harveyi*^{Rf} were cultured as stock in SWC for further testing.

Experimental Design

The experiment followed a completely randomized design. The maintenance treatments included C (control), one week (1W), two weeks (2W), and four weeks (4W). The challenge test treatments comprised C+ (positive control), C- (negative control), 1W, 2W, and 4W. Each treatment in the maintenance and challenge test was conducted in triplicates. Detailed treatment is presented in Table 1.

Table 1. Experimental design and treatment details

Treatment	Description
C+	CD-feeding for four weeks and challenge test
C-	CD feeding for four weeks without challenge test
1W	CAD feeding for one week (first week) and challenged test
2W	CAD feeding for two weeks (first and third week) and challenged test
4W	CAD feeding for four weeks (every other day) and challenged test

The rearing in floating net-cages with a stocking density of 1000 shrimps per bag was maintained for 4 weeks. Treatment diets were given five times a day (06:00, 10:00, 14:00, 18:00, and 22:00) in a restricted manner with a feeding rate of 35 - 50%. Treatment C was given a DC diet for four weeks, treatment 1W was given a CAD diet for one week (first week), treatment 2W was given a CAD diet for two weeks (first and third week), while treatment 4W was given a CAD diet for four weeks (during rearing).

After four weeks of rearing in floating net-cages, observations continued at the challenge test stage. Stocking density was 15 fish per tube and observed for 10 days. The *V. harveyi*^{Rf} challenge test was conducted on the first day at a dose of 10^7 CFU mL^{-1} (LD_{50} dose) using the immersion method. During the challenge test, the diet was given 3 times a day (07:00, 12:00, and 17:00) in a restricted manner. The C+, C-, and 1W treatments were given the CAD diet for 10 days of rearing, the 2W treatment was given the CAD diet for 7 days of rearing, while the 4W treatment was given the CAD diet for 10 days of rearing.

Observation parameters

The parameters observed included growth performance parameters such as biomass growth, specific growth rate, survival, and feed conversion ratio (Tam *et al.,* 2021); immune response parameters such as total hemocytes (Sang *et al.,* 2009), phagocytosis activity (Anderson & Siwicki, 1993), phenoloxidase activity (Hsieh *et al.,* 2008), and respiratory burst (Cheng *et al.,* 2004); and *V. harveyi*^{Rf} resistance. Growth performance parameters were observed from day 0 to day 30. Immune parameters were observed on days 0, 2, and 10 post-challenge. *V. harveyi*^{Rf} resistance was observed after the challenge test. Specifically, phenoloxidase activity was measured using a microplate reader at a wavelength of 490 nm (Hsieh *et al*., 2008), while respiratory burst was measured at a wavelength of 630 nm (Cheng *et al*., 2004).

Data Analysis

The study results were processed and analyzed using Microsoft Excel 2016 and SPSS 20. Before performing ANOVA, assumptions were tested to ensure the data met the necessary requirements, including checks for normality and homogeneity of variances. If the ANOVA results were significant ($P<0.05$), further testing was conducted using the Duncan test at a 95% confidence interval.

RESULTS AND DISCUSSION

The application of medicinal plant ingredients in shrimp farming activities has been shown to significantly improve growth performance (Aftab-Uddin *et al.,* 2017). The growth performance of white leg shrimp during the four weeks of rearing is presented in Table 2. The data indicated that CAD feeding had a

significantly different effect ($P<0.05$) on final biomass, weight gain, specific growth rate, and feed conversion ratio. In general, the values of final biomass, weight gain, specific growth rate, and feed conversion ratio were highest in the 2W and 4W treatments compared to other treatments. The survival rate was lower in the control treatment but did not differ significantly ($P>0.05$) from the other treatments.

This study revealed that the combination of turmeric *C. longa* and kalmegh *A. paniculata* promotes the growth of white leg shrimp (Table 2). Turmeric and kalmegh are known to be effective diet additives due to their content of components such as carotenoids and polyphenols. These components can stimulate enzymes involved in metabolic processes, particularly digestion and nutrient absorption, which may enhance growth (Yusuf *et al.,* 2014; Bohn *et al.,* 2015). Therefore, the combination of turmeric and kalmegh effectively increases the growth of white leg shrimp. This finding aligns with previous research showing that turmeric and kalmegh can promote the growth of white leg shrimp in floating net-cages (Miranti, 2016).

The use of turmeric and kalmegh in aquaculture activities has the potential to control vibriosis disease attacks due to their antibacterial characteristics. These substances can inhibit vibriosis-causing pathogens, including *V. harveyi, V. cholera, V. parahaemolyticus, V. alginolyticus, V. vulnificus,* and *V. fluvialis* (Lawhavinit *et al.,* 2011; Mary & Banu, 2017). Vibriosis, caused by the species in the *Vibrio* genus group, such as *V. harveyi*, is a significant concern in shrimp farming (Liu *et al.,* 2021).

V. harveyi bacteria can produce several toxins and virulence factors that cause host death (Zhang *et al.,* 2020). Infection with this bacterium can lead to up to 100% mortality in shrimp (Manefield *et al.,* 2000).

Table 2. Initial biomass (W0), final biomass (Wt), weight gain (ΔW), specific growth rate (SGR), survival rate (SR), and feed conversion ratio (FCR) of whiteleg shrimp during four weeks of rearing in floating net-cages

Parameter	Treatment			
	K	1W	2W	4W
W0 (g)	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}	0.05 ± 0.00^{a}
Wt (g)	2.60 ± 0.11^{a}	3.42 ± 0.13^{b}	3.93 ± 0.11^{c}	4.12 ± 0.30^{c}
ΔW (g)	2.10 ± 0.11^{a}	2.92 ± 0.13^{b}	3.43 ± 0.11^{c}	3.62 ± 0.30^{c}
SGR (%)	13.47 ± 0.31^{a}	16.08 ± 0.12^{b}	17.2 ± 0.24^{c}	17.45 ± 0.37^{c}
SR (%)	36.87 ± 12.22^{a}	45.77 ± 4.05^{a}	50.00 ± 12.90^{a}	50.10 ± 10.08^{a}
FCR	0.03 ± 0.01^{a}	0.07 ± 0.00^{a}	0.13 ± 0.01^{b}	0.47 ± 0.04^{c}

Data (mean ± SD) in the same row followed by different letters indicate significant differences at the 5% test level (Duncan test), control treatment (C), CAD feeding treatment for one week (1W), CAD feeding treatment for two weeks (2W), and CAD feeding treatment for four weeks (4W).

The intervention of *V. harveyi* also affects the immune system in shrimp (Ayiku *et al.,* 2020). Parameters such as total hemocytes, phagocytic activity, phenoloxidase activity, and respiratory burst increase during *V. harveyi* infection (Rudi *et al.,* 2019). In this study, the total hemocytes, phagocytic activity, phenoloxidase activity, and respiratory burst after the challenge test showed a significant increase compared to the negative control treatment.

The immune response parameters consisting of total hemocytes, phagocytosis activity, phenoloxidase, and respiratory burst of whiteleg shrimp are presented in Figure 1. Total hemocytes before the challenge test in treatments C- and C+ with a value of $6.45 \pm 0.14 \times 10^{6}$ cells mL^{-1} were significantly different ($P < 0.05$) with treatments 1W and 2W showing the results of values of $11.34 \pm 0.23 \times 10^{6}$ cells mL^{-1} and $11.53 \pm 0.11 \times 10^{6}$ cells mL^{-1}, respectively. The highest value of TH before the challenge test was found in the 4W treatment ($21.35 \pm 0.09 \times 10^{6}$ cells mL^{-1}) whose value was significantly different ($P < 0.05$) with all treatments (C+, C-, 1W, 2W). Observations were continued on the 2nd day after the challenge test. Each treatment showed significantly different results ($P<0.05$) with the value of C+ ($10.06 \pm 0.08 \times 10^{6}$ cells mL^{-1}), C- ($10.74 \pm 0.10 \times 10^{6}$ cells mL^{-1}), 1W ($14.51 \pm 0.16 \times 10^{6}$ cells mL^{-1}), 2W ($16.55 \pm 0.13 \times 10^{6}$ cells mL^{-1}), and the highest value was in the 4W treatment ($27.68 \pm 0.33 \times 10^{6}$ cells mL^{-1}). The last observation of total hemocytes was done on the 10th day, with the highest value ($P<0.05$) found in treatment 4W ($14.63 \pm 0.20 \times 106$ cells mL^{-1}), followed by treatment 1W ($12.51 \pm 0.17 \times 10^{6}$ cells mL^{-1}), 2W treatment ($12.5 \pm 0.17 \times 10^{6}$ cells mL^{-1}), C- ($10.15 \pm 0.37 \times 10^{6}$ cells mL^{-1}), and the lowest value of each treatment ($P<0.05$) was C+ ($7.13 \pm 0.2 \times 10^{6}$ cells mL^{-1}).

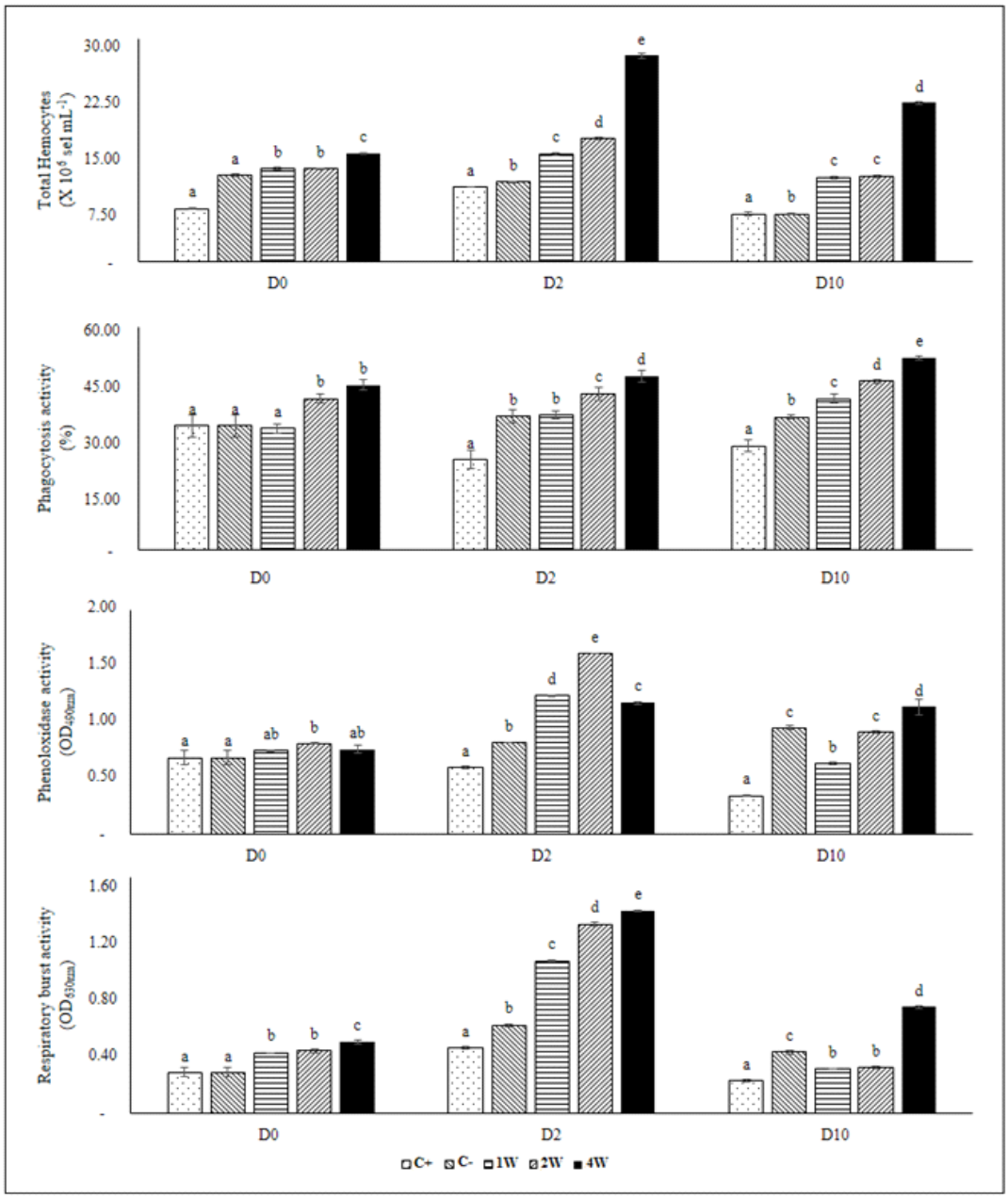

Figure 1. Total hemocytes, phagocytosis activity, phenoloxidase activity, and respiratory burst of whiteleg shrimp during observation of *V. harveyi* Rf challenge test. Data (mean ± SD) with different letters indicate significant differences at the 5% test level (Duncan test). Positive control (C+), negative control (C-), CAD feeding treatment for one week (1W), CAD feeding treatment for two weeks (2W), and CAD feeding treatment for four weeks (4W).

Phagocytosis activity before the challenge test in the C+, C-, and 1W treatments showed results that were not significantly different ($P>0.05$) with values of (33.33 ± 3.06%), (33.33 ± 3.06%), and (32.66 ± 1.15%), respectively. However, the three values were significantly different ($P<0.05$) with the highest value in the 4W treatment (44.33 ± 1.53%) and followed by ($P>0.05$) in the 2W treatment (40.66 ± 1.15%). Phagocytosis activity was also observed on the 2nd day after the challenge test which showed that the highest value ($P<0.05$) was found in the 4W treatment (46.66 ± 1.53%), followed by the 2W treatment (42 ± 1.73%), then the 1W and C- treatments with ($P>0.05$) values of 36.33 ± 1.15% and 36 ± 1.73% respectively, and the lowest value was found in the C+ treatment (24.33 ± 2.52%). Phagocytosis activity observation was also carried out on day 10 with the value of each treatment significantly different ($P<0.05$). The highest value was found in the 4W treatment (51.66 ± 0.58%), followed by the 2W treatment (45.33 ± 0.58%), then the 1W treatment (40.66 ± 1.15%), then the C- treatment (35.66 ± 0.58%) and the lowest value was found in C+ (28 ± 1.73%).

Phenoloxidase activity before the challenge test showed significantly different values ($P<0.05$) highest in the 2W treatment (0.8 ± 0.00 OD_{490} nm), followed by the 4W treatment (0.75 ± 0.4 OD_{490} nm), then 1W (0.74 ± 0.00 OD490 nm), and C- and C+ (0.68 ± 0.07 OD_{490} nm). The phenoloxidase activity value was also observed on day 1 after the highest challenge test ($P<0.05$) was found in the 2W treatment (1.62 ± 0.00 OD_{490} nm), followed by the 1W treatment (1.23 ± 0.01 OD.490 nm), then the 4W treatment (1.17 ± 0.01 OD_{490} nm), the C- treatment (0.82 ± 0.00 OD_{490} nm) and the lowest value ($P<0.05$) was found in the C+ treatment (0.59 ± 0.01 OD_{490} nm). The last observation conducted on the 10th day showed that the highest value ($P<0.05$) was found in the 4W treatment (1.13 ± 0.07 OD_{490} nm), followed by the C- treatment (0.94 ± 0.02 OD_{490} nm), then the 2W treatment (0.91 ± 0.01 OD490 nm), the 1W treatment (0.63 ± 0.01 OD_{490} nm) and the lowest value was found in the C+ treatment (0.34 ± 0.01 OD_{490} nm).

Respiratory burst activity before the challenge test with the highest value ($P < 0.05$) was found in treatment 4W (0.48 ± 0.01 OD630 nm), followed by treatment 2W (0.42 ± 0.07 OD630 nm) and 1W (0.4 ± 0.02 OD630 nm), and the lowest value ($P < 0.05$) was found in treatment C + and C- which amounted to (0.27 ± 0.02 OD630 nm). The highest respiratory burst value on day 2 after the challenge test ($P<0.05$) was found in 4W (1.37 ± 0.004 OD630 nm), then treatment 2W (0.28 ± 0.11 OD630nm), followed by treatment 1W (1.03 ± 0.008 OD630 nm), then treatment C- (0.59 ± 0.01 OD630 nm), and the lowest value in treatment C+ (0.44 ± 0.01 OD630nm). Observation of respiratory burst on day 10 showed the highest value ($P < 0.05$) was found in the 4W treatment (0.71 ± 0.006 OD630 nm), followed by treatment C- (0.41 ± 0.007 OD630 nm), then treatment 2W and 1W with values of 0.3 ± 0.008 and 0.3 ± 0.003 OD630 nm, respectively, while the lowest value ($P < 0.05$) was found in treatment C+ (0.22 ± 0.006 OD630 nm).

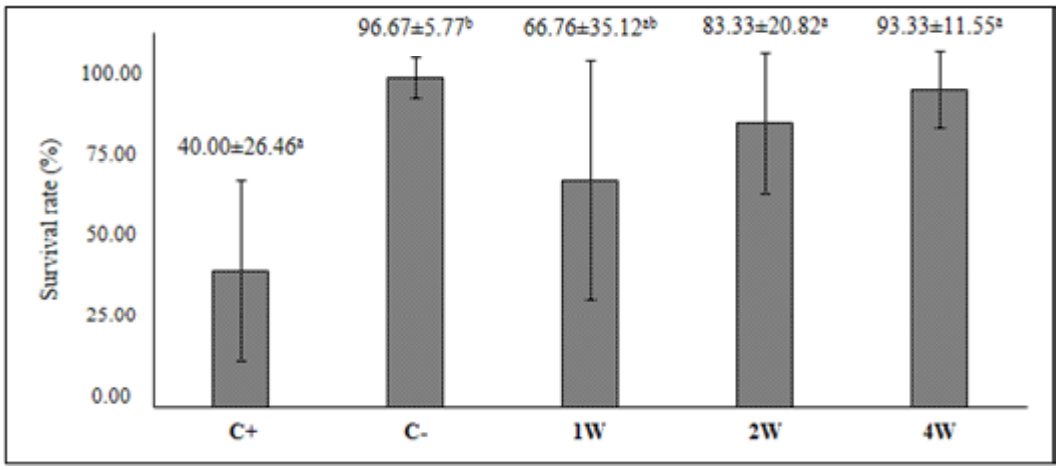

Figure 2. Survival of whiteleg shrimp after *V. harveyi* Rf challenge test. Data (mean ± SD) with different letters indicate significant differences at the 5% test level (Duncan test). Positive control (C+), negative control (C-), CAD feeding treatment for one week (1W), CAD feeding treatment for two weeks (2W), and CAD feeding treatment for four weeks (4W).

Crustaceans possess an innate immune system comprising cellular and humoral defenses. Shrimp hemocytes consist of three cell types based on the presence of cytoplasmic granules: hyaline, semi-granular, and granular cells (Kulkarni *et al.,* 2021). Hemocyte cells play a role in eliminating foreign particles that invade the shrimp body through mechanisms such as phagocytosis, phenoloxidase, and respiratory burst activities (Liu *et al.,* 2020). The observed increase in total hemocytes, phagocytic activity, phenoloxidase activity, and respiratory burst following the test in this study indicates a defensive response to *V. harveyi* infection. These findings align with those reported by Gustilatov *et al.* (2022) in white leg shrimp infected with the pathogen *V. parahaemolyticus*.

The immune response data demonstrated the administration of turmeric and kalmegh controls *V. harveyi* infection. The carotenoids and polyphenols of turmeric and kalmegh play a crucial role in infection control by stimulating the immune system, enhancing phagocytosis, lymphocyte proliferation, as well as increasing T cell interleukin production and B cell antibody production (Tan *et al.,* 2020; Behl *et al.,* 2021). The utilization of carotenoids in the diet has been shown to significantly enhance the immunity of tilapia (*Oreochromis niloticus*) (Hassaan *et al.,* 2021). Similarly, incorporating polyphenols into diets has been reported to improve the immune response of Asian seabass *Lates calcarifer* (Ahmadi *et al.,* 2022).

Although data limitations prevented observation of immune parameters at the gene expression level, supplementations of turmeric and kalmegh signifi-

cantly enhanced shrimp immune responses. Notable improvements were observed in total hemocytes, phagocytic activity, phenoloxidase activity, and respiratory burst, which collectively contributed to increased resistance against vibriosis. Consistent with these findings, the post-challenge survival results (Figure 2) revealed that the highest survival rate occurred in the C- group (93.33 ± 5.77%), followed by 4W treatment (86.67 ± 11.55%), the 2W treatment (76.67 ± 20.82%), the 1W treatment (46.67 ± 35.12%), with the lowest survival rate observed in the C+ group (33.33 ± 26.46%). Significant differences ($P < 0.05$) were identified among treatments.

Therefore, the turmeric and kalmegh treatments demonstrated higher survival rates compared to the positive control during the *V. harveyi* challenge test (Figure 2). This finding indicates that turmeric and kalmegh effectively protect white leg shrimp from vibriosis attacks, aligning with Miranti (2016), who reported that their combination can control *V. harveyi* infection in white leg shrimp.

CONCLUSION

The combination of turmeric (*C. longa*) and kalmegh (*A. paniculata*) at a 2:1 ratio, administered at a dose of 6 ml/kg of diet for either 2 weeks (feeding during the first and third weeks) or 4 weeks (feeding for four weeks), significantly improves the growth performance and immune response of white leg shrimp against *V. harveyi* infection. This treatment results in higher survival rates compared to the positive control, suggesting it could serve as a sustainable alternative to antibiotics for controlling vibriosis in white leg shrimp. Future studies should explore different treatment doses, combinations with other herbs, and alternative delivery methods, such as immersion, to expand the potential applications of this approach. These efforts could help optimize its therapeutic benefits and enhance the use of turmeric and kalmegh in shrimp aquaculture as a sustainable, antibiotic-free solution.

ACKNOWLEDGMENTS

The authors would like to express their sincere gratitude to Adna Sumadikarta, and Yanuar Raharja for their great technical help in this study.

REFERENCES

Aftab-Uddin, S., Siddique, M. A. M., Romkey, S. S., & Shelton, W. L. (2017). Antibacterial function of herbal extracts on growth, survival and immunoprotection in the black tiger shrimp *Penaeus monodon*. *Fish & shellfish immunology*, 65, 52-58. https://doi.org/10.1016/j.fsi.2017.03.050

Ahmadi, A., Bagheri, D., Hoseinifar, S. H., Morshedi, V., & Paolucci, M. (2022). Beneficial role of polyphenols as feed additives on growth performances, immune response and antioxidant status of *Lates Calcarifer* (Bloch, 1790) juveniles. *Aquaculture*, 552, 737955. https://doi.org/10.1016/j.aquaculture.2022.737955

Anderson, D. P., & Siwicki, A. (1995). Basic hematolog and serology for fish health. Manila: Fish Health Section, *Asian Fisheries Society*. https://pubs.er.usgs.gov/publication/95372

Andriani, Y., & Pratama, R. I. (2023). A Review on Herb Utilization in Vannamei Shrimp Cultivation. *Asian Journal of Research in Zoology*, 6(4), 10-17. https://doi.org/10.9734/ajriz/2023/v6i4117

Ayiku, S., Shen, J. F., Tan, B. P., Dong, X. H., & Liu, H. Y. (2020). Effects of dietary yeast culture on shrimp growth, immune response, intestinal health and disease resistance against *Vibrio harveyi*. *Fish & Shellfish Immunology*, 102, 286-295. https://doi.org/10.1016/j.fsi.2020.04.036

Behl, T., Kumar, K., Brisc, C., Rus, M., Nistor-Cseppento, D. C., Bustea, C., Aron, R. A. C,, ... Bungau, S. (2021). Exploring the multifocal role of phytochemicals as immunomodulators. *Biomedicine & Pharmacotherapy,* 133, 110959. https://doi.org/10.1016/j.biopha.2020.110959

Bohn, T., McDougall, G. J., Alegría, A., Alminger, M., Arrigoni, E., Aura, A. M., Brito, C., ... Santos, C. N. (2015). Mind the gap—deficits in our knowledge of aspects impacting the bioavailability of phytochemicals and their metabolites—a position paper focusing on carotenoids and polyphenols. *Molecular Nutrition & Food Research*, 59(7), 1307-1323. https://doi.org/10.1002/mnfr.201400745

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental microbiology*, 8(7), 1137-1144. https://doi.org/10.1111/j.1462-2920.2006.01054.x

Chandrakala, N., & Priya, S. (2017). Vibriosis in shrimp aquaculture a review. International Journal of Scientific Research in Science, *Engineering and Technology*, 3(2), 27-33. https://ijsrset.com/IJSRSET17321

Chen, Y. H., & He, J. G. (2019). Effects of environmental stress on shrimp innate immunity and white spot syndrome virus infection. *Fish & shellfish immunology*, 84, 744-755. https://doi.org/10.1016/j.fsi.2018.10.069

Cheng, W., Hsiao, I. S., Hsu, C. H., & Chen, J. C. (2004). Change in water temperature on the immune re-

sponse of Taiwan abalone *Haliotis diversicolor* supertexta and its susceptibility to *Vibrio parahaemolyticus*. *Fish & shellfish immunology*, 17(3), 235-243. https://doi.org/10.1016/j.fsi.2004.03.007

Effendi, I. (2016). Intensive Culture of White Shrimp *Litopenaeus vannamei* in Sea Net Cages: Location, Physiologycal and Biochemical Studies. [PhD thesis, IPB University]. Postgraduate School. https://repository.ipb.ac.id/handle/123456789/82367

Effendi, I., Suprayudi, M. A., Surawidjaja, E. H., Supriyono, E., & Zairin Jr, M. (2016). Production performance of white shrimp (*Litopenaeus vannamei*) under sea floating net cages with biofloc and periphyton juvenile. *Aquaculture, Aquarium, Conservation & Legislation*, 9(4), 823-832. http://www.bioflux.com.ro/docs/2016.823-832.pdf

FAO. (2022). The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

Gustilatov, M., Ekasari, J., & Pande, G. S. J. (2022). Protective effects of the biofloc system in Pacific white shrimp (*Penaeus vannamei*) culture against pathogenic *Vibrio parahaemolyticus* infection. *Fish & Shellfish Immunology*, 124, 66-73. https://doi.org/10.1016/j.fsi.2022.03.037

Hassaan, M. S., Mohammady, E. Y., Soaudy, M. R., Sabae, S. A., Mahmoud, A. M., & El-Haroun, E. R. (2021). Comparative study on the effect of dietary â-carotene and phycocyanin extracted from *Spirulina platensis* on immune-oxidative stress biomarkers, genes expression and intestinal enzymes, serum biochemical in Nile tilapia, *Oreochromis niloticus*. *Fish & Shellfish Immunology*, 108, 63-72. https://doi.org/10.1016/j.fsi.2020.11.012

Hsieh, S. L., Ruan, Y. H., Li, Y. C., Hsieh, P. S., Hu, C. H., & Kuo, C. M. (2008). Immune and physiological responses in Pacific white shrimp (*Penaeus vannamei*) to *Vibrio alginolyticus*. *Aquaculture*, 275(1-4), 335-341. https://doi.org/10.1016/j.aquaculture.2007.12.019

Ilmiah, Sukenda, Widanarni, & Harris, E. (2012). Isolation and characterization of pathogenic *Vibrio* on tiger grouper *Epinephelus fuscoguttatus*. *Jurnal Akuakultur Indonesia*, 11(1):28-37. https://doi.org/10.19027/jai.11.28-37

Kathleen, M. M., Samuel, L., Felecia, C., Reagan, E. L., Kasing, A., Lesley, M., & Toh, S. C. (2016). Antibiotic resistance of diverse bacteria from aquaculture in Borneo. *International journal of microbiology*, 2016, 2164761. https://doi.org/10.1155/2016/2164761

Kathyayani, S. A., Poornima, M., Sukumaran, S., Nagavel, A., & Muralidhar, M. (2019). Effect of ammonia stress on immune variables of Pacific white shrimp *Penaeus vannamei* under varying levels of pH and susceptibility to white spot syndrome virus. *Ecotoxicology and environmental safety*, 184, 109626. https://doi.org/10.1016/j.ecoenv.2019.109626

Kemal, R., Fauzi, I. A., Nuryati, S., Wardani, W. W., & Suprayudi, M. A. (2023). Evaluation of Selenoprotein supplementation on digestibility, growth, and health performance of Pacific white shrimp *Litopenaeus vannamei*. *Aquaculture Nutrition*, 2023(1), 2008517. https://doi.org/10.1155/2023/2008517

Kulkarni, A., Krishnan, S., Anand, D., Kokkattunivarthil Uthaman, S., Otta, S. K., Karunasagar, I., & Kooloth Valappil, R. (2021). Immune responses and immunoprotection in crustaceans with special reference to shrimp. *Reviews in Aquaculture*, 13(1), 431-459. https://doi.org/10.1016/j.ecoenv.2019.109626

Lawhavinit, O. A., Sincharoenpoka, P., & Sunthornandh, P. (2011). Effects of ethanol tumeric (*Curcuma longa* Linn.) extract against shrimp pathogenic *Vibrio* spp. and on growth performance and immune status of white shrimp (*Litopenaeus vannamei*). *Agriculture and Natural Resources*, 45(1), 70-77. https://doi.org/10.1111/raq.12482

Liu, H., Guo, S., Wang, R., He, Y., Shi, Q., Song, Z., & Yang, M. (2021). Pathogen of *Vibrio harveyi* infection and C-type lectin proteins in whiteleg shrimp (*Litopenaeus vannamei*). *Fish & Shellfish Immunology*, 119, 554-562. https://doi.org/10.1016/j.fsi.2021.10.040

Liu, S., Zheng, S. C., Li, Y. L., Li, J., & Liu, H. P. (2020). Hemocyte-mediated phagocytosis in crustaceans. *Frontiers in Immunology,* 11, 268. https://doi.org/10.3389/fimmu.2020.00268

Loo, K. Y., Letchumanan, V., Law, J. W. F., Pusparajah, P., Goh, B. H., Ab Mutalib, N. S., He, Y. W., & Lee, L. H. (2020). Incidence of antibiotic resistance in *Vibrio* spp. *Reviews in Aquaculture*, 12(4):2590-2608. https://doi.org/10.1111/raq.12460

Manefield M, Harris L, Rice SA, Nys RD, Kjelleberg. 2000. Inhibition of luminescence and virulence in the black tiger prawn (*Penaeus monodon*) pathogen *Vibrio harveyi* by intercellular signal antagonists. *Applied and Environmental Microbiology*, 66(5), 2079-2084. https://doi.org/

10.1128%2Faem.66.5.2079-2084.2000

Mary, R., & Banu, N. (2017). Antiquorum sensing potential of andrographolide from *Andrographis paniculata* in *Vibrio harveyi*. *Research Journal of Pharmacy and Technology*, 10(2), 449-452. http://dx.doi.org/10.5958/0974-360X.2017.00090.7

Miranti, S. (2016). Controlling of *Vibrio harveyi* infection on White Shrimp with *Curcuma longa-Andrographis paniculata* Extract in Diets at Floating Cage Culture, Thousand Islands. [Master's thesis, IPB University]. Postgraduate School. https://repository.ipb.ac.id/handle/123456789/80149

Moghadam, H., Sourinejad, I., & Johari, S. A. (2021). Growth performance, haemato immunological responses and antioxidant status of Pacific white shrimp *Penaeus vannamei* fed with turmeric powder, curcumin and curcumin nanomicelles. *Aquaculture Nutrition*, 27(6), 2294-2306. https://doi.org/10.1111/anu.13363

Munaeni, W., Yuhana, M., Setiawati, M., & Wahyudi, A. T. (2020). Impact of dietary supplementation with *Eleutherine bulbosa* (Mill.) Urb. on intestinal microbiota diversity and growth of white shrimp, *Litopenaeus vannamei*. *Aquaculture*, 528, 735466. https://doi.org/10.1016/j.aquaculture.2020.735466

Rudi, M., Sukenda, S., Wahjuningrum, D., Pasaribu, W., & Hidayatullah, D. (2019). Seaweed extract of Gracilaria verrucosa as an antibacterial and treatment against *Vibrio harveyi* infection of *Litopenaeus vannamei*. *Jurnal Akuakultur Indonesia*, 18(2), 120-129. https://doi.org/10.19027/jai.18.2.11-20

Sang, H. M., & Fotedar, R. (2009). Dietary supplementation of mannan oligosaccharide improves the immune responses and survival of marron, *Cherax tenuimanus* (Smith, 1912) when challenged with different stressors. *Fish & shellfish immunology*, 27(2), 341-348. https://doi.org/10.1016/j.fsi.2009.06.003

Shinn, A. P., Pratoomyot, J., Griffiths, D., Trong, T. Q., Vu, N. T., Jiravanichpaisal, P., & Briggs, M. (2018). Asian shrimp production and the economic costs of disease. *Asian Fisheries Science*, 31, 29-58. https://www.fao.org/3/ca2976en/ca2976en.pdf#page=35

Tam, L. T., Van Cong, N., Thom, L. T., Ha, N. C., Hang, N. T. M., Van Minh, C., Vien, D. T. H., & Hong, D. D. (2021). Cultivation and biomass production of the diatom *Thalassiosira weissflogii* as a live feed for white-leg shrimp in hatcheries and commercial farms in Vietnam. *Journal of Applied Phycology*, 33, 1559-1577. https://doi.org/10.1007/s10811-021-02371-w

Tan, K., Zhang, H., Lim, L. S., Ma, H., Li, S., & Zheng, H. (2020). Roles of carotenoids in invertebrate immunology. *Frontiers in Immunology*, 10, 3041. https://doi.org/10.3389%2Ffimmu.2019.03041

Van-Hai, N. (2015). The use of medicinal plants as immunostimulants in aquaculture: *A review. Aquaculture*, 446, 88-96. https://doi.org/10.1016/j.aquaculture.2015.03.014

Widanarni, W, Widagdo, P., & Wahjuningrum, D. (2013). The application of probiotics, prebiotics, and synbiotics through feed in vannamei shrimp (*Litopenaeus vannamei*) infected with *Vibrio harveyi*. *Jurnal Akuakultur Indonesia*, 11(1), 54-63. https://doi.org/10.19027/jai.11.54-63

Yin, X., Zhuang, X., Liao, M., Cui, Q., Yan, C., Huang, J., Jiang, Z., ... Wang, W. (2023). *Andrographis paniculata* improves growth and non-specific immunity of shrimp *Litopenaeus vannamei*, and protects it from *Vibrio alginolyticus* by reducing oxidative stress and apoptosis. *Developmental & Comparative Immunology*, 139, 104542. https://doi.org/10.1016/j.dci.2022.104542

Yusuf, A. L., Goh, Y. M., Samsudin, A. A., Alimon, A. R., & Sazili, A. Q. (2014). Growth performance, carcass characteristics and meat yield of Boer goats fed diets containing leaves or whole parts of *Andrographis paniculata*. Asian-Australasian *Journal of Animal Sciences*, 27(4), 503. https://doi.org/10.5713%2Fajas.2013.13533

Zarain-Herzberg, M., Campa-Córdova, A. I., & Cavalli, R. O. (2006). Biological viability of producing white shrimp *Litopenaeus vannamei* in seawater floating cages. *Aquaculture*, 259(1-4), 283-289. https://doi.org/10.1016/j.aquaculture.2006.05.044

Zarain-Herzberg, M., Fraga, I., & Hernandez-Llamas, A. (2010). Advances in intensifying the cultivation of the shrimp *Litopenaeus vannamei* in floating cages. *Aquaculture*, 300(1-4), 87-92. https://doi.org/10.1016/j.aquaculture.2009.12.016

Zhang, X. H., He, X., & Austin, B. (2020). *Vibrio harveyi*: a serious pathogen of fish and invertebrates in mariculture. *Marine life science & technology*, 2, 231-245. https://doi.org/10.1007/s42995-020-00037-z