# Effect of supplementation of *Chlorella vulgaris* in feed on growth performance and disease resistance against *Vibrio parahaemolyticus* infection in Pacific white shrimp, *Litopenaeus vannamei*

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**Abstract**. *Vibrio parahaemolyticus* has been reported as a pathogenic bacterium that frequently attacks and causes high mortality in Pacific white shrimp (*Litopenaeus vannamei*). Treating diseases with synthetic antibiotics has an impact on antibiotic resistance, polluting the environment, and endangering consumer health. Therefore, alternative options are needed to control diseases in shrimp, for example, the use of natural ingredients with immunostimulant properties. This study aims to test the supplementation effect of *Chlorella vulgaris* flour in feed to improve growth, immune system and resistance to *V. parahaemolyticus* infection in juvenile of Pacific white shrimp. This study examined four treatments consisting of feed supplemented with 2%, 6%, 10%, and 14% *C. vulgaris* flour (Cv.2%, Cv.6%, Cv.10%, and Cv.14%) respectively, with three replications. Shrimp were reared and fed for 30 days with feed according to the treatment, and on the 31st day they were challenged with *V. parahaemolyticus*. The results showed that Cv.14% resulted in the highest absolute growth, recovery rate, and survival compared to the other three treatments and with the lowest prevalence of vibriosis disease. Furthermore, based on the hemolymph profiles, the Cv.14% treatment resulted a better system-enhancing response than the other treatments. This study shows that Cv.14% treatment is the best dose to improve growth performance and the disease resistance to prevent *V. parahaemolyticus* infection in Pacific white shrimp.
**Keywords**: hemocyte response, immunostimulant, microalgal additive, Pacific white shrimp, vibriosis.

**Introduction**. Pacific white shrimp (*Litopenaeus vannamei*) is the main export commodity of Indonesian fisheries. Based on data from the Indonesian Ministry of Maritime Affairs and Fisheries (KKP), the volume of Indonesian shrimp exports in 2023 reached 220,889 tons with a value of 1.73 billion US dollars (KKP, 2024a, b). The problem experienced in culture of Pacific white shrimp in ponds at this time is the attack of diseases that can result in shrimp harvest failure, thus threatening the development and sustainability of shrimp farming in Indonesia (Kusmarwati et al., 2020). One of the bacterial disease agents that often attacks Pacific white shrimp in ponds is the bacterium *Vibrio parahaemolyticus*. The disease caused by *V. parahaemolyticus* can spread rapidly in ponds and can cause shrimp death up to 85% (Sarjito et al., 2015).

The control of bacterial infectious diseases in shrimp has so far used chemicals in the form of synthetic antibiotics which are not environmentally friendly, have an impact on antibiotic resistance and pollute the cultivation environment (Desbois et al., 2021; Hossain et al., 2022), and can accumulate in the body of aquaculture organisms, potentially endangering consumer health (Li et al., 2021). Therefore, it is necessary to find alternative options in controlling infectious diseases in Pacific white shrimp. The use of natural ingredients such as medicinal plants has high potential to control the impact of bacterial infectious diseases in shrimp (Sethi & Singh, 2015; Bharathi et al., 2019). Natural ingredients such as microalgae can be proposed as an alternative to overcome diseases in shrimp through immunostimulation (Ma et al., 2020), so that it can increase the body's resistance to bacterial infections (Sethi & Singh, 2015; Charoonnart et al., 2018).

One of the microalgae that can be used as an immunostimulant in Pacific white shrimp is *Chlorella vulgaris*. Several research results show that *C. vulgaris* contains bioactive compounds including carotenoids, flavonoids, phenolics, and alkaloids which act as antioxidants, anti-inflammatories and antibacterials (De Fretes et al., 2012; Abdel-Karim et al., 2020; Reis et al., 2022). *C. vulgaris* also contain peridinin chlorophyll protein (PCP) which is useful for increasing the immune system of aquatic organisms (Yanuhar et al., 2024). The results of the study by Darsan et al., (2021) showed that supplementation of *C. vulgaris* in feed can increase antioxidant activity as well as protein and lipid content in fish feed. *C. vulgaris* also contains high essential nutrients, such as vitamins, pigments, proteins needed for growth (Ajiboye et al., 2012).

*C. vulgaris* as direct feed or as a feed additive (supplementation) for various aquatic organisms has been researched. Research on *C. vulgaris* supplementation in feed has been conducted to improve the immune system and growth of freshwater fish (Enyidi, 2017; Aly et al., 2022; Pradhan et al., 2023), marine fish (Saberi et al., 2017; Reis et al., 2022), freshwater prawn *Macrobrachium rosenbergii* (Radhakrishnan et al., 2015; Sukri et al., 2016; Maliwat et al., 2017; Maliwat et al., 2021), and green tiger prawn, *Penaeus semisulcatus* (Al-Musalam et al., 2014). Research on supplementation of *C. vulgaris* in feed to improve growth, immunity and resistance to disease caused by *Vibrio parahaemolyticus* infection in Pacific white shrimp has actually been conducted by Eissa et al., (2024). However, considering that the supplementation of 3% *C. vulgaris* as the highest dose, in Eissa et al., (2024), resulted in better growth and immune response than other treatments, this study was designed by increasing the dose of *C. vulgaris* supplemented in feed on growth performance and disease resistance against *V. parahaemolyticus* infection in Pacific white shrimp. Therefore, this study aims to examine the supplementation effect of *C. vulgaris*, with a higher dose than previous research, in feed on growth performance and disease resistance against *V. parahaemolyticus* infection in Pacific white shrimp.

## Material and Method

***Study period and location***. The study was conducted from March 2023 to June 2023 at the Hatchery and Production Unit Laboratory, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari. Bacterial preparation and hemolymph observation of shrimp were performed at the Productivity and Aquatic Environment Laboratory, Faculty of Fisheries and Marine Science, Halu Oleo University, Kendari.

***Experimental feed***. The *C. vulgaris* flour used was a Seduh brand product. The experimental feed given was commercial feed supplemented with 2, 6, 10, and 14% *C. vulgaris* flour (Cv.2%, Cv.6%, Cv.10%, and Cv.14%) respectively. The commercial feed to be used was first ground into flour, then the *C. vulgaris* flour was mixed into the commercial feed flour according to the experimental design. Carboxyl methyl cellulose (CMC) as much as 1% of the total material was added as a binder, then stirred until evenly mixed. Furthermore, the mixture of ingredients was then added with enough warm water to form a dough. The feed dough was then molded and dried in an oven at a temperature of 40-50$^{o}$C (Darsan et al., 2021).

***Rearing of the test animal***. The Pacific white shrimp used in the study were juvenile-sized, 7-10 g shrimp$^{-1}$. The number of shrimps used in this study was 180. This study used 12 aquariums (35cm L × 35cm W × 40cm H). Before use, the aquariums were cleaned and then dried. The sea water was put into a container until it reached 3/4 of the height of the aquarium. The media was seawater with 20-25 ppt salinity. The water was supplied with oxygen using aeration. The feeding experiment with *C. vulgaris*-supplemented feed was conducted for 30 days. Then on the next 7 days, during the pathogenic bacterial challenge test stage, the feed given was feed without *C. vulgaris* supplementation. Feeding was carried out twice a day, namely in the morning and evening as much as 5% of the weight of the shrimp body biomass. Siphoning of the remaining feed and water replacement was carried out every 2 days. Water replacement was carried out as much as 30% of the water volume in the aquarium.

***Preparation of Vibrio parahaemolyticus***. *V. parahaemolyticus* was obtained from the Takalar Brackish Aquaculture Center, South Sulawesi Province, Indonesia. *V. parahaemolyticus* were rejuvenated in Thiosulfate Bile Salt Sucrose (TCBS) media, then incubated for 1×24 hours at 30°C. After that, bacterial culture was carried out on TSB NaCl 2.5% media which was incubated in a shaker for 1×24 hours at 30°C. Bacteria that had grown on TSB NaCl 2.5% media were put into a 15 mL tube and then centrifuged for 5 minutes at a speed of 3000 rpm. The upper solution was discarded so that bacterial sediment remained. The remaining pellet was washed using PBS solution and then centrifuged again for 5 minutes. The bacterial washing procedure was carried out 3 times. The density of bacteria used in the challenge test was $10^8$ CFU $mL^{-1}$. The density of bacteria used was determined indirectly, referring to Rosmania & Yanti, (2020). This method consists of 4 stages including (1) making a 0.5 McFarland as a standard solution, (2) adjusting the standard solution levels with the help of UV-spectrophotometry, (3) measuring and aligning the turbidity of the bacterial suspension with a standard solution, (4) diluting the bacterial suspension with a ratio of 1:16 to obtain bacteria with a density equivalent to $10^8$ CFU $mL^{-1}$.

***Challenge test with V. parahaemolyticus.*** To determine the immunostimulant potential of feed supplemented with *C. vulgaris*, a bacterial challenge test was conducted on test animals. The challenge test with pathogenic bacteria was carried out on day 31. Bacteria of *V. parahaemolyticus* with a dose of 0.1 mL with a density of $10^8$ CFU $mL^{-1}$ was injected into each test animal. Injection of pathogenic bacteria was carried out on the second abdominal segment. Furthermore, the level of healing, survival, and hemolymph profile of test animals were observed on the 7th day after the challenge test.

***Observation variables***

*Growth, prevalence, recovery rate and survival rate*. The parameters measured in this research were weight gain, prevalence, recovery rate, and survival rate. The observed hemocyte profile parameters were total hemocyte count (THC) and differential hemocyte count (DHC). The hemolymph profile observation were carried out on days 0, 30, 34, and 37 (Figure 1).

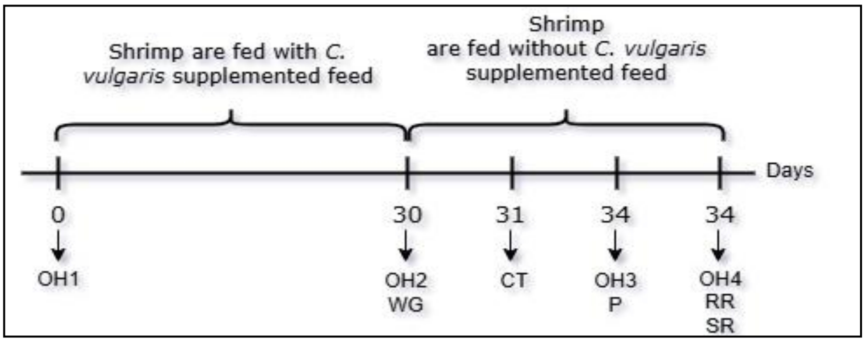


Figure 1. Time schedule of treatments and observation (OH1 - first observation of hemolymph profiles; OH2 - second observation of hemolymph profiles; OH3 - third observation of hemolymph profiles; OH4 - fourth observation of hemolymph profiles; WG - weight gain; CT - challenge test with *V. parahaemolyticus*; P - prevalence; RR - recovery rate; SR - survival rate).

Weight gain was calculated using the formula: Weight gain (g $shrimp^{-1}$) = final mean weight – initial mean weight. The prevalence and recovery rate were calculated using modified formulas from Hasmin et al., (2025). Prevalence (%) = number of sick shrimp /

total number of shrimp × 100. Recovery rate (%) = number of recovered shrimp / number of sick shrimp × 100. Survival rate (%) = number of shrimp at the beginning of rearing / number of shrimp at the end of rearing × 100.

*Hemolymph examination*. Hemolymph samples were collected from three shrimps per treatment. The THC was calculated using the Blaxhall & Daisley, (1973) method. While DHC, consisting of hyaline, semi-granular and granular cells, was calculated according to Martin & Graves, (1985). The characteristics of each cell are that hyaline cells have cytoplasm that appears clear because it does not have granules, semi-granular cells have small granules in small numbers, while granular cells have large and dense granules in the cytoplasm.

***Statistical analysis***. Data on growth, prevalence, recovery rate, survival rate, total hemocyte count (THC), and differential hemocyte count (DHC) were subjected to analysis of variance (ANOVA) at a 95% confidence level ($p < 0.05$) using SPSS 25.0.

## Results

***Growth and health profile***. Supplementation of *C. vulgaris* with different doses in feed significantly ($p < 0.05$) affected the growth, disease prevalence, recovery and survival rate of whiteleg shrimp, as presented in Table 1. Supplementation of 14% *C. vulgaris* in feed resulted in the highest growth, recovery and survival rate parameters in shrimp with values of 3.80±0.31g, 83.33±28.87%, and 93.33±11.55%, respectively. Meanwhile, the lowest disease prevalence was found in shrimp fed with feed containing 14% *C. vulgaris* with a value of 33.33±11.55%.

Table 1
Growth performance parameters, disease prevalence, recovery and survival rate of Pacific white shrimp during the experiment

| *Parameter* | *Treatment* | | | |
|---|---|---|---|---|
| | *Cv.2%* | *Cv.6%* | *Cv.10%* | *Cv.14%* |
| WG (g) | 1.93±0.15$^{a}$ | 2.97±0.29$^{b}$ | 3.11±0.51$^{b}$ | 3.80±0.31$^{c}$ |
| P (%) | 86.67±11.55$^{b}$ | 86.67±11.55$^{b}$ | 73.33±11.55$^{b}$ | 33.33±11.55$^{a}$ |
| RR (%) | 38.33±12.58$^{a}$ | 53.33±5.77$^{ab}$ | 55.56±9.62$^{ab}$ | 83.33±28.87$^{b}$ |
| SR (%) | 46.67±11.55$^{a}$ | 60.00±0.00$^{ab}$ | 66.67±11.55$^{b}$ | 93.33±11.55$^{c}$ |

***Hemolymph examination***. The results of hemolymph examination including the THC and DHC of granular and semi-granular cells, as well as hyaline cells are presented in Table 2.

Table 2
Results of hemolymph examination on the parameters of THC, DHC granular and semi-granular cells, and DHC hyaline cells

| *Parameter/ treatment* | *Before the challenge test* | | *After the challenge test* | |
|---|---|---|---|---|
| | *Day 0* | *Day 30* | *Day 34* | *Day 37* |
| | | *THC (cells mL$^{-1}$)* | | |
| Cv.2% | 4.91±0.21 | 6.31±1.35 | 5.52±0.55 | 9.91±0.40 |
| Cv.6% | 4.91±0.21 | 6.63±1.59 | 5.99±1.31 | 10.19±1.58 |
| Cv.10% | 4.91±0.21 | 7.20±0.94 | 5.43±0.94 | 11.03±0.94 |
| Cv.14% | 4.91±0.21 | 7.57±0.93 | 5.65±0.40 | 12.32±6.59 |
| | | *Granular and semi-granular cells (%)* | | |
| Cv.2% | 63.00±2.65 | 66.00±4.58$^{a}$ | 49.67±5.13$^{a}$ | 56.67±9.87 |
| CV.6% | 63.00±2.65 | 68.67±3.51$^{ab}$ | 57.67±3.51$^{ab}$ | 66.67±3.79 |
| Cv.10% | 63.00±2.65 | 68.67±5.13$^{ab}$ | 59.33±5.51$^{b}$ | 66.33±3.21 |
| Cv.14% | 63.00±2.65 | 74.33±2.89$^{b}$ | 63.00±4.36$^{b}$ | 73.00±3.61 |
| | | *Hyaline cells (%)* | | |
| Cv.2% | 37.00±2.65 | 34.00±4.58 | 50.33±5.13$^{b}$ | 43.33±9.87 |
| CV.6% | 37.00±2.65 | 31.33±3.51 | 42.33±3.51$^{ab}$ | 33.33±3.79 |
| Cv.10% | 37.00±2.65 | 31.33±5.13 | 40.67±5.51$^{a}$ | 33.67±3.21 |
| Cv.14% | 37.00±2.65 | 25.67±2.89 | 37.00±4.36$^{a}$ | 27.00±3.61 |

## Discussion

***Growth and health profile***. The results of this study indicate that Pacific white shrimp fed without *C. vulgaris* produced the growth of 1.82 g, while Pacific white shrimp fed with *C. vulgaris* flour supplementation produced higher growth of 2.97-3.80 g. Among the four treatments of feeding supplemented with *C. vulgaris* flour, the highest of growth was obtained in the *C. vulgaris* flour supplementation treatment of 14% (Cv.14%) compared to other treatments. This is very possible because the nutritional intake contained in the feed is higher with an increase in the ratio of *C. vulgaris* in the feed. *C. vulgaris* microalgae are known to have a high total protein content of 43-58% of dry weight, contain vitamins (Safi et al., 2014), important minerals (Yusof et al., 2011), and all types of essential amino acids (Safi et al., 2013). Supplementation of *C. vulgaris* flour in feed as much as 10-15% *C. vulgaris* has been reported to increase the growth of *Paralichthys olivaceus* fish (Rahimnejad et al., 2017). The weight gain of shrimp in this study was actually lower than the results of the study by Eissa et al., (2024). This is thought to be due to the gap in crude protein content in the basic feed. The protein content of the basic feed in this study was 18.89% (the basic feed used was the same as the feed used by Abidin et al., (2024) while the protein content of the basic feed in the study by Eissa et al., (2024) is reached 38.70%). However, increasing protein intake in feed significantly affects the growth of white shrimp (Araujo et al., 2025).

The recovery rate of Pacific white shrimp in this study was determined through visual observation of clinical signs. Based on clinical assessment, shrimp infected with *V. parahaemolyticus* exhibited pale or reddish body discoloration, reddening of the pleopods, red spots on the dorsal region, and necrotic lesions on the tail. Infected shrimp may also display lethargy and reduced feed intake, which are commonly reported clinical manifestations of vibriosis and *V. parahaemolyticus*-associated disease in penaeid shrimp (Mastan & Begum, 2016). In contrast, recovered shrimp were characterized by the disappearance of red spots on the body, normalization of pleopod coloration, and the absence of visible necrosis on the tail (Figure 2).


Figure 2. Morphology of sick (a) and recovered (b) Pacific white shrimp. Sick shrimp (a) shows reddish body and pale tail due to necrosis.

The treatment of Cv.14% also had an impact on the resistance of shrimp to *V. parahaemolyticus* infection. The treatment of Cv.14% has succeeded in suppressing the number of infected shrimp (prevalence) of *V. parahaemolyticus* infectious disease, which is 33.33% with a recovery rate of 83.33%. While in Cv.2%, the prevalence of *V. parahaemolyticus* infectious disease was the highest, which was 86.67% and with the lowest recovery rate of all treatments, which was 38.33%. The increase in the percentage of *C. vulgaris* flour supplementation (up to 14% in this study) had an impact on increasing the quantity of active compounds and the effect of these active compounds in increasing the immune response. The results of this study indicate that the higher the levels of *C. vulgaris* in the feed, the stronger the shrimp's resistance to disease caused by *V. parahaemolyticus* (the prevalence of the disease decreased) and the rate of recovery from the disease also increased. The content of active compounds in *C. vulgaris* are flavonoids, terpenes, phenolics, and alkaloids (Dineshkumar et al., 2017; Thangaraj et al., 2022), they are thought to have increased the immune response of Pacific white shrimp. Flavonoids and alkaloids are known to be compounds that act as immunomodulators (Behl et al., 2021). Flavonoids also function as natural anti-inflammatory agents (Choy et al., 2019). Supplementation of *C. vulgaris* extract in feed has also been reported to be able to modulate the health status of gilthead seabream (*Sparus aurata*) juveniles (Reis et al., 2022). *Chlorella*-suplemented in Nile tilapia (*Oreochromis niloticus*) diets meaningfully boosted the antioxidative capability of fish and the innate immune indices such as phagocytic activity (PA), respiratori burst activity (RBA) and alternative complement activity (ACH50) (Abdel-Tawwab et al., 2023). Feed supplemented with *C. vulgaris* has been shown to improve the immune system of Gibel carp (*Carassius auratus gibelio*) by increasing the content of immunoglobin M (IgM) in the blood (Zhang et al., 2014).

The survival rate of shrimp in this study is in line with the recovery rate value. The ability of shrimp to defend themselves from the impact of pathogen infection and the ability to heal after infection greatly supports their survival. The survival of shrimp in Cv.14% produced the highest value (93.33%) compared to other treatments. The higher the level of *C. vulgaris* given in the feed, the greater the survival of shrimp. In a similar study conducted by Eissa et al., (2024), supplementation of 3% *C. vulgaris* into the feed has increased the survival rate of Pacific white shrimp compared to treatment with lower doses. Research conducted on Nile tilapia, fish fed with *C. vulgaris* flour supplementation and then challenged with *Aeromonas hyrophilla* had a lower prevalence value with a higher survival rate compared to feed without *C. vulgaris* flour supplementation (Aly et al., 2022).

***Hemolymph examination***. THC showed no significant differences during the maintenance and challenge test periods. However, THC increased with increasing concentrations of *C. vulgaris* flour supplements in the feed. This also indicates that supplementation of *C. vulgaris* flour into the feed successfully increased the immune response of Pacific white shrimp. THC on the 30th day for each treatment with *C. vulgaris* flour supplementation increased. On the 34$^{th}$ day or 3 days after the challenge test, the THC value decreased, which is suspected to be the shrimp's immune response due to *V. parahaemolyticus* infection. The results of observations on the 37th day showed that the THC value increased again in each treatment. The results of previous studies have shown that immunostimulant supplementation in feed causes the production of Pacific white shrimp hemocyte cells to increase 3 days after infection. Hemocyte cells carry out defense activities against foreign substances or pathogens that enter the shrimp's body (Munaeni et al., 2020; Nur et al., 2020). The increase in the immune system as a result of providing feed containing *C. vulgaris* has also been tested on aquaculture organisms other than shrimp. Reis et al., (2022) reported that supplementation of *C. vulgaris* microalgae extract in feed has been beneficial as a modulator of health and inflammatory responses in *Sparus aurata*.

DHC is a calculation of the percentage of each hemocyte cell including granular and semigranular cells and hyaline cells. Hemocyte cells play an important role in the immune system of crustaceans including Pacific white shrimp. The results of observations on the 34th day or 3 days after the challenge test showed that the percentage of hyaline cells increased in all treatments and the highest occurred in the *C. vulgaris* flour supplementation treatment of 14% (Cv.14%). This increase is thought to be caused by the presence of bacteria entering the shrimp's body resulting in a decrease in the percentage of granular cells after the challenge test. This is thought to be related to the activity of granular cells in cytotoxic reactions causing the number of granular cells to decrease. Hauton, (2012) stated that granular cells contribute to producing antimicrobial peptides and are also involved in cytotoxic reactions while hyaline cells play a role in phagocytosis. The hyaline cells in this study appeared to increase on the 34th day and decreased again on the 37th day. This may indicate that the pathogen phagocytosis process is ongoing.

**Conclusions**. The result showed that supplementation of *C. vulgaris* flour in feed can increase the growth of Pacific white shrimp. The treatment of supplementation of *C. vulgaris* flour as much as 14% is the best treatment that can be used to increase immune respon and resistance to prevent *V. parahaemolyticus* infection disease in Pacific white shrimp. These results indicate that *C. vulgaris* has promising potential as a natural immunostimulant feed additive for sustainable Pacific white shrimp culture. Its application may help improve shrimp growth and resistance to *V. parahaemolyticus* while reducing reliance on synthetic antibiotics.

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**Authors Contributions**. Conceptualization: LOBA; Methodology, formal analysis, investigation, and data curation: RSP, YRP, J, AK, LOBA; Writing; RSP, YRP, LOBA; Supervision: LOBA.

**Conflicts of Interest**. The authors declare that there is no conflict of interest.

**Data Availability**. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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**References**

Abdel-Karim, O. H., Gheda, S. F., Ismail, G. A., & Abo-Shady, A. M. (2020). Phytochemical screening and antioxidant activity of *Chlorella vulgaris*. *Delta Journal of Basic and Applied Sciences, 41*, 76-86.

Abdel-Tawwab, M., Khalil, R. H., Selema, T. A. M. A., Elsamanooudy, S. I., El-Werwary, S. O. M., Shady, S. H. H., Monier, M. N., & Ismaiel, M. M. S. (2023). Dietary *Chlorella vulgaris* effectively alleviates oxidative stress, immunosuppression, and enhances the resistance to *Streptococcus agalactiae* infection in cadmium-intoxicated Nile tilapia fingerlings. *Fish and Shellfish Immunology, 136*, 108717.

Abidin, L. O. B., Yustika, Aslin, L. O., Arsal, L. O. M., & Bujang, A. (2024). [Feed efficiency, growth and survival rate of white shrimp (*Litopenaeus vannamei*) fed with feed enriched with tomato fruit flour]. *Jurnal Media Akuatika, 9*(1), 1-10. [in Indonesian]

Ajiboye, O. O., Yakubu, A. F., & Adams, T. E. (2012). A perspective on the ingestion and nutritional effects of feed additives in farmed fish species. *World Journal of Fish and Marine Sciences, 4*(1), 87-101.

Al-Musalam, L. S., Al-Ameeri, A. A., Saheb, A. S., & Al-Yaqout, A. (2014). Effect of herbal feed additive on the growth, survival and immune response of green tiger prawn (*Penaeus semisulcatus*). *Pakistan Journal of Nutrition, 13*(7), 366-371.

Aly, S. M., Eldin, S. M. M., & Abou-El-Atta, M. E., Abdel-Razek, M., & El-Banna, N. I. (2022). Immunomodulatory role of dietary *Chlorella vulgaris* against *Aeromonas hydrophilla* infection in the Nile tilapia (*Oreochromis niloticus*). *Egyptian Journal of Aquatic Biology and Fisheries, 26*(3), 383-401.

Araujo, A. N., Nguyen, K., Strebel, L., Corby, T., Rhodes, M. A., Beck, B. H., Roy, L. A., & Davis, D. A. (2025). Effect of protein intake on growth and nutrient retention of Pacific white shrimp (*Litopenaeus vannamei*) in a green water recirculating aquaculture system. *Aquaculture Nutrition, 2025*, 4942439.

Behl, T., Kumar, K., Brisc, C., Rus, M., Nistor-Cseppento, D. C., Bustea, C., Corb Aron, R. A., Pantis, C., Zengin, G., Sehgal, A., Kaur, R., Kumar, A., Arora, S., Setia, D., Chandel, D., & Bungau, S. (2021). Exploring the multifocal role of phytochemicals as immunomodulators. *Biomedicine and Pharmacotherapy, 133*, 110959.

Bharathi, S., Cheryl, A., Rajagopalasamy, C. B. T., Uma, A., Ahilan, B., & Aanand, S. (2019). Functional feed additives used in fish feeds. *International Journal of Fisheries and Aquatic Studies, 7*(3), 44-52.

Blaxhall, P. C., & Daysley K. W. (1973). Routine haematological methods for use with fish blood. *Journal of Fish Biology, 5*(6), 771-781.

Charoonnart, P., Purton, S., & Saksmerprome, V. (2018). Applications of microalgal biotechnology for disease control in aquaculture. *Biology, 7*(2), 24.

Choy, K. W., Murugan, D., Leong, X. F., Abas, R., Alias, A., & Mustafa, M. R. (2019). Flavonoids as natural anti-inflammatory agents targeting nuclear factor-kappa B (NFκB) signaling in cardiovascular diseases: a mini review. *Frontiers in Pharmacology, 10*, 1295.

Darsan, I. M., Iba, W., & Nur, I. (2021). [Proximate and antioxidant content of aquafeed containing *Chlorella vulgaris* Beyerinck (Beijerinck) 1890 meal]. *Journal of Fishery Science and Innovation, 5*(2), 54-59. [in Indonesian]

Desbois, A. P., Garza, M., Eltholth, M., Hegazy, Y. M., Mateus, A., Adams, A., Little, D. C., Høg, E., Mohan, C. V., Ali, S. E., & Brunton, L. A. (2021). Systems-thinking approach to identify and assess feasibility of potential interventions to reduce antibiotic use in tilapia farming in Egypt. *Aquaculture, 540*, 736735.

De Fretes, H., Susanto, A. B., Prasetyo, B., & Limantara, L. (2012). [Carotenoids from macroalgae and microalgae: health potential, application and biotechnology]. *Jurnal Teknologi dan Industri Pangan, 23*(2), 221-228. [in Indonesian]

Dineshkumar, R., Narendran, R., Jayasingam, P., & Sampathkumar, P. (2017). Cultivation and chemical composition of microalgae *Chlorella vulgaris* and its antibacterial activity against human pathogens. *Journal of Aquaculture and Marine Biology, 5*(3), 00119.

Eissa, E. S. H., Aljarari, R. M., Elfeky, A., El-Aziz, Y. M. A., Munir, M. B., Jastaniah, S. D., Alaidaroos, B. A., Shafi, M. E., El-Hamed, N. N. B. A., AL-Farga, A., Dighiesh, H. S., Okon, O. M., El-Hack, M. E. A., Ezzo, O. H., Eissa, M. E. H., & El-Banna, N. I. (2024). Protective effects of *Chlorella vulgaris* as a feed additive on growth performance, immunity, histopathology, and disease resistance against *Vibrio parahaemolyticus* in the Pacific white shrimp. *Aquaculture International, 32*, 2821-2840.

Enyidi, U. D. (2017). *Chlorella vulgaris* as protein source in the diets of African catfish *Clarias gariepinus*. *Fishes, 2*(4), 17.

Hasmin, Hamzah, M., & Abidin, L. O. B. (2025). Immunostimulation of Nile tilapia through the provision of synbiotic feed (*Eleutherine bulbosa* and probiotic) to prevent motile *Aeromonas* septicemia (MAS) disease. *Journal of Fish Health, 5*(3), 407-417.

Hauton, C. (2012). The scope of the crustacean immune system for disease control. *Journal of Invertebrate Pathology, 110*(2), 251-260.

Hossain, A., Habibullah-Al-Mamun, M., Nagano, I., Masunaga, S., Kitazawa, D., & Matsuda, H. (2022). Antibiotics, antibiotic-resistant bacteria, and resistance genes in aquaculture: risks, current concern, and future thinking. *Environmental Science and Pollution Research International, 29*(8), 11054-11075.

KKP (2024a). [Exports and Imports - KKP RI One Data Portal]. Available at: https://portaldata.kkp.go.id/portals/data-statistik/exim/tbl-statis/d/155. Accessed: August, 2026. [in Indonesian]

KKP (2024b). [Exports and Imports - KKP RI One Data Portal]. Available at: https://portaldata.kkp.go.id/portals/data-statistik/exim/tbl-statis/d/156. Accessed: August, 2026. [in Indonesian]

Kusmarwati, A., Andayani, F., & Yennie Y., 2020 [Prevalence of *Vibrio parahaemolyticus* in whiteleg shrimp at fish processing units in Central Java and East Java]. *JPB Kelautan dan Perikanan, 15*(1), 21-31. [in Indonesian]

Li, J. Y., Wen, J., Chen, Y., Wang, Q., & Yin J. (2021). Antibiotics in cultured freshwater products in Eastern China: Occurrence, human health risks, sources, and bioaccumulation potential. *Chemosphere, 264*(1), 128441.

Ma, K., Bao, Q., Wu, Y., Chen, S., Zhao, S., Wu, H., & Fan, J. (2020). Evaluation of microalgae as immunostimulants and recombinant vaccines for diseases prevention and control in aquaculture. *Frontiers in Bioengineering and Biotechnology, 8*, 590431.

Maliwat, G. C. F., Velasquez, S. F., Robil, J. L., Chan, M., Traifalgar, R. F., Tayamen, M. M., & Ragaza, J. A. (2017). Growth and immune response of giant freshwater prawn *Macrobrachium rosenbergii* (De Man) postlarvae fed diets containing *Chlorella vulgaris* (Beijerinck). *Aquaculture Research, 48*,1666-1676.

Maliwat, G. C. F., Velasquez, S. F., Buluran, S. M. D., Tayamen, M. M., & Ragaza, J. A. (2021). Growth and immune response of pond-reared giant freshwater prawn *Macrobrachium rosenbergii* post larvae fed diets containing *Chlorella vulgaris. Aquaculture and Fisheries, 6*, 465-470.

Martin, G. G., & Graves, B. L. (1985). Fine structure and classification of shrimp hemocytes. *Journal of Morphology, 185*(3), 339-348.

Mastan, S. A., & Begum S. K. A. (2016). Vibriosis in farm reared white shrimp, *Litopenaeus vannamei* in Andhra Pradesh - natural occurrence and artificial challenge. *International Journal of Applied Sciences and Biotechnology, 4*(2), 217-222.

Munaeni, W., Widanarni, Yuhana, M., Setiawati, M., & Wahyudi, A. T. (2020). Effect in white shrimp *Litopenaeus vannamei* of *Eleutherine bulbosa* (Mill.) Urb. powder on immune genes expression and resistance against *Vibrio parahaemolyticus* infection. *Fish and Shellfish Immunology, 102*, 218-227.

Nur, I., Linianti, Munaeni, W., Abidin, L. O. B., & Maulidiyah (2020). Assessment of antibacterial and immunostimulating activity of black cumin (*Nigella sativa*) extract against vibriosis in white shrimp (*Litopenaeus vannamei*). *The Thai Journal of Veterinary Medicine, 50*(4), 549-557.

Pradhan, J., Sahu, S., & Das, B. K. (2023). Protective effects of *Chlorella vulgaris* supplemented diet on antibacterial activity and immune responses in rohu fingerlings, *Labeo rohita* (Hamilton), subjected to *Aeromonas hydrophila* infection. *Life, 13*(4), 1028.

Radhakrishnan, S., Saravana, B. P., Seenivasan, C., & Muralisankar, T. (2015). Effect of dietary replacement of fishmeal with *Chlorella vulgaris* on growth performance, energy utilization and digestive enzymes in *Macrobrachium rosenbergii* postlarvae. *International Journal of Fisheries and Aquaculture, 7*(5), 62-70.

Rahimnejad, S., Lee, S. M., Park, H. G., & Choi, J. (2017). Effects of dietary inclusion of *Chlorella vulgaris* on growth, blood biochemical parameters, and antioxidant enzyme activity in olive flounder, *Paralichthys olivaceus*. *Journal of the World Aquaculture Society, 48*(1), 103-112.

Reis, B., Ramos-Pinto, L., Cunha, S. A., Pintado, M., da Silva, J. L., Dias, J., Conceição, L., Matos, E., & Costas, B. (2022). *Chlorella vulgaris* extracts as modulators of the health status and the inflammatory response of gilthead seabream juveniles (*Sparus aurata*). *Marine Drugs, 20*(7), 407.

Rosmania, & Yanti, F. (2020). [Bacterial counting in the microbiology laboratory using the developed spectrophotometric method]. *Jurnal Penelitian Sains, 22*(2), 76-86. [in Indonesian]

Saberi, A., Zorriehzahra, M. J., Emadi, H., Kakoolaki, S., & Fatemi, S. M. R. (2017). Effects of *Chlorella vulgaris* on blood and immunological parameters of Caspian Sea salmon (*Salmo trutta caspius*) fry exposed to Viral Nervous Necrosis (VNN) virus. *Iranian Journal of Fisheries Sciences, 16*(2), 494-510.

Safi, C., Charton, M., Pignolet, O., Silvestre, F., Vaca-Garcia, C., & Pontalier P. Y. (2013). Influence of microalgae cell wall characteristics on protein extractability and determination of nitrogen-to-protein conversion factors. *Journal of Applied Phycology, 25*(2), 523-529.

Safi, C., Zebib, B., Merah, O., Pontalier, P. Y., & Vaca-Garcia, C. (2014). Morphology, composition, production, processing and applications of *Chlorella vulgaris*: a review. *Renewable and Sustainable Energy Reviews, 35*, 265-278.

Sarjito, Apriliani, M., Afriani, D., & Haditomo, A. H. C. (2015). [Agents causing vibriosis in Pacific white shrimp (*Litopenaeus vannamei*) cultivated intensively in Kendal]. *Jurnal Kelautan Tropis, 18*(3), 189-196. [in Indonesian]

Sethi, J., & Singh, J. (2015). Role of medicinal plants as immunostimulants in health and disease. *Annals of Medicinal Chemistry and Research, 1*(2), 1009.

Sukri, S. A. M., Saad, C. R., Kamarudin, M. S., & Yasin, I. S. M. (2016). Effect of diferent levels of *Chlorella* meal on growth and survival of freshwater prawns *Macrobrachium rosenbergii* juvenile. *Songklanakarin Journal of Science and Technology, 38*(6), 641-644.

Thangaraj, M., Saravana, B. P., Thanasekaran, J., Joen-Rong, S., Manubolu, M., & Pathakoti, K. (2022). Phytochemicals of algae, *Arthospira platensis* (spirulina) *Chlorella vulgaris* (chlorella) and *Azolla pinnata* (azolla). *GSC Biological and Pharmaceutical Sciences, 19*(2), 23-43.

Yanuhar, U., Suryanto, H., Mahasri, G., & Caesar, N. R. (2024). Cloning and characterization of protein fraction specific of *Chlorella vulgaris* endemic East Java. *Evergreen, 11*(1), 82-94.

Yusof, Y. A. M., Basari, J. M. H., Nukti, N. A., Sabuddin, R., Muda, A. R., Sulaiman, S., Makpol, S., & Ngah, W. Z. W. (2011). Fatty acids composition of microalgae *Chlorella vulgaris* can be modulated by varying carbon dioxide concentration in outdoor culture. *African Journal of Biotechnology, 10*(62), 13536-13542.

Zhang, Q., Qiu, M., Xu, W., Gao, Z., Shao, R., & Qi, Z. (2014). Effects of dietary administration of *Chlorella* on the immune status of gibel carp, *Carassius auratus gibelio*. *Italian Journal of Animal Science, 13*(3), 3168.

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