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Original Article

Synbiotic of alginate, spirulina water extract, and *Lactobacillus bulgaricus* promotes survival, growth, and immune parameters based on the gene-expression of *Litopenaeus vannamei* in low-salinity

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Abstract: Synbiotics containing prebiotics and probiotics are commonly applied in aquaculture, which serves mutual benefits. This study focused on applying alginate, spirulina water extract (SWE), and *Lactobacillus bulgaricus* probiotics in *Litopenaeus vannamei* supplementation feed via oral administration at low salinity (1 ppt). The survival rate, growth, and biomass were determined as well as the immune-related gene expression of ProPhenol Oxidase (PO), Superoxide Dismutase (SOD), Lipopolysaccharide Beta Glucan Binding Protein (LGBP) were assessed. Gene-related salinity stressors, namely insulin-like growth Factor (IGF) and Heat Shock Protein (HSP), were also determined. The factorial design with two factors was applied (supplemented and non-supplemented; 1 ppt and 30 ppt mediums) and replicated thrice. 1,000 shrimps (500 ind.m^2) at the initial weight of 0.2±0.05 g were reared in the semi-mass culture at 2 tons medium for 56 days. Growth was monitored weekly. The results show that shrimp fed supplementation of (Alg 3.0 g.kg^{-1} + 5.0 mg SWE.kg^{-1}) in 1 ppt medium ($P \leq 0.05$) reached the best survival rate, biomass, and gene expression (PO, LGBP, HSP, and IGF), except Lectin. These prebiotics of macro and microalga from Indonesia's tropical coast supported the environmentally friendly and sustainable approach. Promising a noteworthy future in culturing *L. vannamei* in low salinity.

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Introduction

As a valuable economic aquatic animal cultivated, the production of *L. vannamei* in 2022 is around 11,237 million tons, describing 51.7% of the global shrimp production (FAO, 2024) and exceeding the caught shrimp for the first time. Whiteleg Shrimp, *Litopenaeus vannamei* is the fastest-growing food sector. As a euryhaline shrimp, it can stand for a broad range of salinities (0.5 to 45 ppt) and is commonly cultured in inland low-salinity water in many countries worldwide (Al-Subiai et al., 2025). Recently, a large amount of *L. vannamei* has been cultured in inland waters at low salinity (< 5 ppt), with poor survival, delayed growth, low-stress tolerance (Su et al., 2023), and decreased immunity (Al-Subiai et al., 2025). In addition, this adaptive response necessitates a substantial quantity of energy obtained from shrimp feed during farming (Yudiati et al., 2024).

Nutritive regulation is a valuable strategy to lighten the contrary effects of low-salt stress (Qiao et al., 2022). Dietary supplementation of nutrients such as amino acids (Xie et al., 2014), protein (Li et al., 2011), lipids (Chen et al., 2015), carbohydrates (Li et al., 2024), vitamins (Zhu et al., 2024) and minerals (Pimentel et al., 2023) can improve the growth performance, antioxidant and immune capacity which regulates the gene expression of the shrimps at low salinity. Furthermore, adding prebiotics such as β-Glucan (Qiao et al., 2022), inulin (Li et al., 2024) as well as synbiotics fertilised with rice bran processed by probiotic bacteria can also affect the shrimp's

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intestinal health at low salinity, which delivers a nutrient-rich medium for the growth contribute of an excessive load up of microorganisms to supporting water quality (Pimentel et al., 2023).

Alginate is a natural polysaccharide consisting of mannuronic and guluronic acid gained from brown seaweeds and is proven as an excellent compound to boost *L. vannamei* immune response (Yudiati et al., 2019; Azhar and Yudiati, 2023). The synbiotics of alginate and *Lactobacillus bulgaricus* probiotics can improve the survival and resistance against pathogenic *Vibrio* spp. of Artemia as biomodel (Yudiati et al., 2021b) and *L. vannamei* (Yudiati et al., 2023a). Lactic acid bacteria (LAB), including *L. bulgaricus*, are gram-positive, non-sporulating, and non-mobile bacteria that can produce lactic acid as the major end product of carbohydrates such as alginate for fermentation. Probiotics are effective not only in disease resistance but also in improving growth performance, activities of digestive enzymes, and immune response (Liu et al., 2022). A previous study on *L. bulgaricus* showed their abilities to attach to the intestinal mucus, race for attachment positions with pathogenic *Vibrio* spp., immune response, and consistently upregulate Lectin, ProPhenol Oxydase (ProPO) as well as Lipopolysaccharide Beta Glucan Binding Protein (LGBP) gene expression (Yudiati et al., 2016; Yudiati et al., 2019; Azhar and Yudiati, 2023). The combination of this pre and probiotics managed to maintain the best cultivation condition even at a lower dose and under-stress salinity (Yudiati et al., 2024).

Spirulina platensis one of the best species of blue-green microalgae, is not only rich in nutrients but also has good immunomodulatory effects (Yudiati et al., 2021a; Azhar and Yudiati, 2023), antioxidant from protein pigment base (Hidayati et al., 2020), and antiviral effects (Liu et al., 2022). The high nutrient levels extracted from water-based spirulina water extract (SWE), such as Beta-glucans (Zahan et al., 2024), protein, vitamins, minerals, and its digestibility/palatability are likely responsible for spirulina's growth-promoting properties, (Bahi et al., 2023) namely Insuline-Like Growth Factor (IGF) gene-expression.

Prebiotics are not merely probiotics; they are also operated in aquaculture. Prebiotics are non-living ingredients indigestible by the host animals but can be digested by gut probiotics such as *Lactobacillus* sp. (Liu et al., 2022). The synbiotics, which is the combination of prebiotics such as alginate, SWE, and probiotics, managed to maintain the best cultivation condition even at a lower dose, enhancing the recovery rate from high virulence of *V. parahaemolyticus* AHPND strain (Azhar and Yudiati, 2023) as well as stress salinity (Yudiati et al., 2024). Finding out the heat shock protein (HSPs) gene expression and insuline-like growth factor (IGF) effect concerning low salinity medium farming is challenging. HSPs and IGF serve as an evolutionarily conserved regulator that plays a crucial role in response to various environmental stressors in almost all organisms, including *L. vannamei*.

As described above, several studies have focused on the effects of alginate, microalgae, or probiotics as dietary supplements. Some previous studies also reported the benefits of those two combinations in combating *Vibrio* spp. However, there are few studies on combining all three synbiotic combinations in *L. vannamei* production. This work is even more noteworthy since it is administered in the low salinity medium (1 ppt). Therefore, this study aimed to evaluate the effects of oral feed supplementation of *L. bulgaricus* combined with alginate and SWE on survival, growth performance, and immune response, complete with heat shock protein and the insuline-like growth factor of *L. vannamei* based on gene expression in low salinity medium.

Materials and Methods

Production of alginate and spirulina hot water extract (SWE): The alginate production was referred to the methods by Yudiati and Isnansetyo (2017). Dried tropical *Sargassum* sp. from Teluk Awur Coast, Jepara, Central Java, Indonesia, was chopped using a commercial blender. Forty grams of *Sargassum* sp. powder was added into 1000 mL of aquadest containing 50 g of Na_2CO_3 and 18.617 g of EDTA.

The extract was then adjusted to pH = 8.5 with HCl, stirred well for 24 h, and filtered. The filtrate was then affixed with 0.13 M KCl and precipitated with 96% ethanol (1:1). The solution was then centrifuged (6 min, 4000 rpm) for separation. The pellet was then left for 24 hours in the drying cabinet. Based on FT-IR spectra, this sodium alginate (Alg) was fit to the standard alginate reference material (Sigma®, USA) (Yudiati et al., 2016), which has a molecular weight of 217.5 KDa and an acetylation level of 89.95% (Yudiati et al., 2018).

Tropical *S. platensis* powder was purchased from the brackish water Aquaculture Development Centre (BADC), Jepara, Indonesia. The hot-water extract of Spirulina was prepared based on Yudiati et al. (2021a) by transferring 10 g of *S. platensis* to 50 ml of deionized water, heating it at 70 for one hour, and leaving it for one night. The extract was centrifuged at 3500 rpm (15 mins) the following day. The supernatant was dried up with a cool dryer. The SWE was then stored in an amber bottle and saved in the refrigerator. The yield of the SWE was 2.50±0.3%.

Preparation of *Lactobacillus bulgaricus* FNCC–0041 and feed formulation: The lactic acid bacteria (LAB), namely *Lactobacillus bulgaricus* FNCC–0041, was provided, re-cultured, and prepared from CV. Hadid Mukti Karya, Indonesia. To re-culture, one dose of FNCC–0041 was cultured in de Man, Rogosa, and Shape Agar/MRS Broth for 24 hrs at 37°C. The culture was centrifuged the next day (4200 rpm, 15 mins), and then the pellet was diluted with sterile seawater. The density was evaluated in a spectrophotometer (600 nm) and adjusted to absorbance 2.0 or the density of 10^8 CFU mL^{-1} (Kongchum et al., 2022). The mixture of alginate, SWE, and LAB was arranged with the formulation of 3 g alginate and 5 mg SWE into 300 mL LAB, then mixed with 1 kg of feed.

Feeding Trial, experimental and design of *L. vannamei*: Shrimps provided from CV. Hadid Mukti Karya was acclimated in indoor concrete cement tanks (8 m^3) for 14 days and fed a commercial diet SGH®. The proximate analysis of SGH® feed had a water content of 11%, protein of 32-36%, crude fiber of 3%, lipid of 6.5-7.0%, ash of 12%, and energy of 16.5-17.0 MJ kg^{-1}. The next day, 1,000 shrimps (500 ind.m^2) at the initial weight of 0.2±0.05 g were reared in the semi-mass culture at 2 tons medium each.

The experiment of different medium shrimp cultures was carried out in a two-level factorial design. The first level was medium shrimp culture, i.e., Seawater at 1 (SW ppt) and 30 ppt (SW 30 ppt). The second level was the shrimp feed supplementation with alginate at 3.0 $g.kg^{-1}$ and SWE at 5.0 $mg.kg^{-1}$ (Alg 3.0 $g.kg^{-1}$ + 5.0 mg $SWE.kg^{-1}$). All treatments were conducted in triplicates. Tanks were fully aerated, and seawater quality was maintained by changing daily by 20%. Shrimps were reared for 56 days. During the experimental period, water temperature ranged from 25-27°C, pH 7.6-8.5, and dissolved oxygen (DO) concentration was 4.41-5.33 mg L^{-1} (DO meter, Hanna Instrument). Shrimp were fed the respective diets at a daily rate of 4% of body weight. Feed was administered four times/day (06.00, 11:00, 16.00 and 21.00). At the end of the experiment, the survival rate and biomass of *L. vannamei* were recorded. Absolute growth, denoted by the shrimp weight gain, was observed at 0, 25, 36, 45, 51, and 56.

The assessment of immune-related gene expression: The total RNA was extracted from shrimp haemolymph with a High Pure Viral-mRNA Extraction Kit (Roche, Germany) based on the manufacturer's procedure. cDNA was then synthesized from the total RNA using AMV Reverse Transcriptase Kit (Roche, Germany) at 42°C for 30 min, followed by transcriptase enzyme inactivated at 94°C for 5 min. The quantity and quality of the extracted RNA were assessed using absorbance at 260 and 280 nm. A 1.7-1.9 ratio was measured by a nanodrop instrument (Thermo Scientific NanoDrop 2000), verifying the purity of RNA. The extract was stored at −80°C until use. The cDNA of extracted total RNA from shrimp gill tissues was synthesized via reverse transcription. The expression of ProPhenol Oxidase, LGBP, Lectin, HSP70, and IGF genes were analyzed comparatively with Sybr Green (Kapa SYBER FAST qPCR Master Mix) (Kapa biosystems) with 7,500 Fast Real-Time PCR (Applied Biosystem)

Table 1. The primers sequence of qRT-PCR for immune-related genes of *Litopenaeus vannamei*.

Gene	Primer	Sequence (5'-3')	Accession no	Reference
proPO	F	TTCAACGGTAGACCCGTGATTCTTC	AY723296.1	(Wang et al., 2007)
	R	TCTTGCCGGGTTTAAGGTGAACAGT		
LGBP	F	CGG CAA CCA GTA CGG AGG ACC		(Cheng et al., 2005)
	R	GTG GAA ATC ATC GGC GAA GGA G		
MnSOD	F	AATTGGAGTGAAAGGCTCTGGCT	DQ005531	(Gómez-Anduro et al., 2006)
	R	ACGGAGGTTCTTGTACTGAAGGT		
IGF-2	F	CTCTGTACAGTCAGCCCAGC	XM02739466	(Sharawy et al., 2022)
	R	CACACCCAGTCAGTCCCAAG		
Lectin C	F	TTTGTAAACAACAGGCAGTTCCAC	EF583939.1	(Zhang et al., 2009)
	R	CTGTCTTTCATCAGAATGCTACCTC		
HSP70-2	F	TCCGAAGGACTGAGCTCTTG		(Jalbout et al., 2003)
	R	CAGCAAAGTCCTTGAGTCCC		
b-actin	F	CCTCCACCATGAAGATCAAGATCAT	AF300705.2	(Sun et al., 2007)
	R	CACTTCCTGTGAACAA TTGATGGTC		

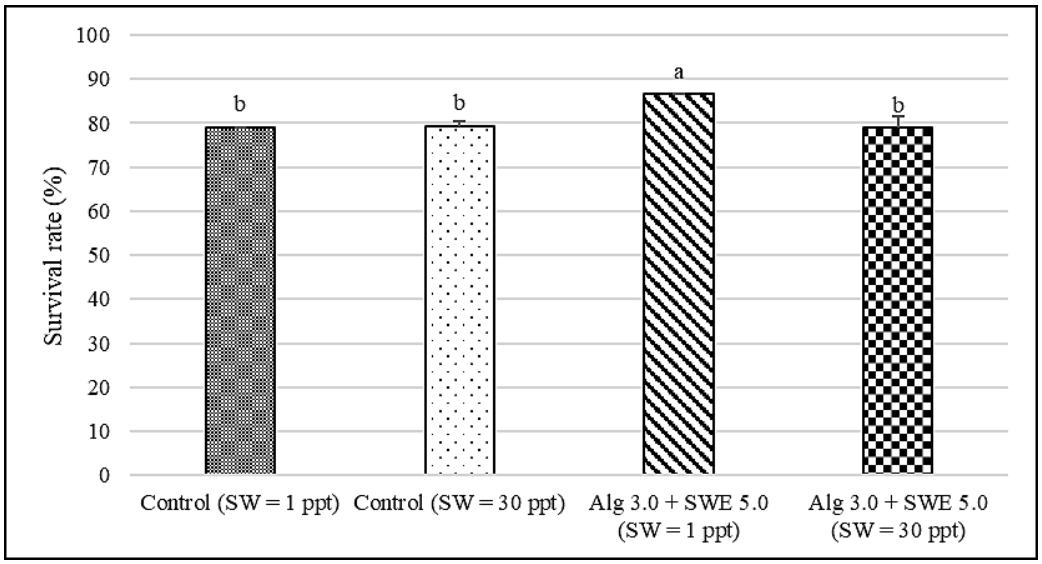

Figure 1. The survival rate of *Litopenaeus vannamei* with different treatments at the end of the experiment (Data with different letters specify a significant difference ($P \leq 0.05$)).

using the specific primers (Table 1). The qPCR program condition was adapted from Yudiati et al. (2016). To determine the gene expression fold level, the amplified data were analyzed by comparative techniques (Azhar and Yudiati, 2023), which were normalized using β-actin as a housekeeping gene (primer Lvbac-F dan Lvbac-R) for internal control. All the gene expressions in different treatments were examined at the end of the experiment.

Statistical analysis: The data were subjected to one-way and two-way analysis of variance (ANOVA) at a significance level of 0.05 using SPSS computer software. Before the analysis, the raw data were normalized using some transformation depending on the data type.

Results

Survival rate and growth of *L. vannamei*: The survival rate of shrimp with different treatments for 56 days of rearing is presented in Figure 1. Based on the results, the survival rate of shrimps from three treatments, namely Control (1 ppt, 30 ppt) and feed supplemented with (Alg 3.0 $g.kg^{-1}$ + 5.0 mg $SWE.kg^{-1}$) in 30 ppt medium, was similar. The best survival rate (86.50%) was achieved from (Alg 3.0 $g.kg^{-1}$ + 5.0 mg $SWE.kg^{-1}$) in 1 ppt medium ($P \leq 0.05$).

The final weight gain (56^{th} day) of shrimps was achieved from supplemented shrimp fed 30 ppt media, while controls (1 and 30 ppt) were the best treatments (Fig. 2). Strengthening this data, the best biomass was achieved from similar treatments as before, namely

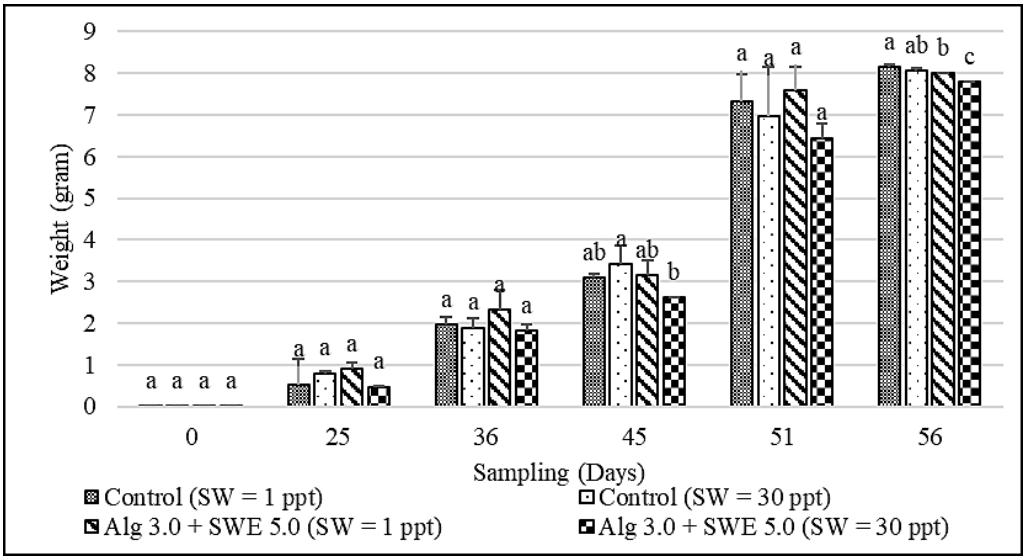

Figure 2. The weight gain of *Litopenaeus vannamei* with different mediums and diet supplementation at 0, 25, 36, 45, 51, and 56 days of the experiment (Data with different letters specify a significant difference ($P \leq 0.05$)).

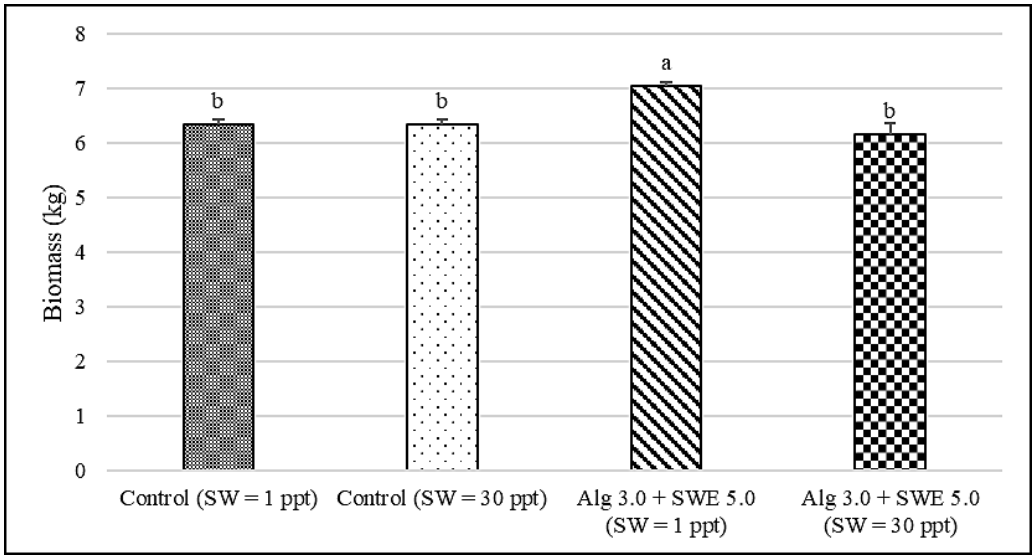

Figure 3. The biomass of *Litopenaeus vannamei* with different mediums and diet supplementation at the end of the experiment. Data with different letters specify a significant difference ($P \leq 0.05$).

supplemented shrimp fed on (Alg 3.0 g.kg^{-1} + 5.0 mg SWE.kg^{-1}) in 1 ppt medium ($P \leq 0.05$) (Fig. 3).

Immune-related (*ProPO, SOD, LGBP, Lectin*), growth (*IGF*), and heat shock protein (*HSP 70*) gene expression of *L. vannamei*: Compared to others, the Alg 3.0 g.kg^{-1} + 5.0 mg SWE.kg^{-1} treatment in 1 ppt medium resulted in the five times upregulated ProPhenol-Oxidase gene expression ($P \leq 0.05$) (Fig. 4) from feed-supplemented shrimp. Superoxide dismutase shows that control without supplementation in 1 ppt medium significantly differed from three other treatments (Fig. 5).

Figure 6 shows that shrimp supplemented with Alg 3.0 g.kg-1 + 5.0 mg SWE.kg^{-1} in 1 ppt reached five times higher Lipopolysaccharide Beta Glucan Binding Protein (*LGBP*) gene expression than the controls. Figure 7 shows Lectin gene expression in shrimp fed supplemented and non-supplemented diets in 1 and 30 ppt water medium. The highest upregulation on mRNA transcript related to lectin was achieved from shrimp fed Alginate 3g.kg^{-1} and SWE 5.0 mg.kg^{-1}, reared in 30 ppt medium.

Insulin-like growth factor (*IGF*) gene expression from shrimp-fed supplementation of alginate, SWE,

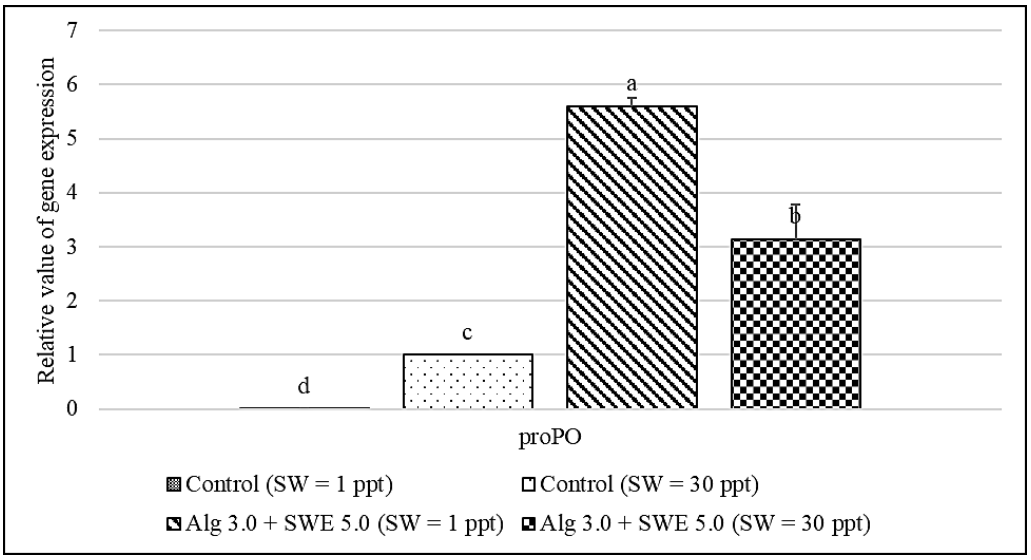

Figure 4. ProPhenol Oxidase gene expression of shrimp reared at different seawater medium and diet supplementation. Data with different letters specify a significant difference ($P \leq 0.05$).

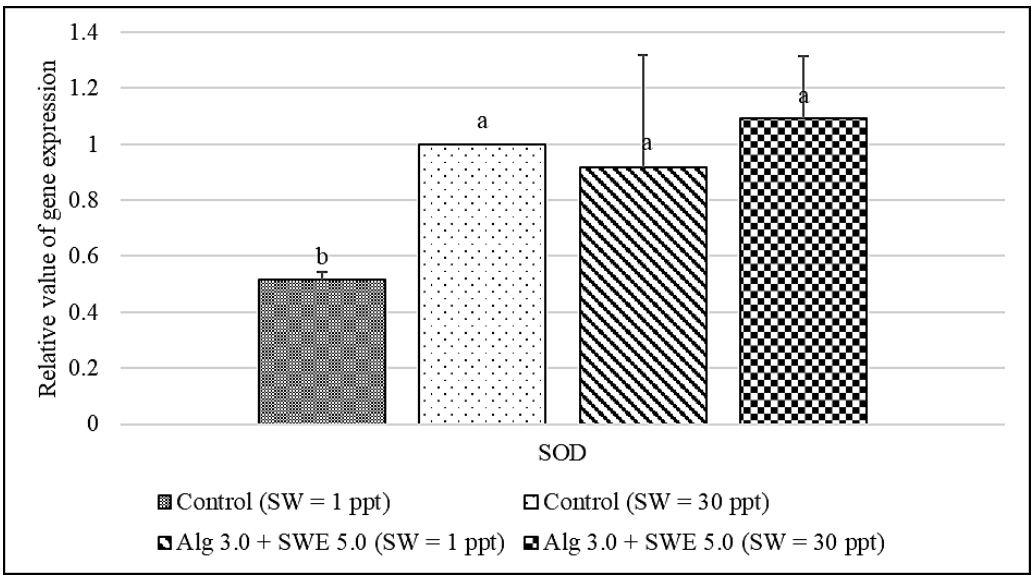

Figure 5. Super Oxide Dismutase gene expression of shrimp reared at different seawater mediums and diet supplementation. Data with different letters specify a significant difference ($P \leq 0.05$).

and *L. bugaricus* probiotics was upregulated more than 4.5 times higher than that of non-supplemented shrimps, both in 1 and 30 ppt medium (Fig. 8). This result is in line with the data on growth (Fig. 2) and biomass (Fig. 3) production of *L. vannamei* farming. The highest *HSP-2* gene expression was reached from shrimp fed Alginate 3g.kg^{-1} and SWE 5.0 mg.kg^{-1}, reared in 1 ppt medium (Fig. 9). HSPs serve as an evolutionarily conserved superfamily in response to various environmental stressors, i.e., low salinity medium in almost all organisms, including *L. vannamei*.

Discussions

In 56 days of rearing, the survival rate of all treatments was almost 80%, indicating good production performance. Surprisingly, the best survival rate was achieved from shrimps supplemented with prebiotics and probiotics in 1 ppt. Excellent nutritional factors from this treatment supported the obstacle of rearing *L. vannamei* in low salinity. Physiologically, low salinity disrupts the *L. vannamei* osmoregulation,

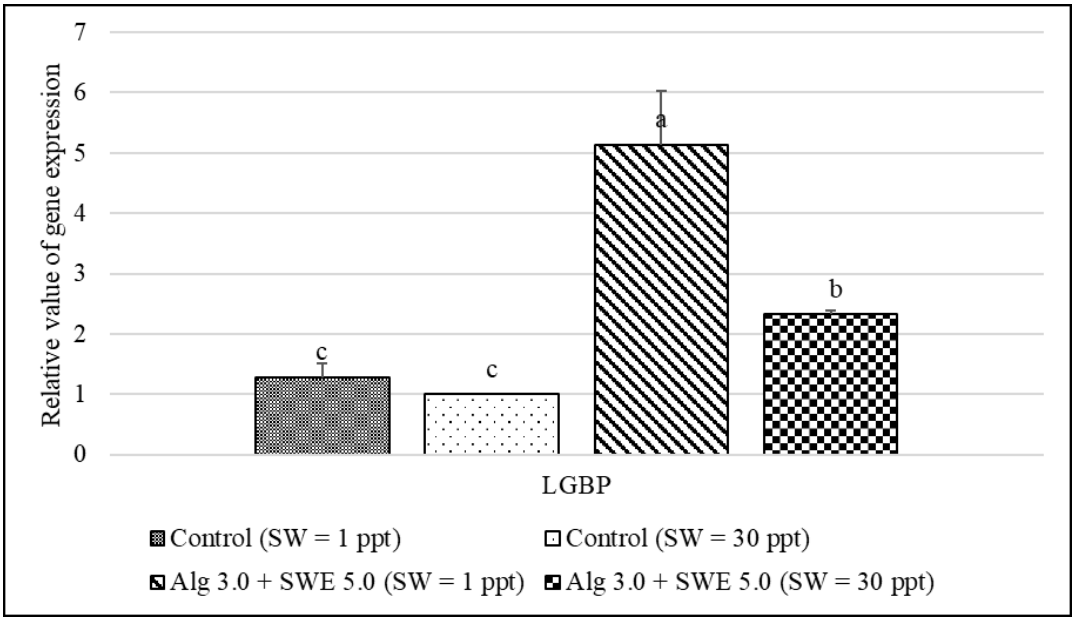

Figure 6. Lipopolysaccharide Beta Glucan Binding Protein gene expression of shrimp reared at different seawater mediums and diet supplementation. Data with different letters specify a significant difference ($P \leq 0.05$).

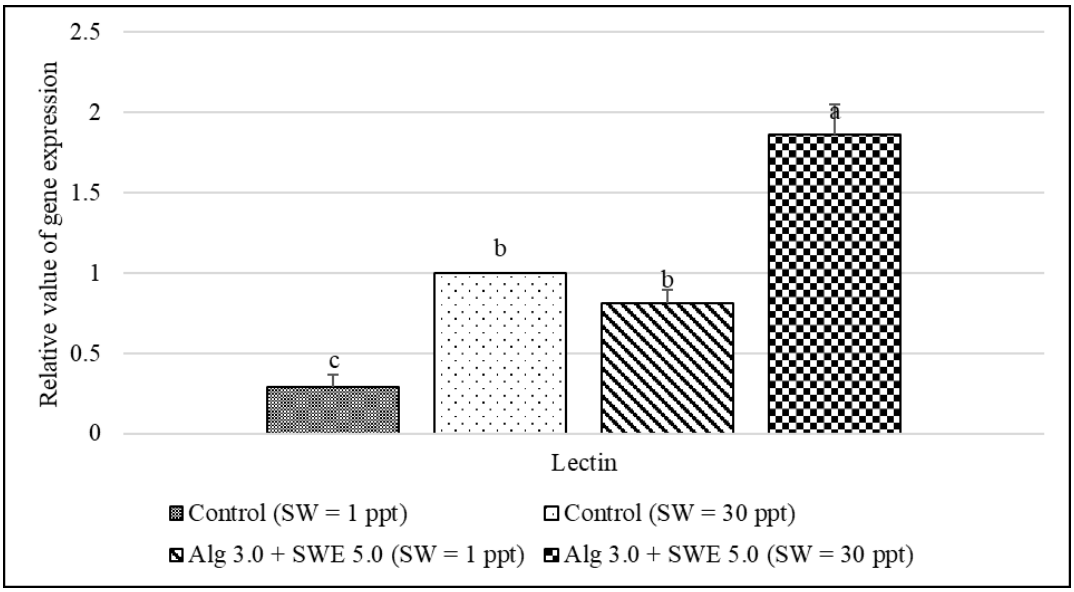

Figure 7. Lectin gene expression of shrimp reared at different seawater mediums and diet supplementation. Data with different letters specify a significant difference ($P \leq 0.05$).

necessitating an increased oxygen supply. Therefore, a high compound rich in antioxidant action to neutralize free radicals is immediately needed (Cao et al., 2022), which is fulfilled by SWE (Hidayati et al., 2020). Prebiotics alginate and SWE also show remarkable results in countering the shock by salinity exposure (Yudiati et al., 2024). It happens inside the shrimp body, and the water medium has a positive impact. Pimentel et al. (2023) reported that carbohydrate sources such as alginate contribute to an excessive load-up of microorganisms to support water quality. As mentioned before, the water quality in all treatments was in appropriate condition during shrimp rearing.

Despite the survival rate, the growth of *L. vannamei* is in accordance. The three combinations containing alginate, SWE, and *L. bulgaricus* achieved the best growth performance. The nutritional quality

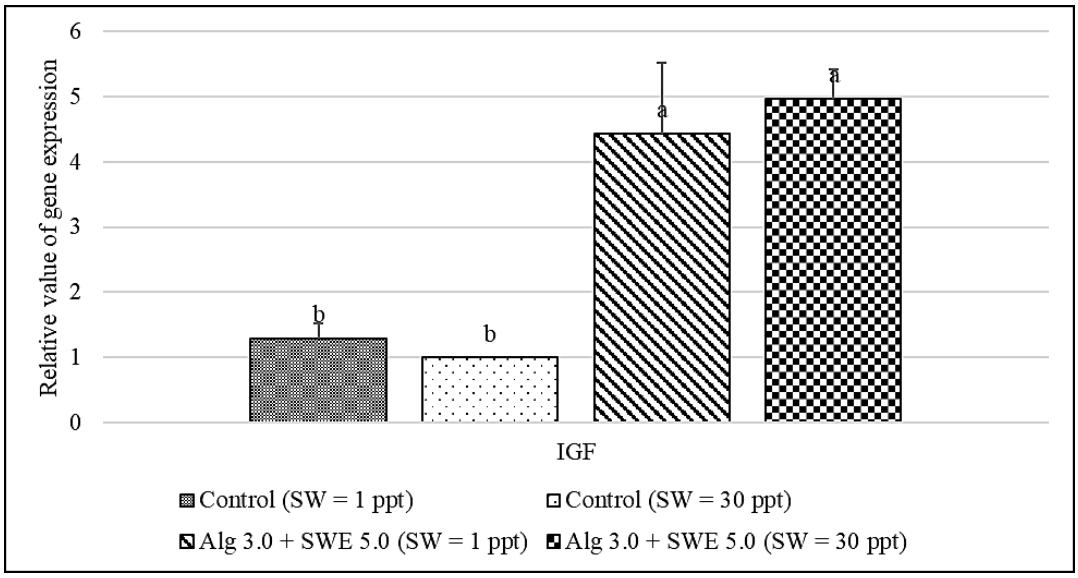

Figure 8. IGF gene expression of shrimp reared at different seawater mediums and diet supplementation. Data with different letters specify a significant difference ($P \leq 0.05$).

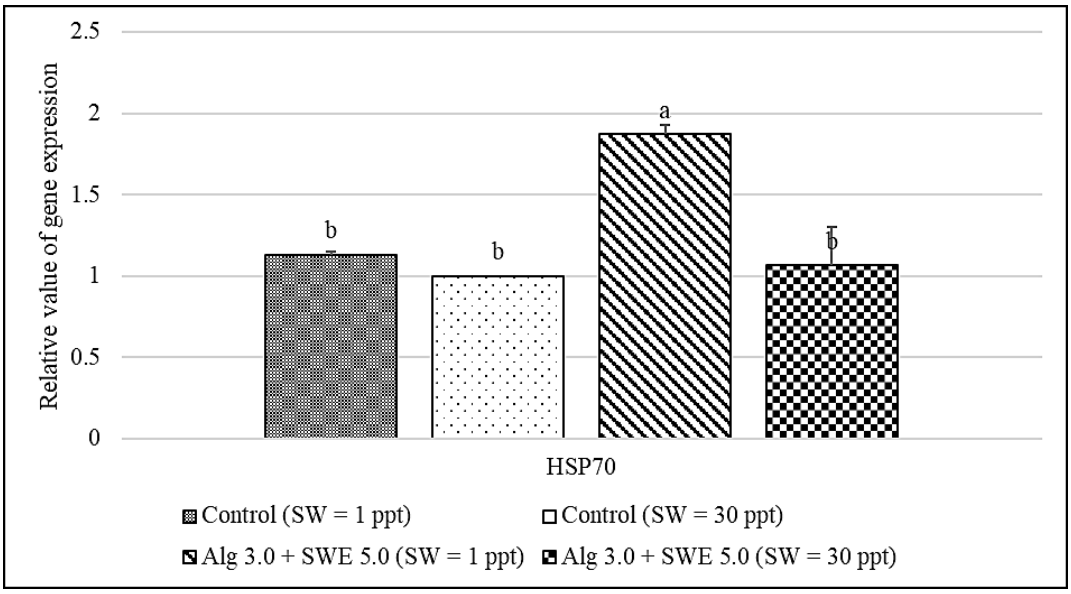

Figure 9. HSP70 gene expression of shrimp reared at different seawater mediums and diet supplementation. Data with different letters specify a significant difference ($P \leq 0.05$).

of aquatic organisms strongly influences growth, particularly in low salinity medium. Previous research noted that supplementation sodium alginate, which contains polysaccharide, managed to perform the best *L. vannamei* growth in a 20-ton mass circle pond at low concentration ($2 g.kg^{-1}$) (Yudiati et al., 2023b). *Lactobacillus bulgaricus* secreted exopolysaccharides (Daba et al., 2021), selectively adhered to mucosal surfaces of shrimp gut, and built cell-bound biosurfactants, displacing the pathogenic strains. These three combinations of pre and probiotics alleviated the undesirable effects of low-salinity stress.

The key parameter of shrimp's non-specific immune system is Phenol Oxidase (PO) (Yudiati et al., 2016; Yudiati et al., 2019; Azhar and Yudiati, 2023). As notable, *L. vannamei* rearing in a medium with low salinity disrupts the PL osmoregulation and increases osmotic pressure, followed by immune system disorder. The supplementation of pre and probiotics in

the shrimp diet upregulated immune-related genes. The pattern recognition receptors of shrimp recognize the L-guluronic acid and D-mannuronic acid as pathogen-associated molecular patterns (PAMPs) of alginate, followed by cell-to-cell communication, which then produces ProPO and PO and regulates the RNA transcripts.

Earlier study denoted that SWE has a high antioxidant activity derived from its phenolic compound (26.64±0.16 mg/GAE samples) and protein-pigmented-phycocyanin content (0.301±0.09 mg/g) (Hidayati et al., 2020). SWE was also accomplished to promote the immune defense for combating three pathogenic *Vibrio* spp. (Yudiati et al., 2021a). Moreover, the shrimp succeeded in fastening the recovery rate of shrimp infected with *Vibrio parahaemolyticus* after being fed on a diet supplemented with alginate and SWE (Azhar and Yudiati, 2023).

β-Glucan is a complex polysaccharide in yeast, grains, mushrooms, and microalgae, including Spirulina. Spirulina polysaccharide extracted with hot water (SWE) is distinguished by PAMPs in molecules that can improve immunity (Qiao et al., 2022). Oral supplementation of alginate and SWE in the shrimp diet consistently improved the gene expression of PO and LGBP (Yudiati et al., 2016; Yudiati et al., 2019; Azhar and Yudiati, 2023). Furthermore, alginate co-activated with *L. bulgaricus* enhanced the ProPO and LGBP gene expression (Suryono et al., 2024).

In invertebrates, lectins are a crucial group of Pattern Recognition Proteins (PRRs), activating the innate immune response. Viana et al. (2024) reported that lectin gene expression remarkably upregulated after the infectious myonecrosis virus IMNV challenge compared to before the challenge. This is similar to the study reported by Yudiati et al. (2016) for the shrimp after challenging with White Spot Syndrome Virus (WSSV) and in AHPND-infected shrimps (Tran-Van et al., 2021). The regulation of lectin gene expression may relate to the pathogenic challenge or medium salinity since the results from this study are slightly different.

Hyposalinity stress has been shown to harm aquaculture species' growth and physiology (Feng et al., 2023). Though *L. vannamei* can be cultured on a large scale in low salinity, this does not imply that this shrimp can generate an ideal physiological response. Shrimp reared at low salinity can have passive effects, including a low survival rate (53.3%) at a salinity of 5 ppt and raises in oxygen utilization and feed quantities (Ye et al., 2009). Some researchers also reported that low salinity could decrease non-specific immune parameters (Esparza-Leal et al., 2019) and even destroy intestinal microbiota structure during aquatic animal culture (Qiao et al., 2022). It has been proven that the IGF gene expression demonstrates the best achievement of supplementing alginate, SWE, and *L. bulgaricus* in 1 and 30 ppt salinity of *L. vannamei* culture.

Aquatic organisms adopt a cascade of protective mechanisms to enhance their survival and cope with the possible threats posed by the unfriendly and even harmful environment. HSPs are the most widely studied proteins activated by various stressors, including salinity. They account for 5-10% of the total protein content and are rapidly synthesized within cells under stress. Therefore, HSPs have been generally employed as important candidates to assist organisms withstanding environmental stress (Gao et al., 2022).

These results outstandingly strengthen the previous study concerning the potency of alginate (Yudiati et al., 2016, 2019, 2021b, 2023b; Suryono et al., 2024) and Spirulina (Yudiati et al., 2021a, 2023a, 2024; Azhar and Yudiati, 2023) as probiotics, in combination with *L. bulgaricus* probiotics (Yudiati et al., 2021a, b, 2023a) to promote the non-specific immune response by cellular, humoral and gene expression based.

Conclusion

The addition of prebiotics from alginate, Spirulina water extract, and *Lactobacillus* probiotics synergically improve the survival rate and growth of *L. vannamei* by upregulating the mRNA transcripts of non-specific innate immune (ProPO, SOD, and LGBP) gene expression to alleviate low-salinity

farming. In addition, this synbiotics addition could enhance HSP and IGF gene expression as crucial keys for low salinity stressors. It is recommended that the alginate and SWE dose added to the diet be 3 $g.kg^{-1}$ and 5.0 $mg.kg^{-1}$ to develop survival, growth, and biomass production. These results can deliver a reference for the practical application in aquaculture, contributing to the healthy, green, environmentally friendly, and sustainable development of the global aquaculture industry of low salinity *L.vannamei* farming.

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References

Al-Subiai S.N., Jang I.K., Bae S.-H., Yoon H.-s., Hussain S., AlNuaimi S., Al-Foudari M. Al-Hasan E. (2025). Enhancing the performance of *Litopenaeus vannamei* nursery and grow-out by modifying Mg/Ca ratios in biofloc systems using low-salinity groundwater of Kuwait Desert. Aquaculture, 594: 741405.

Azhar N., Yudiati E. (2023). Outbreak simulation of *Litopenaeus vannamei* recovery rate with oral alginate and spirulina diet supplementation against *Vibrio parahaemolyticus* AHPND. Aquaculture International, 31(3): 1659-1676.

Bahi A., Ramos-Vega A., Angulo C., Monreal-Escalante E. Guardiola F.A., (2023). Microalgae with immunomodulatory effects on fish. Reviews in Aquaculture, 15(4): 1522-1539.

Chen K., Li E., Xu C., Wang X., Lin H., Qin J.G. Chen L. (2015). Evaluation of different lipid sources in diet of pacific white shrimp *Litopenaeus vannamei* at low salinity. Aquaculture Reports, 2: 163-168.

Cheng W., Liu C.-H., Kuo C.-M. Chen J.-C. (2005). Dietary administration of sodium alginate enhances the immune ability of white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Fish and Shellfish Immunology, 18(1): 1-12.

Daba G.M., Elnahas M.O., Elkhateeb W.A. (2021). Contributions of exopolysaccharides from lactic acid bacteria as biotechnological tools in food, pharmaceutical, and medical applications. International Journal of Biological Macromolecules, 173: 79-89.

Esparza-Leal H.M., Ponce-Palafox J.T., Cervantes-Cervantes C.M., Valenzuela-Quiñónez W., Luna-González A., López-Álvarez E.S., Vázquez-Montoya N., López-Espinoza M., Gómez-Peraza R.L. (2019). Effects of low salinity exposure on immunological, physiological and growth performance in *Litopenaeus vannamei*. Aquaculture Research, 50(3): 944-950.

FAO. (2024). Fishery and aquaculture statistics – Yearbook 2021. FAO Yearbook of Fishery and Aquaculture Statistics, Romehttps://doi.org/10.4060/cc9523en

Feng H., Wang X., Zhou H., Mai K., He G., Liu C. (2023). Involvement of insulin-like growth factor binding proteins (IGFBPs) and activation of insulin/IGF-like signaling (IIS)-target of rapamycin (TOR) signaling cascade in pacific white shrimp, *Litopenaeus vannamei* exposed to acute low-salinity. Aquaculture Reports, 30: 101627.

Gao Y., Li J.N., Pu J.J., Tao K.X., Zhao X.X., Yang Q.Q. (2022). Genome-wide identification and characterization of the HSP gene superfamily in apple snails (Gastropoda: Ampullariidae) and expression analysis under temperature stress. International Journal of Biological Macromolecules, 222: 2545-2555.

Gómez-Anduro G.A., Barillas-Mury C.-V., Peregrino-Uriarte A.B., Gupta L., Gollas-Galván T., Hernández-López J., Yepiz-Plascencia G. (2006). The cytosolic manganese superoxide dismutase from the shrimp *Litopenaeus vannamei*: Molecular cloning and expression. Developmental and Comparative Immunology, 30(10): 893-900.

Hidayati J.R., Yudiati E., Pringgenies D., Oktaviyanti D.T., Kusuma A.P. (2020). Comparative study on antioxidant activities, total phenolic compound and pigment contents of tropical *Spirulina platensis*, *Gracilaria arcuata* and *Ulva lactuca* extracted in different solvents polarity. E3S Web of Conferences, 147: 03012.

Jalbout M., Bouaouina N., Gargouri J., Corbex M., Ben Ahmed S., Chouchane L. (2003). Polymorphism of the stress protein HSP70-2 gene is associated with the susceptibility to the nasopharyngeal carcinoma. Cancer Letters, 193(1): 75-81.

Kongchum P., Chimtong S., Prapaiwong N. (2022). Association between single nucleotide polymorphisms of nLvALF1 and PEN2-1 genes and resistance to *Vibrio parahaemolyticus* in the Pacific white shrimp *Litopenaeus vannamei*. Aquaculture and Fisheries, 7(4):

373-381.

Li E., Arena L., Lizama G., Gaxiola G., Cuzon G., Rosas C., Chen L., Van Wormhoudt A. (2011). Glutamate dehydrogenase and Na+-K+ ATPase expression and growth response of *Litopenaeus vannamei* to different salinities and dietary protein levels. Chinese Journal of Oceanology and Limnology, 29(2): 343-349.

Li Z., Chang T., Han F., Fan X., Liu W., Wu P., Xu C., Li E. (2024). Effects of myo-inositol on growth and biomarkers of environmental stress and metabolic regulation in Pacific white shrimp (*Litopenaeus vannamei*) reared at low salinity. Comparative Biochemistry and Physiology Part D: Genomics and Proteomics 50: 101216.

Liu L., Cai X., Ai Y., Li J., Long H., Ren W., Huang A., Zhang X., Xie Z.-Y. (2022). Effects of *Lactobacillus pentosus* combined with *Arthrospira platensis* on the growth performance, immune response, and intestinal microbiota of *Litopenaeus vannamei*. Fish and Shellfish Immunology, 120: 345-352.

Pimentel O.A.L.F., de Oliveira V.Q., de Andrade R.J.V., Oliveira C.Y.B.d., dos Santos E.P., Pereira A.M.L., Brito L.O., Gálvez A.O. (2023). Plankton community in *Penaeus vannamei* nurseries with synbiotic system under different frequencies of adjustment on Ca:Mg:K ratio in low salinity water. Aquaculture and Fisheries, https://doi.org/10.1016/j.aaf.2023.09.003

Qiao Y., Zhou L., Qu Y., Lu K., Han F., Li E. (2022). Effects of different dietary β-Glucan levels on antioxidant capacity and immunity, gut microbiota and transcriptome responses of white shrimp (*Litopenaeus vannamei*) under low salinity. Antioxidants, 11(11).

Sharawy Z.Z., Ashour M., Labena A., Alsaqufi A.S., Mansour A.T., Abbas E.M. (2022). Effects of dietary Arthrospira platensis nanoparticles on growth performance, feed utilization, and growth-related gene expression of Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture, 551: 737905.

Su C., Li J., Zhang M., Pan L., Wang Y., Ding Y., Chen Z. Lu M. (2023). Dietary cholesterol enhances osmoregulation, antioxidant defenses and immune response of *Litopenaeus vannamei* to alleviate the macromolecular damage induced by salinity stress. Aquaculture, 563: 738861.

Sun J., Wang L., Wang B., Guo Z., Liu M., Jiang K., Luo Z. (2007). Purification and characterisation of a natural lectin from the serum of the shrimp *Litopenaeus vannamei*. Fish and Shellfish Immunology, 23(2): 292-299.

Suryono C.A., Santoso A., Yudiati E., Yulianto B., Sunaryo S., Azhar N., Alghazeer R. (2024). The impact of varying alginate co-activation with probiotics on the artemia bioencapsulation to enhance immunity against *Vibrio* spp. Indonesian Journal of Marine Sciences. https://doi.org/10.14710/ik.ijms.29.3.372-384

Tran-Van H., Nguyen P.T.T., Mai Q.-G., Tran T.L. (2021). Production and purification of polyclonal antibody against shrimp lectin LvLTLC1. International Aquaculture Research, 13(4): 233-240.

Viana J.T., Rocha R.D.S. Maggioni R. (2024). Immunological lectins in shrimp *Penaeus vannamei* challenged with infectious myonecrosis virus (IMNV) under low-salinity conditions. Fish and Shellfish Immunology, 148: 109471.

Wang Y.C., Chang P.S., Chen H.Y. (2007). Tissue expressions of nine genes important to immune defence of the Pacific white shrimp *Litopenaeus vannamei*. Fish and Shellfish Immunology, 23(6): 1161-1177.

Xie S.-w., Tian L.-x., Jin Y., Yang H.-j., Liang G.-y., Liu Y.-J. (2014). Effect of glycine supplementation on growth performance, body composition and salinity stress of juvenile Pacific white shrimp, *Litopenaeus vannamei* fed low fishmeal diet. Aquaculture, 418-419: 159-164.

Ye L., Jiang S., Zhu X., Yang Q., Wen W., Wu K. (2009). Effects of salinity on growth and energy budget of juvenile *Penaeus monodon*. Aquaculture, 290(1): 140-144.

Yudiati E., Isnansetyo A., Murwantoko, Ayuningtyas, Triyanto Handayani C.R. (2016). Innate immune-stimulating and immune genes up-regulating activities of three types of alginate from *Sargassum siliquosum* in Pacific white shrimp, *Litopenaeus vannamei*. Fish and Shellfish Immunology, 54: 46.

Yudiati E., Isnansetyo A. (2017). Characterizing the Three Different Alginate Type of *Sargassum siliquosum*. Ilmu Kelaut, 22(1): 7.

Yudiati E., Pringgenies D., Djunaedi A., Arifin Z., Sudaryono A. (2018). Free Radicals Scavenging Activities of Low Molecular Weight Sodium Alginate (LMWSA) from *Sargassum polycystum*, Produced by Thermal Treatment. Aquacultura Indonesiana, 19(1): 21-27.

Yudiati E., Isnansetyo A., Murwantoko, Triyanto Handayani C.R. (2019). Alginate from *Sargassum siliquosum* simultaneously stimulates innate immunity,

upregulates immune genes, and enhances resistance of Pacific white shrimp (*Litopenaeus vannamei*) against white spot syndrome virus (WSSV). Marine Biotechnology, 21(4): 503.

Yudiati E., Sedjati S., Azhar N., Oktarima W.A., Arifin Z. (2021a). Spirulina water extract and *Lactobacillus bulgaricus* FNCC– 0041, *Streptococcus thermophilus* FNCC–0040 secretion as immunostimulants in gnotobiotic *Artemia* challenge tests against pathogenic *Vibrio parahaemolyticus, V. vulnificus,* and *V. harveyi*. IOP Conference Series: Earth and Environmental Science, 890(1): 012018.

Yudiati E., Wijayanti D.P., Azhar N., Chairunnisa A.I., Sedjati S., Arifin Z. (2021b). Alginate oligosaccharide/polysaccharide and lactic acid bacteria (*Lactobacillus bulgaricus* FNCC–0041 & *Streptococcus thermophilus* FNCC–0040) as immunostimulants against pathogenic *Vibrio* spp. using *Artemia* bio model. IOP Conference Series: Earth and Environmental Science, 919(1): 012060.

Yudiati E., Arifin Z., Santoso A., Hidayati J.R., Alghazeer R., Azhar N. (2023a). Artemia with Synbiotics Enrichment Improves Resistance Against *Vibrio parahaemolyticus* AHPND of *Litopenaeus vannamei Larvae*. Buletin Oseanografi Marina, 12(3): 8.

Yudiati E., Widiasa I.N., Sunaryo S., Ridhuwan R., Tadeus D.Y., Arifin Z., Mangkusasmito F., Sugiyanto S., Setyawan D.A. (2023b). Oral supplementation of low alginate dose in diet stimulates immune response of *Litopenaeus vannamei* at concrete circle pond mass culture. IOP Conference Series: Earth and Environmental Science, 1260(1): 012006.

Yudiati E., Kurniawan P.N.R., Putri A.N., Al Falah I.H., Azhar N., Sedjati S., Arifin Z., Santosa G.W. (2024). Benefits of Alginate and Spirulina Supplementation to the Growth and Resistance Against Salinity Exposure in *Litopenaues vannamei* Post-Larvae. Ribarstvo, 82(2): 65-72.

Zahan N., Hossain M.A., Islam M.R., Saha J., Akter T., Fatema U.K., Haque F. (2024). Effects of dietary *Spirulina platensis* on growth performance, body composition, haematology, immune response, and gut microflora of stinging catfish *Heteropneustes fossilis*. Aquaculture Reports, 35: 101997.

Zhang Y., Qiu L., Song L., Zhang H., Zhao J., Wang L., Yu Y., Li C., Li F., Xing K., Huang B. (2009). Cloning and characterization of a novel C-type lectin gene from shrimp *Litopenaeus vannamei*. Fish and Shellfish Immunology, 26(1): 183-192.

Zhu G., Lu K., Lai Y., Wang L., Wang F., Li N., Peng Y., Gong H. (2024). Effects of dietary 25-hydroxyvitamin D3 on growth, calcium-phosphorus metabolism, lipid metabolism and immunity of *Litopenaeus vannamei* at low salinity. Aquaculture Reports, 35: 101965.