GROWTH PERFORMANCE, SURVIVAL RATE, AND RESISTANCE AGAINST AHPND OF *Litopenaeus vannamei* JUVENILES FED WITH SYNBIOTIC BIO-ENCAPSULATED ARTEMIA

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ABSTRACT

The whiteleg shrimp (*Litopenaeus vannamei*) is a highly valued aquaculture species globally, yet its production faces challenges due to disease outbreaks, notably acute hepatopancreatic necrosis disease (AHPND). This study aimed to evaluate the growth and survival of *L. vannamei* juveniles fed with synbiotic bio-encapsulated *Artemia* and their resilience against AHPND-causing *Vibrio parahaemolyticus* and salinity stress. The experiment employed a completely randomized design with two treatments: one with synbiotic-enriched *Artemia* (600 ppm alginate and *Lactobacillus bulgaricus*) and a control without synbiotic. Each treatment was replicated five times, using 600 juveniles at a density of 30 post-larvae per L over a 14-day rearing period. Growth and survival metrics were recorded, followed by challenge tests for AHPND and salinity shock. The juveniles' survival rate was recorded 54 hours post-infection with *Vp*AHPND and every 10 minutes for 230 minutes after salinity exposure until 100% mortality. Results indicated higher survival (92.0 ± 9%), length gain (243.33 ± 18.80 mm), specific growth rate (18.44±2.01%), and stress tolerance in juveniles fed synbiotic encapsulated *Artemia* compared to the control. The survival rates for the challenge test with AHPND and salinity shock were similarly improved under synbiotic treatment, suggesting that synbiotics significantly benefit nursery production of *L. vannamei*. This study highlights the potential of synbiotic application in enhancing the resilience and growth of *L. vannamei* against common stressors in aquaculture, indicating its potential to support more sustainable shrimp farming practices.

KEYWORDS: salinity; shrimp; synbiotic; *Vibrio*

*ABSTRAK: **Kinerja Pertumbuhan, Tingkat Kelangsungan Hidup, dan Resistansi terhadap AHPND pada Benih** Litopenaeus vannamei **yang Diberi Pakan dengan** Artemia **yang Dibioenkapsulasi Sinbiotik***

*Udang vaname **(Litopenaeus vannamei)** adalah komoditas budidaya bernilai tinggi di seluruh dunia, namun produksinya menghadapi tantangan akibat wabah penyakit, terutama serangan acute hepatopancreatic necrosis disease (AHPND). Penelitian ini bertujuan untuk mengevaluasi pertumbuhan dan kelangsungan hidup benih **L. vannamei** yang diberi pakan **Artemia** yang*

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*dibioenkapsulasi dengan sinbiotik serta ketahanannya terhadap **Vibrio parahaemolyticus** penyebab AHPND dan stres salinitas. Eksperimen ini menggunakan rancangan acak lengkap dengan dua perlakuan: satu dengan **Artemia** diperkaya sinbiotik (600 ppm alginat dan **Lactobacillus bulgaricus**) dan kontrol tanpa sinbiotik. Setiap perlakuan diulang lima kali, dengan menggunakan 600 ekor benih udang pada kepadatan 30 ekor pascalarva per L selama 14 hari periode pemeliharaan. Parameter pertumbuhan dan kelangsungan hidup dicatat, diikuti dengan uji tantang terhadap AHPND dan kejutan salinitas. Tingkat kelangsungan hidup benih dicatat 54 jam pasca-infeksi dengan VpAHPND dan setiap 10 menit selama 230 menit setelah paparan salinitas hingga mortalitas mencapai 100%. Hasil menunjukkan kelangsungan hidup yang lebih tinggi (92,0 ± 9%), peningkatan panjang (243,33 ± 18,80 mm), laju pertumbuhan spesifik (18,44 ± 2,01%), dan toleransi stres yang lebih baik pada benih udang yang diberi pakan **Artemia** berenkapsulasi sinbiotik dibanding kontrol. Tingkat kelangsungan hidup pada uji tantang dengan AHPND dan kejutan salinitas juga meningkat dengan perlakuan sinbiotik, menunjukkan bahwa sinbiotik memberikan manfaat signifikan pada produksi **L. vannamei** fase pendederan. Penelitian ini menunjukkan adanya potensi aplikasi sinbiotik dalam meningkatkan ketahanan dan pertumbuhan **L. vannamei** terhadap stresor umum dalam akuakultur, serta potensinya untuk mendukung kegiatan budidaya udang yang lebih berkelanjutan.*

***KATA KUNCI: salinitas; sinbiotik; udang;** Vibrio*

INTRODUCTION

The white leg shrimp (*Litopenaeus vannamei*) is one of the main productive crustaceans among fishery trading commodities globally and is the most important species of shrimp culture in Indonesia. According to Food and Agriculture Organization (2020), white shrimp production makes up approximately 52.9% of crustaceans with a quantity of 4,966,200 MT in aquaculture worldwide, and Asian countries generate 80 % of the world's shrimp production (Diwan *et al.*, 2022).

Healthy and high-quality *L. vannamei* post-larvae that are free from diseases originating from advanced hatcheries and from the nurseries with good sanitation, adequate feeding, proper water quality, and quality control are needed to provide a successful shrimp farming to rear in the grow-out phase (Rodríguez-Olague *et al.*, 2021). Remarkably, the shrimp PLs stage needs a nutritious feed throughout the nursery stage to insure high-quality marine nutritional elements (Ayisi *et al.*, 2017). *Artemia* is one of the highly essential live foods consumed in aquaculture production. Nauplii developing from the viable dormant cyst of *Artemia* is the best and most suitable larval live food that transformed the hatchery invention of aquaculture organisms (Dhont & Sorgeloos, 2002). However, when used as a singular food source, *Artemia* nauplii often do not provide adequate nutritional support for larval development. Moreover, *Artemia* needs appropriate nutrition immediately after hatching or they die within several days (Nishida *et al.*, 2023). Several researchers have reported *Artemia* enrichment by *Spirulina* water extract (Yudiati *et al.*, 2024), *Chlorogonium capillatum* (Nishida *et al.*, 2023), and *Isochrysis galbana* (Martelli *et al.*, 2020) can successfully supply basic nutrients for the development of *Artemia* nauplii.

Bacterial diseases such as acute hepatopancreatic necrosis diseases (AHPND) have caused massive losses for global penaeid shrimp aquaculture (Kumar *et al.*, 2020). Once the shrimp including PLs are infected with AHPND-causing *Vibrio parahaemolyticus*, these bacteria initially colonize the shrimp's gut and

digestive tract. Subsequently, bacterial toxins such as specific binary pirA-pirB release into the hepatopancreas and induce the exuviation of the tubule epithelial cells (Chin *et al.*, 2024b). The most deadly strain is the early *L. vannamei* juvenile mortality syndromethat causes total mortality in 24 hours of infection (Choi *et al.*, 2017). Some evidence shows that the health status of shrimp is closely interrelated to gut microbiota (Chin *et al.*, 2024a) through maintenance of the immune system (Azhar & Yudiati, 2023). It is not adequate to evaluate the performance of PL shrimp by determining their survival and growth alone, but by assessing their quality as well. This can be completed through *V. parahaemolyticus* AHPND challenge and stress, as well as the salinity stress test (Yudiati *et al.*, 2024).

Alginate, the polysaccharide which water based extracted from *Sargassum* sp. cell wall is recognized as an immunostimulant (Azhar & Yudiati, 2023; Yudiati *et al.*, 2016; Yudiati *et al.*, 2019). Alginate also has a potency as a prebiotic (Yudiati *et al.*, 2020) and it has worked synergically as a synbiotic with *Lactobacillus bulgaricus* (lactic acid bacteria, probiotics) (Yudiati *et al.*, 2021c). Furthermore, Chin *et al.* (2024a) showed that synbiotic of *Lactobacillus plantarum* L20 and *Sargassum polycystum* can increase gut bacterial diversity, immune response, and impart some *V. parahaemolyticus* AHPND resistance. We have evaluated sodium alginate in different doses demonstrating good potency as a synbiotic and was synergetic with *Spirulina* water extract to counteract *V. parahaemolyticus* AHPND in early stage *L. vannamei* PL (Yudiati *et al.*, 2024) and *Artemia* (Yudiati *et al.*, 2021b; Yudiati *et al.*, 2021c). The key mechanism is the enhancement of the immune system and balancing of gut microbiota that may provide a blockade against pathogen invasion, initiate host nutrient absorption through extracellular product secretion, and/or accelerate immune response (Libertucci & Young, 2019).

Global climate change, has increased seasonal temperature and salinity variability, especially in Indonesia and other tropical areas becoming more unpredictable (Yudiati *et al.*, 2023a). Among the various environmental stress test methods, such as variable pH, oxygen, and temperature exposure, salinity shock valuation is the most appropriate and useful instrument in penaeid PLs (Istiqomah *et al.*, 2024). However, there is still a little information regarding the performance *L. vannamei* PL fed on *Artemia* bioencapsulated with the synbiotic of sodium alginate (prebiotics) and *L. bulgaricus* (lactic acid bacteria, probiotics) in inhibiting the virulence *V. parahaemolyticus* which causes AHPND and salinity stress. This study aims to find out the survival and growth of *L vannamei* PLs in 14 days of rearing fed on synbiotic and non-synbiotic *Artemia* bioencapsulation and the resistance against *Vp*AHND, consequently stress salinity at the end of the experiment.

MATERIALS AND METHODS

Sodium Alginate Production

This research was conducted in Biology Laboratory, E Building, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang from July to August 2023. The sodium alginate used in this research was sourced from seaweed extraction based on a method previously reported by Azhar & Yudiati (2023), with several modifications. Briefly, a total of 10 g of dry *Sargassum* sp. powder was mixed with 12.5 g Na_2CO_3, and dissolved in 250 mL in distilled water. The pH of the solution was adjusted to 8.5 with HCl solvent and filtered. Then, 0.13 M KCl was added to the solution after filtration and precipitated with 96% absolute ethanol for 24 hours at 60°C in the oven (Memmert UN 30). The precipitate was then centrifuged (Thermofisher, Megafuge ST1) at 3,500 rpm for 20 minutes (Thermofisher, Megafuge ST1). The precipitate or solid material was used as alginate and dried at temperature 60°C for 60 minutes. Alginate powder was stored in the refrigerator (Samsung, RT28) at a temperature of 4°C until used. The previous data have shown that this prepared sodium alginate was equivalent to the relatively expensive alginate by Sigma®, USA (Yudiati *et al.*, 2016).

Synbiotic Preparation and *Artemia* Bioencapsulation

Lactobacillus bulgaricus, lactic acid bacteria, were collected and supplied by Tropical Marine Biotechnology Laboratory, Faculty of Fisheries and Marine Science, Diponegoro University. One colony of lactic acid bacteria (LAB) was cultured on de Man, Rogosa and Shape Agar media (Merck, US). The growing colonies (one scoop of inoculation needle) were then inoculated in 100 mL of nutrient broth (Merck, US) and incubated in an incubator (Memmert, IN) at 37°C overnight (Yudiati *et al.*, 2021b; Yudiati *et al.*, 2021c). The synbiotic was made by mixing LAB and sodium alginate with a final concentration of 600 ppm based on the best results from our previous research (Yudiati *et al.*, 2023a). The synbiotic was prepared by mixing 20 mL of LAB liquid culture (concentration 10^8 cfu.mL^{-1}), 0.018 g of sodium alginate, and 180 mL of sterile seawater with a final concentration of 600 ppm based on the best results from our previous research (Yudiati *et al.*, 2023a). Incubation of the LAB and alginate was then carried out using a stirrer (Thermo Scientific Series SP8885710) at a speed of 150 rpm at room temperature for 24 hours (Yudiati *et al.*, 2021b; Yudiati *et al.*, 2021c).

***Artemia* Bioencapsulation**

Bioencapsulated *Artemia* is carried out using the method from Yudiati *et al.* (2023a). One g of *Artemia* cyst (Supreme Plus®, Golden West *Artemia*) was measured and hatched in 1,000 mL sterilized seawater and aerated strongly. After 16 hours, the nauplii hatched. These nauplii were then collected by plankton filter, and ready to use (Rudtanatip *et al.*, 2019). The bioencapsulation of *Artemia* nauplii was done by immersion. Approximately 250-500 nauplii *Artemia* were immersed in 50 mL of fermented probiotics and 600 ppm alginate for one hour (Yudiati *et al.*, 2023a). These encapsulated *Artemia* were then ready to use as natural feed for *L. vannamei* juveniles.

Feeding Trial and Experimental Design

This experimental laboratory study used a completely randomized design (CRD) with control and alginate treatments that were replicated five times. Based on the previous study (Yudiati *et al.*, 2023a), the best treatment was 600 ppm alginate and LAB. Therefore, the treatment in this work was *Artemia* encapsulated with a synbiotic formulation involving 600 ppm alginate and LAB, while the control was *Artemia* without any synbiotics encapsulation.

There were two sets of experiments of 14 days of rearing. The first set was to determine growth performance. This point was to measure the length of juveniles. The second set was to determine the survival rate of *L. vannamei* juveniles to bacterial challenges, and salinity stress. The acclimatization of two sets of treatments contain 600 *L. vannamei* juveniles (0.05 mg in weight and 7 mm in length) was done in a 46 L container. The first set of trials containing 300 PL15 of growth performance–were selected, stocked, and set up. On the second set, 300 PL15 for survival rate, bacterial challenges, and stress salinity were also set up. Both sets were conducted in round bottom flask (1000 mL in volume) with 25 ppt seawater media. All juveniles in both experiments were reared and started feeding with *Artemia* in control (non-synbiotic) and synbiotic encapsulation concurring with the treatments. The density of each flask was 30 PLs. The PLs were reared in 14 days and fed five times per day (07.00, 10.00, 13.00, 16.00, and 19.00). For water quality maintenance, the shrimp feces was siphoned every day and then refilled with fresh seawater. At the end of 14 days of rearing, five juveniles from the first set were selected randomly and measured under the microscope to assess their weight at various rearing times. Length was also measured microscopically using Image J software, and recorded at days 0, 3, 6, 10, and 14 days of rearing. After 14 days of rearing, the juveniles from the second set of experiments were counted for the survival rate. All the

surviving juveniles in every replicate were mixed and randomly chosen for the next tests which are *Vp*AHPND and salinity stress. The 10 surviving juveniles from the second set were challenged against *Vibrio parahaemolyticus* AHPND. Another 10 PLs from the same set were designed for salinity stress with 0 ppt salinity exposure.

Challenge Test

The *V. parahaemolyticus* (*Vp*AHPND) strain was sourced from the Tropical Marine Biotechnology Laboratory, Diponegoro University. The bacterial colonies were grown in alkaline peptone water agar (Merck, US). The *Vp*AHPND was then propagated in liquid alkaline peptone water (APW) agar which was incubated at room temperature for 24 hours. The *Vp*AHPND solution was centrifuged at 4000 rpm for 15 minutes. The *Vp*AHPND pellet was diluted with sterile marine water and its density was measured with a spectrophotometer (wavelength 600 nm). The *Vp*AHPND concentrations were calculated based on the method of Kongchum *et al.* (2022), where an optical density of 2.0 is equivalent to 10^9 CFU mL^{-1}. The *Vp*AHPND challenge test was performed following the procedure previously proposed by Balcázar *et al.* (2007) with several modifications. Balcázar *et al.* (2007) used *V. parahaemolyticus* PS-017 at a slightly different concentration (10^6 CFU mL^{-1}) with different weights of *L. vannamei*. A total of 10 PL from each flask in both treatments was taken and placed into another round bottom flask which had been filled with 99 mL of sterile seawater. One mL of *Vp*AHPND (10^7 CFU mL^{-1}) was added to each flask. The PL immersion with *Vp*AHPND was carried along the experiments, until the juvenile mortality reached 100%. Survival rates were monitored every six hrs until reaching 100% mortality.

Salinity Shock Test

The stress salinity test was adapted by Yudiati *et al.* (2024). At 14 days of rearing, 10 survived mix juveniles were taken and randomly moved to a 50 mL falcon tube (50 mL freshwater, 0 ppt) corresponding to the treatments. The mortality of PL was monitored and counted every 10 minutes until 100% mortality was reached.

Ethical Statement

The use of shrimps in this work follows the ethical protocol of animal welfare for research at Diponegoro University and SNI 8037.1: 2014 (SNI, 2014).

Data Analysis

All data including survival rate, growth performance, mortality, and salinity stress was definite by one-way analysis of variance (ANOVA) to determine whether the treatment had a significant effect ($\alpha=0.05$).

RESULTS AND DISCUSSION

Survival Rate

Litopenaeus vannamei PLs fed on *Artemia* synbiotic reached a better survival rate at (92.0 ± 9%) ($\alpha=0.05$) when compared with control (87.3 ± 11%) during 14 days of rearing (Figure 1). Therefore, a combination of alginate and *L. bulgaricus* synbiotic effectively improved the survival of shrimp PLs during the rearing period.

Sodium alginate is an insoluble part of algae's cell wall. Alginate is a polysaccharide and is characterized specifically by mannuronic and guluronic monosaccharides (Yudiati & Isnansetyo, 2017). Alginate polysaccharides are structured chemically analogously to the bacterial cell walls. When this structure is detected by host or shrimp receptors, the immune response is then initiated by hemocyte proliferation by cell-to-cell communication, leading to phagocytic activity. Phenol oxidase (PO) plays a key role in shrimp's innate immunity. Phenol oxidase can primarily be found in the hemolymph cells including hemocytes and

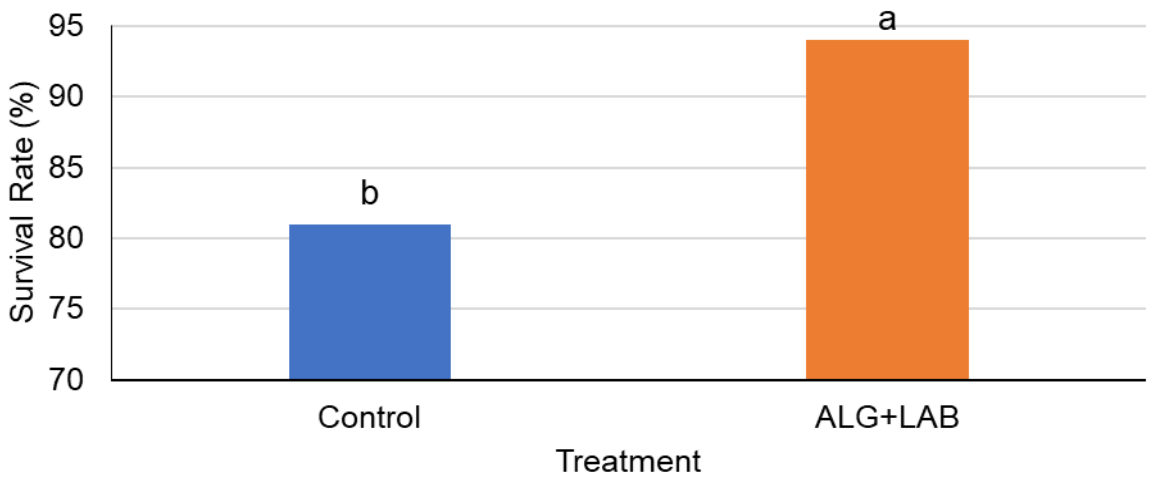

Figure 1. The survival rate of *Litopenaeus vannamei* post-larvae fed *Artemia* encapsulated synbiotic and non-synbiotic (control) at 14 days of rearing. Different letters on each bar represent a significant statistical difference ($P<0.05$)

it influences melanogenesis by transforming phenols into quinones and polymerizing them for melanin formation (Goh *et al.*, 2023). Melanin assists in preventing pathogens from growing and reproducing, leading to produce reactive oxygen species (ROS) (Azhar & Yudiati, 2023). To fight pathogens ie. *Vibrio* spp., the phagocytosis is correlated with producing highly microbiocidal ROS (Cheng *et al.*, 2005) Excess ROS can trigger severe non-specific cellular damage which is then followed by subsequent macromolecules cellular problems such as carbohydrates, proteins, and lipids, as well as nucleotides being oxidized (Tassanakajon *et al.*, 2013). In terms of related-immune gene expression such as lectin, phenol oxidase, and lipopolysaccharide glucan binding protein by alginate supplementation was widely reported as being upregulated (Azhar & Yudiati, 2023; Chin et al., 2024a; Yudiati et al., 2019). As shown from Figure 1, since the immune parameters include phagocytic activity, phenol oxidase, super oxide dismutase to combat the ROS, as well as the related-immune gene expression was accelerated, the survival rate of *L. vannamei* with synbiotic was also improved.

Length Gain and Specific Growth Rate

Figure 2 depicted that *L. vannamei* PLs fed on *Artemia* encapsulated synbiotic reached a higher length gain ($\alpha=0.05$), compared to control. After 3rd day of rearing, the PL fed with *Artemia* encapsulated synbiotic gave better data synchronously. On the 14th day of rearing, the length of PLs of *Artemia* synbiotic encapsulated reached 243.33 mm+18.80, while the length of PLs of *Artemia* control was 210.0 ± 22.15 mm. The specific growth rate of shrimp fed on Artemia synbiotic encapsulation shows better growth (18.44 ± 2.01%) which is significant ($P<0.05$) when compared to the control (15.77 ± 2.37%) over time (Figure 3).

Previous researchers showed that probiotics play a vital role in the digestion of protein, starch, and lipids in the gastrointestinal tract of shrimp's gut, enhancing feed digestion and absorption of feed which results in better shrimp growth (Mirbakhsh *et al.*, 2023). The combination of natrium alginate and *L. bulgaricus* LAB in this research shows similarly remarkable results and synergetic results in synbiotic, particularly in growth performance. A study by Prabawati *et al.* (2022) denoted that the synbiotic of *Pleurotus eryngii* mushroom and *L. plantarum* incorporated with feed increase *L. vannamei* growth performance and reduce the endanger of infectious diseases (*Vibrio*). Our alginate is extracted from the sea wall of Indonesian tropical *Sargassum* sp. that fits the standard alginate (Sigma, USA) (Yudiati *et*

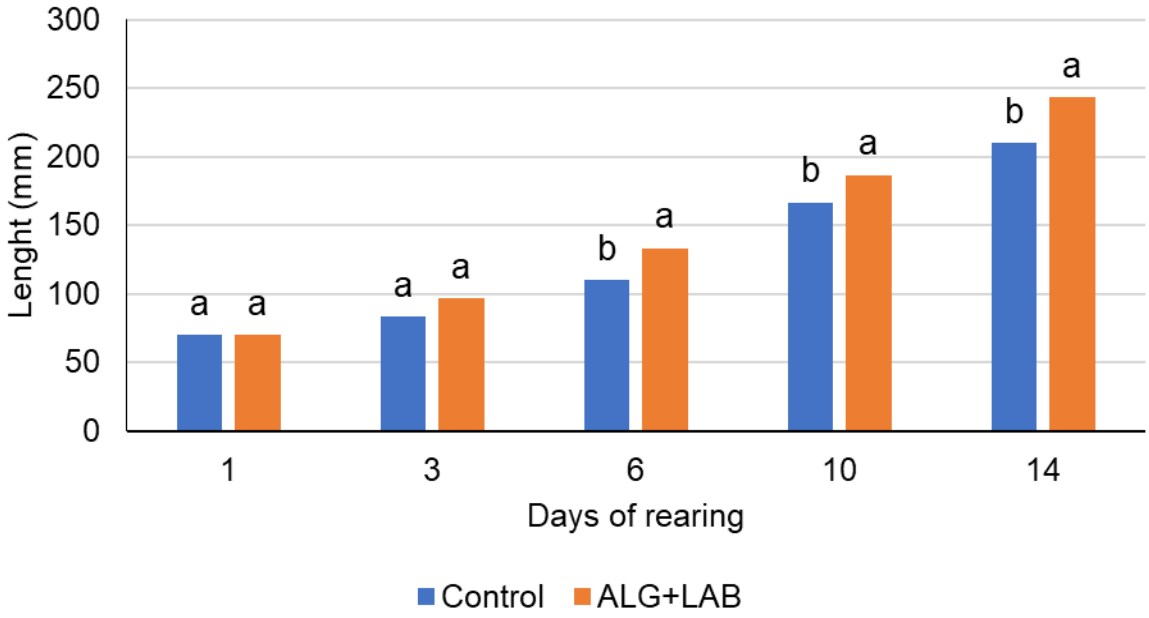

Figure 2. Length gain of *Litopenaeus vannamei* post-larvae fed *Artemia* synbiotic and non-synbiotic (control) encapsulation at 1, 3, 6, 10, and 14 days of rearing. Different letters on each at the same days of rearing represent a significant statistical difference (P<0.05)

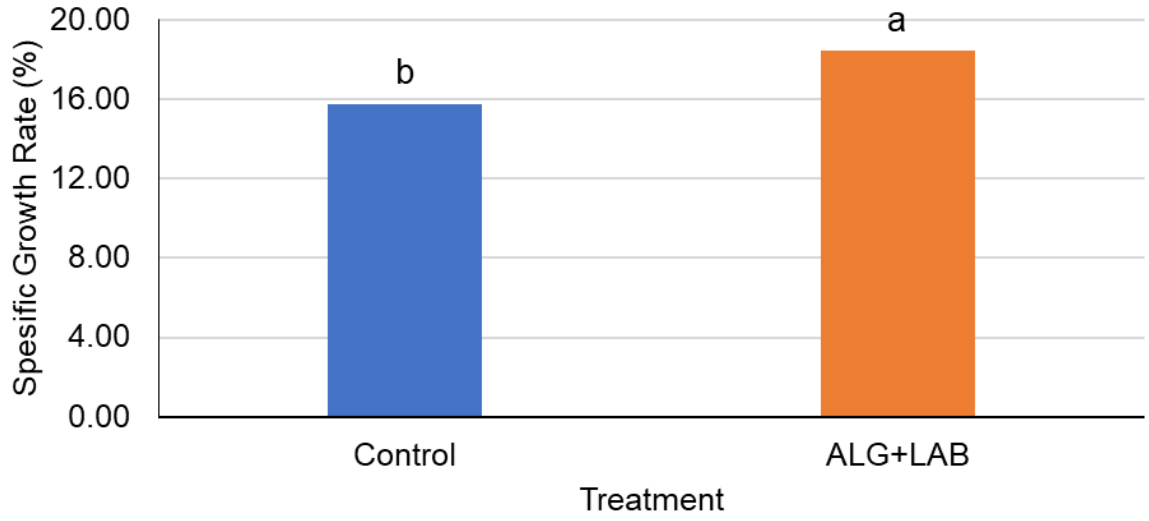

Figure 3. Specific growth rate of *Litopenaeus vannamei* post-larvae fed with *Artemia* synbiotic and non-synbiotic encapsulation at the end of the rearing time. Different letters on each bar represent a significant statistical difference (P<0.05)

al., 2016). Similar to alginate, the mushroom *Pleurotus* species are rich in non-digestible oligosaccharides such as xylose, glucose, mannose, fructose, sucrose, trehalose, xylose, and β-glucan (Prabawati *et al.*, 2022). The richness of bioactive compounds from alginate and mushrooms provides the *Lactobacillus* spp. probiotics' nutrients and be able to promote shrimp growth.

In terms of shrimp health, administering probiotic-supplemented diets is effective in promoting considerably higher growth rates within experimental groups compared to control groups. This beneficial effect

expands beyond simple growth, as proven by observations demonstrating significant prevention of the adverse impacts of stressors (Pooljun *et al.*, 2020). The complex mechanisms underlying probiotics' growth-promoting effects include various aspects of shrimp physiology. Improvements in the gut microbiome, improved immune responses, changes in intestinal and hepatopancreatic morphology, and elevated enzymatic activity, have all been identified as supporting factors (Tamilselvan & Raja, 2024).

Furthermore, probiotic supplementation has been linked to noticeable changes in gene expression, providing further understanding of the complex processes by which these microbes positively impact shrimp growth. The elevated gene expression including lysozyme and prophenoloxidase as well as decreased cumulative mortality in larvae (Goh *et al.*, 2023).

Survival Rate of *Litopenaeus vannamei* Post-Larvae After the Challenge Test

The survival rate of post-*Vp*AHPND challenged test shows that PLs fed *Artemia* encapsulated with symbiotic reached the better survival rate, initially at 24 hours post infection (HPI), synchronously until 54 HPI (Figure 4).

In this present research, the PLs resistance to severe *Vp*AHPND was reached from *L. vannamei* PL fed on *Artemia* with synbiotic (alginate and *L. bulgaricus*) encapsulation, which has a high relationship with a non-specific immune system in penaeid shrimps. This finding is in agreement with Prabawati *et al.* (2022) who reported that *L. vannamei* fed with synbiotic (*P. eryngii* and *L. plantarum*) significantly lower cumulative mortality due to the major increase in immune responses such as phenoloxidase, lysozyme activity, and super-oxide dismutase (Yudiati *et al.*, 2023a; Yudiati *et al.*, 2023b). The gene expressions of immune-relaled genes including penaeidin 3 (PEN3), penaeidin 3 (PEN4), LGBP, and GPX were also upregulated (Tseng *et al.*, 2023).

Vibrio parahaemolyticus was identified as the causative agent of acute hepatopancreatic necrosis which caused inflicted massive economic losses and posed a considerable threat to the shrimp culture with 100% mortality in first 35 days of the post larva stage caused by severe hepatopancreas atrophy (Choi *et al.*, 2017). Our previous works noticed

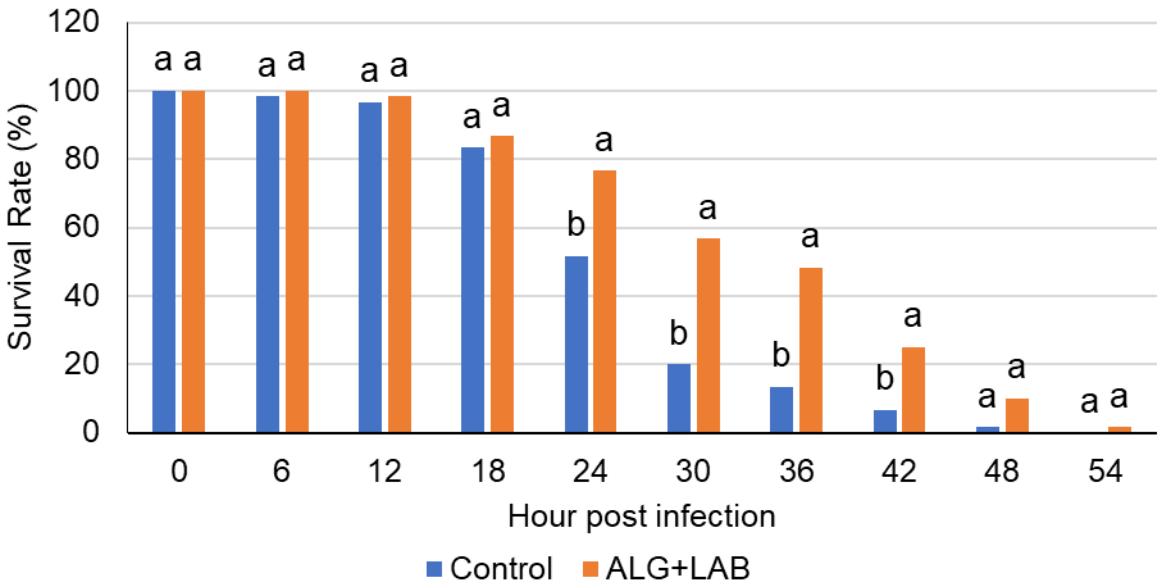

Figure 4. The survival rate of *Litopenaeus vannamei* post-larvae fed *Artemia* encapsulated synbiotic and non-synbiotic (control) at every six hours of *Vibrio parahaemolyticus* AHPND post-infection. Different letters on each bar at the same hour post-infection represent a significant statistical difference ($P<0.05$)

the infection of that highly pathogenic bacteria associated with AHPND infection 10^6 CFU.mL^{-1} by immersion methods, altered the water medium color into red (Azhar & Yudiati, 2023). Firstly, the pathogenic bacteria colonize the stomach gut, and digestive tract of shrimp, where then reaching the hepatopancreas, the bacteria would provoke the sloughing of tubule epithelial cells via the production of pirA-B toxin, the specific gene from *Vp*AHPND, eventually leading to shrimp mortality (Kumar *et al.*, 2020). Typical but abandoned techniques, such as the use of antibiotics and disinfectants, had been unsuccessfully in managing AHPND. It was denoted that *Vibrio harveyi, Vibrio vulnificus*, and *V. parahaemolyticus* isolated from *L. vannamei* grower pond were resistant to some beta-lactam antibiotics (Amoxicilin, Ampicilin, Co-Amoxiclav) (Yudiati *et al.*, 2021a).

Evidently, there is a bactericidal effect of exopolysaccharide from lactic acid bacteria (Daba *et al.*, 2021) including *L. bulgaricus* (Al-Nabulsi *et al.*, 2022; Yudiati *et al.*, 2023a). Furthermore, their use has been linked to changes in the host gastrointestinal microbiota and immunity as indicated by an excessive population of *L. plantarum* L20 that had colonized the *L. vannamei* gastrointestinal tract, enhancing microvilli and intestinal wall thickness which could reduce the infiltration and development of *V. parahaemolyticus* (Chin *et al.*, 2024b).

Among lactic acid bacteria, *Lactobacillus* spp. are beneficial to the host, and the most effective and widely used to enhance aquatic and marine animals' health and control diseases (Tseng *et al.*, 2023). Lactic acid bacteria (*L. bulgaricus*) as probiotics possess the anti-inflammatory properties of *Lactobacillus*-derived exopolysaccharides (EPSs) (Zhang *et al*., 2024). In their research, (Chin *et al.*, 2024b) reported that *Bacillus* sp. and *Lactobacillus* sp. proved that those are effective probiotics for better growth rate and disease resistance in *Penaeus monodon* shrimp against *V. parahaemolyticus* AHPND. Similar to this research, *Lactobacillus* sp., as a probiotic strain, was reported to inhibit the activity of pathogenic AHPND-causing bacteria in *L. vannamei* (Yudiati *et al.*, 2023a) by producing various antibacterial compounds (Kumar *et al.*, 2021). Probiotics validate remarkable effects on the immune defence of the host's gastrointestinal system, which play an important role in protection against diseases and directing control of inflammation inside the digestive tract (Tamilselvan & Raja, 2024). In agreeing with this present synbiotic research, Chin *et al.* (2024a) reported that *P. monodon* fed on synbiotic of *S. polycystum* and *L. plantarum* L20 was resistant against AHPND-causing *V. parahaemolyticus*.

Survival Rate of *Litopenaeus vannamei* Post-Larvae after Salinity Shock

Litopenaeus vannamei PL's fed *Artemia* synbiotic reached a better survival rate from 10 to 230 minutes after salinity stress when compared with control without synbiotic, significantly ($P<0.05$) (Figure 4). Therefore, the synbiotic supplementation of 600 ppm alginate (prebiotic) and *L. bulgaricus* as probiotics in encapsulated *Artemia* effectively improved the survival of shrimp after the challenge test.

The salinity shock in this research applied the difference enormous of osmotic exposure, ie. from 25 ppt to 0 ppt. Based on Istiqomah *et al.* (2024), Pramudyo *et al.* (2024), and Yudiati *et al.* (2024), the osmotic stress on shrimp is concerning the immune system. The data from Figure 5, are consistent with the survival rate and growth data from Figure 1, 2, and 3. This is due to the fact of boosting immune system hits from *L. vannamei* PLs fed *Artemia* encapsulated with synbiotic of alginate and *L. bulgaricus* LAB. The 600 ppm optimal dose of synbiotic gives rise to the best survival rate in acute osmotic (salinity) stress. As with the previous works, the immune response parameters are indicated by the activity of two enzymes involved, namely super oxide dismutase and catalase to counter the production of radical species production caused by bacterial infection (Azhar & Yudiati, 2023; Tamilselvan & Raja, 2024). Moreover, the gene-related immune expression of LGBP, peroxinectin, prophenoloxidase, toll-like receptor, penaeidin is also likely to be upregulated (Azhar & Yudiati, 2023; Chin *et al.*, 2024a).

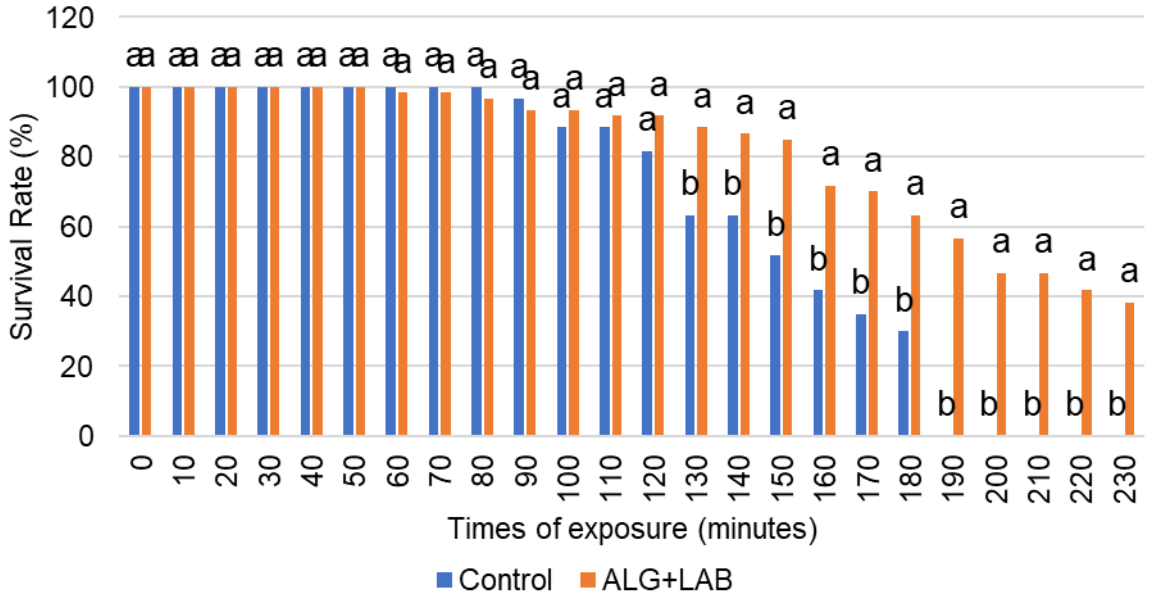

Figure 5. The survival rate of *Litopenaeus vannamei* post-larvae fed *Artemia* encapsulated synbiotic and non-synbiotic (control) every ten minutes after salinity shock. Different letters on each bar at the same times of exposure (minutes) represent a significant statistical difference ($P<0.05$)

CONCLUSION

Providing *Artemia* with synbiotic alginate (prebiotic) and *L. bulgaricus* (probiotic) to *L. vannamei* juveniles was able to increase survival, growth, and resistance to *V. parahaemolyticus* infection, and increasing tolerance of exposure to salinity during 14 days of rearing, in a specific dose. The rearing time of this study was limited (14 days), and the longer periods of rearing time (more than 28 days) will be more adequate.

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CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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