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Review Article

Assessing Various Administration Strategies for dsRNA Vaccine Delivery: A Concise Review of VP15-WSSV Research Progress in Tiger Shrimp *Penaeus monodon*

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Abstract

RNAi technology offers a novel powerful approach to silence gene expression by introducing dsRNA into the cell to degrade the mRNA at the post-transcriptional stage. A dsRNA administration delivery is one of the main considerations in applying the dsRNA vaccine for controlling pathogen infections. This mini-review aimed to evaluate three different methods (immersion, injection, and oral administration) of VP15-dsRNA vaccine delivery to the tiger shrimp *Penaeus monodon*. A desk study method was applied to this mini-review. The immersion was generally conducted for the shrimp larval stage and seemed to be a simple technique for a large number in a small tank. The VP15-dsRNA immersion improved the survival of larvae by 3.9% compared to the control. The injection was an effective way to deliver dsRNA to the cell, but it is difficult to apply to a large number of individuals. The VP15-dsRNA injection increased significantly ($P<0.05$) survival, proPO, and THC. A higher survival rate (75%) was observed in shrimp injected with in vivo and in vitro VP15-dsRNA compared to the control. Oral administration of VP15-dsRNA-enriched pellets was a useful method for larvae, juveniles, and broodstocks; however, it had limitations due to the leaching of the pellet into the water. The VP15-dsRNA vaccine application on the feed significantly ($P<0.05$) enhanced the 26.7% survival rate compared to the control, supported also by a higher Total Haemocyte Count. The three VP15-dsRNA delivery methods have advantages and limitations, but they provide potential approaches to increase tiger shrimp resistance to control pathogen infection.

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1. Introduction

Penaeus monodon, commonly known as tiger shrimp, is an economically important indigenous crustacean species widely cultivated in Indonesia's brackish water aquaculture systems. Since the 1990s, penaeid shrimp aquaculture has been severely affected by habitat degradation and recurring outbreaks of viral diseases (Walker and Mohan, 2009). The most infectious virus that affects tiger shrimp in pond culture and hatcheries is the White Spot Syndrome Virus (WSSV), called White Spot Disease (WSD) (Chakrobortty *et al*., 2020; Islam *et al*., 2024; Kaikkolante *et al*., 2025; Kanitchinda *et al*., 2024; Koesharyani *et al*., 2023; Li *et al*., 2024; Parenrengi *et al*., 2021a; Priya and Sudhakaran, 2025; Ziarati *et al*., 2025). The infection affects all cultured penaeid shrimp, leading to significant economic repercussions in global aquaculture production (Asche *et al*., 2020; Chen *et al*., 2024; Cox *et al*., 2024; Lee *et al*., 2024; Yun *et al*., 2025). The WSSV virion's circular double-stranded DNA genome consists of an enveloped nucleocapsid (Van Hulten *et al*., 2002). Viral proteins (VP) of the WSSV protein structures, such as VP15, VP24, and VP26, are located inside the nucleocapsid, whereas VP19 and VP28 are located in the nucleocapsid envelope. The VP15, the smallest nucleocapsid protein, was recently found in WSSV. Based on its capacity for DNA binding, VP15 is identified as a major viral protein involved in WSSV genomic packaging (Boonyakida *et al*., 2020; Kwankaew *et al*., 2018; Sangsuriya *et al*., 2011; Tsai *et al*., 2006).

A strategy for preventing disease outbreaks and occurrences in aquaculture is the biotechnological application to improve shrimp tolerance to infections. For instance, the use of genetic engineering using RNA interference (RNAi) technology is known to be a powerful strategy for enhancing shrimp disease resistance (Kulkarni *et al*., 2021; Men *et al*., 2025; Parenrengi *et al*., 2020, 2017; Westenberg *et al*., 2005). RNA interference (RNAi) is a molecular technique used to silence the expression of virulence-related genes in pathogens, thereby enhancing disease resistance in shrimp (Chaimongkon *et al*., 2020; Fajardo *et al*., 2024; Hsu *et al*., 2022; Liu *et al*., 2025; Weerachatyanukul *et al*., 2021). There are several phases in the RNAi pathway, which are unique to crustacean species (He *et al*., 2015; Reshi *et al*., 2014). When dsRNA is injected into a cell, the enzyme Dicer converts it into small interfering RNAs (siRNAs). These siRNAs are integrated into the RNA-induced silencing complex (RISC), where the guide strand directs the complex to a complementary messenger RNA (mRNA) target, leading to its degradation and resulting in the suppression of gene expression at the post-transcriptional level. Employing recombinant protein WSSV on *P. chinensis* (Kim *et al*., 2004), kuruma shrimp (*Marsupenaeus japonicus*) (Boonyakida *et al*., 2022), and antiviral dsRNA on *Litopenaeus vannamei* (Robalino *et al*., 2004) for inducing immune responses has been performed through vaccination approaches. The delivery system of RNAi technology is one of the important parts of successfully applying the dsRNA vaccine (Jonjaroen *et al*., 2025; Liu *et al*., 2025; Nie *et al*., 2022; Parenrengi *et al*., 2022; Phromma-in *et al*., 2025). Three delivery methods (immersion, injection, and oral administration) are generally applied to the dsRNA vaccine and need to be assessed to understand their limitations and advantages. Despite the growing interest in RNAi-based strategies, a comprehensive review focusing specifically on the VP15-dsRNA application in *P. monodon* remains lacking. All our progress results and the other related studies have been discussed to formulate the decision and the recommendation for future studies.

This mini-review aimed to present recent achievements of dsRNA vaccine delivery to the tiger shrimp and to evaluate their survival and immune response using three different methods (immersion, injection, and oral administration). The review provided some updated important issues not only in assessing the delivery system of dsRNA but also in disseminating our research progress on the dsRNA application to the tiger shrimp.

2. Materials and Methods

2.1 Materials

2.1.1 The equipments

A thorough literature search was conducted utilizing several reputable scientific databases to find pertinent research on the VP15 of WSSV and its use with dsRNA at various delivery systems in *P. monodon*. Microsoft Office 16 (Microsoft Corporation, USA) was used for data synthesis, which helped in organizing and understanding the information that was retrieved, including SmartArt in Microsoft Word for producing schematic illustrations and Microsoft Excel for creating the graphical figures. These tools were employed to allow the accurate and transparent depiction of conceptual frameworks and data visualizations.

2.1.2 The materials

Mendeley Desktop (Mendeley Ltd., UK) was used to effectively manage and arrange the references, which made the citation process easier and guaranteed accurate referencing throughout the evaluation. The artificial intelligence of ChatGPT (OpenAI Inc.,

USA) was used for language refinement and summarization in preparing this manuscript. All tools collectively supported the systematic approach undertaken to compile, analyze, and present the findings from the selected studies.

2.1.3 Ethical approval

This desk research did not involve any research with animals. As such, ethical approval from an institutional review board or ethics committee was not required.

2.2 Methods

This mini-review employed a desk research method by a comprehensive literature search based on the PRISMA (Preferred Reporting Items for Systematic Literature Reviews and Meta-Analyses) approach with slight adjustments. This method was applied across multiple journal databases to find relevant research for searching, selecting, and analysing. The PRISMA flow chart of this mini-review is shown in Figure 1. The following flow-step for this method:

Search. The search process was conducted using recognized databases, including Scopus, ScienceDirect, and Google Scholar, to identify primary studies. This assessment involved a brief examination of the document's key components: the title, abstract, introduction, methods, results, discussion, and conclusion. For inclusion in the analysis, at least one of these sections needed to satisfy one or more of the specified criteria. To develop the topic research strategy, an experienced librarian relied on the following keywords: shrimp, tiger shrimp, White Spot Disease, WSSV, RNAi, VP genes, characteristic of VP15, dsRNA production, dsRNA vaccine, *in vitro* and *in vivo* production, delivery system, immersion, injection, and oral administration. To conduct a more comprehensive review, studies dated between 2004 and 2025 were included.

Eligibility criteria. The included studies were all published in peer-reviewed journals and written in English and Indonesian (reputable national journal, RNJ) under the Ministry of Maritime Affairs and Fisheries.. The main criterion was aquaculture studies on crustacean species, so studies on the finfish species were excluded. The review focused on the research progress of the gene encoding VP15 of WSSV and its application to the tiger shrimp using dsRNA in three common delivery methods: immersion, injection, and oral administration. Therefore, a research question has been formulated: What is the research progress on the application of the VP15 dsRNA and its delivery system to tiger shrimp? Here, the purpose of this mini-review was to evaluate the progress of research on different delivery systems of application of VP15-dsRNA to *P. monodon*.

Study selection. Firstly, relevant studies regarding RNAi technology for the crustacean species shrimp were collected to gain a general insight into the background of this review, including viral proteins (VPs) of WSSV for all shrimp species. Some information regarding the VPs like V15, VP19, VP24, VP26, VP28, VP53A, and the other VPs of WSSV, as well as the species of *P. monodon, P. vannamei, P. chinensis, M. japonicus, M. rosenbergii, F. merguiensis*, and the other crustacean species, was included in the first selection. Secondly, all collected publications were then filtered according to the selection criteria of VP, especially using the VP15-dsRNA. A specific application of VP15-dsRNA included component technology, such as the characteristics and production of dsRNA by *in vitro* and *in vivo* selection criteria. The two main criteria of the articles were selected for future selection. The next selection was performed by using the criteria of dsRNA delivery system, emphasizing of evaluation of three delivery systems, in selected methods of immersion, injection, and oral administration. Therefore, the out-of-scope items from the three delivery systems were not included for selection. Finally, the review was closed by constructing a conclusion and formulating recommendations for the implications of dsRNA application, as well as suggestions for future research. The brief schematic concepts of this mini-review are illustrated in Figure 2.

Data collection. The study characteristics were extracted from each of the full-text articles, including the authors, publication year, research field, type of study, crustacean species, type of VP, type of dsRNA production, and delivery methods. Due to the mini-review focusing on progress research by using the tiger shrimp as a host and VP15-dsRNA as a vaccine material, as well as three delivery methods were applied as a filter for data collection. According to these criteria, the study conducted by the relevant subject was included; however, the data collection was limited. From the studies on the efficacy of VP-dsRNA on different dsRNA production, larval stage, dosages, and method of delivery system were extracted to categorize the effect of application of VP15-dsRNA on the *P. monodon*. Studies that had been analysed by ANOVA were used as a criterion for further analysis.

Synthesis of results. A narrative synthesis approach was employed to summarize and interpret the findings from the included studies. The synthesis emphasized identifying the common characteristics of the gene encoding VP15-WSSV, updating progress in re-

search, advantages, and limitations of the three delivery systems for VP15-dsRNA application to *P. monodon*. The dominant results of our progress research, more than 50%, were discussed in comparison with the related results of the relevant studies. The findings from our research, along with pertinent studies in the field, have been critically analyzed to create conclusions and to propose directions for future investigations. The data from the references were descriptively discussed and illustrated by the Figures. The quality of each study was quantitatively assessed by determining the results of its data analysis and qualitatively evaluated by identifying the journal in which the article was published.

The results of this mini-review were presented, focusing on our research progress on the VP15-dsRNA application in *P. monodon*.

3.1.1 Characteristics of VP15-WSSV

The nucleocapsid major protein of 15 kDa (VP15) has been verified by some techniques, including engineering of recombinants, SDS–PAGE (Figure 3), South-Western blotting (Figure 4), protein sequencing, and immunodetection. In Indonesia, a gene encoding VP15 was successfully cloned into the L4440 Cloning vector (containing a T7 promoter) and transformed into the bacteria *Escherichia coli*

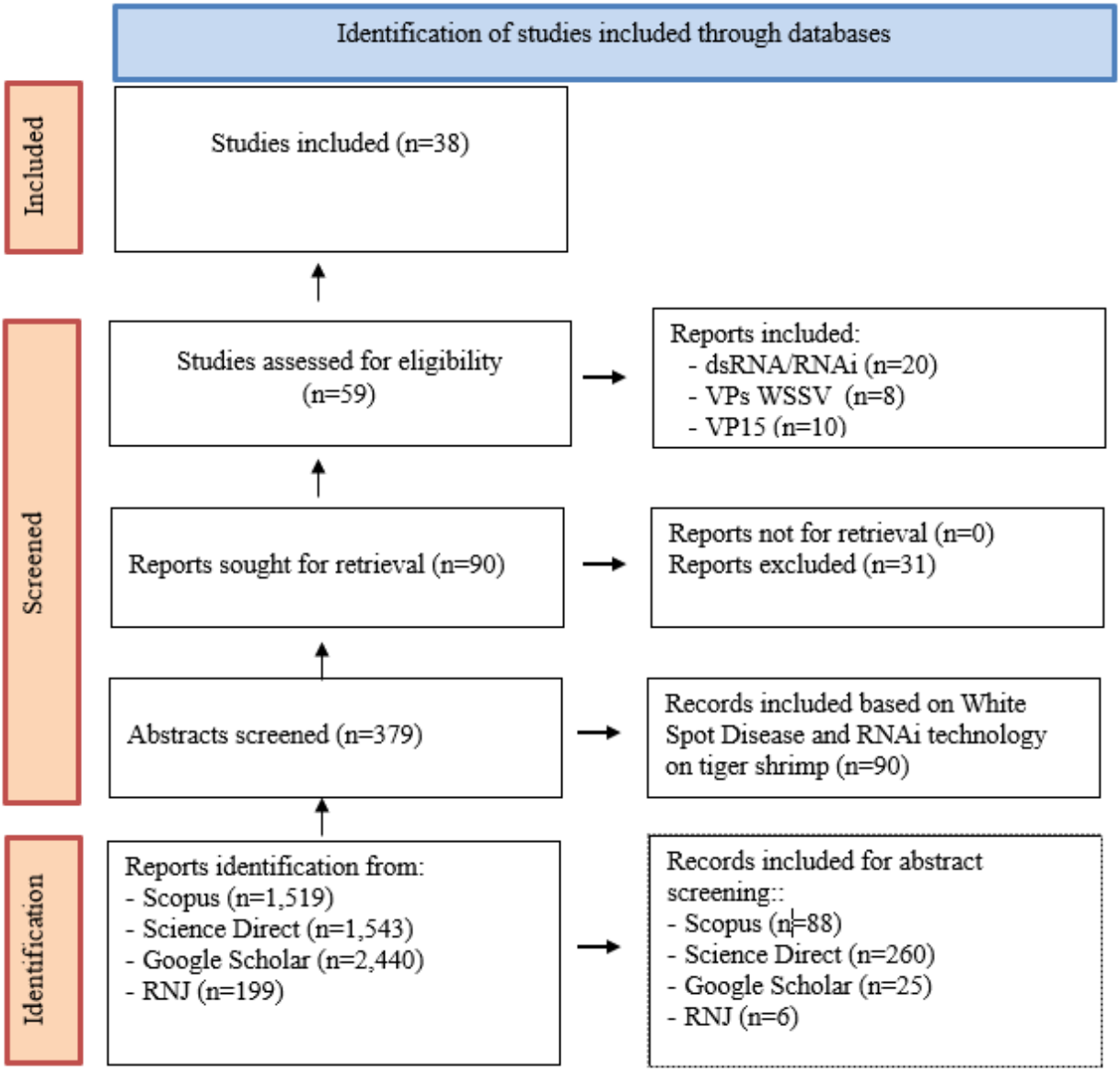


Figure 1. PRISMA flow chart illustrating journal article identification, screening, and selection.

3. Results and Discussion

3.1 Results

DH5α (Figure 5). The gene encoding VP-15 collected from *P. monodon* consisted of 246 bp of nucleotide sequence and 80 amino acid deductions (Figure 6).

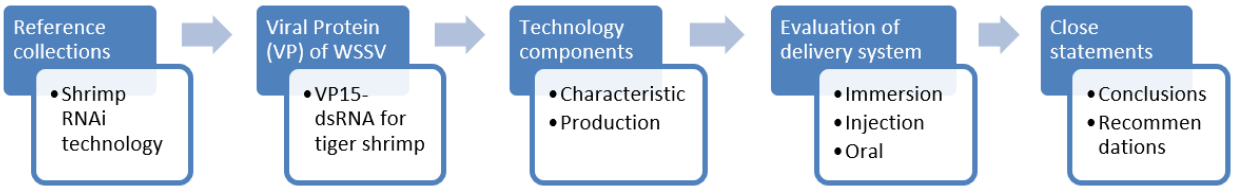


Figure 2. A schematic concept of this mini-review on the research progress of VP15-dsRNA.

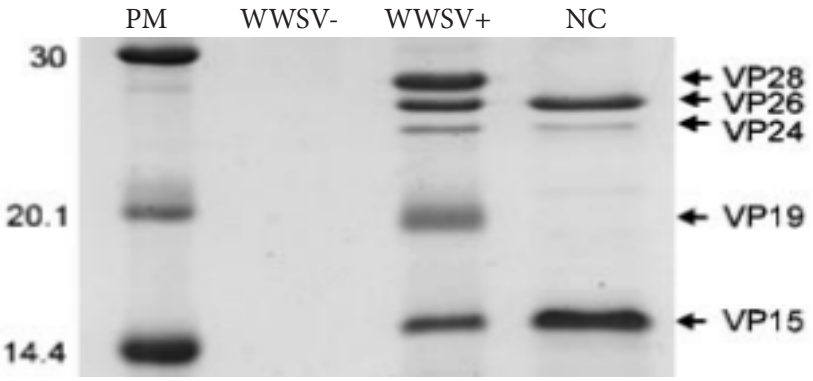


Figure 3. The SDS-PAGE (sodium dodecyl-sulfate polyacrylamide gel electrophoresis) of purified WSSV. PM=protein marker (kDa); NC=purified WSSV nucleocapsids (Van Hulten *et al*., 2002).

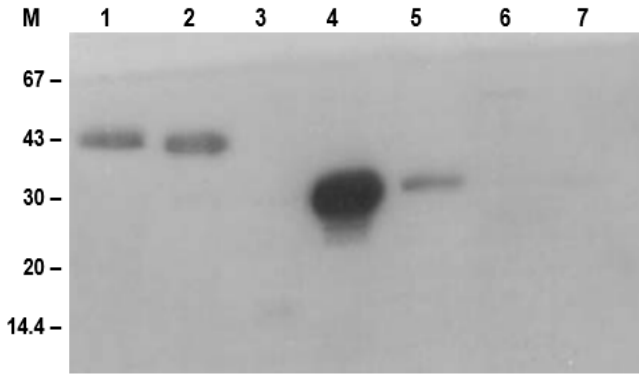


Figure 4. South-Western analysis of VP15 in different protein samples. M=marker, 1=MBV-VP15 crude soluble protein extract, 2=MBP-VP15 purified protein, 3=MBV crude soluble protein extract, 4=GST-VP15 crude soluble protein extract, 5=GST-VP15 purified protein, 6=GST crude soluble protein extract, and 7=MBV-VP28 crude soluble protein extract (Witteveldt *et al*., 2005).

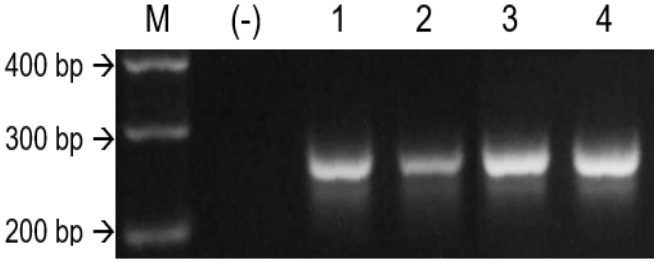


Figure 5. Electrophoresis of the VP15-WSSV gene isolated from bacteria E. coli DH5α. M=DNA marker; 1-4=VP15 WSSV and (-)=negative control (Parenrengi *et al*. (2019) with modification).

3.1.2 Production of VP15-dsRNA

The VP15-dsRNA vaccine production could be performed by *in vitro* and *in vivo* procedures. Both approaches have been reported to be a useful way of preparing the dsRNA vaccine. The *in vivo* production was usually used by inactive bacterial recombinants. A method of heat-killing bacteria by immersion for 5 minutes at 80°C was reported to be an optimal time and a simple technique for inactivating the bacteria carrying VP15-dsRNA (Figure 7).

3.1.3 Delivery of dsRNA VP15 on tiger shrimp

An administration delivery method is one of the main considerations in applying the dsRNA vaccine for controlling pathogen infections. The popular techniques to deliver the VP15-dsRNA vaccine to the tiger shrimp are immersion, injection, and oral administration.

3.1.3.1 Immersion

Delivery of dsRNA vaccine by immersion administration technique was generally applied for the larval stage of tiger shrimp. It seemed to be a simpler technique for a large number of individuals in a small tank. An example study reported that the post-larvae (PL-2) were vaccinated with VP15-dsRNA in a concentration of 1.3×10^8 CFU/mL by immersion method in a density of 1,000 larvae/1 L seawater in a plastic bag filled with oxygen: seawater (1:5) for 30 minutes. After the PL-12 larvae were challenged with WSSV,

the dsRNA treatment exhibited a higher survival than the control group (Figure 8). The improvement of larval survival rate in this study was 3.9% (28.3% for vaccinated and 24.4% for control).

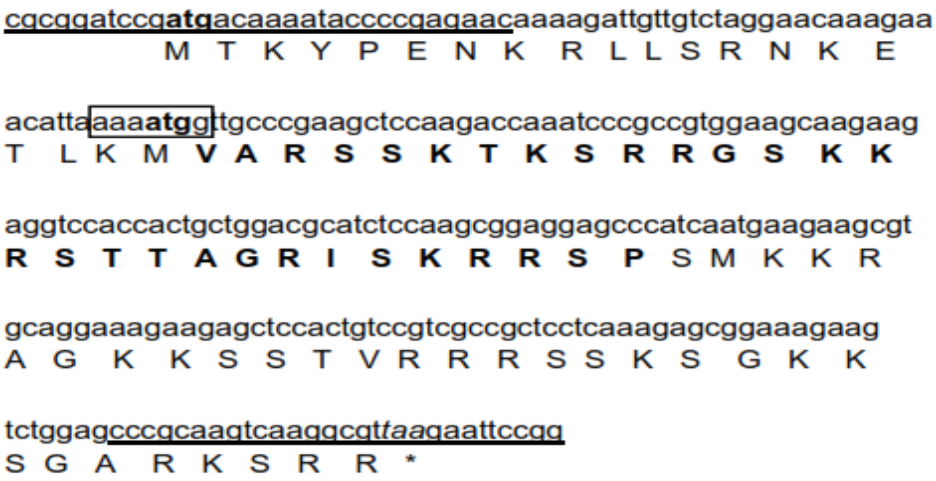


Figure 6. Nucleotide sequence and amino acid deduction of the VP15 gene isolated from tiger shrimp. The Kozak context was boxed, the N-terminal sequence was bold amino acids, the forward and reverse primers were underlined nucleotides, the start codon was bold nucleotides, and the stop codon was italic nucleotides (Parenrengi *et al*., 2017).

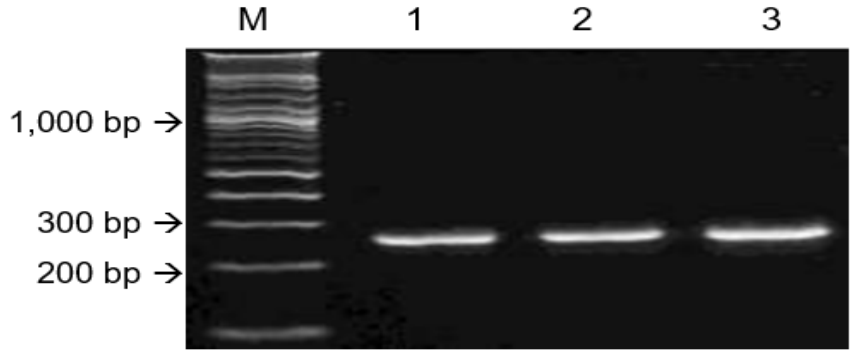


Figure 7. Electrophoresis of a gene encoding VP15 dsRNA from the DNA plasmid of inactivated bacteria by the heat-killed bacteria method. Lines 1-3=bacteria carrying VP15 of WSSV, and M=DNA marker (Parenrengi *et al*., 2020).

3.1.3.2 Injection

The injection method to deliver the dsRNA vaccine to tiger shrimp was mostly conducted by the intramuscular technique. Some papers reported the effective method of injection to increase the survival of tiger shrimp and other crustacean species. The injected tiger shrimp (body weight of 32.5±1.83 g) showed significantly ($P<0.05$) higher survival (75%) compared with the control shrimps (Figure 9). The VP-15 dsRNA treatment, both *in vitro* and *in vivo,* showed relatively lower mortality from day 5 to day 10. After day 10, survival remained stable until the end of the experiment. The THC of tiger prawns after the challenge test indicated that the *in vivo* dsRNA treatment had a higher THC average of 5.70×10^7 cells/mL than the *in vitro* dsRNA application was 3.52×10^7 cells/mL, and the lowest was in the control treatment was 3.32×10^7 cells/mL (Figure 10). The *in vivo* dsRNA vaccine injection revealed the greatest proPO (0.138), followed by the *in vitro* dsRNA treatment with a 0.093 confidence level, and the lowest in the control treatment (0.061).

The other result of vaccinated shrimps by a single dose and challenged with WSSV at 10 dpv via intramuscular injection at a dose of 2.69×10^3 DNA copies/shrimp showed the highest RPS (60%) compared with VP19 (45%) and control (40%) (Figure 11). The survival rate of tiger shrimp (weighing 15.8±3.50 g)

was significantly (P<0.05) affected by intramuscular vaccination at different dosages. The cumulative survival rate was higher for a dose of 0.02 g/shrimp than for the control and other doses. Additionally, all treatments for tiger prawns resulted in 100% death by the seventh day, but at a dose of 0.02 g, there were 15% alive until the research ended (Figure 12).

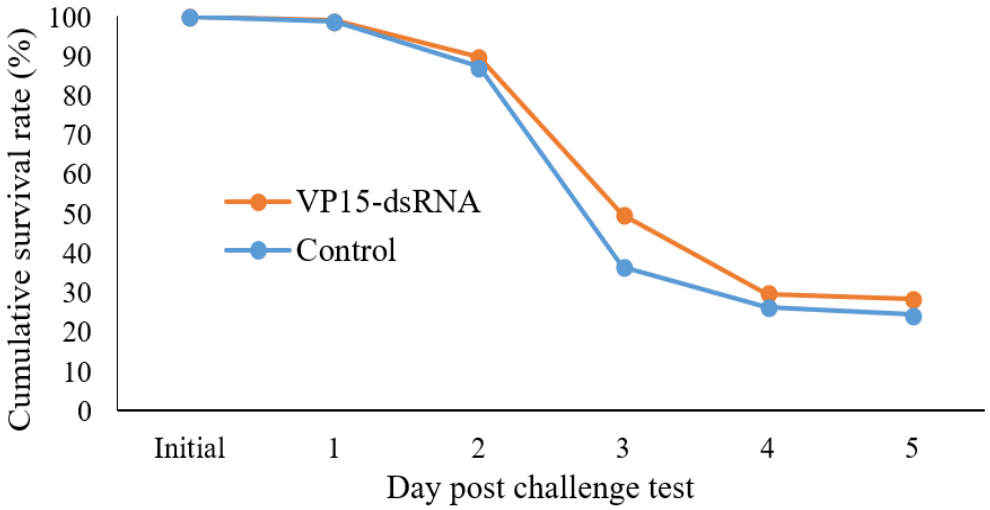


Figure 8. The cumulative tiger shrimp survival rate of larvae immersed with VP15-dsRNA after WSSV challenge test (Parenrengi *et al*., 2020).

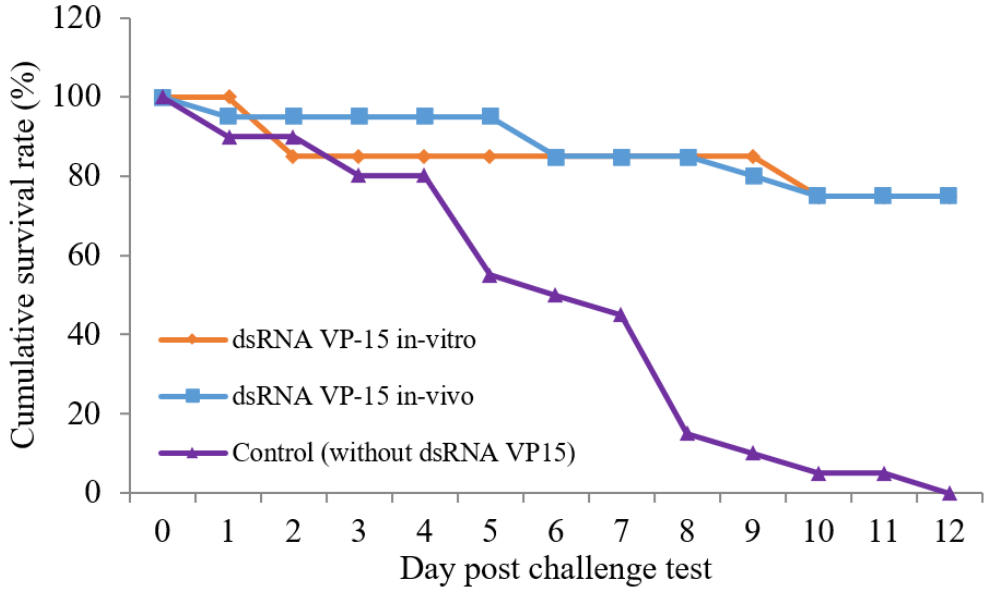


Figure 9. The cumulative survival rate of VP15-dsRNA-injected tiger shrimp after the WSSV challenge test (Parenrengi *et al*., 2019).

The dose of 0.02 g/shrimp (390×10^4 cells/mL) had the highest THC cell at the end of observation (5-dpc), followed by 0.2 g/shrimp (270×10^4 cells/mL), 2.0 g/shrimp (240×10^4 cells/mL), and the control (120×10^4 cells/mL). ProPO activity in the tiger shrimp hemolymph showed that it increased during the three-day post-challenge test but subsequently fell until the end of the trial. (Figure 13).

3.1.3.3 Oral

The use of VP15-dsRNA on tiger shrimp larvae (10^8 cells inactive bacteria/0.02 g feed) in different stages showed a significant (P<0.05) survival post-challenge test. The cumulative survival until the 14th day of observation showed that the application of dsRNA from mysis to PL-12 showed the highest survival rate of 26.0% compared to other treatments, which were 19.2%, 15.6%, and 9.87%, for the treatment of post-larvae, control, and zoea stages, respectively (Figure 14). The study's findings indicated that adding dsRNA to the larval diet from mysis to post-larva (PL12) could boost survival by 12.1% when compared to controls.

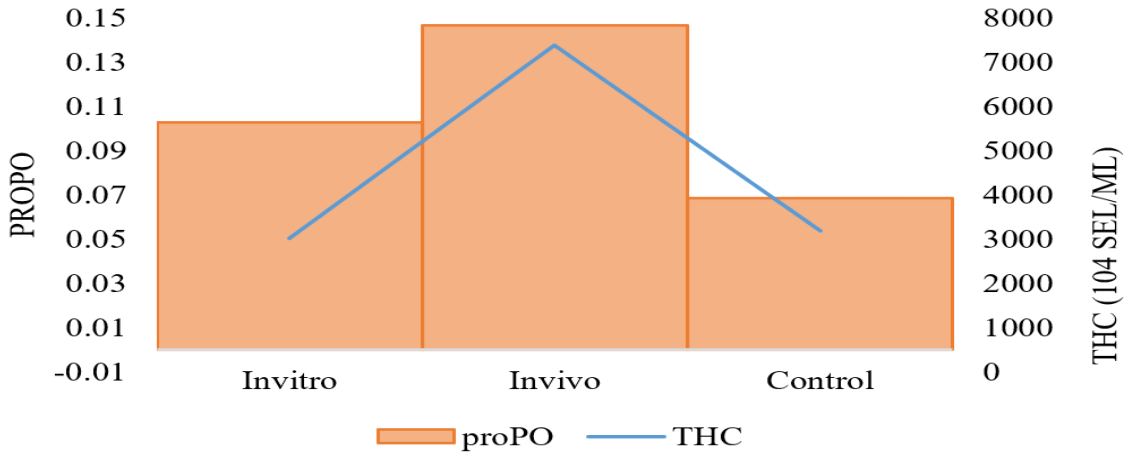


Figure 10. The average proPO activity and THC value of tiger shrimp injected with VP15 dsRNA post-challenge test with WSSV (Parenrengi *et al*., 2019 with modification).

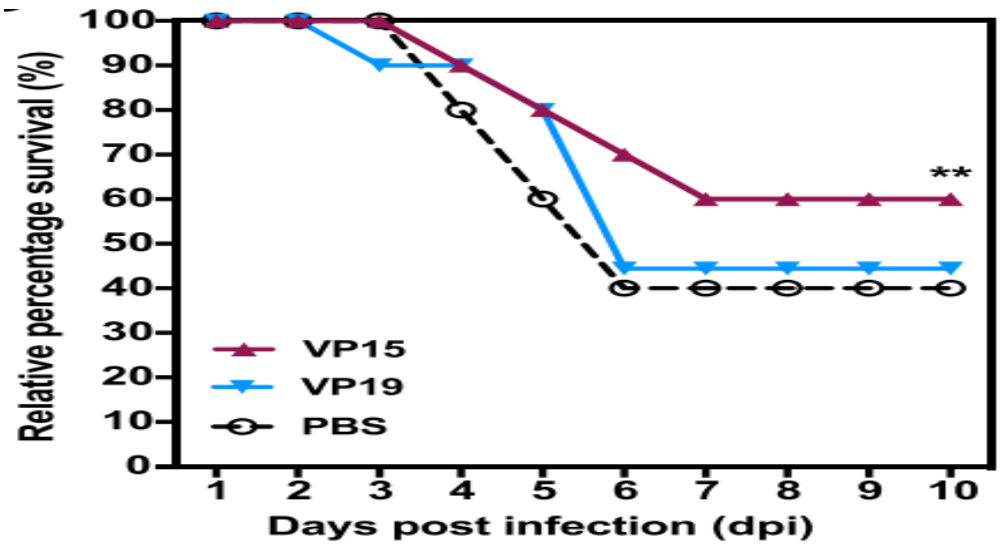


Figure 11. Relative percentage survival (RPS) of vaccinated shrimp post WSSV infection (Boonyakida *et al*., 2020).

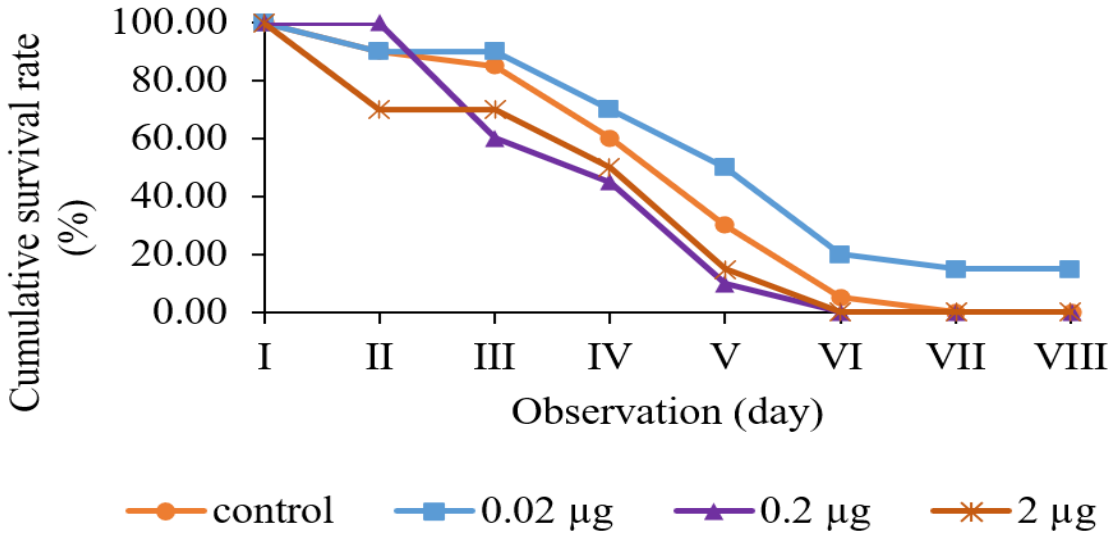


Figure 12. The effect of different VP15-dsRNA doses on the survival rate of vaccinated tiger shrimp after the WSSV challenge test (Parenrengi *et al*., 2021b).

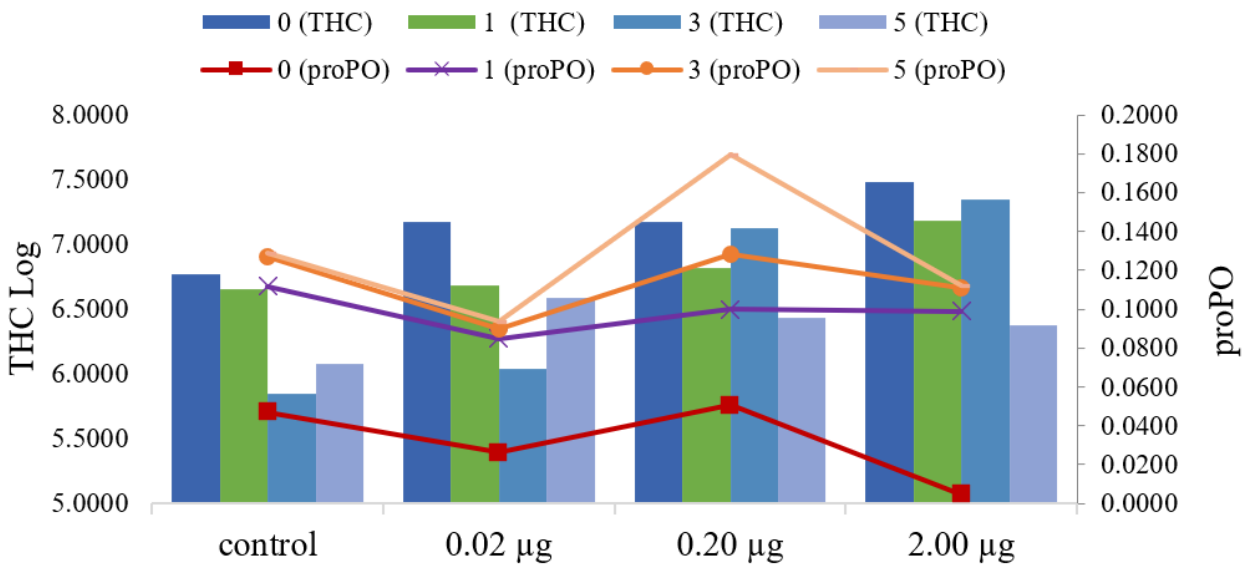


Figure 13. The effect of different VP15-dsRNA doses on the vaccinated tiger shrimp's THC number (in log value) and proPO activity both before and after the challenge test (0-5 days of observation) (Parenrengi *et al*. 2021b with modification).

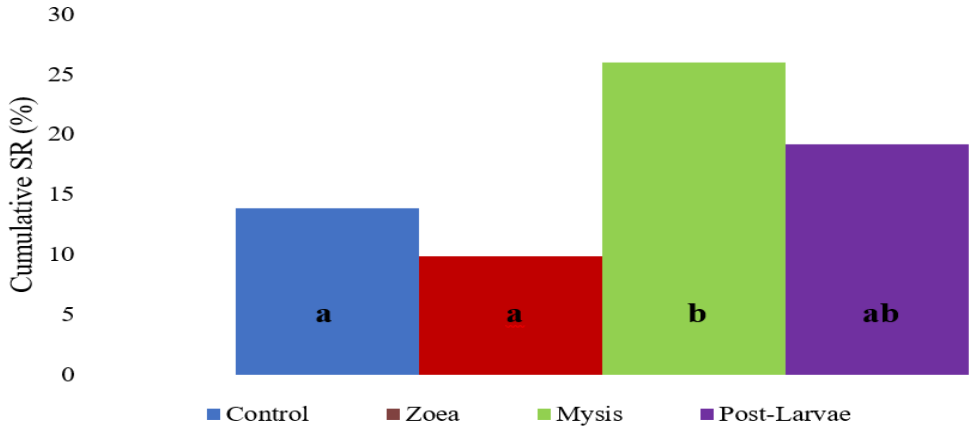
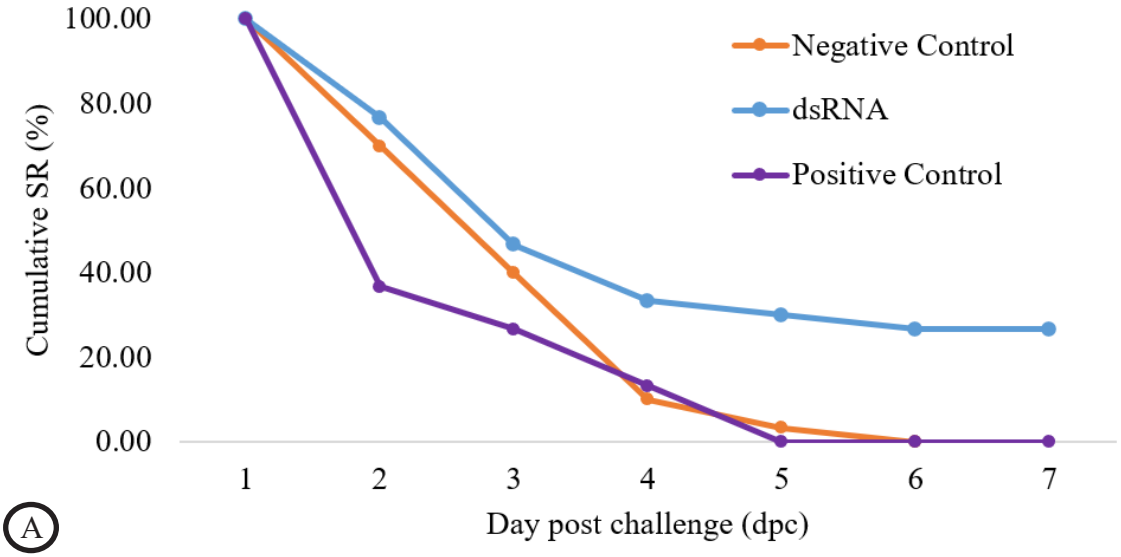


Figure 14. The cumulative survival rate of dsRNA application treatment at various larval stages on day 14 after the challenge test. Significant differences in the survival rate ($P<0.05$) were indicated by the different letters (Parenrengi *et al*., 2020).



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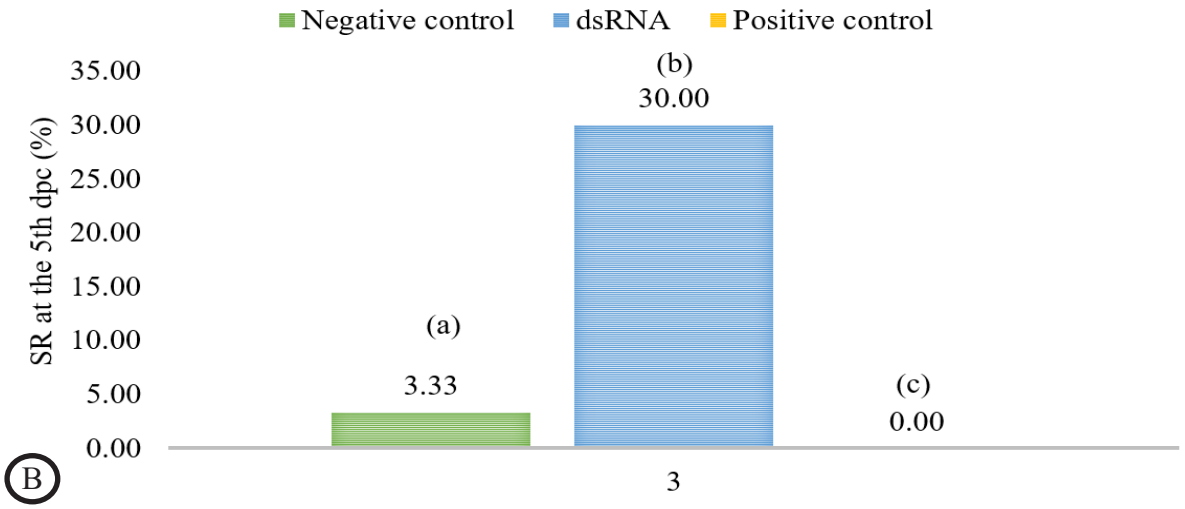
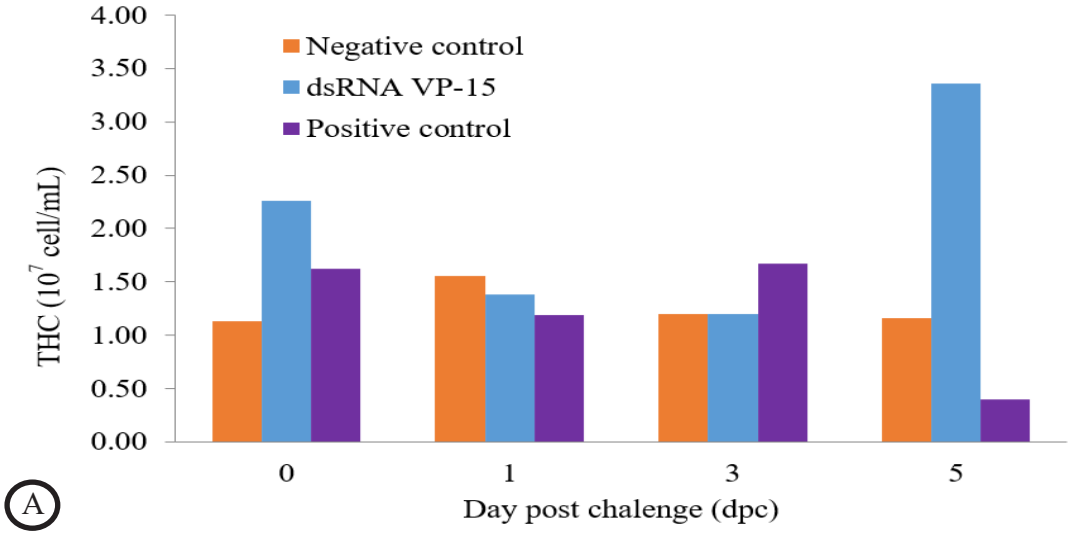


Figure 15. The cumulative survival rate of VP15-dsRNA-vaccinated tiger shrimp administered orally (A) and the survival average on the 5th day post-challenge (B). Significant differences ($P<0.05$) in the survival rate were indicated by the different letters.at the 5th dpc (Parenrengi *et al*., 2022).



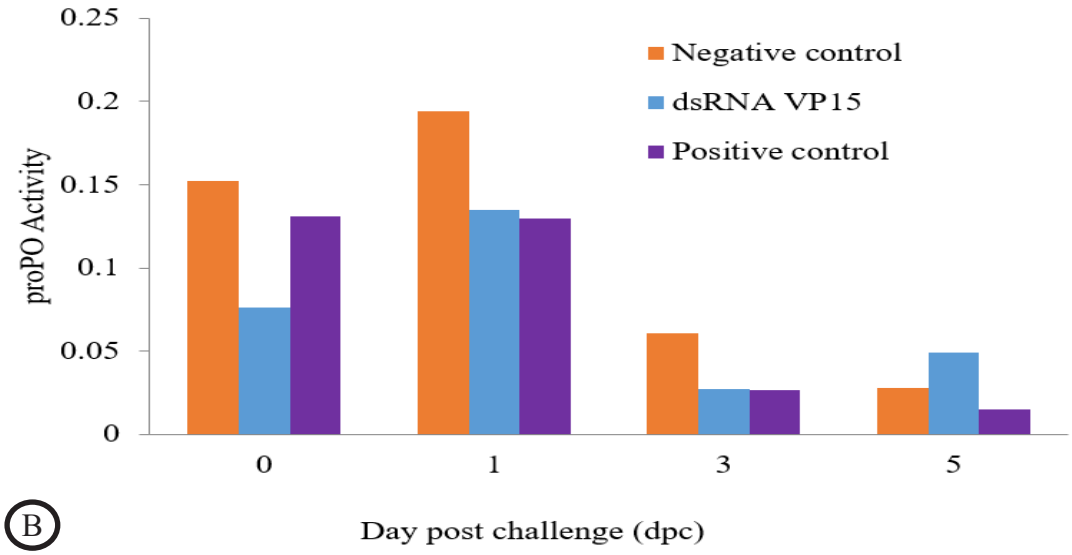


Figure 16. The THC (A) and proPO (B) of tiger shrimp before and the 1st, 3rd and 5th-day post-challenge test (Parenrengi *et al*., 2022).

Application of VP15-dsRNA (10^8 cells inactive bacteria/0.02 g feed) was reported to the juvenile tiger shrimp in the size of 25.2±4.2 g and 13.6±0.7cm. The survival rate of the vaccinated tiger shrimp was much higher than that of the two control treatments, with 30% survival on the 5-day post-challenge and quite stable at 27.3% until the end of the experiment (Figure 15).

The result of different concentrations of VP15-dsRNA by oral administration showed that the highest average THC content (1.98×10^7 cells/mL) was found in the feed enriched with dsRNA, which was followed by the negative control (1.31×10^7 cells/mL) and the positive control (1.09×10^7 cells/mL) (Figure 16A). ProPO activity on the 5 dpc showed greater values in vaccinated tiger shrimp than in both controls. However, the statistical analysis showed that the average proPO activity throughout the treatments did not differ significantly ($P>0.05$) (Figure 16B).

3.2 Discussion

3.2.1 Characteristics of VP15-WSSV and dsRNA production

Some researchers have reported the success in isolating and characterizing the VP15 gene of WSSV. The gene encoding VP15-WSSV isolated in the years 2012, 2013, and 2014 from tiger shrimp in Indonesian pond culture exhibited a high nucleotide similarity; however, those isolates in 2013 revealed some differences among the other isolates (Parenrengi *et al*., 2017). The nucleocapsid major protein of 15 kDa (VP15) has been identified and characterized from infected crayfish, and it was recommended that VP15 is a potential candidate DNA-binding protein in the WSSV nucleocapsid (Van Hulten *et al*., 2002). The position of the DNA fragment of VP15 isolated from Indian tiger shrimp, in a length of 245 bp (Sarathi *et al*., 2010), was quite similar to the research above, which consisted of 80 amino acids and in length of 243 bp (Parenrengi *et al*., 2018; 2017).

Studies have shown that the gene encoding VP15, as a basic, histone-like DNA-binding protein, was found to interact with itself, producing homomultimers, and had a significant propensity for binding supercoiled DNA, implying a role in packaging the viral genome within the nucleocapsid (Witteveldt *et al*., 2005). The gene for VP15 was successfully cloned into the L4440 cloning vector and expressed in *E. coli*, enabling future studies on its function and potential applications. The analysis of VP15 gene sequences from WSSV isolates in Indonesia demonstrated substantial nucleotide similarity, with some differences identified in individual isolates, showing genetic diversity among WSSV strains. The VP15 protein's DNA-binding capabilities and structural traits highlight its potential role in WSSV structural integrity and replication, making it a target for future virological research and therapeutic interventions.

Before applying, the dsRNA has to be produced to provide the vaccine material. The procedure of dsRNA production *in vivo* had several stages based on the procedure developed by Parenrengi *et al.* (2020). After cloning the gene encoding VP15 of WSSV into the L4440 RNAi vector, a T7-VP15 gene construct was subsequently transformed into the *E. coli* DH5α bacteria. A culture of recombinant bacteria on liquid LB media was used to prepare dsRNA vaccine material, which was then incubated overnight. Bacteria were harvested by centrifugation, and the pellet, as a DNA template, was applied to verify the gene constructs in plasmids.

The successful production of *in vivo* dsRNA using bacteria offers a promising perspective on a bigger scale for immersion applications and shrimp feed enrichment. Using bacterial cells to produce RNAi on a large scale is a hopeful and affordable approach (Ongvarrasopone *et al*., 2007). In shrimp facilities like grow-out ponds or hatcheries, RNAi can be effectively introduced by delivering the molecule through the feed, either by macromolecule coating or binding RNAi or by inactive bacterial cells expressing RNAi (Escobedo-Bonilla, 2011). Studies have shown that this approach effectively induces an immune response in shrimp, enhancing their survival rates upon WSSV challenge.

3.2.2 Immersion delivery method

The potential for employing dsRNA by immersion to enhance the resistance of tiger shrimp larvae to WSSV was indicated by the 3.9% improvement in survival for VP15-dsRNA (equivalent to a 4.6% increase in the percentage of enhancement). However, to develop the appropriate RNAi technology for tiger shrimp, modifications to the process, dosage, immersion duration, and larval stage were necessary. The greater survival rate of dsRNA-vaccinated tiger shrimp compared to unvaccinated tiger shrimp indicates significant protection against WSSV infection. This hypothesis is confirmed by the fact that the VP15 gene is highly similar to histone proteins and that this protein is reported to be involved in the WSSV DNA binding, nucleoprotein core formation, systemic infection, and immune system stimulation in tiger shrimp (Underwood *et al*., 2013). A similar finding was reported in VP24-dsRNA by larval immersion, which showed higher survival in vaccinated tiger shrimp

(86.9%) compared to the control shrimp (83.1%) 10 days after the challenge test (Parenrengi *et al*., 2020).

According to the most recent research on immersion techniques, in order to completely elicit gene silencing, nanoparticle additions including chitosan, liposomes, and cationic dendrimers may be necessary to stabilize dsRNA in the soaking solution and enhance a delivery system (Phromma-in *et al*., 2025). They discovered that when pre-soaked shrimp were challenged with WSSV, the cumulative mortality dropped from 47% in the control group to 16.6%, and the virus levels in the gills plummeted by 87% in the low-dose group.

3.2.3 Injection delivery method

The use of VP28 and VP28+VP29 mixture vaccines by injection on tiger shrimp resulted in increased relative percentage survival (RPS) values of 44 percent and 33 percent, 2 days after the challenge test, respectively, when compared to the control (Witteveldt *et al*., 2004). The other study found that injecting the VP28 and VP26 vaccines into shrimp *P. japonicas* boosted resistance to WSSV by 95 and 80 percent, respectively (Namikoshi *et al*., 2004), while injecting the VP28-dsRNA vaccination into *Macrobrachium rosenbergii* increased resistance by 44.5 percent (Jariyapong *et al*., 2015).

A significant effect on the THC and proPO activity using the VP15-dsRNA was exhibited in the tiger shrimp (Parenrengi *et al*., 2019). The VP24-dsRNA vaccine application showed a significant influence on the THC in haemocytes, then a dose of 0.2 g/shrimp was observed as the optimal dose (THC of 1.55×10^{7} cells/mL) (Mulyaningrum *et al*., 2018). Tiger shrimp proPO gene expression was not significantly affected by the VP28-dsRNA up to 24 hours after the challenge test, but it increased threefold at 48 hours later (Paria *et al*., 2013).

The different dosage study suggested that the 0.02 g VP15-dsRNA vaccination could boost tiger shrimp survival rates by up to 15% (Parenrengi *et al*., 2021b). According to certain research, a recombinant DNA vaccine construct could protect against WSSV infection in tiger shrimp by up to 62% (Krishnan *et al*., 2009). By administering an injection of the VP24 vaccine (*in vivo*), tiger shrimp cumulative mortality rose to 37% (Sarathi *et al*., 2010), whereas tiger shrimp survival improved to 50% at a dose of 2.5 g/g shrimp (Puneeth *et al*., 2017). In white shrimp *L. vannamei*, the introduction of dsRNA vaccinations at a dose of 2.0 g enhanced the survival rate against IHNV by 100% (Loy *et al*., 2012). On the ninth day following the WSSV challenge test, injection with plasmids expressing WSV-477, VP39, and VP28 showed survival rates of 90%, 60%, and 50% in comparison to the controls (0%) (Akhila *et al*., 2015).

The different doses of VP15-dsRNA have been studied in their effect on the tiger shrimp. Tiger shrimp showed a trend of declining THC levels post-WSSV challenge test (Parenrengi *et al*., 2021b). On average, the proPO activity value was comparable across dosages; the highest proPO activity was obtained at 0.2 g, followed by the control, 2.0 g, and 0.02 g. However, according to the statistical analysis, proPO activity in tiger shrimp was unaffected by the different dosages of VP15-dsRNA. Based on the recent work, proPO activity in tiger shrimp reached its peak (0.042) when VP24-dsRNA was applied at a dose of 0.2 g (Mulyaningrum *et al*., 2018). A previous study also showed that VP15 was found to be an effective candidate against WSSV infection when it was used in Kuruma shrimp, as evidenced by an increase in RPS against WSSV (Boonyakida *et al*., 2020). The other study showed that the polyanhydride nanoparticle delivery platform could effectively administer dsRNA antivirals to shrimp by the injection approach (Phanse *et al*., 2022). In a recent study by Suksai *et al*. (2025), the injection of composite polymer-clay nanoparticles, based on a bentonite biopolymer (BenPol), into shrimp demonstrated a potential strategy for dsRNA delivery in shrimp aquaculture.

The aforementioned study on dsRNA injection showed a dosage-dependent increase in total hemocyte count (THC), with shrimp receiving 0.02 g/shrimp having the greatest THC. This suggested that the dose of VP15-dsRNA may promote a more robust immune response in terms of hemocyte proliferation. While THC levels were impacted by the VP15-dsRNA doses, proPO activity, another immunological metric, did not significantly change across the different dosages, suggesting that proPO activity was mostly unaffected. According to these results, VP-dsRNA can improve some immunological parameters, but its effects might differ depending on the immune system's components. This underscores the complexity of immune modulation in shrimp (Chen *et al*., 2024).

3.2.4 Oral delivery method

The oral delivery of the vaccine is expected to be an effective approach for widespread application (Boonyakida *et al*., 2022; Jonjaroen *et al*., 2025). A vaccination strategy needs the use of dsRNA administered orally, and *in vivo* dsRNA production is simpler and more easily done in large quantities. A straightforward and efficient method for implementing RNAi

technology in shrimp facilities, such as hatcheries or grow-out ponds, is by feeding animals in large quantities. This can be done by using either a macromolecule that encapsulates binding RNAi or inactive bacterial cells that express RNAi (Escobedo-Bonilla, 2011).

The oral administration of VP15-dsRNA to the larvae and juveniles (Parenrengi *et al*., 2022) of tiger shrimp revealed survival and immune response improvement. Similar findings were reported by the application of VP19 and VP28. Oral administration of the VP19-dsRNA vaccine via tiger shrimp feed improved survival following the WSSV challenge test, with an RPS value of 77% on day 7th and 29% on day 21st (Witteveldt *et al*., 2004). In WSSV infection trials, shrimp given the codon-optimized VP28 line mixed with their feed had the highest survival rate (87%); therefore, this line may be useful for WSSV control in shrimp populations (Kiataramgul *et al*., 2020). These findings suggested that while VP15-dsRNA showed efficacy, other constructs like VP19 and VP28 may offer higher levels of protection, indicating that the choice of dsRNA sequence is crucial for optimizing vaccine efficacy. Overall, these studies underscore the potential of dsRNA-based vaccines in aquaculture, highlighting the need for further research to identify the most effective dsRNA sequences and dosages for combating WSSV in shrimp populations.

The comparative effectiveness of oral, injectable, and immersion dsRNA delivery techniques has not been thoroughly investigated in shrimp aquaculture. Although the effects of different delivery systems cannot be directly compared, the progress results showed that the use of the injection method provided the highest survival rate, reaching an increase of 75%, then the oral method (27.3%), and the lowest immersion method, only around 3.9%. There was no THC and proPO data for the immersion treatment of larvae. The increase in THC value was higher in the injection method compared to the oral method, namely 100-225% and 51.1-81.6%, respectively. The trend of proPO activity also showed a higher increase in the injection method (52.5-471.5%) compared to the control (75.0-226.0%). Every technique has distinct benefits and drawbacks that may affect how well RNAi treatments are delivered as a basic therapy application (Fajardo *et al*., 2024; Liu *et al*., 2025). By using the findings of research advancements, this brief review provides the present comparison of the three dsRNA delivery techniques. Conclusions can be utilized as a guide for future dsRNA vaccine applications, even though the study is currently primarily focused on the development of VP15-dsRNA research on tiger shrimp. It would be intriguing to find out in future research why and how VP15 functions in shrimp immunological memory. Additionally, significant suggestions can be made for future, more thorough research.

4. Conclusions

Based on the progress studies' results, it could be concluded that the delivery of VP15-dsRNA using the three administration methods provides potential approaches to increase tiger shrimp resistance to control pathogen infection. The immersion method was generally applied for the larval stage, and it seemed to be a simple technique for a large number of individuals. The injection technique was an effective way to deliver dsRNA to the cell; however, it did not seem suitable for the enormous delivery and continuous mass RNAi vaccine production required for large animal populations in grow-out or hatcheries. The oral administration using pellet-enriched dsRNA may be used for larvae, juveniles, and broodstock, but it has limitations since the pellet leaches into the water. The dsRNA is recommended to be applied according to the developmental stages of shrimp, and oral delivery appears to be effective across all stages and mass-scale uses. Future studies are necessary to improve the technical application of RNAi technology on the tiger shrimp, including dsRNA concentration, period of application, protection time, application to the pond rearing culture, and dsRNA encapsulation.

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Authors' Contributions

AP, SL, and ES conducted the literature search and wrote the manuscript. RR contributed to improving the manuscript. SS, HH, and YA supervised the process.

Conflict of Interest

The authors declare that they have no competing interests.

Declaration of Artificial Intelligence (AI)

The authors acknowledge the use of ChatGPT for language refinement and summarization in preparing this manuscript. All AI-generated content was

rigorously reviewed, edited, and validated to ensure accuracy and originality. Full responsibility for the manuscript's final content rests with the authors.

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