Sains Malaysiana 52(7)(2023): 1915-1923
http://doi.org/10.17576/jsm-2023-5207-02

Survival and Immune Responses of F_1 Transgenic Tiger Shrimp *Penaeus monodon* against White Spot Syndrome Virus (WSSV)

(Kemandirian dan Tindak Balas Kekebalan Udang Harimau Transgen F1 *Penaeus monodon* terhadap Virus Sindrom Bintik Putih (WSSV))

Andi Parenrengi[1,*], Andi Tenriulo[1], Bunga Rante Tampangallo[1], Herlinah Herlinah[1], Rosmiati Rosmiati[1], Emma Suryati[1], Alimuddin Alimuddin[2], Samuel Lante[1], Agus Nawang[1], Suwardi Suwardi[1] & Andi Aliah Hidayani[3]

[1]*Research Center for Fishery, National Research and Innovation Agency (BRIN), Cibinong 16911, Indonesia*
[2]*Department of Aquaculture, IPB University, Bogor 16680, Indonesia*
[3]*Department of Fisheries, Hasanuddin University, Makassar 90245, Indonesia*

Received: 14 December 2022/Accepted: 7 July 2023

ABSTRACT

Transgenic technology has been applied to tiger shrimp *Peneus monodon* to produce a resistant strain to white spot syndrome virus (WSSV) by antiviral gene overexpression. The founders (F_0) transgenic tiger shrimp have been successfully bred to produce the first generation (F_1). The present study aimed to evaluate the survival and immune responses of F_1 transgenic tiger shrimp against WSSV. The F_1 transgenic and non-transgenic shrimp (control) with an average weight of 7.93±1.49 g were collected from the controlled brackish water ponds and stocked in a 20 L fiberglass tank with a density of 5 shrimp/tank. The transgenic shrimps were confirmed by a PCR assay. The shrimp were intramuscularly injected with WSSV to evaluate the performance between transgenic and non-transgenic. The survival (SR) was observed daily after the challenge test. Measurement of immune responses, namely total haemocyte count (THC), differential haemocyte count (DHC), prophenoloxidase (proPO) activity, and RNA content in haemolymph was conducted on before challenge, and on the 1st, 3rd, and 5th days after challenge. The survival and immune responses were statistically analysed by the t-student test. The results showed that the SR of transgenic (52.0%), with a relative percentage survival of 47.82%, was greater ($P<0.05$) than the control shrimp (8.0%). The THC, proPO, and RNA content of the transgenic shrimp was higher ($P<0.05$) than the non-transgenic shrimp. The results suggested that the transgenic tiger shrimp, due to antiviral gene overexpression, increased the resistance against WSSV.

Keywords: Challenge test; F_1 transgenic tiger shrimp; immune response; survival rate; white spot syndrome virus

ABSTRAK

Teknologi transgen telah digunakan pada udang harimau *Peneus monodon* untuk menghasilkan strain tahan terhadap virus sindrom bintik putih (WSSV) oleh ekspresi berlebihan gen antiviral. Pengasas (F_0) udang harimau transgen telah berjaya dibiakkan untuk menghasilkan generasi pertama (F_1). Kajian ini bertujuan untuk menilai kemandirian dan tindak balas imun udang harimau transgen F_1 terhadap WSSV. Udang transgen dan bukan transgen F_1 (kawalan) dengan berat purata 7.93±1.49 g telah dikumpul daripada kolam air payau terkawal dan disimpan dalam tangki gentian kaca 20 L dengan ketumpatan 5 ekor udang/tangki. Udang transgen telah disahkan oleh ujian PCR. Udang telah disuntik secara intramuskul dengan WSSV untuk menilai prestasi antara transgen dan bukan transgen. Kemandirian (SR) diperhatikan setiap hari selepas ujian cabaran. Pengukuran tindak balas imun, iaitu jumlah bilangan hemosit (THC), kiraan hemosit pembezaan (DHC), aktiviti profenoloksidase (proPO) dan kandungan RNA dalam hemolimfa telah dijalankan pada sebelum cabaran dan pada hari ke-1, ke-3, dan ke-5 selepas cabaran. Kemandirian dan tindak balas imun dianalisis secara statistik oleh ujian t-pelajar. Keputusan menunjukkan bahawa SR transgen (52.0%), dengan peratusan kemandirian relatif 47.82%, adalah lebih besar ($P<0.05$) daripada udang kawalan (8.0%). Kandungan THC, proPO dan RNA udang transgen adalah lebih tinggi ($P<0.05$) berbanding udang bukan transgen. Hasilnya mencadangkan bahawa udang harimau transgen, disebabkan ekspresi berlebihan gen antivirus, meningkatkan daya tahan terhadap WSSV.

Kata kunci: Kadar kemandirian; tindak balas imun; udang harimau transgen F_1; ujian cabaran; virus sindrom bintik putih

Introduction

Tiger shrimp *Penaeus monodon,* which is of Indo-West Pacific origin (Khafage, Taha & Attallah 2019) and has been the main shrimp species cultured for several decades in the eastern hemisphere due to its large final size and rapid growth rate compared to other penaeid species (Coman et al. 2005). However, since the 1990s, the tiger shrimp aquaculture industry has been confronted with serious viral disease problems of white spot disease caused by WSSV infection (Sánchez-Paz 2010). Therefore, to supply high-quality seeds for domestication and cultivation, in-depth investigations on the improvement of SPF (specific pathogen-free) and SPR (specific pathogen resistance) seeds are required.

Several studies have demonstrated the domestication of black tiger shrimp broodstock, including the development of SPF broodstock domestication techniques in ponds and controlled tanks (Suwoyo et al. 2020). The successful domestication of tiger shrimp in Australia has significantly contributed to the development of tiger shrimp aquaculture. Coman et al. (2013) reported that the domesticated tiger shrimp up to generation 8 (F_8) had demonstrated increases in the production of egg and nauplii of the stocks throughout subsequent generations. However, the SPR tiger shrimp production is still limited to be assessed. A transgenic-based approach could be potentially applied to develop disease-resistant tiger shrimp.

The development of fish transgenesis provides excellent research models for fundamental scientific study, biotechnology, and environmental toxicity (Wakchaure et al. 2015). Yazawa et al. (2005) used chloramphenicol acetyltransferase (CAT) and green fluorescence protein (GFP) as marker genes to evaluate gene transfer strategies for black tiger shrimp. As a part of efforts to improve tiger shrimp resistance, a basic study and application of antiviral gene transfer were successfully conducted. Previous research showed that the promoter (pProAV) and antivirus (PmAV) gene could be isolated from the tiger shrimp survivors (Parenrengi et al. 2009), as well as the gene construct of pProAV-EGFP could be introduced into shrimp to study promoter activity (Parenrengi et al. 2018). The pProAV-PmAV 'all shrimp gene construct' was transferred to tiger shrimp embryos at a rate of 75%, and the transgenic shrimp increased its resistance to 24.5% against WSSV and 67% against *Vibrio harveyi* (Parenrengi, Tampangallo & Tenriulo 2014).

The transgenic tiger shrimp founder (F_0) was assessed to confirm the insertion of pProAV-PmAV before grow-out in the controlled tanks or ponds to get the broodstock for producing the transgenic tiger shrimp generation-1 (F_1). Production of F_1 transgenic tiger shrimp broodstock has been successfully conducted in controlled ponds with survival rates of 51.7-81.5% (Suwoyo & Sahabuddin 2017; Suwoyo et al. 2020). A challenge test is an effective approach to evaluate the tiger shrimp performance against pathogens. Therefore, the performance of F_1 transgenic shrimp needs to be evaluated, especially to determine its resistance to WSSV. This present study aimed to evaluate the survival rate and immune responses of the F_1 transgenic tiger shrimp by a WSSV challenge test.

Materials and Methods

Animal testing

The F_1 transgenic tiger shrimp of approximately two months of age were collected from the controlled brackish water ponds at The Tiger Shrimp Station, RIBAFE in Barru, South Sulawesi, Indonesia. The shrimp were cultured according to the standard protocol of shrimp growth-out at ponds (Suwoyo & Sahabuddin 2017; Suwoyo et al. 2020) and screened through PCR technique to verify the transgenic tiger shrimp marker (Parenrengi et al. 2021), while the F_1 non-transgenic tiger shrimp as a control group. A total of 40 selected F_1 tiger shrimp with a total length of 9.15±0.58 cm and an average weight of 7.93±1.49 g were acclimatised in the indoor system using eight aquariums containing 20 L seawater, with a density of 5 shrimp.

Challenge test with WSSV

A challenge test was conducted on the F_1 transgenic (A) and non-transgenic shrimp as a control treatment (B). Each treatment had four replications (three for survival and one for immune response observation). The WSSV was collected from tiger shrimp infected with white spot disease before being injected by the intramuscular method in a dose of 100 µL/shrimp (Mulyaningrum et al. 2018). During the acclimatisation and the challenge test, a commercial shrimp pellet was used to feed the shrimp twice a day at 3.5% of body weight.

Survival and immune responses observation

Survival rate (SR) was observed daily post-challenge tests (dpc), and immune responses, i.e.,: total haemocyte count (THC), differential haemocyte count (DHC), prophenoloxidase (proPO), and RNA content in the haemolymph were observed before and 1st, 3rd, and

5th dpc. Each parameter consisted of three individual replicates.

The THC was determined by drawing 0.1 mL haemolymph from the 2nd abdominal segment using a 1.0 mL syringe attached to a 26-gauge needle with a 3.8% sodium citrate anticoagulant (Van-De Braak et al. 2002). A handshaking of a figure-eight shape was applied to homogenise the mixture. After discarding the first drop, the second was dripped down onto a haemocytometer. The total number of haemocyte cells was counted under a microscope at a 100-fold magnification and estimated by a formula by Van-De Braak et al. (2002).

The activity of proPO was measured using a GeneQuant 1300 (GE Healthcare, USA) according to the dopachrome formation induced by L-dihydroxy phenylalanine (L-DOPA) (Sigma-Aldrich, USA). In addition, the activity of proPO was measured according to the standard procedure developed by Liu and Chen (2004).

DHC was observed using a haemocytometer (Neubauer Improved, China) under a microscope (Olympus BX40, Japan) to morphologically identify the type of cells (hyaline, granular, and semi-granular (Rowley & Pope 2012). They were calculated based on the composition of the haemocyte cell type.

RNA concentration was measured according to the reference of Parenrengi, Tonnek and Tenriulo (2013). RNA concentration was measured using GeneQuant 1300 (GE Healthcare, USA). A total of 7 µL of DNA or RNA was inserted into a 5 mm cuvette using TE buffer as the standard solution. The measurement was carried out at an absorption wavelength of 260 nm (A_{260}) to obtain the RNA concentration (Linacero, Rueda & Vazquez 1998).

DATA ANALYSIS

The survival rate, immune responses (THC and proPO activity), and RNA concentration were analysed by t-student test using an SPSS program at a confidence level of 0.05, while DHC was descriptively presented.

Results and Discussion

SURVIVAL RATE

The mortality of non-transgenic shrimp sharply increased on the 2nd dpc and continued until the end of the study (Figure 1). The trend of high survival in F_1 transgenic shrimp has shown since the 2nd dpc, and even up to the 5th dpc, the non-transgenic shrimp almost all died. At the 5th dpc, the transgenic shrimp showed a greater resistance ($P<0.05$) to infection of WSSV (52.0% SR) than the control shrimp (8.0% SR), equal to 47.82% of relative percentage survival. These data indicated the increased survival of transgenic shrimp by 44%, which may be the effect of overexpression of the PmAV gene carried by transgenic tiger shrimp.

Our previous study on transgenic shrimp larvae (F_0) showed increased resistance to WSSV infection, indicating that the transgenic tiger shrimp larvae showed a better survival percentage compared to the control group. Parenrengi et al. (2021) reported that the survival of transgenic (95.6%) was significantly higher than non-transgenic tiger shrimp larvae (71.1%) at five days post-challenge test. The survival increment of transgenic shrimp suggested that the PmAV antiviral gene overexpression plays a strategic role in enhancing the resistance of tiger shrimp to WSSV infection. Resistant improvement of *L. vannamei* by transferring a TSV-CP gene was reported by Lu and Sun (2005), in which the transgenic exhibited a greater survival (83%) than the control shrimp (44%).

Transgenic approaches provide two major advantages over traditional selective breeding: The capacity to use genes and DNA constructs across species barriers and the ability to enhance growth, disease resistance, and other qualities in a very short period (Xiaojun & Jianhai 2003). In immunity enhancement, fish protection occurs during the larval development stage before the immune system matures (Dunham 2009). The transfer of an antimicrobial peptide gene of cecropin to channel fish *Ictalurus punctatus* increased the bacterial disease resistance up to 2-4 times compared to the control fish group (Dunham et al. 2002). The performance of the transgenic catfish carrying the preprocecropin gene construct in combating *Flavobacterium columnare* was much better (100% survival) than the non-transgenic fish (27.3%). Furthermore, when exposed to *Edwardsiella ictaluri*, the transgenic catfish had a higher survival rate (40.7%) than non-transgenic fish (14.8%). Moreover, Sarmasik, Warr and Chen (2002) reported that the F_2 transgenic medaka fish had high resistance to *Pseudomonas fluorescens* (SR 90-100%) compared to the control fish (SR 60%), and to *Vibrio anguillarum*, the transgenic fish survived 70-90%, while the control only survived around 60%. A study of challenge tests also reported that the F_1 and F_2 transgenic rainbow trout families exhibited resistance to infection of IHNV and *Aeromonas salmonicida* (Chiou et al. 2014).

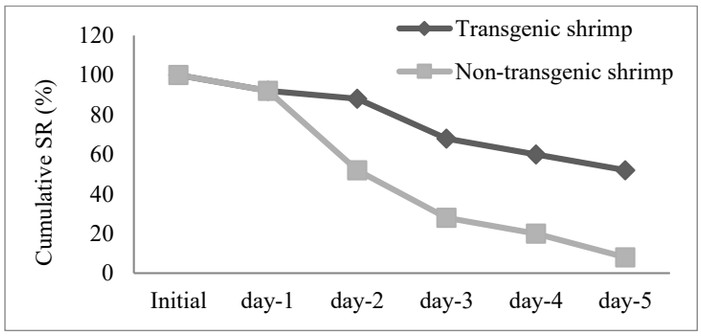

FIGURE 1. The cumulative survival rate of transgenic and non-transgenic tiger shrimp after the WSSV challenge test

TOTAL HAEMOCYTE COUNT

THC is one of the haemolymph parameters that can be applied to detect the level of shrimp resistance (Novriadi et al. 2021). The result of the THC observation indicated that a higher THC in transgenic shrimp had been shown since the beginning of the challenge test compared with the control shrimp (Figure 2). The average value of the THC post-challenge test of transgenic tiger shrimp (2.19×10^7 cells/mL) was significantly higher ($P<0.05$) than the non-transgenic tiger shrimp (1.10×10^7 cells/mL). The present study pointed out that the transgenic tiger shrimp can fight pathogens, in particular by increasing the amount of THC in the haemolymph. Our previous study also showed that the transgenic tiger shrimp had higher total THC (1.57×10^7 cells/mL) than non-transgenic tiger shrimp (1.13×10^7 cells/mL) when challenged with *Vibrio harveyi* (Parenrengi, Tampangallo & Tenriulo 2014). The role of the antiviral gene in promoting the formation of haemolymph cells for the body's defenses may cause the increased total haemolymph cells in transgenic shrimp.

The resistance pattern of THC observation tended to decrease on the 1st dpc, then increased until the 3rd dpc and decreased again in 5th dpc. Most crustaceans, including transgenic and non-transgenic shrimp, resist after exposure to pathogens, as indicated by an increase in THC in the haemolymph for self-protection to survive. Improvement of THC is a form of resistance against pathogens during the experimental period. Our previous study also reported a similar pattern of total haemocyte between transgenic and non-transgenic shrimp when challenged with *Vibrio harveyi* (Parenrengi, Tampangallo & Tenriulo 2014). A similar finding on the THC of dsRNA vaccinated tiger shrimp post-challenge test has been reported by Parenrengi et al. (2022). Haemocyte migration from the body's circulatory system to the tissues because of infected cells is a common reason for the decreased THC post-challenge test results (Yeh et al. 2009). The increased amount of haemocyte cells aids in the inhibition or damage of infections that enter the body. In several studies, the improvement of THC in tiger shrimp also has been reported by using the dsRNA vaccine. The highest THC in tiger shrimp by applying the dsRNA VP-24 vaccine, as much as 1.55×10^7 cells/mL, has been reported by Mulyaningrum et al. (2018). Kanagu et al. (2010) used vitamins C, E, and β-1,3 glucan to increase shrimp THC to 1.7×10^7, 1.5×10^7, and 18×10^6 cells/mL, respectively. The use of nucleotides in the white shrimp diet enhanced the total number of haemocytes by up to 87% compared to control shrimp, and these nucleotides can also improve cell multiplication for growth (Manoppo et al. 2011). Application of seaweed extract of *Codium* sp. to the feed increased the THC of tiger shrimp (Siswati, Anshary & Parenrengi 2021).-

DIFFERENTIAL HAEMOCYTE COUT

Differential haemocyte count (DHC) is known as a comparison of the three distinct shrimp haemocyte cell types (semi-granular, granular, and hyaline) (Kakoolaki et al. 2011). The hyaline and granular cells decreased at the 1st dpc and subsequently increased at the 3rd and the 5th dpc, while the hyaline cell increased at the 1st dpc and then decreased at the 3rd and the 5th dpc for both non-transgenic and transgenic shrimp (Table 1). This trend illustrated the change in differential haemocyte count during the challenge test, in which the hyaline and granular cells may have an essential role in the immune

responses of shrimp. The hyaline cells, the smallest type among the haemocytes, are identified as active phagocytes, while granular cells are identified to contain protease inhibitors, agglutinins, antimicrobial peptides, and cell adhesion/degranulation/factor (Kulkarni et al. 2021).

Before the challenge test, the percentage of semi-granular cells was higher in non-transgenic shrimp than in transgenic shrimp (Table 1). The finding of a lower percentage of semi-granular cells in transgenic shrimp could indicate the possibility that the overexpression of the antiviral gene triggered the maturation of hemocyte cells more quickly than in control shrimp during the grow-out in the pond. The granular cell is commonly known as a mature blood cell, which is accumulated in the connective tissue, so it is easily released into the haemolymph for self-protection (Van-De Braak et al. 2002).

The percentage of granular cells had the highest proportion among the three haemocyte cells (Figure 3). Transgenic shrimp had granular cells of 77%, higher than those in non-transgenic shrimp (66%). The non-transgenic shrimp showed a higher percentage of semi-granular cells than the transgenic shrimp, while no significant difference was observed in the semi-granular cell for both treatments. The highest percentage of granular cells in this study indicated that transgenic shrimp carrying the PmAV gene could increase the immune system by inducing haemocyte improvement. It has been determined that granular cells play a crucial part in the cytotoxic activity to produce and release the proPO system (Kakoolaki et al. 2011). The peptidoglycan from the bacterium *Bifidobacterium thermophilum* also improved the granular cell and stimulated phagocytosis in the kuruma shrimp (*Penaeus japonicas*) (Itami et al. 1998).

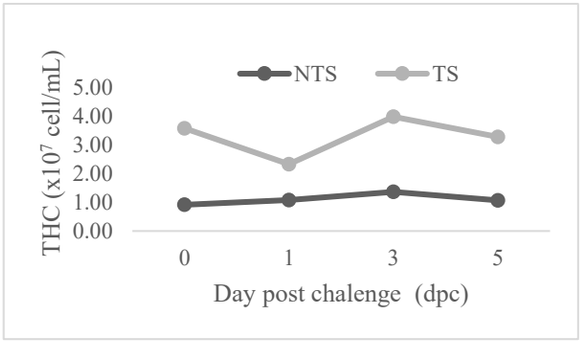

FIGURE 2. THC before, 1st, 3rd, and 5th dpc of transgenic and non-transgenic shrimp after the WSSV challenge test. NTS=Non-transgenic shrimp, TS=transgenic shrimp, and the average THC with different letters were significantly different (P<0.05)

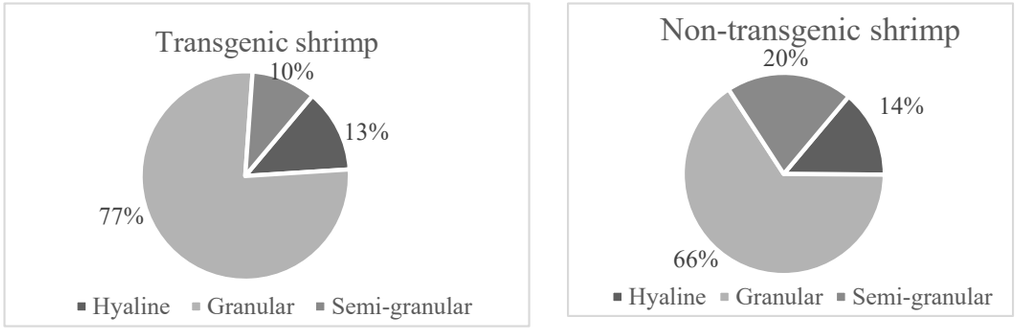

FIGURE 3. The average differential haemocyte count of transgenic and non-transgenic tiger shrimp post-challenge test

PROPHENOLOXIDACE ACTIVITY

The prophenoloxidase (proPO) activity was relatively stable until the 3rd dpc for both non-transgenic and transgenic shrimp, and proPO activity increased in transgenic shrimp but decreased in non-transgenic shrimp on the 5th dpc (Figure 4). The average proPO activity was statistically higher ($P<0.05$) in transgenic (0.0615) than in non-transgenic shrimp (0.0102). Increased proPO activity in haemolymph indicates an increased immune response against pathogens. The activation cascade of proPO plays an essential role in shrimp's immune response to pathogenic infection (Amparyup, Charoensapsri & Tassanakajon 2013). One of the most effective shrimp immune responses against pathogens is activating the proPO system by activating the PO enzyme as a cellular melanotic encapsulation or melanisation (Tassanakajon et al. 2018). Whilst, decreased proPO activity can cause failed phagocytosis and tissue damage (Amparyup, Charoensapsri & Tassanakajon 2013).

A similar finding of the proPO activity in transgenic shrimp was obtained in vaccinated shrimp when challenged with WSSV. The use of the dsRNA vaccine on tiger shrimp significantly increased the proPO activity compared with the control group (without the dsRNA vaccine) (Mulyaningrum et al. 2018; Parenrengi et al. 2019). On the other hand, the WSSV challenge test to dsRNA VP28 vaccinated tiger shrimp did not show a significantly increased value in the expression of the proPO gene up to 24 h, but a threefold expression enhancement of vaccinated tiger shrimp was observed at 48 h post-challenge test (Paria et al. 2013).

Overall, the immune responses of proPO and THC were higher in transgenic shrimp than the non-transgenic shrimp even before the WSSV challenge. An explanation for this may be that introducing the antiviral gene could induce gene expression in general terms of immune responses and particular expression of antiviral

TABLE 1. The differential haemocyte count of transgenic and non-transgenic tiger shrimp post-challenge test

Shrimp	Cell type	Initial	1st dpc	3rd dpc	5th dpc
Transgenic	Hyaline (%)	14.3	10.1	18.4	8.7
	Granular (%)	81.0	71.9	74.5	81.1
	Semi-granular (%)	4.7	18.0	7.1	10.2
Non-transgenic	Hyaline (%)	16.1	12.5	13.2	14.2
	Granular (%)	70.6	45.7	69.4	77.0
	Semi-granular (%)	13.3	41.8	17.4	8.8

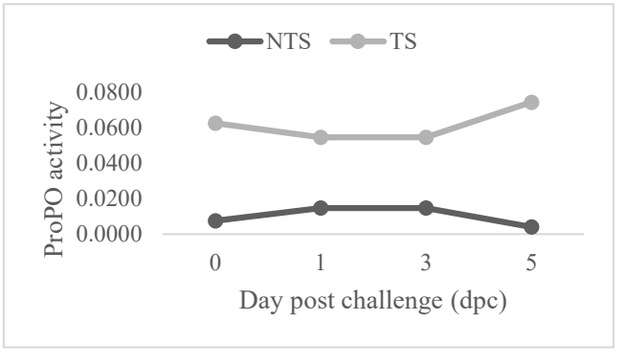

FIGURE 4. ProPO activity before, 1st, 3rd, and 5th dpc of transgenic and non-transgenic shrimp after the WSSV challenge test. NTS=Non-transgenic shrimp, TS=transgenic shrimp, and the average proPO activity with different letters were significantly different ($P<0.05$)

genes against pathogens during the grow-out at the pond, indicated by higher survival. The previous study showed that the survival of transgenic tiger shrimp (called transfection tiger shrimp) was 81.5%, higher than the control groups (71.5%) for 90 days of culture in the pond (Suwoyo et al. 2020).

TOTAL RNA CONTENT

The total RNA content in the haemolymph of transgenic was higher than the non-transgenic tiger shrimp, where the decreased concentration occurred at the 1st dpc and the 5th dpc, but increased at the 3rd dpc (Figure 5). A similar trend was obtained in the THC observation. The decrease in RNA content can be caused by the pressure of the pathogen infecting the tiger shrimp. The present study showed that the transgenic exhibited a higher RNA content than the non-transgenic tiger shrimp during the challenge test. On average, the total RNA concentration was statistically higher ($P<0.05$) in the transgenic shrimp (59.90 µg/mL) compared with non-transgenic shrimp (34.00 µg/mL).

Results of the present study indicated that the transgenic tiger shrimp might have the ability to induce gene expression in general and, in particular, overexpression of antiviral genes against viral pathogens by the increase of RNA concentration. The enhancement of transgenic tiger shrimp resistance to the WSSV has been reported by overexpression of the antiviral gene on the larvae (Parenrengi et al. 2021). The RNA concentration has been widely applied as an indicator for growth markers in shrimp (Haryanti et al. 2006; Moss, Harbor & Loa 1994; Parenrengi, Tonnek & Tenriulo 2013). The introduction of the antivirus gene PmAV controlled by an antiviral promoter of tiger shrimp was supposed to have an important key to increase the RNA concentration. However, the pathway of the relationship between overexpression of antiviral genes in increasing RNA concentration in tiger shrimp still requires a more comprehensive and in-depth study.

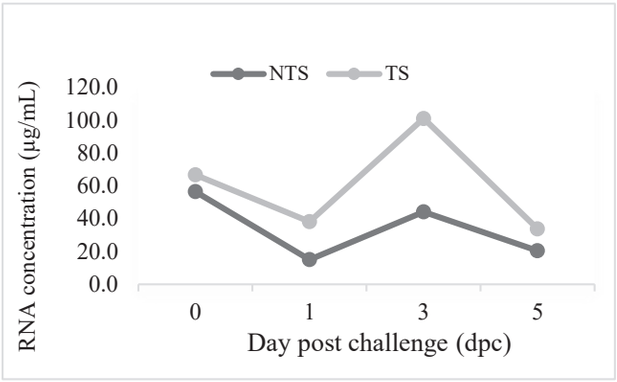

FIGURE 5. Total RNA concentration before, 1st, 3rd, and 5th dpc of transgenic and non-transgenic shrimp post-challenge test. NTS=Non-transgenic shrimp, TS=transgenic shrimp, and the average RNA concentration with different letters were significantly different ($P<0.05$)

CONCLUSION

The F_1 transgenic tiger shrimp exhibited a higher survival rate than the non-transgenic tiger shrimp. The higher THC, proPO activity, and RNA content in haemolymph were also showed in the transgenic shrimp compared to the control shrimp. The results indicated that the antiviral gene overexpression of the F_1 transgenic tiger shrimp increased the resistance against WSSV.

ACKNOWLEDGMENTS

The research was supported by the Ministry of Marine Affairs and Fisheries (MMAF) of the Republic of Indonesia through the DIPA-BRPBAP of Research Institute for Coastal Aquacultre. The authors thank researchers and technicians for their assistance in conducting the research.

REFERENCES

Amparyup, P., Charoensapsri, W. & Tassanakajon, A. 2013. Prophenoloxidase system and its role in shrimp immune responses against major pathogens. *Fish and Shellfish Immunology* 34: 990-1001. https://doi.org/10.1016/j.fsi.2012.08.019

Chiou, P.P., Chen, M.J., Lin, C.M., Khoo, J., Larson, J., Holt, R., Leong, J.A., Thorgarrd, G. & Chen, T.T. 2014. Production of homozygous transgenic rainbow trout with enhanced disease resistance. *Marine Biotechnology* 16(3): 299-308. https://doi.org/10.1007/s10126-013-9550-z

Coman, G.J., Arnold, S.J., Wood, A.T. & Preston, N.P. 2013. Evaluation of egg and nauplii production parameters of a single stock of domesticated *Penaeus monodon* (Giant Tiger Shrimp) across generations. *Aquaculture* 400-401: 125-128. https://doi.org/10.1016/j.aquaculture.2013.03.015

Coman, G.J., Crocos, P.J., Arnold, S.J., Keys, S.J., Preston, N.P. & Murphy, B. 2005. Growth, survival and reproductive performance of domesticated Australian stocks of the giant tiger prawn, *Penaeus monodon*, reared in tanks and raceways. *Journal of the World Aquaculture Society* 36(4): 464-479. https://doi.org/10.1111/j.1749-7345.2005.tb00394.x

Dunham, R.A. 2009. Transgenic fish resistant to infectious diseases, their risk and prevention of escape into the environment and future candidate genes for disease transgene manipulation. *Comparative Immunology, Microbiology and Infectious Diseases* 32(2): 139-161. https://doi.org/10.1016/j.cimid.2007.11.006

Dunham, R.A., Warr, G.W., Nichols, A., Duncan, P.L., Argue, B., Middleton, D. & Kucuktas, H. 2002. Enhanced bacterial disease resistance of transgenic channel cat fish *Ictalurus punctatus* possessing cecropin genes. *Marine Biotechnology* 4: 338-344. https://doi.org/10.1007/s10126-002-0024y

Haryanti, Mahardika, K., Moria, S.B. & Permana, I.G.N. 2006. Study on fry performance of black tiger shrmp *Penaeus monodon* with special reference to its morphology and RNA/DNA ratio analysis. *Indonesian Aquaculture Journal* 1(2): 159-164. http://ejournal-balitbang.kkp.go.id/index.php/iaj/article/view/2657/2171

Itami, T., Asano, M., Tokushige, K., Kubono, K., Nakagawa, A., Takeno, N., Nishimura, H., Maeda, M., Kondo, M. & Takahashi, Y. 1998. Enhancement of disease resistance of kuruma shrimp, *Penaeus japonicus*, after oral administration of peptidoglycan derived from *Bifidobacterium thermophilum*. *Aquaculture* 164(1-4): 277-288. https://doi.org/10.1016/S0044-8486(98)00193-8

Kakoolaki, S., Soltani, M., Ebrahimzadeh Mousavi, H.A., Sharifpour, I., Mirzargar, S., Afsharnasab, M. & Motalebi, A.A. 2011. The effect of different salinities on mortality and histopathological changes of SPF imported *Litopenaeus vannamei*, experimentally exposed to White Spot Virus and a new defferential hemocyte staining method. *Iranian Journal of Fisheries Sciences* 10(3): 447-460.

Kanagu, L., Senthilkumar, P., Stella, C. & Jaikumar, M. 2010. Effect of vitamins C dan E and B-1,3 Glucan as immunodulator in *P. monodon* diseases management. *Middle-East Journal of Scientific Research* 6(5): 537-543.

Khafage, A.R., Taha, S.M. & Attallah, M.A. 2019. Presence of tiger shrimp *Penaeus monodon* Fabricius, 1798 (Penaeidae) in the Egyptian commercial shrimp catch, Alexandria, Egypt. *Egyptian Journal of Aquatic Research* 45: 183-187. https://doi.org/10.1016/j.ejar.2019.05.002

Kulkarni, A., Krishnan, S., Anand, D., Kokkattunivarthil Uthaman, S., Otta, S.K., Karunasagar, I. & Kooloth Valappil, R. 2021. Immune responses and immunoprotection in crustaceans with special reference to shrimp. *Reviews in Aquaculture* 13(1): 431-459. https://doi.org/10.1111/raq.12482

Linacero, R., Rueda, J. & Vazquez, A. 1998. Quantification of DNA. In *Molecular Tools for Screening Biodiversity: Plants and Animals*. Netherlands: Springer. p. 528.

Liu, C.H. & Chen, J.C. 2004. Effect of ammonia on the immune response of white shrimp *Litopenaeus vannamei* and its susceptibility to *Vibrio alginolyticus*. *Fish and Shellfish Immunology* 16: 321-334. https://doi.org/10.1016/S1050-4648(03)00113-X

Lu, Y. & Sun, P.S. 2005. Viral resistance in shrimp that express an antisense Taura syndrome virus coat protein gene. *Antiviral Research* 67(3): 141-146. https://doi.org/10.1016/j.antiviral.2005.06.007

Manoppo, H., Sukenda, Djokosetiyanto, D., Sukadi, M.F. & Harris, E. 2011. Enhancement of non-specific immune response, resistance and growth of (*Litopenaeus vannamei*) by oral administration of nucleotide. *Jurnal Akuakultur Indonesia* 10(1): 1. https://doi.org/10.19027/jai.10.1-7

Moss, S.M., Harbor, F. & Loa, H. 1994. Use of nucleic acids as indicators of growth in juvenile white shrimp, *Penaeus vannamei*. *Marine Biology* 120: 359-367.

Mulyaningrum, S.R.H., Parenrengi, A., Tampangallo, B.R. & Trismawanti, I. 2018. Respons imun udang windu *Penaeus monodon* terhadap vaksin dsRNA VP-24 pada dosis berbeda. *Jurnal Riset Akuakultur* 13(1): 77-84. https://doi.org/10.15578/jra.13.1.2018.77-84

Novriadi, R., Ilham, I., Roigé, O. & Segarra, S. 2021. Effects of dietary nucleotides supplementation on growth, total haemocyte count, lysozyme activity and survival upon challenge with *Vibrio harveyi* in pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture Reports* 21(June): 100840. https://doi.org/10.1016/j.aqrep.2021.100840

Parenrengi, A., Tampangallo, B.R. & Tenriulo, A. 2014. Analysis of immune responses on transgenic tiger shrimp (*Penaeus monodon*) against pathogenic bacterium *Vibrio harveyi*. *Indonesian Aquaculture Journal* 9(1): 23-32. https://doi.org/10.15578/iaj.9.1.2014.23-32

Parenrengi, A., Tonnek, S. & Tenriulo, A. 2013. Analisis rasio RNA/DNA udang windu *Penaeus monodon* hasil seleksi tumbuh cepat. *Jurnal Riset Akuakultur* 8(1): 1-12. https://

doi.org/10.15578/jra.8.1.2013.1-12

Parenrengi, A., Tenriulo, A., Suryati, E., Rosmiati, R., Lante, S., Azis, A.A. & Alimuddin, A. 2022. Application of dsRNA VP15-WSSV by oral vaccination to increase survival rate and response immunes of tiger shrimp *Penaeus monodon*. *Indian Journal of Animal Research* 56(7): 893-898. https://doi.org/10.18805/IJAR.BF-1460

Parenrengi, A., Tenriulo, A., Alimuddin, A. & Sukenda, S. 2021. Enhancement of tiger shrimp *Penaeus monodon* resistance to white spot syndrome virus by overexpression of antiviral gene. *International Journal of Agriculture and Biology* 25(2): 277-284. https://doi.org/10.17957/IJAB/15.1667

Parenrengi, A., Tenriulo, A., Mulyaningrum, S.R.H., Lante, S. & Nawang, A. 2019. Pengaruh aplikasi dsRNA VP-15 *in vitro* dan *in vivo* terhadap sintasan dan respons imun udang windu *Penaeus monodon*. *Jurnal Riset Akuakultur* 14(4): 213. https://doi.org/10.15578/jra.14.4.2019.213-223

Parenrengi, A., Mulyaningrum, S.R.H., Tenriulo, A. & Nawang, A. 2018. Gen penyandi viral protein 15 (VP-15) WSSV dan aplikasinya sebagai vaksin rekombinan pada udang windu. *Jurnal Riset Akuakultur* 13(1): 57. https://doi.org/10.15578/jra.13.1.2018.57-65

Parenrengi, A., Alimuddin, A., Sukenda, S., Sumantadinata, K., Yamin, M. & Tenriulo, A. 2009. Cloning of ProAV promoter isolated from tiger prawn *Penaeus monodon*. *Indonesian Aquaculture Journal* 4(1): 1-7. https://doi.org/10.15578/iaj.4.1.2009.1-7

Paria, A., Greeshma, S.S., Chaudhari, A., Makesh, M., Purushothaman, C.S. & Rajendran, K.V. 2013. Nonspecific effect of double-stranded (ds) RNA on prophenoloxidase (proPO) expression in *Penaeus monodon*. *Applied Biochemistry and Biotechnology* 169: 281-289. https://doi.org/10.1007/s12010-012-9964-5

Rowley, A.F. & Pope, E.C. 2012. Vaccines and crustacean aquaculture-A mechanistic exploration. *Aquaculture* 334-337: 1-11. https://doi.org/10.1016/j.aquaculture.2011.12.011

Sánchez-Paz, A. 2010. White spot syndrome virus: An overview on an emergent concern. *Veterinary Research* 41(6): 43. https://doi.org/10.1051/vetres/2010015

Sarmasik, A., Warr, G. & Chen, T.T. 2002. Production of transgenic medaka with increased resistance to bacterial pathogens. *Marine Biotechnology* 4(3): 310-322. https://doi.org/10.1007/s10126-002-0023-z

Siswati, Anshary, H. & Parenrengi, A. 2021. The effect extract bioactive compounds seaweed *Codium* sp. on total hemocyte count (THC) of tiger shrimp (*Penaeus monodon*). *International Journal of Scientific and Research Publications (IJSRP)* 11(2): 94-100. https://doi.org/10.29322/ijsrp.11.02.2021.p11011

Suwoyo, H.S. & Sahabuddin. 2017. Performa pertumbuhan calon induk udang windu *Penaeus monodon* transfeksi pada generasi yang berbeda. *Jurnal Ilmu dan Teknologi Kelautan Tropis* 9(1): 185-200.

Suwoyo, H.S., Sahabuddin, Sahrijannah, A., Septiningsih, E. & Mulyaningrum, S.R.H. 2020. Grow-out of transfection and non transfection black tiger shrimp broodstock, *Penaeus monodon* in concrete pond. *IOP Conference Series: Earth and Environmental Science* 584: 012015. https://doi.org/10.1088/1755-1315/584/1/012015

Tassanakajon, A., Rimphanitchayakit, V., Visetnan, S., Amparyup, P., Somboonwiwat, K., Charoensapsri, W. & Tang, S. 2018. Shrimp humoral responses against pathogens: Antimicrobial peptides and melanization. *Developmental and Comparative Immunology* 80: 81-93. https://doi.org/10.1016/j.dci.2017.05.009

Van-De Braak, C.B.T., Botterblom, M.H.A., Liu, W., Taverne, N., Van Der Knaap, W.P.W. & Rombout, J.H.W.M. 2002. The role of the haematopoietic tissue in haemocyte production and maturation in the black tiger shrimp (*Penaeus monodon*). *Fish and Shellfish Immunology* 12(3): 253-272. https://doi.org/10.1006/fsim.2001.0369

Wakchaure, R., Ganguly, S., Qadri, K., Praveen, P.K. & Mahajan, T. 2015. Importance of transgenic fish to global aquaculture: A review. *Fisheries and Aquaculture Journal* 06(04). https://doi.org/10.4172/2150-3508.1000e124

Xiaojun, Z. & Jianhai, X. 2003. Transgenic shrimp: Current status and future perspectives. *China Biotechnology* 23(12): 36-42.

Yazawa, R., Watanabe, K., Koyama, T., Ruangapan, L., Tassanakajon, A., Hirono, I. & Aoki, T. 2005. Development of gene transfer technology for black tiger shrimp, *Penaeus monodon*. *Journal of Experimental Zoology Part A: Comparative Experimental Biology* 303(12): 1104-1109. https://doi.org/10.1002/jez.a.235

Yeh, S.P., Chen, Y.N., Hsieh, S.L., Cheng, W. & Liu, C.H. 2009. Immune response of white shrimp, *Litopenaeus vannamei*, after a concurrent infection with white spot syndrome virus and infectious hypodermal and hematopoietic necrosis virus. *Fish and Shellfish Immunology* 26: 582-588. https://doi.org/10.1016/j.fsi.2008.09.010

*Corresponding author; email: andi053@brin.go.id