



# Reverse-gavage feeding as a novel administration to investigate the immunomodulatory effects of *Radix Scutellaria* water extract on *Macrobrachium rosenbergii* immunity

Soni Andriawan<sup>1,2</sup> · Hung Tran Bao<sup>1</sup> · Wahyu Purbiantoro<sup>1,3</sup> · Ating Yuniarti<sup>4</sup> · Hso Chi Chaung<sup>5,6</sup> · Tsair-Bor Yen<sup>1,5</sup> · Ta-Chih Cheng<sup>1,5</sup>

Received: 23 April 2022 / Accepted: 10 January 2023 / Published online: 21 January 2023  
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2023

## Abstract

The application of herbal medicine is one of the world's most ancient approaches to treating diseases. This study aimed to examine the immunomodulatory effect of *Radix Scutellariae* water extract (RSWE) on the *Macrobrachium rosenbergii* immunity by reverse gavage feeding (RGF). In this study, *M. rosenbergii* was administered RSWE by RGF to stimulate its immunity. Furthermore, total hemocyte count (THC), phenoloxidase (PO), respiratory burst (RB), transglutaminase (TG), and lysozyme activity in hemocytes were evaluated to determine the immunological responses. Meanwhile, midgut and hepatopancreas were isolated to observe immune-related genes (LGPB, PE, and proPO). The study employed four treatments (0, 1, 5, and 10 µg of RWSE), and each treatment consisted of 3 replications. All data were analyzed using SPSS 17.0 (one-way ANOVA and Duncan test) to evaluate the differences among treatments. The results showed that the 5 µg RSWE was the optimum dosage to enhance the immune responses and induce the expression of immune-related genes in the hepatopancreas and midgut of *M. rosenbergii*. Finally, the RGF method can be used to investigate the immunomodulatory effect of RSWE on prawn, and the immune response associated with each dose can be compared precisely.

**Keywords** Reverse gavage feeding · *Radix Scutellariae* water extract · Hemolymph · Digestive organs · Immune responses

## Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii*, is a commercially important freshwater crustacean species (Lalrinsanga et al. 2014; Nguyen et al. 2019). However, the rapid development of prawn industry has promoted the incidence of infectious diseases,

---

Handling Editor: Gavin Burnell

✉ Ta-Chih Cheng  
cheng.tachih@gmail.com

Extended author information available on the last page of the article

mainly caused by viruses and bacteria (Chen et al. 2001a; Hsu et al. 2005; Suanyuk and Dangwetngam 2014). *Lactococcus garvieae* has proven to be one of the significant coccus pathogens for prawns, in which *L. garvieae* infection in *M. rosenbergii* causes cumulative mortality of around 30 to 75% (Chen et al. 2001b; Chen and Wang 2001). Several strategies have been implemented to deal with bacterial infections (Serrano 2005). However, antibiotics have adverse biosafety and biosecurity implications (Guo et al. 2011; Lakshmi et al. 2013; Sørsum et al. 2006). Therefore, a solution is needed to solve the problem of antibiotics, such as plant-based medicines recently used as therapeutic alternatives (Disch et al. 2017; Valladão et al. 2015).

In aquaculture, medicinal herbs have been reported to promote growth, improve immunity, and possess antimicrobial properties (Direkbusarakom et al. 1998; Jian and Wu 2004; Sivaram et al. 2004; Yahaya et al. 2015). Radix Scutellariae is the dried root of the medicinal herb *Scutellariae baicalensis* Georgi. In addition, it shows various therapeutic effects (e.g., anti-inflammation, anti-cancer, and anti-virus-related diseases), and it has been proved that flavonoids are the most abundant constituents and stimulate these therapeutic effects. The major bioactive flavones in Radix Scutellariae existing are aglycones (baicalin, wogonin, oroxylin A) and glycosides (baicalin, wogonoside, oroxylin A-7-glucuronide) (Li et al. 2011). Meanwhile, the main bioactive component in the water extract of Radix Scutellaria (WERS) is baicalin (Yan et al. 2022). Noticing the promising potential of Radix Scutellariae, several studies have been conducted to evaluate the effect of Radix Scutellariae supplementation on aquatic animals, such as olive flounder (Cho et al. 2013) and tilapia (Yin et al. 2006). However, to the best of our knowledge, no studies have considered the effects of Radix Scutellariae on prawns.

Injection, immersion, and oral administrations are the most common delivery routes of plant extracts in aquaculture (Reverter et al. 2014). However, as previously reviewed, each method has advantages and disadvantages (Assefa and Abunna 2018; Galindo-Villegas and Hosokawa 2004). In the present study, through the prawn's anus, WERS was directly introduced to the region of the anterior midgut connecting with the hepatopancreas for stimulating immune responses. This method is called reverse gavage feeding (RGF) (Aranguren and Lightner 2009; Tran et al. 2013; Xie et al. 2017). Delivering WERS to the midgut directly at least partially avoids the first-pass metabolism effect and the possibility of WERS-food interactions when applied WERS incorporation in the feed. In addition, the RGF method is less traumatic than oral gavage and makes it possible to deliver a dose of WERS into the midgut precisely. Therefore, the immunomodulatory effects of WERS associated with each dose on prawn immunity can be accurately compared. For this reason, it is necessary to investigate the immunomodulatory effects of WERS when applied to prawns by RGF.

## Materials and methods

### Prawn acclimatization

Selected *M. rosenbergii* ( $20.0 \pm 2.0$  g) were purchased from a local commercial shrimp farm (Pingtung, Taiwan). Two weeks before treatment, prawns were maintained at room temperature in a 400 l tank (25 prawns/tank) fitted with a freshwater circulator and fed with commercial feed twice daily, equal to 5% of prawn body weight.

## Plant extraction

Selected *Radix Scutellariae* was purchased from a local market (Pingtung, Taiwan). Following a previous method to prepare WERS (Zhao et al. 2016), 10 g of *Radix Scutellariae* powder was boiled in 150 ml of double-distilled water at 95 °C for 15 min. Then, the supernatant was obtained by centrifugation at 1000×g at 4 °C for 10 min before evaporating using a freeze dryer (EYELA FDU-1100). Lyophilized powder of WERS was then stored at 4 °C until used.

## Reverse gavage feeding and sample collection

The selected prawns were divided into 4 treatment groups, and each treatment consisted of 3 replications (25 prawns/tank). Before WERS administration, prawns were fasted for 24 h to ensure their intestines were free of feces. Subsequently, a 10 µl mixed solution of PBS and red dye (30: 1 v/v) containing different doses of WERS (0, 1, 5, and 10 µg) was slowly introduced to the anterior midgut via anal route by RGF. The use of red dye to facilitate observation and ensure that the solution sent into the intestine through the anus can reach the anterior midgut.

Based on a previous study (Xie et al. 2017), sampling was carried out 24 h after RGF, consisting of four time intervals (3, 6, 12, and 24 h after RGF). Hemolymph (100 µl) was transferred to a 1.5-ml Eppendorf tube containing 900 µl cooled anticoagulant (0.34 g EDTA, 0.8 g sodium citrate, and 10 µl Tween 80 in 100 ml of distilled H<sub>2</sub>O, at pH 7.45 with the osmolality adjusted to 490 mOsm kg<sup>-1</sup> with NaCl). In addition, digestive organs (hepatopancreas and midgut) were taken out from the prawns' bodies and stored at -20 °C in RNAlater™ solution until use.

## Measurement of the immune responses

Total hemocyte count (THC) was determined by counting the number of hemocytes in the anticoagulant and hemolymph mixture on the hemocytometer fields under a microscope. Hemocytes found on the fields of the hemocytometer were calculated in three replications.

The respiratory burst (RB) activity was determined using the reduction of nitroblue tetrazolium (NBT) to formazan as a measure of superoxide anion (O<sub>2</sub><sup>-</sup>) production (Song and Hsieh 1994) with some modification. RB activity was expressed as the amount of NBT reduction in 100 µl of hemolymph.

The hydroxamate-based colorimetric method was used to determine the TG activity in *M. rosenbergii* hemolymph (Gorman and Folk 1980). Initially, 50 µL of hemolymph was incubated with 150 µl of Tris–acetate buffer, 12.5 µl of hydroxylamine hydrochloride 2 M, and 37.5 µl of Z-Glu-Gly at 37 °C for 10 min. Then, 250 µl of coloring agent (contained 15 g trichloroacetic acid and 5 g iron (III) chloride hexahydrate in 100 ml of ddH<sub>2</sub>O) was added and centrifuged at 10,000 rpm for 5 min. Finally, the supernatant was measured colorimetrically at 525 nm with a microplate reader.

Lysozyme activity was determined by a turbidimetric assay which used a suspension of *Micrococcus lysodeikticus* as a substrate in a reaction mixture (Shugar 1952). Hemolymph (10 µl) was mixed with 200 µl of *M. lysodeikticus* in PBS (0.02% (w/v)) at 25 °C. Then, the absorbance at 450 nm was immediately recorded with a microplate reader every minute. Lysozyme from white egg (Amresco, USA) was used as control. Lysozyme activity was quantified in absorbance reduction per minute.

## RNA extraction, cDNA synthesis, and qRT-PCR analysis of immune related genes

Total RNA was extracted from the digestive organs (hepatopancreas and midgut) using Trizol® reagent (Ambion®, USA). The RNA samples were suspended in 30 µl DECP-treated water and kept at –80 °C until use. Total RNA concentration was qualified and quantified using BioSpectrometer ((Eppendorf BioSpectrometer® fitted with Hellma TrayCell Cap, Germany). According to the manufacturer's instructions, first-strand cDNA was synthesized from 2 µg of total RNA using M-MuLV Reverse Transcriptase (Lucigen®, USA). The cDNA was subsequently stored at –20 °C.

Quantitative real-time PCR (qRT-PCR) was performed to investigate the expression of immune-related genes in the prawn digestive organs after WERS administration by RGF (Table 1). qRT-PCR was performed using the ABI StepOnePlus™ Real-Time PCR System (Applied Biosystems™, USA). The amplification was performed in a total volume of 10 µl, containing 1 µl of cDNA template, 5 µl of TB Green Premix Ex Taq II (Tli RNaseH Plus) (Takara Bio Inc., Japan), 0.2 µl of each primer, 0.2 µl of ROX reference dye (50x), and 3.4 µl of DEPC treated water. The real-time PCR program was 95 °C for 1 min, followed by 40 cycles of 95 °C for the 15 s and 60 °C for 1 min. Dissociation and melting curves of amplification products were performed at the end of each PCR to confirm that only one PCR product was amplified and detected. The relative expression of related genes were calculated by the  $2^{-\Delta\Delta CT}$  method (Livak and Schmittgen 2001).

## Statistical analysis

ANOVA (one-way analysis of variance) was applied to evaluate data obtained from the experiments. Multiple comparisons (Duncan test) were conducted to investigate significant differences among treatments using SPSS 17. Results were present as mean ± SD. Differences between different groups at each time point were considered significant at  $p < 0.05$ .

## Results

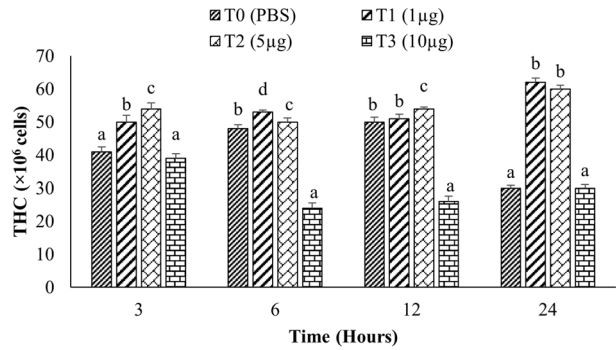
### Effect of Radix Scutellariae water extract (RSWE) on the immune responses

As shown in Fig. 1, after administration with 1 and 5 µg of WERS by RGF, THC levels increased significantly ( $p < 0.05$ ) compared with the other treatments for 24 h (Fig. 1).

**Table 1** RT-qPCR primers of immune-related genes

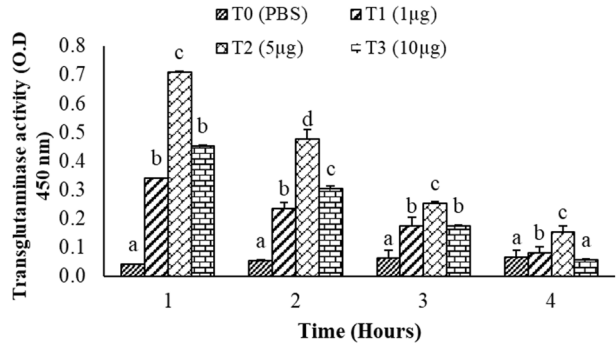
Target genes	Genebank	Primer	Sequence (5'-3')
LGBP (LPS-β-glucan Binding Protein)	<a href="#">GQ228481</a>	F	AGGAACCGGCGGTTTCTT
		R	GGTGTGGACCAAGGCTTGT
PE (peroxinectin)	<a href="#">JN572543.1</a>	F	CACTGCTGCCTCCGTTTC
		R	AGGGCTTGTGGATTATTCTG
proPO (prophenoloxidase)	<a href="#">AY947400</a>	F	ACACTGAAGGACATAAGGCGAGAT
		R	AGTAGAGTTCCAAGTCGGAGATGCT
Mrβ-actin	<a href="#">AY651918.1</a>	F	CCCGACGGTCACTTGTTT
		R	CGTGGATGCCGCAGGATT

**Fig. 1** Total hemocyte count in hemolymph of *M. rosenbergii* in response to *S. radix* water extract (RSWE) administration at 3, 6, 12, and 24 h post-RGF. Data are presented as mean  $\pm$  SD ( $n=3$ ). Different letters indicate significant differences among treatments at each time point ( $p < 0.05$ )

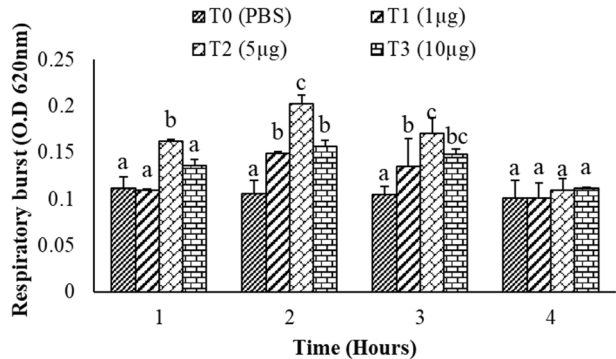


Moreover, RSWE administration by RGF was shown to enhance the TG levels of *M. rosenbergii*, which persist for up to 24 h, and administration of 5  $\mu$ g RSWE showed the highest TG activity (Fig. 2). Furthermore, RSWE administration by RGF was shown to enhance the RB levels of *M. rosenbergii*. However, administration of high doses of RSWE (10  $\mu$ g) by RGF showed excessive RB activity, in which RB activity increased significantly ( $p < 0.05$ ) 3 h after RGF and persisted for up to 24 h (Fig. 3). In addition, RSWE administration by RGF was shown to enhance the lysozyme levels of *M. rosenbergii* but administration of high doses of RSWE (5 and 10  $\mu$ g) by RGF showed an earlier increase in lysozyme activity than low doses of RSWE (Fig. 4).

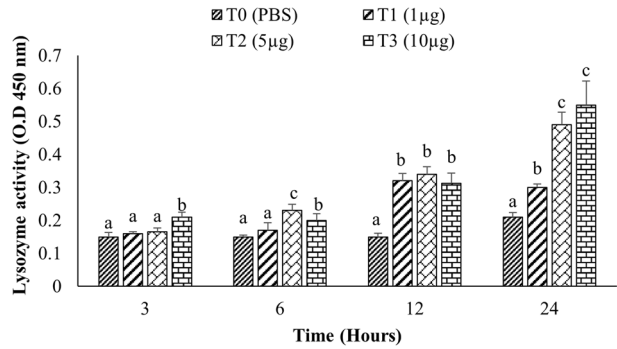
**Fig. 2** Transglutaminase activity in hemolymph of *M. rosenbergii* in response to *S. radix* water extract (RSWE) administration at 3, 6, 12, and 24 h post-RGF. Data are presented as mean  $\pm$  SD ( $n=3$ ). Different letters indicate significant differences among treatments at each time point ( $p < 0.05$ )



**Fig. 3** Respiratory burst activity in hemolymph of *M. rosenbergii* in response to *S. radix* water extract (RSWE) administration at 3, 6, 12, and 24 h post-RGF. Data are presented as mean  $\pm$  SD ( $n=3$ ). Different letters indicate significant differences among treatments at each time point ( $p < 0.05$ )



**Fig. 4** Lysozyme activity in hemolymph of *M. rosenbergii* in response to *S. radix* water extract (RSWE) administration at 3, 6, 12, and 24 h post-RGF. Data are presented as mean  $\pm$  SD ( $n=3$ ). Different letters indicate significant differences among treatments at each time point ( $p < 0.05$ )

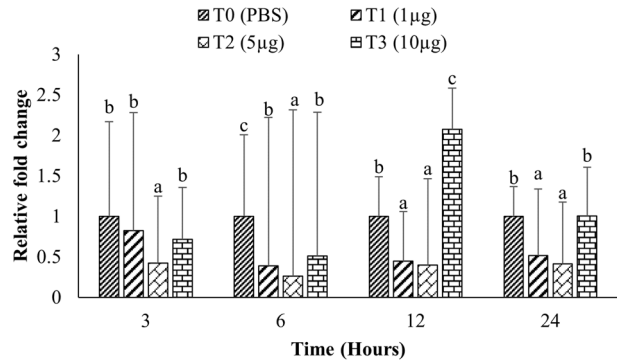


## Effect of Radix Scutellariae water extract (RSWE) on the immune-related genes

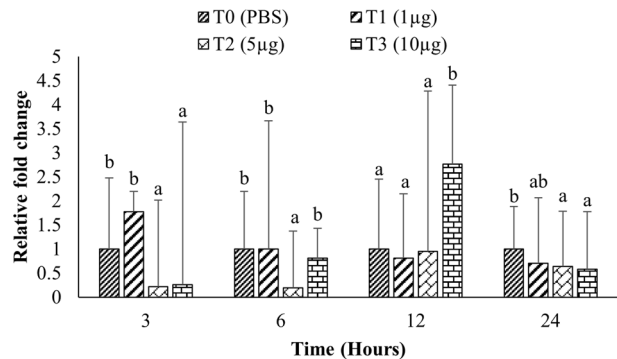
### Hepatopancreas

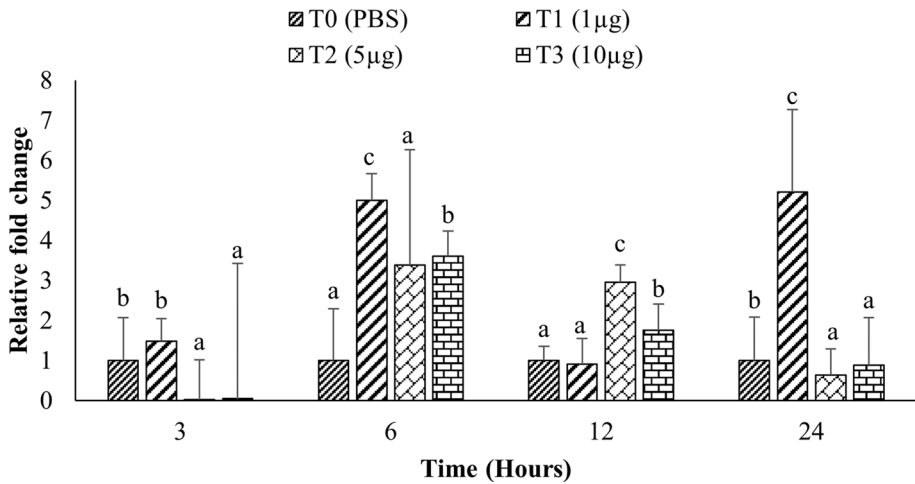
The expressions of LGBP in *M. rosenbergii* HP were decreased post-RGF in all treatments for 24 h, except for an increase of LGBP (2.1-fold) at 12 h post-RGF in T3 (Fig. 5). The downregulation of PE in T2 and T3 was observed as early as 3 h (0.2-fold) post-RGF (Fig. 6). An increase in PE was only shown 12 h post-RGF in T3 (non-significant).

**Fig. 5** Expression of LGBP in *M. rosenbergii* hepatopancreas post-RGF in response to *S. radix* water (RSWE) extract administration at 3, 6, 12, and 24 h post-RGF. Data are presented as mean  $\pm$  SD ( $n=3$ ). Different letters indicate significant differences among treatments at each time point ( $p < 0.05$ )

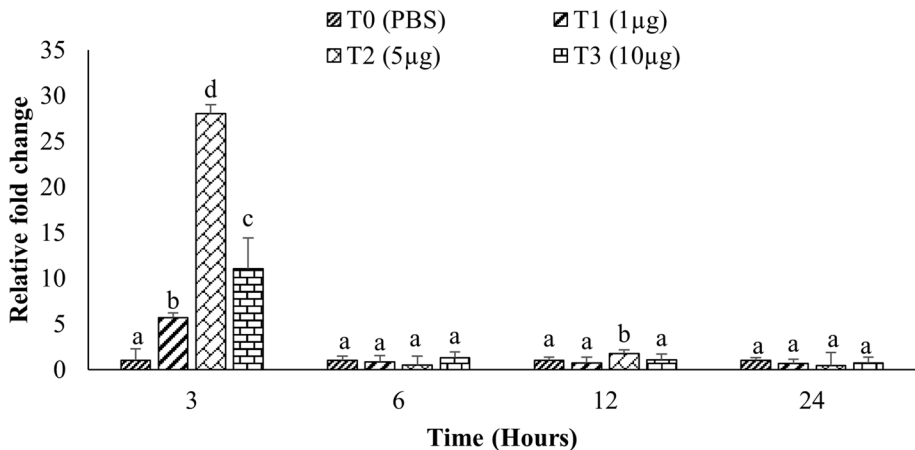


**Fig. 6** Expression of PE in *M. rosenbergii* hepatopancreas post-RGF in response to *S. radix* water (RSWE) extract administration at 3, 6, 12, and 24 h post-RGF. Data are presented as mean  $\pm$  SD ( $n=3$ ). Different letters indicate significant differences among treatments at each time point ( $p < 0.05$ )





**Fig. 7** Expression of LGBP in *M. rosenbergii* midgut post-RGF in response to *S. radix* water (RSWE) extract administration at 3, 6, 12, and 24 h post-RGF. Data are presented as mean ± SD (n=3). Different letters indicate significant differences among treatments at each time point (p < 0.05)

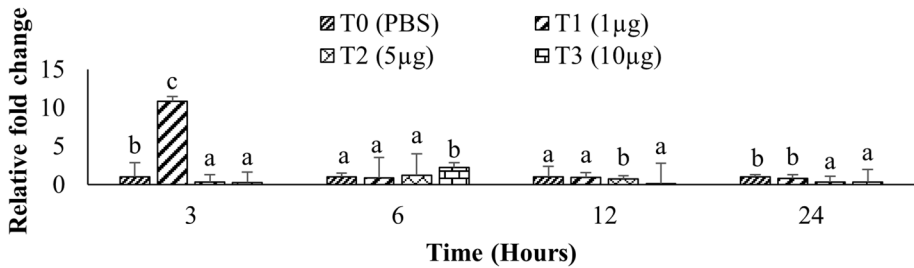


**Fig. 8** Expression of proPO in *M. rosenbergii* midgut post-RGF in response to *S. radix* water (RSWE) extract administration at 3, 6, 12, and 24 h post-RGF. Data are presented as mean ± SD (n=3). Different letters indicate significant differences among treatments at each time point (p < 0.05)

### Midgut

The expressions of LGBP in *M. rosenbergii* MG were downregulated as early as 3 h post-RGF at T2 and T3, while T1 expression was not significantly different to the control group (Fig. 7). The upregulation of LGBP was observed in all treatments at 6 h. Furthermore, an increase of LGBP in MG was also seen at 12 and 24 h post-RGF in T2 and T1 (2.6- and 5.35-fold, respectively). The expression of proPO was upregulated in all treatments for 3 h and only T2 for 12 h (1.77-fold), while at 6 and 24 h showed no significance among treatments (Fig. 8). The upregulation of PE in T1 was observed as early as 3 h (10.64-fold) post-RGF (Fig. 9). An increase in PE also was shown at 6 h post-RGF in T3 (Fig. 9).





**Fig. 9** Expression of PE in *M. rosenbergii* midgut post-RGF in response to *S. radix* water (RSWE) extract administration at 3, 6, 12, and 24 h post-RGF. Data are presented as mean  $\pm$  SD ( $n=3$ ). Different letters indicate significant differences among treatments at each time point ( $p < 0.05$ )

## Discussion

Most of the immune responses occur in hemolymph, which contains three different types of hemocytes, namely semigranular, granular, and hyaline (Interaminense et al. 2019). Meanwhile, the digestive tract of prawn consists of three main parts, the foregut, midgut, and hindgut (McGaw and Curtis 2013). The midgut is the main absorptive area of the digestive tract, which represents a vital route of pathogen entry (Barreto et al. 2018; Rosa and Barracco 2010) and is closely associated with hemocytes. Another essential organ is the hepatopancreas, the prawn's primary digestive gland similar to the liver in vertebrates. The hepatopancreas, which occupies a substantial part of the posterior cephalothorax and surrounds the posterior stomach and anterior midgut, is an essential site for the expression of immune-related genes in prawn (Du et al. 2013; Robalino et al. 2007; Zeng et al. 2013).

## Effect of *Radix Scutellariae* water extract (RSWE) on the immune responses

### Total hemocyte count (THC)

The circulating hemocytes play primary roles in crustaceans' immune response to invaded microbial pathogens and external stimulations. Hemocytes conduct various physiological and pathological functions, such as antigen recognition, phagocytosis, encapsulation, nodule formation, and releasing of humoral factors (Chang et al. 2015). However, the number of hemocytes is related to many factors, such as environmental stresses (Perazzolo et al. 2002), infections (Feng et al. 2008), immunostimulant administration (Chen et al. 2014), and endocrine activities (Estrada et al. 2016). In the present study, we evaluated the effects of RSWE administration by RGF on the THC of *M. rosenbergii*. As shown in Fig. 1, after administration with 1 and 5  $\mu$ g of RSWE by RGF, THC levels increased significantly compared with the other treatments for 24 h (Fig. 1). The similar results described by Wu et al. (Wu et al. 2015) showed that the water extract of *Gynura bicolor* could enhance THC of *Litopenaeus vannamei* at 7th day post-feeding treatment. Noni leaves extract was also shown to increase *M. rosenbergii* THC at 3rd day post-feeding treatment (Halim et al. 2017). It is supposed that RSWE administration by RGF may trigger immune cell proliferation in prawns, thereby increasing the secretion of cytokines (e.g., astakine), which can increase hemocyte synthesis in hematopoietic tissues.



## Transglutaminase (TG)

Coagulation is initiated by releasing factors and enzymes from hemocytes, which effectively polymerizes the hemolymph clotting protein (Martin et al. 1991). In crustaceans, clotting is mediated through coagulates presented in the plasma and TG within circulating hemocytes (Liu et al. 2011). TG prevents hemocyte loss during the microbial invasion and inhibits their growth in the hemocoel (Fagutao et al. 2012). Besides, hemolymph clotting is responsible for binding and killing pathogens in horseshoe crabs. This process involves inflammatory responses, wound closure, and healing (Theopold et al. 2004). In the present study, RSWE administration by RGF was shown to enhance the TG levels of *M. rosenbergii*, which persist for up to 24 h, and administration of 5 µg WERS showed the highest TG activity (Fig. 2). A previous study using *Eichhornia crassipes* supplemented feed to enhance the immune system of *M. rosenbergii* also showed a similar result (Chang et al. 2013). Together with an increase in THC, our data support a functional involvement of TG in the acceleration of hemolymph coagulation of *M. rosenbergii*.

## Respiratory burst (RB)

RB is the rapid release of reactive oxygen species (ROS) from phagocytes during phagocytosis. Increased RB activity is related to increased bactericidal activity by phagocytes or hemocytes within the cellular immune system (Song and Hsieh 1994). However, overproduction of ROS may induce oxidative stress that can potentially damage host cells (Pohanka 2013). In this study, RSWE administration by RGF was shown to enhance the RB levels of *M. rosenbergii*. However, administration of high doses of RSWE (10 µg) by RGF showed excessive RB activity, in which RB activity increased significantly 3 h after RGF and persisted for up to 24 h (Fig. 3). A previous study showed that RB activity of *L. vannamei* was increased at 24 h post-feeding with *P. binghamiae* hot water extract at the concentration of 10 µg/g (Chen et al. 2014). Furthermore, crude extract of *Cardiospermum halicacabum* leaves supplemented with commercial feed enhanced the RB activity of *Penaeus monodon* from 48 to 120 h after feeding (Rajasekar et al. 2011). These results suggest that the appropriate administration of RSWE by RGF can effectively induce RB activity of hemocytes in prawns.

## Lysozyme

Lysozyme is found in hemolymph and most shrimp tissues, but lysozyme production is related to the number of hemocytes in the hemolymph (Burge et al. 2007; Mai and Hu 2009; Qiao et al. 2013). Lysozyme is an antimicrobial agent, and it is proven that the increased activity of lysozyme can protect the host by acting as an essential defense enzyme against infectious diseases (Ragland and Criss 2017; Saurabh and Sahoo 2008). In this study, RSWE administration by RGF was shown to enhance the lysozyme levels of *M. rosenbergii*. However, administration of high doses of RSWE (5 and 10 µg) by RGF showed an earlier increase in lysozyme activity than low doses of WERS (Fig. 4). An increase in lysozyme activity was also found in *P. monodon* and *L. vannamei*, given guava leaf extract and zingerone, respectively (Chang et al. 2012; Yin et al. 2014). In contrast, *Radix Scutellariae* could not enhance lysozyme activity in tilapia, *Oreochromis niloticus*

(Yin et al. 2006). These results suggest that administering high doses RSWE by RGF can effectively induce RB activity of hemocytes in prawns.

### Effect of *Radix Scutellariae* water extract (RSWE) on the immune related-genes

Lipopolysaccharide- and  $\beta$ -1,3-glucan-binding protein (LGBP).

LGBP was mainly expressed in the hepatopancreas (Pan et al. 2005; Yeh et al. 2009) and is involved in cellular and humoral defenses using PAMP (pathogen-associated molecular patterns) and stimulates a cascade of responses, such as phagocytosis, clotting activation, antibacterial peptides production, and proPO cascades (Iwanaga 1994; Lai et al. 2011; Roux et al. 2002; Yeh et al. 2009). In our study, the significant upregulation of the LGBP gene in hepatopancreas was observed at 12 h after administration of 10  $\mu$ g RSWE by RGF (Fig. 5). A previous study reported that LGBP expression was increased in the hepatopancreas of *L. vannamei* at 72 h and 7 days post-feeding treatment of diets containing  $\beta$ -1,3-glucan (Wang et al. 2008). In addition, LGBP expression in the hepatopancreas of *M. rosenbergii* was upregulated at 12 h after LPS or poly I:C administration (Yeh et al. 2009). Meanwhile, at 6 h after RSWE administration by RGF, the expression of LGBP in the midgut was significantly upregulated, and this trend persisted for prawn groups with 5 and 10  $\mu$ g RSWE (Fig. 7). The increased expression of LGBP in the midgut originates from the infiltration of hemocytes (Silveira et al. 2018). Hemocytes perform a migratory behavior to digestive tissues and contribute to gut immunity (Arts 2006; Silveira et al. 2018; Yeh et al. 2009). Based on the statements above, we speculated that the increased LGBP in MG was due to their content of hemocytes. These results suggest that the difference in LGBP expression in the digestive organs was modulated by types of immunostimulants, delivery method, and migratory behavior of hemocytes.

### Prophenoloxidase (proPO)

The proPO-activating system is believed to be an essential innate defense in invertebrate immunity (Charoensapsri et al. 2011). Activation of proPO is shown to generate cytotoxic products, melanin production (melanization), and encapsulation of pathogen (Cerenius et al. 2008). The shrimp's proPO expression was found in the hemolymph, gill, stomach, hepatopancreas, and intestine (Ai et al. 2008; Charoensapsri et al. 2009; Pang et al. 2014). It showed that proPO plays a central role in killing and eliminating invading pathogens (Tassanakajon et al. 2018). Besides, upregulation of proPO expression in diverse tissues was used as a hemocyte marker to determine hemocyte infiltration into various tissues (Soonthornchai et al. 2010).

In the present study, proPO was expressed in midgut *M. rosenbergii*. However, in the midgut, the expression of proPO showed a rapid on–off response after stimulation with RSWE by RGF (Fig. 8). This result is consistent with previous studies on *Morinda citrifolia* (Halim et al. 2017), *Musa acuminata* (Rattanavichai and Cheng 2015), and *Solanum nigrum* (Harikrishnan et al. 2011). In contrast, another study showed that the regulatory mechanism for proPO gene expression after stimulation with pUC57-CpG is a long-lasting response (Yi et al. 2014). Besides, the present study considered the upregulation of proPO in the midgut caused by infiltrating hemocytes in the midgut because proPO is synthesized in granular hemocytes and converted into an active PO form by a serine proteinase (Okumura 2007).

## Peroxinectin (PE)

Peroxinectin is a cell adhesion protein essential to invertebrates for many physiological processes, including immune responses (Johansson 1999). In addition, active PE can bind to integrin receptors on the cell surface to encourage the degranulation process in a positive reaction loop and play a multifunctional agent during pathogen invasion (Sricharoen et al. 2005). In the present study, the expression of PE in the hepatopancreas of *M. rosenbergii* was significantly upregulated after administration of RSWE, particularly when stimulated with 1 and 10  $\mu\text{g}$  RSWE at 3 and 12 h after RGF (Fig. 6). Meanwhile, the expression of PE in the midgut has a similar pattern to the expression of proPO in the midgut (Figs. 8 and 9). These circumstances are understandable since PE is a protein associated with the proPO system, and its cell adhesion activity is triggered when the proPO system is activated (Sri-tunyalucksana et al. 2001).

Overall, there was no correlation between the administered doses of RSWE by RGF with the immune responses and the expression of immune-related genes on *M. rosenbergii*. Moreover, there is no single dose of RSWE that can simultaneously boost all immune responses in circulating hemocytes and the expression of immune-related genes in the digestive organs of *M. rosenbergii*. However, stimulation with 5  $\mu\text{g}$  RSWE by RGF consistently increased immune responses and immune-related genes in *M. rosenbergii*. In contrast, administration of 10  $\mu\text{g}$  RSWE may inhibit some immune responses and immune-related genes in *M. rosenbergii*.

## Conclusion

The results demonstrated that administration of RSWE by RGF improved immune responses (THC, TG, RB, and lysozyme activity) and induced the expression of immune-related genes (LGBP, proPO, and PE) in the hepatopancreas and midgut. The RGF method can be used to investigate the immunomodulatory effect of RSWE on shrimp, and the immune response associated with each dose can be compared precisely.

**Acknowledgements** The authors are grateful to the Double Degree program, Faculty of Fisheries and Marine Sciences, Brawijaya University, Indonesia, and the Laboratory of Molecular Fish Immunology and Genetics, Department of Tropical Agriculture and International Cooperation, NPUST, Taiwan.

**Author contribution** Soni Andriawan: concept, methods, critical evaluation, initial draft writing, and writing—review and editing. Hung Tran Bao: methodology, writing—review and editing. Ating Yuniarti: writing—review. Wahyu Purbiantoro: writing—review and editing. Hso Chi Chung: writing—review. Tsair-Bor Yen: writing—review. Ta-Chih Cheng: concept, methods, critical evaluation, initial draft writing, and writing—review and editing.

**Funding** The Research Center for Animal Biologics supported this research under project number 107–3017-F-020–001.

**Data availability** All the required data are available in the manuscript.

**Code availability** Not applicable.

## Declarations

**Ethics approval** Not applicable.

**Human and animal ethics** Not applicable.

**Consent to participate** Not applicable.

**Consent for publication** According to the publication.

**Competing interests** The authors declare no competing interests.

## References

- Ai H-S, Huang Y-C, Li S-D, Weng S-P, Yu X-Q, He J-G (2008) Characterization of a prophenoloxidase from hemocytes of the shrimp *Litopenaeus vannamei* that is down-regulated by white spot syndrome virus. *Fish Shellfish Immunol* 25(1–2):28–39
- Aranguren F, Lightner D (2009) Reverse gavage: a new standardized technique for infecting shrimp suitable for challenge tests [Abstracts]. *Aquaculture America 2009*, Seattle, Washington
- Arts JA (2006) Immune defence white spot syndrome virus infected shrimp, *Penaeus monodon*
- Assefa A, Abunna F (2018) Maintenance of fish health in aquaculture: review of epidemiological approaches for prevention and control of infectious disease of fish. *Vet Med Int* 2018:1–10
- Barreto C, Coelho JdR, Yuan J, Xiang J, Perazzolo LM, Rosa RD (2018) Specific molecular signatures for type ii crustins in penaeid shrimp uncovered by the identification of crustin-like antimicrobial peptides in *Litopenaeus vannamei*. *Mar Drugs* 16(1):31. <https://doi.org/10.3390/md16010031>
- Burge EJ, Madigan DJ, Burnett LE, Burnett KG (2007) Lysozyme gene expression by hemocytes of Pacific white shrimp, *Litopenaeus vannamei*, after injection with *Vibrio*. *Fish Shellfish Immunol* 22(4):327–339
- Cerenius L, Lee BL, Soderhall K (2008) The proPO-system: pros and cons for its role in invertebrate immunity. *Trends Immunol* 29(6):263–271. <https://doi.org/10.1016/j.it.2008.02.009>
- Chang Y-P, Liu C-H, Wu C-C, Chiang C-M, Lian J-L, Hsieh S-L (2012) Dietary administration of zingerone to enhance growth, non-specific immune response, and resistance to *Vibrio alginolyticus* in Pacific white shrimp (*Litopenaeus vannamei*) juveniles. *Fish Shellfish Immunol* 32(2):284–290. <https://doi.org/10.1016/j.fsi.2011.11.017>
- Chang C-C, Tan H-C, Cheng W (2013) Effects of dietary administration of water hyacinth (*Eichhornia crassipes*) extracts on the immune responses and disease resistance of giant freshwater prawn, *Macrobrachium rosenbergii*. *Fish Shellfish Immunol* 35(1):92–100. <https://doi.org/10.1016/j.fsi.2013.04.008>
- Chang C-C, Tsai W-L, Jiang J-R, Cheng W (2015) The acute modulation of norepinephrine on immune responses and genes expressions via adrenergic receptors in the giant freshwater prawn, *Macrobrachium rosenbergii*. *Fish Shellfish Immunol* 46(2):459–467. <https://doi.org/10.1016/j.fsi.2015.07.015>
- Charoensapsri W, Amparyup P, Hirono I, Aoki T, Tassanakajon A (2009) Gene silencing of a prophenoloxidase activating enzyme in the shrimp, *Penaeus monodon*, increases susceptibility to *Vibrio harveyi* infection. *Dev Comp Immunol* 33(7):811–820
- Charoensapsri W, Amparyup P, Hirono I, Aoki T, Tassanakajon A (2011) PmPPAE2, a new class of crustacean prophenoloxidase (proPO)-activating enzyme and its role in PO activation. *Dev Comp Immunol* 35(1):115–124. <https://doi.org/10.1016/j.dci.2010.09.002>
- Chen W, Wang CH (2001) The susceptibility of the giant freshwater prawn *Macrobrachium rosenbergii* to *Lactococcus garvieae* and its resistance under copper sulfate stress. *Dis Aquat Organ* 47(2):137–144. <https://doi.org/10.3354/dao047137>
- Chen SC, Lin YD, Liaw LL, Wang PC (2001b) *Lactococcus garvieae* infection in the giant freshwater prawn *Macrobrachium rosenbergii* confirmed by polymerase chain reaction and 16S rDNA sequencing. *Dis Aquat Organ* 45(1):45–52. <https://doi.org/10.3354/dao045045>
- Chen Y, Chen J, Lin Y, Yeh S, Chao K, Lee C (2014) White shrimp *Litopenaeus vannamei* that have received *Petalonia binghamiae* extract activate immunity, increase immune response and resistance against *Vibrio alginolyticus*. *J Aquat Res Dev* 5(6):1
- Chen S-J, Lin YD, Liaw LL, Wang PC (2001a) *Lactococcus garvieae* infection in the giant freshwater prawn *Macrobrachium rosenbergii* confirmed by polymerase chain reaction and 16S rDNA sequencing. 45. <https://doi.org/10.3354/dao045045>
- Cho SH, Jeon GH, Kim HS, Kim DS, Kim C (2013) Effects of Dietary *Scutellaria baicalensis* Extract on Growth, Feed Utilization and Challenge Test of Olive Flounder (*Paralichthys olivaceus*). *Asian Australas J Anim Sci* 26(1):90–96. <https://doi.org/10.5713/ajas.2012.12147>
- Direkbusarakom S, Ezura Y, Yoshimizu M, Herunsalee A (1998) Efficacy of Thai traditional herb extracts against fish and shrimp pathogenic bacteria. *Fish Pathol* 33(4):437–441

- Disch L, Drewe J, Fricker G (2017) Dissolution testing of herbal medicines: challenges and regulatory standards in Europe, the United States, Canada, and Asia. *Dissolut Technol* 24(2):6–12. <https://doi.org/10.14227/DT240217P6>
- Du J, Zhu H, Liu P, Chen J, Xiu Y, Yao W, ... Wang W (2013) Immune responses and gene expression in hepatopancreas from *Macrobrachium rosenbergii* challenged by a novel pathogen *spiroplasma* MR-1008. *Fish Shellfish Immunol* 34(1):315–323. <https://doi.org/10.1016/j.fsi.2012.11.009>
- Estrada N, Velázquez E, Rodríguez-Jaramillo C, Ascencio F (2016) Carbohydrate Moieties and cytoenzymatic characterization of hemocytes in whiteleg shrimp *Litopenaeus vannamei*. *Int J Cell Biol* 2016:9032181. <https://doi.org/10.1155/2016/9032181>
- Fagutao FF, Maningas MBB, Kondo H, Aoki T, Hirono I (2012) Transglutaminase regulates immune-related genes in shrimp. *Fish Shellfish Immunol* 32(5):711–715
- Feng S, Zhan W, Xing J, Li J, Yang K, Wang J (2008) Hematological changes in white spot syndrome virus-infected shrimp, *Fenneropenaeus chinensis* (Osbeck). *J Ocean Univ China* 7(3):287–293
- Galindo-Villegas J, Hosokawa H (2004) Immunostimulants: towards temporary prevention of diseases in marine fish. *Advances en Nutricion. Acuicola VII Memorias del VII Simposium Internationale de Nutricion Acuicola*, 16–19
- Gorman JJ, Folk JE (1980) Transglutaminase amine substrates for photochemical labeling and cleavable cross-linking of proteins. *J Biol Chem* 255(3):1175–1180
- Guo JJ, Her BY, Chou RL, Chen TI (2011) Screening of Modern Herbal Medicines in White Shrimp (*Litopenaeus vannamei*) against *Vibrio harveyi* Infection (Vol. 63)
- Halim AM, Lee P-P, Chang Z-W, Chang C-C (2017) The hot-water extract of leaves of noni, *Morinda citrifolia*, promotes the immunocompetence of giant freshwater prawn, *Macrobrachium rosenbergii*. *Fish Shellfish Immunol* 64:457–468
- Harikrishnan R, Balasundaram C, Jawahar S, Heo M-S (2011) *Solanum nigrum* enhancement of the immune response and disease resistance of tiger shrimp, *Penaeus Monodon* against *Vibrio Harveyi*. *Aquaculture* 318(1–2):67–73
- Hsu JP, Huang C, Liao GM, Hsuan SL, Hung HH, Chien MS (2005) Engulfed pathogen-induced apoptosis in haemocytes of giant freshwater prawn, *Macrobrachium rosenbergii*. *J Fish Dis* 28(12):729–735
- Interaminense JA, Vogeley JL, Gouveia CK, Portela RS, Oliveira JP, Silva SMBC, ... Bezerra RS (2019) Effects of dietary *Bacillus subtilis* and *Shewanella* algae in expression profile of immune-related genes from hemolymph of *Litopenaeus vannamei* challenged with *Vibrio parahaemolyticus*. *Fish Shellfish Immunol* 86:253–259. <https://doi.org/10.1016/j.fsi.2018.11.051>
- Iwanaga S (1994) Clotting cascade in the immune response of horseshoe crab. *Phylogenetic perspectives in immunity: the insect host defense*, 79–96
- Jian J, Wu Z (2004) Influences of traditional Chinese medicine on non-specific immunity of Jian carp (*Cyprinus carpio* var. Jian). *Fish Shellfish Immunol* 16(2):185–191
- Johansson MW (1999) Cell adhesion molecules in invertebrate immunity. *Dev Comp Immunol* 23(4–5):303–315. [https://doi.org/10.1016/s0145-305x\(99\)00013-0](https://doi.org/10.1016/s0145-305x(99)00013-0)
- Lai X, Kong J, Wang Q, Wang W, Meng X (2011) Cloning and characterization of a  $\beta$ -1, 3-glucan-binding protein from shrimp *Fenneropenaeus chinensis*. *Mol Biol Rep* 38(7):4527–4535
- Lakshmi B, Viswanath B, Sai Gopal DVR (2013) Probiotics as antiviral agents in shrimp aquaculture. *J Pathog* 2013:13, Article 424123. <https://doi.org/10.1155/2013/424123>
- Lalrinsanga P, Pillai BR, Patra G, Mohanty S, Naik NK, Das RR, ... Nelliyouara R (2014) Yield characteristics and morphometric relationships of giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879). *Aquacult Int* 22(3):1053–1066
- Li C, Lin G, Zuo Z (2011) Pharmacological effects and pharmacokinetics properties of *Radix Scutellariae* and its bioactive flavones. *Biopharm Drug Dispos* 32(8):427–445. <https://doi.org/10.1002/bdd.771>
- Liu C-H, Chang C-C, Chiu Y-C, Cheng W, Yeh M-S (2011) Identification and cloning of a transglutaminase from giant freshwater prawn, *Macrobrachium rosenbergii*, and its transcription during pathogen infection and moulting. *Fish Shellfish Immunol* 31(6):871–880
- Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2<sup>-</sup> $\Delta\Delta$ CT method. *Methods* 25(4):402–408
- Mai W, Hu C (2009) cDNA cloning, expression and antibacterial activity of lysozyme C in the blue shrimp (*Litopenaeus stylirostris*). *Prog Nat Sci* 19(7):837–844
- Martin GG, Omori JEHS, Chong C, Hoodbhoj T, McKrell N (1991) Localization and roles of coagulogen and transglutaminase in hemolymph coagulation in decapod crustaceans. *Comp Biochem Physiol B: Comp Biochem* 100(3):517–522. [https://doi.org/10.1016/0305-0491\(91\)90213-W](https://doi.org/10.1016/0305-0491(91)90213-W)
- McGaw II, Curtis DL (2013) A review of gastric processing in decapod crustaceans [journal article]. *J Comp Physiol B* 183(4):443–465. <https://doi.org/10.1007/s00360-012-0730-3>

- Nguyen TMT, Chen T-Y, Shiau C-Y, Cheng Y-T, Chang Y-W (2019) Study on biochemical divergences of the meat and egg of freshwater prawns (*Macrobrachium rosenbergii*). *Food Sci Nutr* 7(6):2017–2023. <https://doi.org/10.1002/fsn3.1031>
- Okumura T (2007) Effects of lipopolysaccharide on gene expression of antimicrobial peptides (penaeidins and crustin), serine proteinase and prophenoloxidase in haemocytes of the Pacific white shrimp. *Litopenaeus Vannamei Fish & Shellfish Immunology* 22(1):68–76. <https://doi.org/10.1016/j.fsi.2006.03.013>
- Pan D, He N, Yang Z, Liu H, Xu X (2005) Differential gene expression profile in hepatopancreas of WSSV-resistant shrimp (*Penaeus japonicus*) by suppression subtractive hybridization. *Dev Comp Immunol* 29(2):103–112
- Pang Z, Kim SK, Yu J, Jang IK (2014) Distinct regulation patterns of the two prophenoloxidase activating enzymes corresponding to bacteria challenge and their compensatory over expression feature in white shrimp (*Litopenaeus vannamei*). *Fish Shellfish Immunol* 39(2):158–167. <https://doi.org/10.1016/j.fsi.2014.04.026>
- Perazzolo L, Gargioni R, Ogliari P, Barracco M (2002) Evaluation of some hemato-immunological parameters in shrimp *Farfantepenaeus paulensis* submitted to environmental and physiological stress. 214. [https://doi.org/10.1016/S0044-8486\(02\)00137-0](https://doi.org/10.1016/S0044-8486(02)00137-0)
- Pohanka M (2013) Role of oxidative stress in infectious diseases. A review. *Folia Microbiol (praha)* 58(6):503–513. <https://doi.org/10.1007/s12223-013-0239-5>
- Qiao G, Kim S-K, Cho Y-R, Kim S, Jang I-K (2013) Expression of c-type lysozyme from the fleshy shrimp *Penaeus chinensis* is upregulated following *Vibrio anguillarum* and lipopolysaccharide injection. *Fisheries and Aquatic Sciences* 16(4):267–272
- Ragland SA, Criss AK (2017) From bacterial killing to immune modulation: recent insights into the functions of lysozyme. *PLoS Pathog* 13(9):e1006512
- Rajasekar T, Usharani J, Sakthivel M, Deivasigamani B (2011) Immunostimulatory effects of *Cardiospermum halicacabum* against *Vibrio parahaemolyticus* on tiger shrimp *Penaeus monodon*. *J Chem Pharm Res* 3(5):501–513
- Rattanavichai W, Cheng W (2015) Dietary supplement of banana (*Musa acuminata*) peels hot-water extract to enhance the growth, anti-hypothermal stress, immunity and disease resistance of the giant freshwater prawn, *Macrobrachium rosenbergii*. *Fish Shellfish Immunol* 43(2):415–426. <https://doi.org/10.1016/j.fsi.2015.01.011>
- Reverter M, Bontemps N, Lecchini D, Banaigs B, Sasal P (2014) Use of plant extracts in fish aquaculture as an alternative to chemotherapy: current status and future perspectives. *Aquaculture* 433:50–61
- Robalino J, Almeida JS, McKillen D, Colglazier J, Trent HF, Chen YA, ... Gross PS (2007) Insights into the immune transcriptome of the shrimp *Litopenaeus vannamei*: tissue-specific expression profiles and transcriptomic responses to immune challenge. 29(1):44–56. <https://doi.org/10.1152/physiolgenomics.00165.2006>
- Rosa RD, Barracco MA (2010) Antimicrobial peptides in crustaceans. *Invertebr Surviv J* 7(2):262–284
- Roux MM, Pain A, Klimpel KR, Dhar AK (2002) The lipopolysaccharide and  $\beta$ -1, 3-glucan binding protein gene is upregulated in white spot virus-infected shrimp (*Penaeus stylirostris*). *J Virol* 76(14):7140–7149
- Saurabh S, Sahoo P (2008) Lysozyme: an important defence molecule of fish innate immune system. *Aquac Res* 39(3):223–239
- Serrano PH (2005) Responsible use of antibiotics in aquaculture. *FAO*
- Shugar D (1952) The measurement of lysozyme activity and the ultra-violet inactivation of lysozyme. *Biochim Biophys Acta* 8(C):302–309
- Silveira AS, Matos GM, Falchetti M, Ribeiro FS, Bressan A, Bachère E, ... Rosa RD (2018) An immune-related gene expression atlas of the shrimp digestive system in response to two major pathogens brings insights into the involvement of hemocytes in gut immunity. *Dev Comp Immunol* 79:44–50
- Sivaram V, Babu M, Immanuel G, Murugadass S, Citarasu T, Marian MP (2004) Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. *Aquaculture* 237(1–4):9–20
- Song Y-L, Hsieh Y-T (1994) Immunostimulation of tiger shrimp (*Penaeus monodon*) hemocytes for generation of microbicidal substances: analysis of reactive oxygen species. *Dev Comp Immunol* 18(3):201–209. [https://doi.org/10.1016/0145-305X\(94\)90012-4](https://doi.org/10.1016/0145-305X(94)90012-4)
- Soonthornchai W, Rungrasamee W, Karoonthaisiri N, Jarayabhand P, Klinbunga S, Söderhäll K, Jiravanichpaisal P (2010) Expression of immune-related genes in the digestive organ of shrimp, *Penaeus monodon*, after an oral infection by *Vibrio harveyi*. *Dev Comp Immunol* 34(1):19–28. <https://doi.org/10.1016/j.dci.2009.07.007>
- Sørnum M, Johnsen PJ, Aasnes B, Rosvoll T, Kruse H, Sundsfjord A, Simonsen GS (2006) Prevalence, persistence, and molecular characterization of glycopeptide-resistant enterococci in Norwegian poultry and poultry farmers 3 to 8 years after the ban on avoparcin. *Appl Environ Microbiol* 72(1):516–521. <https://doi.org/10.1128/AEM.72.1.516-521.2006>



- Sricharoen S, Kim JJ, Tunkijjanukij S, Soderhall I (2005) Exocytosis and proteomic analysis of the vesicle content of granular hemocytes from a crayfish. *Dev Comp Immunol* 29(12):1017–1031. <https://doi.org/10.1016/j.dci.2005.03.010>
- Sritunyalucksana K, Wongsuebsantati K, Johansson MW, Söderhäll K (2001) Peroxinectin, a cell adhesive protein associated with the proPO system from the black tiger shrimp, *Penaeus Monodon*. *Dev Comp Immunol* 25(5):353–363. [https://doi.org/10.1016/S0145-305X\(01\)00009-X](https://doi.org/10.1016/S0145-305X(01)00009-X)
- Suanyuk N, Dangwetngam M (2014) Identification and pathology of *Lactococcus garvieae* isolated from cultured and wild giant freshwater prawns (*Macrobrachium rosenbergii* de Man) in Thailand. *Thai J Vet Med* 44(3):325
- Tassanakajon A, Rimphanitchayakit V, Visetnan S, Amparyup P, Somboonwiwat K, Charoensapsri W, Tang S (2018) Shrimp humoral responses against pathogens: antimicrobial peptides and melanization. *Dev Comp Immunol* 80:81–93. <https://doi.org/10.1016/j.dci.2017.05.009>
- Theopold U, Schmidt O, Söderhäll K, Dushay MS (2004) Coagulation in arthropods: defence, wound closure and healing. *Trends Immunol* 25(6):289–294
- Tran L, Nunan L, Redman RM, Mohney LL, Pantoja CR, Fitzsimmons K, Lightner DV (2013) Determination of the infectious nature of the agent of acute hepatopancreatic necrosis syndrome affecting penaeid shrimp. *Dis Aquat Org* 105(1):45–55 (<https://www.int-res.com/abstracts/dao/v105/n1/p45-55/>)
- Valladao G, Gallani S, Pilarski F (2015) Phytotherapy as an alternative for treating fish disease. *J Vet Pharmacol Ther* 38(5):417–428
- Wang Y-C, Chang P-S, Chen H-Y (2008) Differential time-series expression of immune-related genes of Pacific white shrimp *Litopenaeus vannamei* in response to dietary inclusion of  $\beta$ -1, 3-glucan. *Fish Shellfish Immunol* 24(1):113–121
- Wu C-C, Chang Y-P, Wang J-J, Liu C-H, Wong S-L, Jiang C-M, Hsieh S-L (2015) Dietary administration of *Gynura bicolor* (Roxb. Willd.) DC water extract enhances immune response and survival rate against *Vibrio alginolyticus* and white spot syndrome virus in white shrimp *Litopenaeus vannamei*. *Fish Shellfish Immunol* 42(1):25–33. <https://doi.org/10.1016/j.fsi.2014.10.016>
- Xie S, Li F, Zhang X, Zhang J, Xiang J (2017) Peritrophin-like protein from *Litopenaeus vannamei* (LvPT) involved in white spot syndrome virus (WSSV) infection in digestive tract challenged with reverse gavage [journal article]. *Chin J Ocean Limnol* 35(6):1524–1530. <https://doi.org/10.1007/s00343-017-6109-2>
- Yahaya R, Dash GK, Abdullah MS, Mathews A (2015) *Clinacanthus nutans* (burm. F.) Lindau: a useful medicinal plant of south-east Asia. *Int J Pharmacogn Phytochem Res* 7(6):1244–1250
- Yan X, Zhang Y, Peng Y, Li X (2022) The water extract of *Radix scutellariae*, its total flavonoids and baicalin inhibited CYP7A1 expression, improved bile acid, and glycolipid metabolism in T2DM mice. *J Ethnopharmacol* 293:115238. <https://doi.org/10.1016/j.jep.2022.115238>
- Yeh M-S, Chang C-C, Cheng W (2009) Molecular cloning and characterization of lipopolysaccharide- and beta-1,3-glucan-binding protein from the giant freshwater prawn *Macrobrachium rosenbergii* and its transcription in relation to foreign material injection and the molt stage. *Fish Shellfish Immunol* 27(6):701–706. <https://doi.org/10.1016/j.fsi.2009.08.014>
- Yi Q, Liu R, Sun R, Wang L, Zhou Z, Wang M, Liu Y, Sun J, Madzak C, Song L (2014) The protection of CpG ODNs and *Yarrowia lipolytica* harboring VP28 for shrimp *Litopenaeus vannamei* against White spot syndrome virus infection. *Isj-Invertebr Surviv J* 11:119–131
- Yin G, Jeney G, Racz T, Xu P, Jun X, Jeney Z (2006) Effect of two Chinese herbs (*Astragalus radix* and *Scutellaria radix*) on non-specific immune response of tilapia, *Oreochromis niloticus*. *Aquaculture* 253(1–4):39–47. <https://doi.org/10.1016/j.aquaculture.2005.06.038>
- Yin XL, Li ZJ, Yang K, Lin HZ, Guo ZX (2014) Effect of guava leaves on growth and the non-specific immune response of *Penaeus monodon*. *Fish Shellfish Immunol* 40(1):190–196. <https://doi.org/10.1016/j.fsi.2014.07.001>
- Zeng D, Chen X, Xie D, Zhao Y, Yang C, Li Y, ... Chen X (2013) Transcriptome analysis of Pacific white shrimp (*Litopenaeus vannamei*) hepatopancreas in response to Taura syndrome virus (TSV) experimental infection. *PLoS One* 8(2):e57515. <https://doi.org/10.1371/journal.pone.0057515>
- Zhao Q, Chen X-Y, Martin C (2016) *Scutellaria baicalensis*, the golden herb from the garden of Chinese medicinal plants. *Sci Bull* 61(18):1391–1398. <https://doi.org/10.1007/s11434-016-1136-5>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.



## Authors and Affiliations

Soni Andriawan<sup>1,2</sup> · Hung Tran Bao<sup>1</sup> · Wahyu Purbiantoro<sup>1,3</sup> · Ating Yuniarti<sup>4</sup> · Hso Chi Chaung<sup>5,6</sup> · Tsair-Bor Yen<sup>1,5</sup> · Ta-Chih Cheng<sup>1,5</sup>

Soni Andriawan  
soniandriawan1992@gmail.com

Hung Tran Bao  
tbhungle@gmail.com

Wahyu Purbiantoro  
wahy033@brin.go.id

Ating Yuniarti  
ating\_y@ub.ac.id

Hso Chi Chaung  
hcchaung@mail.npust.edu.tw

Tsair-Bor Yen  
tbyen@mail.npust.edu.tw

- <sup>1</sup> Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
- <sup>2</sup> Faculty of Agriculture and Animal Science, Department of Aquaculture, University of Muhammadiyah Malang, Malang, East Java 65144, Indonesia
- <sup>3</sup> National Research and Innovation Agency (BRIN), Jakarta, Indonesia
- <sup>4</sup> Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, East Java 65145, Indonesia
- <sup>5</sup> Research Center for Animal Biologics, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan
- <sup>6</sup> Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology, No. 1, Shuefu Road, Neipu, Pingtung 91201, Taiwan