Journal of Marine Science and Technology

Volume 25 | Issue 2 Article 26

# SIMPLE HEAT PROCESSING OF BROWN SEAWEED SARGASSUM CRISTAEFOLIUM SUPPLEMENTATION IN DIET CAN IMPROVE GROWTH, IMMUNE RESPONSES AND SURVIVAL TO VIBRIO ALGINOLYTICUS OF WHITE SHRIMP, LITOPENAEUS VANNAMEI

Yu-Hung Lin
*Department of Aquaculture, National Pingtung University of Science and Technology, Taiwan, R.O.C.*,
yuhunglin@mail.npust.edu.tw

Yi-Che Su
*Department of Aquaculture, National Pingtung University of Science and Technology, Taiwan, R.O.C.*

Winton Cheng
*Department of Aquaculture, National Pingtung University of Science and Technology, Taiwan, R.O.C.*,
winton@mail.npust.edu.tw

Follow this and additional works at: https://jmstt.ntou.edu.tw/journal

Recommended Citation
Lin, Yu-Hung; Su, Yi-Che; and Cheng, Winton (2017) "SIMPLE HEAT PROCESSING OF BROWN SEAWEED SARGASSUM CRISTAEFOLIUM SUPPLEMENTATION IN DIET CAN IMPROVE GROWTH, IMMUNE RESPONSES AND SURVIVAL TO VIBRIO ALGINOLYTICUS OF WHITE SHRIMP, LITOPENAEUS VANNAMEI," *Journal of Marine Science and Technology*: Vol. 25: Iss. 2, Article 26.
DOI: 10.6119/JMST-016-1129-1
Available at: https://jmstt.ntou.edu.tw/journal/vol25/iss2/26

This Research Article is brought to you for free and open access by Journal of Marine Science and Technology. It has been accepted for inclusion in Journal of Marine Science and Technology by an authorized editor of Journal of Marine Science and Technology.

Journal of Marine Science and Technology, Vol. 25, No. 2, pp. 242-248 (2017)
DOI: 10.6119/JMST-016-1129-1

# SIMPLE HEAT PROCESSING OF BROWN SEAWEED *SARGASSUM CRISTAEFOLIUM* SUPPLEMENTATION IN DIET CAN IMPROVE GROWTH, IMMUNE RESPONSES AND SURVIVAL TO *VIBRIO ALGINOLYTICUS* OF WHITE SHRIMP, *LITOPENAEUS VANNAMEI*

Yu-Hung Lin, Yi-Che Su, and Winton Cheng

Key words: heat process, seaweed meal, white shrimp; immunostimulant.

## ABSTRACT

This study evaluated the effects of different processed brown seaweed on the growth, nonspecific immune responses, and resistance to *Vibrio alginolyticus* of white shrimp (*Litopenaeus vannamei*). A basal diet was supplemented with 0.25 and 0.5 g $kg^{-1}$ of a seaweed hot-water extract (HWE), 2.5 g $kg^{-1}$ of a heat-treated seaweed meal (HSM), and an untreated seaweed meal (USM). A basal diet without seaweed product supplementation served as the control. In total, five experimental diets were each fed to triplicate groups of white shrimp (initial wt: 5.58 ± 0.03 g) in an indoor static rearing system for 16 wks. After the 16-wk feeding trial, the shrimp were challenged with the pathogen *V. alginolyticus*. Weight gain and feed efficiency were higher ($P < 0.05$) in shrimp that were fed diets containing 0.25 and 0.5 g $kg^{-1}$ of the HWE and HSM than in shrimp that were fed other diets. Shrimp that were fed the HSM diet showed higher phenoloxidase activity than did shrimp that were fed the diets with 0.25 g $kg^{-1}$ of the HWE and USM and the control diet. Superoxide anion production was higher in shrimp that were fed the HSM diet than in shrimp that were fed the control diet. From 96 to 168 h after the *V. alginolyticus* challenge, the survival of shrimp that were fed the HSM diet was significantly higher than that of the shrimp that were fed other diets. The results indicate that the HSM can enhance the growth, nonspecific immune responses, and resistance to *V. alginolyticus* of white shrimp.

*Paper submitted 02/15/16; revised 10/27/16; accepted 11/29/16. Author for correspondence: Yu-Hung Lin (e-mail: yuhunglin@mail.npust.edu.tw); Winton Cheng (e-mail: winton@mail.npust.edu.tw).*
*Department of Aquaculture, National Pingtung University of Science and Technology, Taiwan, R.O.C.*

## I. INTRODUCTION

White shrimp, *Litopenaeus vannamei*, is the most crucial cultured shrimp species in the world. The annual aquaculture production of white shrimp increased from 8,286 MT in 1980 to 3,314,447 MT in 2013 (FAO, 2015). However, commercial white shrimp farming has been severely hit by epidemics associated with viruses and *Vibrio* infections, which have caused serious economic losses worldwide. The emergence of new diseases caused by *Vibrio* spp., such as acute hepatopancreatic necrosis syndrome, is causing high mortalities in commercial crops and consequently large economic losses in carciniculture (da Silva et al., 2013).

Conventional strategies for disease control in pond culture rely on the use of antibiotics and chemical disinfectants. However, because of the induction of bacterial resistance and environment pollution, in recent years, the use of immunostimulants has been proposed as a potential method for activating protection against microbial infections in aquatic culture animals (Anderson, 1992; Sakai, 1999; Bricknell and Dalmo, 2005; Huynh et al., 2011).

Some polysaccharides isolated from brown and red seaweeds have shown antiviral activities against viruses responsible for fish and shrimp diseases (Cheng et al., 2004; Cheng et al., 2005; Hou and Chen, 2005; Yeh et al., 2006; Cheng et al., 2007; Fu et al., 2007; Siiriustananun et al., 2011). Among them, algal polysaccharides, such as carrageenan, alginates, fucoidan, and laminaran, have been discussed as prominent compounds (Cheng et al., 2005; Deachamag et al., 2006; Liu et al., 2006; Cheng et al., 2007; Kitikiew et al., 2013). Our previous study demonstrated that administering a hot-water extract (HWE) of brow seaweed, *Sargassum cristaefolium*, at 2 g $kg^{-1}$ to the shrimp diet induced immune modulation and enhanced the immune ability of *L. vannamei*, in addition to increasing its resistance to *V. alginolyticus* infection (Chang et al., 2013).

**Table 1. Feed formulation and proximate composition of the experimental diets.**

| | Diet | | | | |
|---|---|---|---|---|---|
| | Control | 0.25 g/kg Hot-water seaweed extract | 0.5 g/kg Hot-water seaweed extract | 2.5 g/kg Heat-treated seaweed meal | 2.5 g/kg Untreated seaweed meal |
| Ingredients (g $kg^{-1}$) | | | | | |
| Fish meal[1] | 615 | 615 | 615 | 615 | 615 |
| α-starch[1] | 192 | 192 | 192 | 192 | 192 |
| Squid cream[1] | 26 | 26 | 26 | 26 | 26 |
| Defatted soybean meal[1] | 87 | 87 | 87 | 87 | 87 |
| Shrimp shell meal[1] | 51 | 51 | 51 | 51 | 51 |
| Cellulose[1] | 2.5 | 2.25 | 2.0 | 0 | 0 |
| Hot-water seaweed extract (HWE) | - | 0.25 | 0.5 | - | - |
| Heat-treated seaweed meal (HSM) | - | - | - | 2.5 | - |
| Untreated seaweed meal (USM) | - | - | - | - | 2.5 |
| Mineral mixture[2] | 12 | 12 | 12 | 12 | 12 |
| Vitamin mixture[2] | 15 | 15 | 15 | 15 | 15 |
| Proximate composition (g $kg^{-1}$) | | | | | |
| Moisture | 26 | 26 | 26 | 26 | 27 |
| Crude protein | 429 | 434 | 436 | 433 | 437 |
| Ether extract | 41 | 45 | 41 | 38 | 41 |

[1] Fish meal, Pesquera Diamante, Peru; α-starch, Trust River Trading Co., Ltd., Taiwan; squid cream, Power Omega, Korea; defatted soybean meal, TTET Union, Taiwan; and shrimp shell meal, Shye Yih Feeding Co., Taiwan; cellulose, Sigma Chemical, USA.

[2] Mineral and vitamin mixtures were provided by Shinta Feed Company, Pingtung, Taiwan.

Notably, the extraction of seaweeds is associated with increased costs such as the costs associated with the electricity used for heating and drying. Cost reduction for feed ingredients is a critical issue for the shrimp industry. Simplifying seaweed processing is meaningful as an incentive for the shrimp feed industry.

*S. cristaefolium* is distributed extensively along the coasts of the South China Sea and Taiwan. However, little information is available concerning its biological activity. Therefore, this study evaluated the effects of dietary heat-processed brown seaweed (*S. cristaefolium*) on the growth of *L. vannamei* and its immune responses and resistance to *V. alginolyticus*.

## II. MATERIALS AND METHODS

### 1. Processing of *S. cristaefolium*

*S. cristaefolium* was obtained from Pingtung, Taiwan. Fresh samples were thoroughly washed with tap water, rinsed twice with distilled water to remove other suspended particles, and then air dried in an oven at 50°C. The dried seaweed was frozen in liquid nitrogen and ground to a powder (serving as an untreated seaweed meal [USM]). The seaweed meal was boiled in hot water (4.0 g in 100 mL distilled water at 100°C) for 10 min. It was lyophilized using a freeze dryer (Eyela, FDU-1100, Tokyo, Japan), thus resulting in a heat-treated seaweed meal (HSM). The other parts of the seaweed meal were boiled following the same protocol as for the HSM. After boiling, the extract was filtered, and the solution was centrifuged at $10^4 \times g$ for 10 min. The supernatant containing the hot water-soluble extract was lyophilized using a freeze dryer (Eyela, FDU-1100, Tokyo, Japan). Three varieties of the seaweed products, namely the USM, HSM, and HWE, were stored at -20°C until use.

### 2. Diet Preparation

The diet formulation and proximate composition (AOAC, 1995) are shown in Table 1. A basal diet was supplemented with 0.25 and 0.5 g $kg^{-1}$ of the HWE and 2.5 g $kg^{-1}$ of the HSM and USM. A basal diet without seaweed product supplementation served as the control. The ingredients of the experimental diet were mixed, after which cold water was added to the mixture; subsequently, the mixture was further mixed until a stiff dough resulted. The dough was then passed through a mincer with die, and the resulting strands were dried using an electrical fan at 20°C. After drying, the strands were broken down, sieved into pellets (2.0 mm in diameter), and stored at -20°C until use.

### 3. Experimental Procedure

White shrimp, *L. vannamei*, obtained from brackish water (20%-23‰) ponds at the Department of Aquaculture, National Pingtung University of Science and Technology, were acclimated to room temperature (27°C ± 1°C) and a salinity of 20‰ in an indoor cement pond (6 × 2 × 1.5 m) for 2 wks before experimentation. During the acclimation period, shrimp were fed the control diet twice daily, and 50% of the water was changed weekly to maintain water quality. Only shrimp in the intermolt stage (stage C) were used for the subsequent tests. The molt

stage was determined by examination of the uropoda in which partial retraction of the epidermis could be distinguished (Robertson et al., 1987). At the beginning of the experiment, 15 fiber-reinforced plastic tanks (500 L) were each stocked with 40 shrimp with an average weight of 5.58 ± 0.03 g. Each experimental diet was fed to three groups of shrimp. Each tank received continuous aeration. In each tank, waste from uneaten feed and fecal pellets were removed through siphoning every day; 10% of the water was changed every day to maintain water quality. During the experiments, the water temperature was maintained at 27°C ± 1°C, pH at 7.8-8.2, and salinity at 20‰. Shrimp were fed their respective diets at a rate of 30 g $kg^{-1}$ body weight per day. This daily ration was subdivided into two equally sized feedings provided at 0900 and 1600 h. Shrimp were weighed every two weeks, and the daily ration was adjusted accordingly (3% body weight). The duration of the study was 16 wks.

At the end of the feeding trial, the shrimp were weighed. The percentages of body weight gain (WG) in each aquarium [100 × (final body weight − initial body weight)/initial body weight], feed efficiency (FE) [(final body weight − initial body weight)/feed intake], and survival [100 × (final shrimp number/initial shrimp number)] were calculated.

### 4. Susceptibility of Shrimp to *V. alginolyticus*

A known pathogenic strain, *V. alginolyticus*, isolated from diseased *L. vannamei*, which displayed symptoms of anorexia, inactivity, poor growth, and necrotic musculature, was used for the study. The pathogen was cultured according to the method described by Chang et al. (2013).

Challenge trials were conducted on shrimp following the 16-wk feeding trial by injecting them with 20 μL of a bacterial suspension (at $1.0 \times 10^8$ cfu $mL^{-1}$); thus, each shrimp received an injection of $1.0 \times 10^6$ cfu into the ventral sinus of the cephalothorax. Moreover, 10 shrimp were randomly collected from the control group and then injected with 20 μL saline as the unchallenged control. Experimental shrimp (10 shrimp in each aquarium) were kept in 60-L glass aquaria containing 40 L of water at 20‰ and 27°C ± 1°C. Each test involved three treatments. In addition, 20% of the water was renewed daily, and the experiment lasted 168 h. Shrimp were fed twice daily with the experimental diets after challenge.

### 5. Immune Parameters of the Shrimp

After the shrimp were fed different diets, their immune parameters were determined. Hemolymph (100 μL) was withdrawn from the ventral sinus of each shrimp into a 1-mL sterile syringe (25-gauge) containing 0.9 mL anticoagulant solution (30 mM trisodium citrate, 0.34 M sodium chloride, and 10 mM EDTA, at a pH of 7.55 and with the osmolality adjusted with glucose to 780 mOsm $kg^{-1}$). They were divided into two parts. A drop of the anticoagulant-hemolymph mixture (100 μL) was placed on a hemocytometer to measure the total hemocyte count by using an inverted phasecontrast microscope (Leica DMIL, Leica Microsystems, Wetzlar, Germany). The remainder of the hemolymph mixture was used for subsequent tests. Hemocyte phenoloxidase (PO) activity was measured spectrophotometrically by recording the formation of dopachrome, according to the procedures of a previous study (Herñandez-López et al., 1996). L-3,4-dihydroxy-phenylalanine and trypsin served as a substrate and an elicitor, respectively. The details of the measurement were described previously (Liu and Chen, 2004). The optical density of the shrimp PO activity is expressed as dopachrome formation in 50 μL of hemolymph.

The respiratory bursts of the hemocytes were quantified using the reduction of nitroblue tetrazolium (NBT) to formazan as a measure of superoxide anion ($O_2^-$) formation. The optical density at 630 nm was measured using a microplate reader (Model VERSAmax, Molecular Devices, Sunnyvale, CA, USA). The quantified respiratory bursts are expressed as NBT-reduction per 10 μL of hemolymph. Moreover, hemocyte superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide radical-dependent reactions by using the Ransod kit (Randox, Crumlin, UK) on the basis of the methods described in the instructions. Total protein was quantified using a Bio-Rad Protein Assay Kit (no. 500-0006, Bio-Rad Laboratories, USA) with bovine serum albumin (Sigma Chemical, USA) as a standard.

For the phagocytic activity and clearance efficiency tests, 20 μL of a bacterial suspension ($1 \times 10^8$ cfu $mL^{-1}$), resulting in $2 \times 10^6$ cfu $shrimp^{-1}$, was injected into the ventral sinus of two shrimp from each treatment. After injection, the shrimp were kept for 3 h in separate tanks containing 40 L of seawater (20‰) at 27°C ± 1°C. Subsequently, 200 μL of hemolymph was collected from the ventral sinus and mixed with 200 μL of sterile anticoagulant. This mixture was divided into two equal subsamples: one to measure phagocytic activity and the other to measure clearance efficiency. The methods for the measurements of phagocytic activity and clearance efficiency were described previously (Liu and Chen, 2004). Two hundred hemocytes were counted. Phagocytic activity, defined as the phagocytic rate (PR), can be expressed as follows:

$$PR = [(\text{phagocytic hemocytes})/(\text{total hemocytes})] \times 100$$

The clearance efficiency, defined as percentage inhibition (PI) of *V. alginolyticus*, can be calculated as follows:

$$PI = 100 - [(\text{cfu in the test group})/(\text{cfu in the control group})] \times 100$$

### 6. Statistical Analysis

Each experimental diet was fed to the three shrimp groups in accordance with a completely randomized design. The results were analyzed through one-way analysis of variance (ANOVA). When the ANOVA identified differences among the groups, multiple comparisons were performed among the means by using the Student-Newman-Keuls test. Statistical significance was determined by setting the aggregate type I error at $P < 0.05$.

**Table 2. Initial body weight, final body weight, weight gain, feed efficiency and survival of white shrimp fed different diets for 16 weeks.**

| | Diet code | | | | |
|---|---|---|---|---|---|
| | Control | 0.25 g kg$^{-1}$ HWE[1] | 0.5 g kg$^{-1}$ HWE | 2.5 g kg$^{-1}$ HSM | 2.5 g kg$^{-1}$ USM |
| Initial body weight (g/shrimp) | 5.66 ± 0.06 | 5.58 ± 0.02 | 5.50 ± 0.09 | 5.56 ± 0.06 | 5.58 ± 0.13 |
| Final body weight (g/shrimp) | 10.58 ± 0.23$^{a}$ | 12.05 ± 0.37$^{b}$ | 11.85 ± 0.15$^{b}$ | 11.82 ± 0.56$^{b}$ | 10.71 ± 0.41$^{a}$ |
| Weight gain (g) | 4.92 ± 0.26$^{a}$ | 6.47 ± 0.39$^{b}$ | 6.35 ± 0.19$^{b}$ | 6.27 ± 0.49$^{b}$ | 5.13 ± 0.48$^{a}$ |
| FE | 0.56 ± 0.04$^{a}$ | 0.80 ± 0.01$^{b}$ | 0.75 ± 0.02$^{b}$ | 0.78 ± 0.01$^{b}$ | 0.51 ± 0.01$^{a}$ |
| Survival | 71.4 ± 2.9 | 70.5 ± 1.6 | 71.4 ± 2.9 | 72.4 ± 4.4 | 74.3 ± 4.9 |

Data in the same column with different letters are significantly ($p < 0.05$) among different treatments. Values are means ± S.E. from three groups of shrimp fed on a same experimental diet (n = 3 tanks).

[1] HWE: hot water extract of seaweed meal; HSM: heat-treated seaweed meal; USM: untreated seaweed meal.

**Table 3. Hemocytic phenoloxidase (PO), superoxide anion ($O_2^-$) production, superoxide dismutase (SOD), phagocytic activity and clearance efficiency of white shrimp fed different diets for 16 weeks.**

| | Diet code | | | | |
|---|---|---|---|---|---|
| | Control | 0.25 g kg$^{-1}$ HWE[1] | 0.5 g kg$^{-1}$ HWE | 2.5 g kg$^{-1}$ HSM | 2.5 g kg$^{-1}$ USM |
| PO (O.D. 490 nm per $10^7$ cell) | 0.78 ± 0.47$^{a}$ | 0.81 ± 0.33$^{a}$ | 1.06 ± 0.86$^{ab}$ | 1.89 ± 0.62$^{b}$ | 0.78 ± 0.13$^{a}$ |
| $O_2^-$ production (per $10^7$ cell) | 1.96 ± 0.34$^{a}$ | 2.11 ± 0.73$^{ab}$ | 2.25 ± 0.25$^{ab}$ | 2.70 ± 0.32$^{b}$ | 2.18 ± 0.34$^{ab}$ |
| SOD (unit mg$^{-1}$ protein) | 2.28 ± 0.80$^{a}$ | 3.18 ± 0.37$^{ab}$ | 3.00 ± 0.26$^{ab}$ | 4.79 ± 0.82$^{b}$ | 2.36 ± 0.70$^{a}$ |
| Phagocytic activity (%) | 8.72 ± 1.70$^{a}$ | 12.17 ± 3.00$^{a}$ | 11.56 ± 1.50$^{a}$ | 21.11 ± 3.30$^{b}$ | 12.28 ± 3.36$^{a}$ |
| Clearance efficiency (%) | 0.00 ± 0.00$^{a}$ | -1.95 ± 1.57$^{a}$ | 7.80 ± 5.76$^{a}$ | 40.98 ± 9.68$^{b}$ | -1.71 ± 7.36$^{a}$ |

Data in the same column with different letters are significantly ($p < 0.05$) among different treatments. Values are means ± S.E. from three groups of shrimp fed on a same experimental diet (n=3 tanks).

[1] HWE: hot water extract of seaweed meal; HSM: heat-treated seaweed meal; USM: untreated seaweed meal.

**Table 4. Effects of dietary different treated *Sargassum cristaefolium* administrations on the survival of shrimp *Litopenaeus vanamei* challenged with *Vibrio alginolyticus.***

| Bacterial dose (CFU/shrimp) | Treatments | Survival (%), time after challenge (h) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 24 | 48 | 72 | 96 | 120 | 144 | 168 |
| Saline | - | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| 2 ×10$^6$ | Control | 63.3 ± 3.3$^{a}$ | 43.3 ± 3.3 | 30.0 ± 0.0$^{a}$ | 26.7 ± 3.3$^{a}$ | 26.7 ± 3.3$^{a}$ | 26.7 ± 3.3$^{a}$ | 26.7 ± 3.3$^{a}$ |
| 2 × 10$^6$ | 0.25 g kg$^{-1}$ HWE[1] | 66.7 ± 3.3$^{ab}$ | 50.0 ± 3.3 | 40.0 ± 3.3$^{ab}$ | 33.3 ± 6.7$^{a}$ | 33.3 ± 6.7$^{a}$ | 33.3 ± 6.7$^{a}$ | 33.3 ± 6.7$^{a}$ |
| 2 × 10$^6$ | 0.5 g kg$^{-1}$ HWE | 76.7 ± 3.3$^{ab}$ | 56.7 ± 6.7 | 50.0 ± 0.0$^{bc}$ | 43.3 ± 3.3$^{a}$ | 43.3 ± 3.3$^{a}$ | 43.3 ± 3.3$^{a}$ | 43.3 ± 3.3$^{a}$ |
| 2 × 10$^6$ | 2.5 g kg$^{-1}$ HSM | 80.0 ± 0.0$^{b}$ | 66.7 ± 6.7 | 63.3 ± 3.3$^{c}$ | 63.3 ± 3.3$^{b}$ | 63.3 ± 3.3$^{b}$ | 63.3 ± 3.3$^{b}$ | 63.3 ± 3.3$^{b}$ |
| 2 × 10$^6$ | 2.5 g kg$^{-1}$ USM | 66.7 ± 3.3$^{ab}$ | 43.3 ± 3.3 | 33.3 ± 6.7$^{a}$ | 30.0 ± 3.3$^{a}$ | 30.0 ± 3.3$^{a}$ | 30.0 ± 3.3$^{a}$ | 30.0 ± 3.3$^{a}$ |

Data in the same column with different letters are significantly ($p < 0.05$) among different treatments. Values are means ± S.E. from three groups of shrimp fed on a same experimental diet (n = 3 tanks).

[1] HWE: hot water extract of seaweed meal; HSM: heat-treated seaweed meal; USM: untreated seaweed meal.

## III. RESULTS

The final weight, WG, and FE were higher ($P < 0.05$) in shrimp that were fed diets containing 0.25, 0.5 g kg$^{-1}$ of the HWE and HSM than in shrimp that were fed other diets (Table 2). Moreover, survival outcomes did not differ among the treatments ($P > 0.05$).

Shrimp that were fed the HSM diet exhibited higher PO activity than did shrimp that were fed diets containing 0.25 g kg$^{-1}$ of the HWE and USM and the control diet (Table 3). Superoxide anion production was higher in shrimp that were fed the HSM diet than in shrimp that were fed the control diet. Hemolymph superoxide dismutase activity was higher in shrimp that were fed the HSM diet than in shrimp that were fed the USM diet or the control diet. Shrimp that were fed the HSM diet exhibited higher phagocytic activity and clearance efficiency than did shrimp that were fed other dietary treatments.

Table 4 presents the survival rate of white shrimp (*L. vannamei*) that were fed different diets and then challenged with *V. alginolyticus*. All the unchallenged control shrimp survived.

By contrast, mortalities were recorded after 24 h for the challenged shrimp in all experimental groups. From 96 to 168 h after challenging, the survival of shrimp that were fed the HSM diet was significantly higher than that of the shrimp that were fed other diets.

## IV. DISCUSSION

This study determined that supplementing heat-processed seaweed meals (both the HSM and HWE) in diets improved the growth performance of white shrimp, compared with the USM. This clearly demonstrates that subjecting the seaweed meal to the heat process may release some active compounds associated with shrimp growth. Seaweed is typically considered a natural food for shrimp (Lombardi et al., 2006). Shrimp may be able to use the released nutrients from seaweed for growth. However, this differs from results derived from observations of land animals. For example, Gardiner et al. (2008) and Turner et al. (2002) have reported growth depression in pigs that were fed diets with seaweed extracts. This phenomenon might be due to the presence of phenolic acids, chelated minerals, or indigestible polysaccharides. Moreover, in crustaceans, the activities of carbohydrases such as alginase, agarase, inulinase, and xylanase have not been demonstrated (Vonk and Western, 1984). This suggests that nutrients such as proteins, lipids, or some unknown growth factors benefit shrimp growth.

The growth performance levels of white shrimp that were fed the HSM showed a similar trend regarding immune responses and disease resistance (Tables 2-4). Karunasagar et al. (1994) and Song and Sung (1990) have demonstrated the benefits of growth enhancement in immunostimulated shrimp larvae and juveniles. Azad et al. (2005) attributed the growth enhancement effects of dietary seaweed product to the efficient nutrient digestion and assimilation caused by the activation of fixed phagocytes in the hepatopancreas that secrete hydrolytic enzyme in the digestive gland. This may explain why the shrimp that were fed the HSM diet exhibited the best growth performance. However, the HWE seaweed did not engender the same immunostimulant effect for white shrimp during the 16-wk feeding period. Some water-insoluble compounds, such as lipids, aromatic chemicals, ketenes, or carotenoids, were identified in seaweed (Peinadoa et al., 2014). After hot-water extraction, crude protein and lipids decreased from 5.94% and 1.25% to 2.05% and 0.80%, respectively (data not shown) in our study. This suggests that some nutrients were lost during the extraction procedure. However, the compositions of the lost nutrients were not identified. Further research regarding the composition of the lost nutrients is therefore necessary.

Our previous study demonstrated that a brown seaweed (*S. cristaefolium*) HWE can enhance immunity and resistance to white shrimp pathogens for a short-term (18-d) feeding period (Chang et al., 2013). However, in this long-term (16-wk) study, dietary HWE did not exhibit the same immunostimulant effect as that observed in the previous study. This might be due to the adaption to the immunostimulant by the shrimp over this long duration. However, the fact that the HSM retained the immunostimulant effect even for a 16-wk feeding period is remarkable. The reason for this phenomenon requires further investigation.

The five commonly used immune parameters, namely hemocytic PO, superoxide anion ($O_2^-$) production, SOD, phagocytic activity, and clearance efficiency, were used in this study as immune responses of white shrimp that were fed different processed seaweed products. Notably, circulating hemocytes play a central role in the immune response in crustaceans. Hemocytes are generally classified into three morphologically different cell types: hyaline cells, semigranular cells, and granular cells (Tsing et al., 1989). Hemocytes are involved in the production of melanin through the prophenoloxidase (proPO) system; melanin is stored and produced by both semigranular and granular cells (Johansson and Söderhäll, 1989). In addition, hyaline and semigranular cells are involved in phagocytosis (Walton and Smith, 1999; Giulianini et al., 2007). The elimination of phagocyted particles involves the generation of reactive oxygen intermediates (ROIs) such as $O_2^-$, hydrogen peroxidase ($H_2O_2$), hydroxyl radicals ($OH^-$), and singlet oxygen ($^1O_2$). The process by which ROIs are produced is known as the respiratory burst, and it plays a major role in microbicidal activity (Muñoz et al., 2000; Rodríguez and Moullac, 2000). ROIs do not distinguish between self-tissues and microbes; therefore, they are capable of causing tissue injury if they enter the extracellular environment. The harmful effects of ROIs on the host itself are neutralized by antioxidant defense mechanisms, which include antioxidant molecules and enzymes such as SOD (Campa-Córdova et al., 2002). These immune parameters showed the best performance in the shrimp that were fed the HSM diet compared with other dietary treatments. Fish treated with immunostimulants show increased immune response parameters (Sakai, 1999). Several seaweed polysaccharides contain sulfate residues. The sulfated polysaccharides from the brown seaweeds *Sargassum wightii* and *Halophila ovalis* (Yuvaraj et al., 2013) and the red seaweeds *Gracilaria cornea* (Coura et al., 2015) and *Caulerpa racemosa* (Ribeiro et al., 2014) have been identified to demonstrate immunostimulatory activities.

The shrimp that were fed the HSM diet exhibited increased resistance to the pathogen *Vibrio alginolyticus*. These results are in agreement with those of previous studies. A study reported that the injection of HWEs from *Sargassum duplicatum* (brown seaweed) and *Gracilaria tenuistipitata* (red seaweed) increased the survival of *L. vannamei* after *V. alginolyticus* infection (Hou and Chen, 2005). Moreover, oral administration of fucoidan, a polysaccharide extracted from brown seaweeds, has been reported to reduce the impact of white spot syndrome virus in *L. vannamei* (Cruz-Súarez et al., 2007), *Penaeus monodon* (Chotigeat et al., 2004), and *Marsupenaeus japonicus* (Takahashi et al., 1998). Sodium alginate derived from *Macrocystis pyrifera* and administered orally and by injection has also been reported to increase the resistance of *L. vannamei* to *V. alginolyticus* infection (Cheng et al., 2004; Cheng et al., 2005). *Litopenaeus indicus* juveniles reared in water inoculated with

*V. parahaemolyticus* and fed with *Artemia* enriched with butanol crude extracts from *Ulva lactuca* and *S. wightii* showed increased survival rates (Immanuel et al., 2004). In addition, a polysaccharide from *S. fusiforme* was reported to increase the resistance of *Fenneropenaeus chinensis* against *V. harveyi* when administered into the feed (Huang et al., 2006). The use of different compounds derived from seaweeds for increasing immune responses or disease resistance in shrimp is considered an alternative to the prophylactic use of chemicals.

In conclusion, the results indicate that both HWE and HSM diet supplementation can enhance the growth performance of white shrimp. The HSM, but not the HWE, can improve nonspecific immune responses and resistance to *V. alginolyticus* of the shrimp. Therefore, 2.5 g $kg^{-1}$ of the HSM is recommended for use in white shrimp diet to enhance growth, nonspecific immune responses, and resistance to *V. alginolyticus*.

## REFERENCES


Anderson, D. P. (1992). Immunostimulants, adjuvants, and vaccine carriers in fish: application to aquaculture. Annual Review of Fish Diseases 2, 281-307.

Association of Official Analytical Chemists (AOAC) (1995). Official Methods of Analysis, 16th ed. AOAC, Arlington, VA.

Azad, I. S., A. Panigrahi, C. Gopal, S. Paulpandi, C. Mahima and P. Ravichandran (2005). Routes of immunostimulation via-á-vis survival and growth of *Penaeus monodon* postlarvae. Aquaculture 248, 227-234.

Bricknell, I. and R. A. Dalmo (2005). The use of immunostimulants in fish larval aquaculture. Fish and Shellfish Immunology 19, 457-472.

Campa-Córdova, A. I., N. Y. Hernández-Saavedra, R. De Philippis and F. Ascencio (2002). Generation of superoxide anion and SOD activity in haemocytes and muscle of American white shrimp (*Litopenaeus vannamei*) as a response to β-glucan and sulphated polysaccharide. Fish and Shellfish Immunology 12, 353-366.

Chang, C. C., L. H. Gao and W. Cheng (2013). Effect of dietary administration of hot-water extracts of *Sargassum cristaefolium* on the immunity of white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Journal of the Fisheries Society of Taiwan 40, 27-41.

Cheng, A. C., C. W. Tu, Y. Y. Chen, F. H. Nan and J. C. Chen (2007). The immunostimulatory effects of sodium alginate and iota-carrageenan on orange-spotted grouper *Epinephelus coioides* and its resistance against *Vibrio alginolyticus*. Fish and Shellfish Immunology 22, 197-205.

Cheng, W., C. H. Liu, S. T. Yeh and J. C. Chen (2004). The immune stimulatory effect of sodium alginate on white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Fish and Shellfish Immunology 17, 41-51.

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

Chiu, C. H., Y. K. Guu, C. H. Liu, T. M. Pan and W. Cheng (2007). Immune responses and gene expression in white shrimp, *Litopenaeus vannamei*, induced by *Lactobacillus plantarum*. Fish and Shellfish Immunology 23, 364-377.

Chotigeat, W., S. Tongsupa, K. Supamataya and A. Phongdara (2004). Effect of fucoidan on disease resistance of black tiger shrimp. Aquaculture 233, 23-30.

Coura, C. O., R. B. Souza, J. A. G. Rodrigues, E. S. O. Vanderlei, I. W. F. de Araújo, N. A. Ribeiro, A. F. Frota, K. A. Ribeiro, H. V. Chaves, K. M. A. Pereira, R. M. S. da Cunha, M. M. Bezerra and B. N. M. Benevides (2015). Mechanisms involved in the anti-inflammatory action of a polysulfated fraction from *Gracilaria cornea* in rats. PLOS One E 10, e0119319.

Cruz-Súarez, L. E., J. Hernández, M. Porchas-Cornejos, D. E. Coronado-Molina, M. L. Linné-Unzueta-Bustamante, M. Nieto-López, M. Tapia-Salazar and D. Ricque-Marie (2007). The antiviral effect of algal fucoidans. Aqua Culture Asia Pacific Magazine 3, 19.

da Silva, B. C., F. N. Vieira, J. L. P. Mouriño, G. S. Ferreira and W. Q. Seiffert (2013). Salts of organic acids selection by multiple characteristics for marine shrimp nutrition. Aquaculture 384-387, 104-110.

Deachamag, P., U. Intaraphad, A. Phongdara and W. Chotigeat (2006). Expression of a phagocytosis activating protein (PAP) gene in immunized black tiger shrimp. Aquaculture 255, 165-172.

FAO Yearbooks of Fisheries Statistics (2015). Food Agriculture Organization of the United Nations, Rome.

Fu, Y. W., W. Y. Hou, S. T. Yeh, C. H. Li and J. C. Chen (2007). The immunostimulatory effects of hot-water extract of *Gelidium amansii* via immersion, injection and dietary administration on white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Fish and Shellfish Immunology 22, 673-685.

Gardiner, G. E., A. J. Campbell, J. V. O'Doherty, E. Pierce, P. B. Lynch, F. C. Leonard, C. Stanton, R. P. Ross and P. G. Lawlor (2008). Effect of *Ascophyllum nodosum* extract on growth performance, digestibility, carcass characteristics and selected intestinal microflora populations of grower-finisher pigs. Animal Feed Science and Technology 141, 259-273.

Giulianini, P. G., M. Bierti, S. Lorenzon, S. Battistella and E. A. Ferrero (2007). Ultrastructural and functional characterization of circulating hemocytes from the freshwater crayfish *Astacus leptodactylus*: Cell types and their role after in vivo artificial non-self challenge. Micron 38, 49-57.

Herñandez-López, J., T. Gollas-Galván and F. Vargas-Albores (1996). Activation of the prophenoloxidase system of the brown shrimp (*Penaeus californensis* Holmes). Comparative on Biochemistry and Physiology 113, 61-66.

Hou, W. Y. and J. C. Chen (2005). The immunostimulatory effect of hot-water extract of *Glacilaria tenuistipitata* on the white shrimp *Litopenaeus vannamei* and its resistance against *Vibrio alginolyticus*. Fish and Shellfish Immunology 19, 127-138.

Huynh, T. G., S. T. Yeh, Y. C. Lin, J. F. Shyu, L. L. Chen and J. C. Chen (2011). White shrimp *Litopenaeus vannamei* in seawater containing *Sargassum hemiphullum* var. chinese powder and its extract showed increased immunity and resistance against *Vibrio alginolyticus* and white spot syndrome virus. Fish and Shellfish Immunology 31, 286-293.

Huang, X., H. Zhou and H. Zhang (2006). The effect of *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and immune activity of the shrimp *Fenneropenaeus chinensis*. Fish and Shellfish Immunology 20, 750-757.

Immanuel, G., V. C. Vincybai, V. Sivaram, A. Palavesam and M. P. Marian (2004). Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. Aquaculture 236, 53-65.

Johansson, M. W. and K. Söderhäll (1989). Cellular immunity in crustaceans and the proPO system. Parasitology Today 5, 171-176.

Karunasagar, I., R. Pai, G. R. Malathi and I. Karunasagar (1994). Mass mortality of *Penaeus monodon* due to antibiotic-resistant *Vibrio harveyi* infection. Aquaculture 128, 203-209.

Kitikiew, S., J. C. Chen, D. F. Putra, Y. C. Lin, S. T. Yeh and C. H. Liou (2013). Fucoidan effectively provokes the innate immunity of white shrimp *Litopenaeus vannamei* and its resistance against experimental *Vibrio alginolyticus* infection. Fish and Shellfish Immunology 34, 280-290.

Liu, C. H. and J. C. Chen (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.

Liu, C. H., S. P. Yeh, C. M. Kuo, W. Cheng and C. H. Chou (2006). The effect of sodium alginate on the immune response of tiger shrimp via dietary administration: Activity and gene transcription. Fish and Shellfish Immunology 21, 442-452.

Lombardi, J. V., H .L. de Almeida Marques, R. T. L. Pereira, O. J. S. Barreto and E. J. de Paula (2006). Cage polyculture of the Pacific white shrimp *Litopenaeus vannamei* and the Philippines seaweed *Kappaphycus alvarezii*. Aquaculture 258, 412-415.

Muñoz, M., R. Cedeño, J. Rodríguez, W. P. W. van der Knaap, E. Mialhe and E. Bachère (2000). Measurement of reactive oxygen intermediate produc-

tion in haemocytes of the penaeid shrimp, *Penaeus vannamei*. Aquaculture 191, 89-107.

Peinadoa, I., J. Girónb, G. Koutsidisa, J. M. Ames (2014). Chemical composition, antioxidant activity and sensory evaluation of five different species of brown edible seaweeds. Food Research International 66, 36-44.

Ribeiro, N. A., T. M. Abreu, H. V. Chaves, M. M. Bezerra, H. S. A. Monteiro, R. J. B. Jorge and N. M. B. Benevides (2014). Sulfated polysaccharides isolated from the green seaweed *Caulerpa racemosa* plays antinociceptive and anti-inflammatory activities in a way dependent on HO-1 pathway activation. Inflammation Research 63, 569-580.

Rodríguez, J. and G. Le Moullac (2000). State of the art of immunological tools and health control of penaeid shrimp. Aquaculture 191, 109-119.

Robertson, L., W. Bray, J. Leung-Truillo and A. Lawrenve (1987). Practical molt staging of *Penaeus setiferus* and *Penaeus stylirostris*. Journal of the World Aquaculture Society 18, 180-185.

Sakai, M. (1999). Current research status of fish immunostimulants. Aquaculture 172, 63-92.

Siiriustananun, N., J. C. Chen, Y. C. Lin, S. T. Yeh, C. H. Kiuo, L. L. Chen, S. S. Sim and S. L. Chiew (2011). Dietary administration of a *Gracilaria tenuistipitata* extract enhances the immune response and resistance against *Vibrio alginolyticus* and white spot syndrome virus in the white shrimp *Litopenaeus vannamei*. Fish and Shellfish Immunology 31, 848-855.

Song, Y. L. and H. H. Sung (1990). Enhancement of growth in tiger shrimp (*Penaeus monodon*) by bacterin prepared from *Vibrio vulnificus*. Bulletin of the European Association of Fish Pathologists 10, 98-99.

Takahashi, Y., K. Uehara, R. Watanabe, T. Okumura, T. Yamashita, H. Omura, T. Yomo, T. Kawano, A. Kanemitsu, H. Narasaka, N. Suzuki and T. Itami (1998). Efficacy of oral administration of fucoidan, a sulfated polysaccharide, in controlling white spot syndrome in kuruma shrimp in Japan, In: Advances in Shrimp Biotechnology, National Center for Genetic Engineering and Biotechnology, Flegel TW, Bangkok, Thailand, 171-173.

Tsing, A., J. M. Arcier and M. Brehélin (1989). Hemocytes of Penaeid and Palaemonid shrimps: morphology, cytochemestry, and hemograms. Journal of Invertebrate Pathology 53, 64-77.

Turner, J. L., S. S. Dritz, J. J. Higgins and J. E. Minton (2002). Effects of *Ascophyllum nodosum* extract on growth performance and immune duction of young pigs challenged with *Salmonella typhimurium*. Journal of Animal Science 80, 1947-1953.

Yeh, S. T., C. S. Lee and J. C. Chen (2006). Administration of hot-water extract of brown seaweed *Sargassum duplicatum* via immersion and injection enhances the immune resistance of white shrimp *Litopenaeus vannamei*. Fish and Shellfish Immunology 20, 332-345.

Vonk, H. J. and R. H. Western (1984). Comparative Biochemistry and Physiology of Enzymatic Digestion. Academic Press, London.

Walton, A. and V. J. Smith (1999). Primary culture of the hyaline haemocytes from marine decapods. Fish and Shellfish Immunology 9, 181-194.

Yuvaraj, N., P. Kanmani, R. Satishkumar, A. Paari, V. Pattukumar and V. Arul (2013). Antinociceptive and anti-inflammatory activities of *Sargassum wightii* and *Halophila ovalis* sulfated polysaccharides in experimental animal models. Journal of Medicinal Food 16, 740-748.