Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(5): 1501 – 1516 (2025)
www.ejabf.journals.ekb.eg

Utilization of Crustacean Shell Extract as a Natural Antioxidant Source in Functional Feed to Enhance Growth and Immunity of Whiteleg Shrimp (*Penaeus vannamei*)

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ARTICLE INFO

Article History:
Received: July 21, 2025
Accepted: Sep. 13, 2025
Online: Sep. 27, 2025

Keywords:
Crustacean shell extract, *Penaeus vannamei*, Antioxidant activity, Phagocytic activity

ABSTRACT

Astaxanthin is a natural antioxidant that plays a crucial role in enhancing the immune response of aquatic organisms. This study evaluated the effects of crustacean shell extract on the growth performance, survival rate, and immune response of the whiteleg shrimp (*Penaeus vannamei)*, and identified the primary bioactive compounds through gas chromatography-mass spectrometry (GC-MS) analysis. The extract was obtained *via* maceration using 96% ethanol and incorporated into feed at a dose of 2g/ kg for 56 days of rearing. Results showed that the inclusion of crustacean shell extract significantly (P<0.05) improved the specific growth rate (5.10%/day), survival rate (96%), and phagocytosis activity (92%) compared to the control group. The GC-MS analysis identified dominant bioactive compounds with antioxidant properties, including 9,12-octadecadienoic acid (linoleic acid methyl ester; 5.93% area) from the whiteleg shrimp carapace (PUFA omega-6), 5,8,11,14,17-eicosapentaenoic acid methyl ester (EPA; 2.81% area) from mud crab shell (PUFA omega-3), and 5,8,11,14,17-eicosapentaenoic acid methyl ester (EPA; 1.11% area) from swimming crab shell (PUFA omega-3). These compounds are recognized for their strong antioxidant, immunostimulant, and anti-inflammatory properties. These findings suggest that crustacean shell extract holds significant potential as a natural functional feed additive to sustainably enhance the growth performance, survival, and immune system of the whiteleg shrimp.

INTRODUCTION

The whiteleg shrimp (*Penaeus vannamei*) is one of the main products in the aquaculture industry in Indonesia and globally. In 2019, total whiteleg shrimp production worldwide reached more than 6.5 million tons and contributed about 83% of the total global aquaculture shrimp production **(Emerenciano *et al.*, 2022)**. This species has various advantages, including fast growth rate, adaptability to intensive farming systems, feed conversion efficiency, and high consumer preference for its meat taste, making it very suitable for intensive and super-intensive farming systems. However, the success of intensive farming still faces technical and ecological challenges, particularly related to

declining water quality, increased stress, and high prevalence of bacterial and viral infections, which cause significant economic losses **(Kumar *et al*., 2022)**. One consequence of continuous environmental stress is the onset of oxidative stress, which is a state of imbalance between the production of reactive oxygen species (ROS) and the capacity of the biological antioxidant system, which can damage cell structure and reduce the physiological performance of whiteleg shrimp.

One of the main problems in intensive farming systems is oxidative stress caused by the accumulation of free radicals in the aquatic environment. Oxidative stress can damage cell membranes and tissues, reduce physiological capacity, and cause disturbances in the shrimp's immune system, which ultimately reduces productivity and increases mortality **(Ayuni *et al*., 2020)**. In addition, high stocking densities are known to exacerbate this condition by increasing the rate of free radical production. Meanwhile, salinity and water quality in general also have a significant effect on shrimp survival and growth **(Gao *et al*., 2017; Hasbullah *et al*., 2018)**.

Astaxanthin is a high-value carotenoid pigment known for its commercial applications in various industries, including aquaculture, food, cosmetics, nutraceuticals, and pharmaceuticals **(Lim *et al*., 2017)**. Astaxanthin (C40H52O4 or 3,3'-dihydroxy-β-carotene-4,4'-dione) is a pigment from the carotenoid group that is widely found in algae, shrimp, and crabs. Astaxanthin, as the main pigment, plays a role in boosting the immune system, accelerating tissue repair, and protecting cells from oxidative stress **(Hidayati *et al*., 2023**). In addition to supporting growth and survival, antioxidant compounds are also important in strengthening non-specific immune responses, particularly through increased phagocytosis activity by hemocytes, which is a key parameter of shrimp immune resistance **(Anderson & Siwicki, 1995)**.

Crustacean shell extract functions as a functional feed additive in the whiteleg shrimp and has been proven *in vitro* and in other aquatic species, but its application in shrimp aquaculture remains under-explored. Fishery waste, such as swimming crab shell, mud crab shell and shrimp shells, contains bioactive compounds such as astaxanthin, polyunsaturated fatty acids (PUFAs), phenolic compounds, and cyclic peptides, which exhibit antioxidant, antimicrobial, anti-inflammatory, and immunostimulatory activities **(Gao *et al*., 2017)**. In intensive aquaculture, oxidative stress due to high stocking density and fluctuating water quality can disrupt physiological performance and immunity, which directly affects survival and growth **(Hasbullah *et al*., 2018; Ayuni *et al*., 2020)**. Studies on the direct application of crustacean shell extract in Pacific whiteleg shrimp (*Penaeus vannamei*) feed are still limited, particularly regarding improvements in growth performance, survival rates, phagocytosis activity, and its effects on aquaculture water quality. Crustacean shells are a source of bioactive compounds, including natural antioxidants, which have the potential to support shrimp health and to reduce the environmental impact of fishery waste. Therefore, this study aimed to evaluate and analyze the potential of crustacean shell extract as a feed additive to improve growth performance, survival rates, phagocytosis activity, and water quality in sustainable aquaculture systems.

MATERIALS AND METHODS

Research materials

This study utilized three types of crustacean shell waste: mud crab (*Scylla serrata*), swimming crab (*Portunus pelagicus*), and whiteleg shrimp (*Penaeus vannamei*), each weighing 10kg. Mud crab and swimming crab shells were sourced from Maros Regency, while the whiteleg shrimp carapaces were obtained from the industrial area of Makassar City, South Sulawesi. Supporting materials included 96% ethanol and laboratory equipment such as a blender, oven, 60-mesh sieve, Buchner funnel, Whatman filter paper, rotary evaporator, digital scale (0.001 g accuracy), measuring glassware, and Erlenmeyer flasks. All extraction processes were conducted at the Fishery Product Technology Laboratory, Hasanuddin University.

Research metdods

1. Crustacean shell extraction

Mud crab shells (MCS), swimming crab shells (SCS) and whiteleg shrimp carapaces (WSC) were cleaned, washed with running water, then dried in an oven at 50°C for 72 hours. Once dried, the shells were crushed, ground, and sieved with a 60 mesh sieve to produce a fine powder. A total of 1kg of powder from each type was extracted using 96% ethanol (1:5 b/v ratio), stirred for 10–15 minutes, then left for 24 hours at room temperature. The filtrate was then filtered with Whatman No. 1 paper and a vacuum Buchner funnel, then evaporated with a rotary evaporator to obtain a thick extract, which is stored in a closed container at low temperature.

2. Gas chromatography-mass spectrometry (GC-MS) analysis

The identification of bioactive compounds was performed using a Gas Chromatography-Mass Spectrometry (GC-MS Ultra QP2010 Shimadzu) device. A total of 0.5mL of extract was mixed with 5mL of methanol, then homogenized for 1 minute using a vortex and placed in a vial. The analysis was performed in splitless mode with an injector temperature of 250°C, pressure of 76.9 kPa, carrier gas flow rate of 1.4mL/ min, and split ratio of 1:10. The column used was SH-Rxi-5Sil MS (length: 30m; inner diameter: 0.25mm). The temperature program started at 70°C for 2 minutes, then increased to 200°C at a rate of 10°C/ minute, then continued to 280°C with an increase of 50°C/ minute and held for 9 minutes, so that the total analysis time reached 30 minutes. Detection was performed in the mass range of m/z 400–700, with an ion source temperature of 200°C and an interface temperature of 280°C. Compound identification was performed by matching the mass spectrum to the NIST 20 database in accordance with the standard operating procedures of the Chromatography Laboratory at the Ujung Pandang State Polytechnic.

3. Test feed preparation

Mud crab shell (MCS), swimming crab shell (SCS) and whiteleg shrimp carapace (WSC) were each extracted at a weight of 2g/ kg of feed, in accordance with the specified

formulation. The extracts were then gradually mixed with other crushed raw materials, starting from the smallest to the largest amount. The raw materials were stirred until evenly mixed, then 10–12% water was added to create a dough. The dough was formed into pellets, then dried in an electric oven at 50°C for 4–6 hours until it had a dry and solid texture, before being used in the experimental shrimp cultivation.

4. Test animals

Juvenile whiteleg shrimp, with an initial weight of approximately 3 grams, were reared in circular experimental tanks, with 40 shrimp per unit. Feed was provided at a rate of 5% of body weight per day, adjusted periodically. The rearing period lasted for 56 days. To maintain water quality, 10–20% of the water was changed daily to maintain stable environmental conditions in the aquarium.

5. Experimental design and statistical analysis

This study used a completely randomized design (CRD) with 4 treatments and 3 replicates. The treatments consisted of: treatment A which is control, without extract, treatment B, which was mud crab shell extract (MCS 2 g/kg feed), treatment C, which is swimming crab shell extract (SCS 2g/ kg feed), and treatment D, which is whiteleg shrimp carapace extract (WSC 2g/ kg feed). Data were analyzed using analysis of variance (ANOVA), and if significant differences were found, Tukey's test was performed at a 5% significance level for the parameters of specific growth rate (SGR), survival rate, and phagocytosis activity of the whiteleg shrimp.

6. Parameters and data analysis

a. Specific growth rate (SGR in g/day)

SGR (g/day) and survival rate (SR,%) were calculated using the formula proposed by **Zainuddin *et al*. (2019)**:

$$SGR = \frac{Ln\ Wt - Ln\ W_0}{t} x\ 100\%$$

where: Wo = Initial shrimp weight (g)
Wt = Final shrimp weight (g)
t = Grow-out time (days)

b. Survival rate (SR,%)

Survival rate (SR %) was calculated as the proportion of shrimp that survived until the end of the experimental period, using the following formula:

$$SR = Nt / No \times 100\%$$

Nt = Initial number of shrimp
No = Final number of shrimp

c. *Phagocytosis activity*

Phagocytosis activity was measured according to **Anderson and Siwicki (1995)**. A 0.1mL hemolymph sample mixed with 0.1mL anticoagulant was incubated with 50µL *Staphylococcus aureus* suspension (10^7 cells/mL in PBS) in a microplate for 20 minutes at room temperature. A 5µL aliquot was placed on a glass slide, smeared, fixed with methanol (5–10 minutes), and stained with Giemsa (15–20 minutes). The slides were observed under a light microscope at 400x magnification to count phagocytic cells engulfing bacteria out of 100 cells observed. The phagocytosis index was calculated as the percentage of active phagocytic cells relative to the total cells observed.

$$\text{AF} = \frac{\text{Number of phagocytic cells performing phagocytosis}}{\text{Total number of hemocytes}} x\ 100\%$$

RESULTS

1. GC-MS analysis of mud crab shell extract (*Scylla serrata*)

The results of the GC-MS test of mud crab shell extract are shown in Fig. (1) which displays the percentage of different areas.

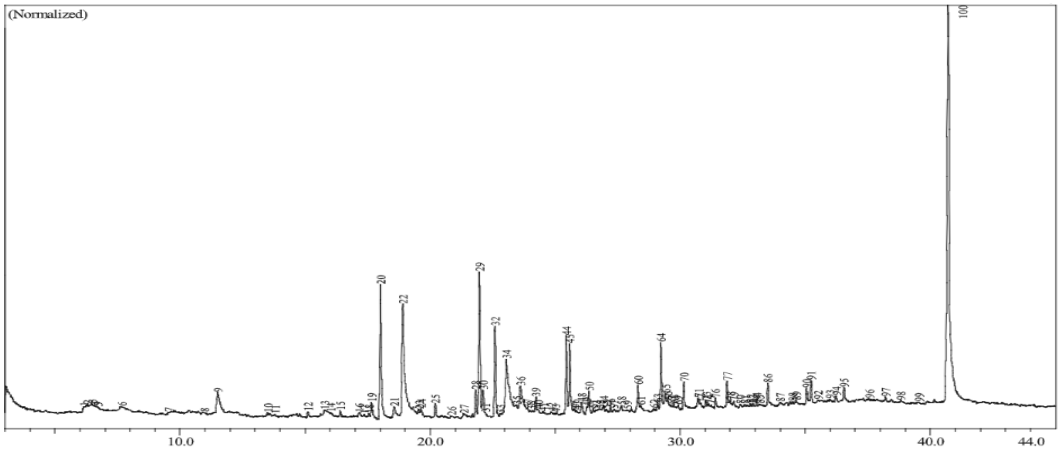

Fig. 1. GC-MS test results of mud crab shell (*Scylla serrata*)

The GC-MS chromatogram of mud crab shell extract (Fig. 1) shows 100 compound peaks, including four main bioactive compounds that exhibit strong antioxidant activity (Table 1), namely Peak 44 (2.61%) 5,8,11,14-Eicosatetraenoic acid methyl ester, Peak 45 (2.81%) Eicosapentaenoic acid methyl ester, Peak 64 (2.44%) Docosahexaenoic acid methyl ester, and Peak 91 (0.83%) Squalene. These compounds include omega-6 PUFAs, omega-3 PUFAs, and natural triterpenoids that have been proven to be effective in neutralizing free radicals, maintaining cell membrane stability, reducing oxidative stress, and supporting the immune system and physiological health of shrimp.

Table 1. Primary bioactive compounds with strong antioxidant activity identified from mud crab shell extract based on GC-MS analysis

Peak	R Time	Area (%)	Compound Name	Compound Class	Primary Biological Activity
44	25.452	2.61	5,8,11,14-Eicosatetraenoic acid, methyl ester (Arachidonic Acid	PUFA, Omega-6	Antioxidant, anti-inflammatory
45	25.589	2.81	5,8,11,14,17-Eicosapentaenoic acid, methyl ester, (all-Z)-	PUFA, Omega-3	Antioxidant, anti-inflammatory, immunostimulant
64	29.232	2.44	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester (DHA)	PUFA, Omega-3	Antioxidant, neuroprotective, immunoprotective
91	35.235	0.83	Squalene	Natural Triterpenoid	Antioxidant, anti-aging

Source: Chromatography Laboratory, Ujung Pandang State Polytechnic.

2. GC-MS analysis of swimming crab shell extract (*Portunus pelagicus*)

The results of the GC-MS test of crab shell extract are shown in Fig. (2), which shows the percentage of different areas.

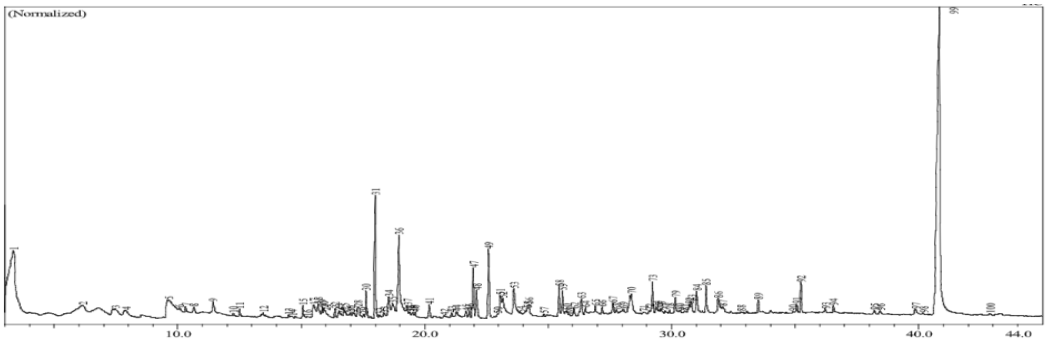

Fig. 2. GC-MS analysis of swimming crab (*Portunus pelagicus*) shell extract

The chromatogram of the GC-MS analysis of the crab shell extract shows 100 peaks (Fig. 2), with 5 (five) main bioactive compounds dominated by strong antioxidant compounds (Table 2), namely: (Peak 11; 0.22%) Phenol, 2,4-bis(1,1-dimethylethyl)-, (Peak 34; 1.25%) Palmitoleic acid, (Peak 59; 1.11%) Eicosapentaenoic acid methyl ester, (Peak 73; 1.16%), Docosahexaenoic acid methyl ester, and (Peak 92; 0.94%) Squalene. These compounds come from the phenol, omega-3 PUFA, MUFA, and triterpenoid groups, which are known to have strong antioxidant potential. Compounds from these groups have a strong ability to neutralize free radicals, protect cell structures from oxidative stress, and support the physiological functions of aquatic organisms.

Table 2. Primary bioactive compounds with strong antioxidant activity identified from swimming crab shell *(Portunus pelagicus*) extract based on GC-MS

Peak	R Time (Minutes	Area (%)	Compound Name	Compound Class	Primary Biological Activity
11	12.508	0.22	Phenol, 2,4-bis(1,1-dimethylethyl)-	Phenolic compound	Antioxidant, antibacterial
34	18.540	1.25	Palmitoleic acid	Unsaturated fatty acid (MUFA)	Antioxidant, anti-inflammatory,
59	25.580	1.11	5,8,11,14,17-Eicosapentaenoic acid, methyl ester (EPA)	PUFA Omega-3	Antioxidant, anti-inflammatory, immunostimulant
73	29.223	1.16	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester (DHA	PUFA Omega-3	Antioxidant
92	35.233	0.94	Squalene	Triterpenoid (carotenoid)	Antioxidant, anticancer, anti-aging, immunostimulant

Source: Chromatography Laboratory, Ujung Pandang State Polytechnic.

3. GC-MS analysis of whiteleg shrimp carapace extract (*Penaeus vannamei*)

The results of the GC-MS test of the whiteleg shrimp carapaces are shown in Fig. (3) below, which shows the percentage of different areas.

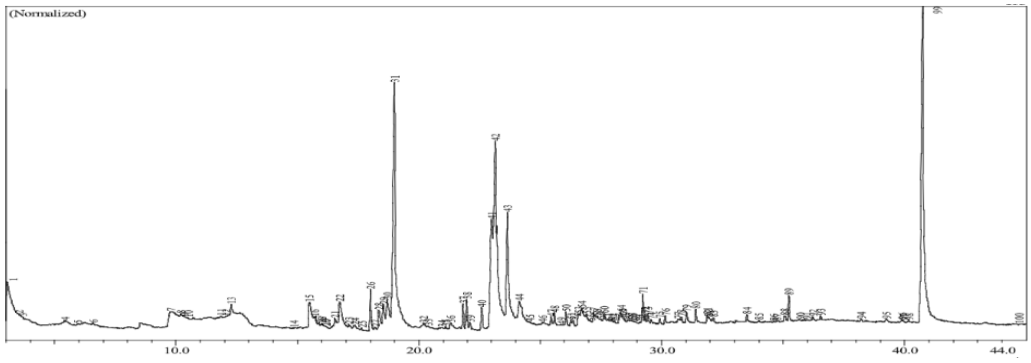

Fig. 3. GC-MS test results of whiteleg shrimp (*Penaeus vannamei*) carapace extract

The chromatogram from the GC-MS analysis of the whiteleg shrimp carapace extract shows 100 compound peaks (Fig. 3), with 5 (five) compounds identified as having strong antioxidant activity (Table 3). The main compounds exhibiting antioxidant activity include: (Peak 30; 1.94%), Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-, (Peak 41; 5.93%), 9,12-Octadecadienoic acid (Z,Z) - or linoleic acid, (Peak 44; 2.57%), 9(E),11(E)-Conjugated Linoleic Acid, (Peak 54; 1.32%) Doconexent, and (Peak 71; 0.77%), docosahexaenoic acid methyl ester.

Table 3. The main bioactive compounds with strong antioxidant activity identified from whiteleg shrimp (*Penaeus vannamei*) carapace extract based on GC-MS analysis

Peak	R Time	Area %	Compound Name	Compound Class	Primary Biological Activity
30	18.691	1.94	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-	Cyclic peptide	Antioxidant, immunostimulant
41	23.003	5.93	9,12-Octadecadienoic acid (Z,Z)- (Linoleic acid methyl ester)	PUFA Omega-6	Antioxidant, anti-inflammatory
44	24.138	2.57	9(E),11(E)-Conjugated linoleic acid	CLA (isomer PUFA)	Antioxidant, anti-inflammatory
54	26.707	1.32	Doconexent	PUFA Omega-3	Antioxidant
71	29.224	0.77	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	PUFA Omega-3	Antioxidant, immunostimulant

Source: Chromatography Laboratory, Ujung Pandang State Polytechnic.

4. Specific growth rate (SGR) of whiteleg shrimp (*Penaeus vannamei*)

The specific growth rate (SGR) of the whiteleg shrimp (%/day) during cultivation is shown in Fig. (4).

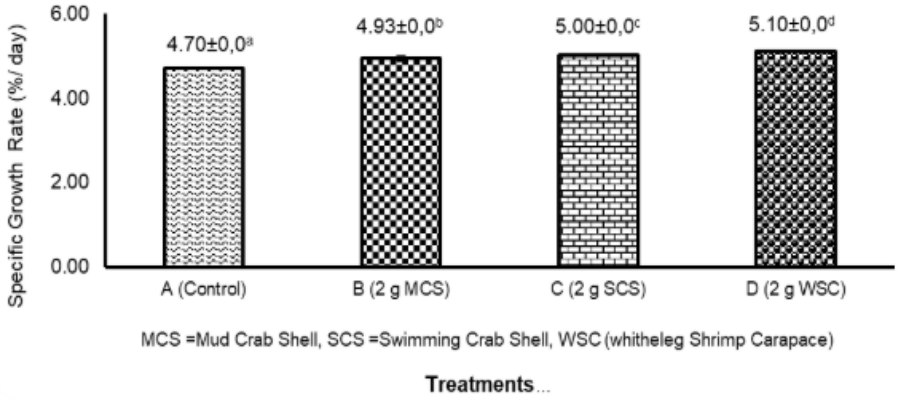

Fig. 4. Specific growth rate of whiteleg shrimp during maintenance (%/day)

According to Fig. (4), the specific growth rate (SGR) of the whiteleg shrimp showed significant differences between treatments ($P< 0.05$) after feed was supplemented with crustacean shell extract. Treatment D, which used the whiteleg shrimp carapace extract (WSC 2g/ kg feed), showed the highest growth rate of 5.10%/day, followed by the swimming crab shell treatment (SCS 2g/ kg feed) with a growth rate of 5.00%/day, and mud crab shell (MCS 2g/ kg feed) at 4.93%/day; all three treatments showed higher growth rates than the control (without extract), which was only 4.70%/day.

5. Survival rate

The average survival rate of whiteleg shrimp during maintenance is shown in Fig. (5).

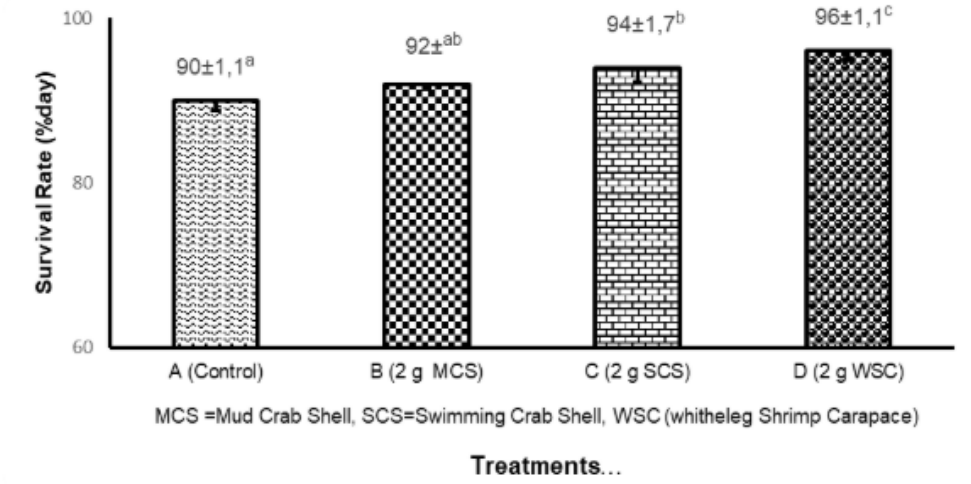

Fig. 5. Average survival rate of whiteleg shrimp during 56 days

The administration of carotenoid extract from crustacean shells significantly increased the survival rate of the whiteleg shrimp ($P< 0.05$). Treatment using the whiteleg shrimp carapace (WSC 2g/ kg feed) produced the highest survival rate (96%), followed by swimming crab shell (SCS, 94%), then mud crab shell (MCS, 92%), and finally the control (90%). Duncan's multiple range test showed that the whiteleg shrimp carapace extract at a concentration of (WSC 2g/ kg feed) had a significant difference compared to the control. These findings indicate that even though the doses used were identical, the effectiveness of the extract varied depending on its source, especially in increasing shrimp resistance to environmental stress.

6. Phagocytosis activity

The average phagocytic activity (%) of the whiteleg shrimp (*Penaeus vannamei*) following administration of crustacean shell extract and subsequent challenge with *Vibrio harveyi* is presented in Fig. (6).

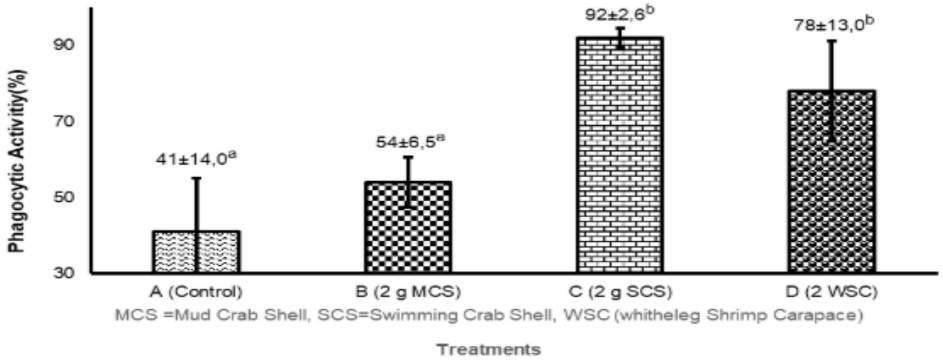

Fig. 6. Results of measuring the phagocytic activity of whiteleg shrimp after a challenge test using *Vibrio harveyi* bacteria

Based on Fig. (6), the analysis of phagocytosis activity showed a significant difference between treatments ($P< 0.05$) on the phagocytosis activity of whiteleg shrimp blood cells. The highest increase in phagocytic activity occurred in the swimming crab shell (SCS) extract treatment at a dose of 2g/kg feed at 92.0%, followed by the whiteleg shrimp carapace (WSC) carapace extract at 78%, then mud crab shell extract (MCS) at 54.0%, and the lowest was found in the control (without treatment) at 41%. Statistical tests indicated that the treatments with the whiteleg shrimp carapace (WSC) and swimming crab shell (SCS) extracts were significantly different compared to the control and mud crab shell (MCS).

DISCUSSION

1. GC-MS analysis of shell extract from mud crab (*Scylla serrata*)

The mud crab shell extract shows great potential as a source of antioxidant compounds, with the discovery of four main components through GC-MS analysis (Table 1), namely Arachidonic acid methyl ester (Peak 44; 2.61%), Eicosapentaenoic acid methyl ester or EPA (Peak 45; 2.81%), Docosahexaenoic acid methyl ester or DHA (Peak 64; 2.44%), and Squalene (Peak 91; 0.83%). These components belong to the group of polyunsaturated fatty acids (PUFA) and natural triterpenoids, which are known to have high antioxidant activity. The significant content of these four compounds indicates their major contribution to protection against oxidative stress.

Omega-3 fatty acids such as EPA and DHA play a crucial role in preventing the formation of free radicals and increasing cell membrane stability, thereby supporting the immunity and metabolic health of aquatic organisms **(Prasetyo *et al*., 2020; Saini *et al*., 2020)**. Arachidonic acid (omega-6), although pro-inflammatory in certain situations, functions as a bioactive compound that helps reduce oxidative stress and increase cell resistance to oxidative damage **(Calder, 2013)**. All the three not only have antioxidant properties, but also support immunostimulatory and anti-inflammatory functions, thus playing an important role in improving the non-specific immune system in shrimp and fish.

Squalene identified at Peak 91 (0.83%) is a natural triterpenoid that functions as a free radical scavenger and has anti-aging effects. This compound can stabilize the lipid structure within cell membranes and also increase tissue resistance to environmental stress **(Tyagi *et al*., 2021)**. The bioactive potential of squalene is not limited to its antioxidant effects, but also supports cell regeneration and maintains cellular balance. The combination of these four compounds provides a synergistic contribution to antioxidant protection in the use of functional feed for shrimp or other aquatic organisms.

2. GC-MS analysis of shell extract swimming crab (*Portunus pelagicus*)

GC-MS analysis of crab shell extract identified five major compounds exhibiting strong antioxidant activity (Table 2), namely Peak 11 (Phenol, 2,4-bis(1,1-dimethylethyl)-; 0.22%), Peak 34 (Palmitoleic acid; 1.25%), Peak 59 (5,8,11,14,17-Eicosapentaenoic acid methyl ester / EPA; 1.11%), Peak 73 (4,7,10,13,16,19-

Docosahexaenoic acid methyl ester / DHA; 1.16%), and Peak 92 (Squalene; 0.94%). The phenolic compound in Peak 11 functions as an efficient hydrogen donor in stopping oxidative chain reactions **(Tyagi *et al*., 2021; Hidayati *et al*., 2023)**. Palmitoleic acid, a monounsaturated fatty acid (MUFA) identified at Peak 34, is known to have antioxidant properties and contributes to maintaining cell membrane integrity and metabolic function. PUFA compounds such as EPA and DHA identified in Peaks 59 and 73 not only act as antioxidants but also have immunomodulatory activity that is crucial in supporting the non-specific immune system in aquatic organisms **(Prasetyo *et al*., 2020; Saini *et al*., 2020)**. Squalene (Peak 92), a natural triterpenoid compound, has been proven effective against free radicals and has potential as an anti-aging compound.

Squalene, a lipophilic isoprenoid compound, functions as an antioxidant that breaks the chain of free radicals and protects cell membranes from oxidative stress. In addition, squalene is known to have immunostimulatory, anti-inflammatory, and anticancer effects, and can enhance phagocytosis in the context of non-specific immunity **(Permadi & Putri, 2024)**. The presence of this compound increases the potential of rajungan extract as a functional additive in shrimp feed.

In addition to the main compound, rajungan shell extract also contains unsaturated fatty acids (oleic, linoleic), phenolic compounds (ferulic, cinnamic), and flavonoids (quercetin, naringin), which play a role in fighting free radicals and reducing oxidative stress. The composition of these compounds supports the use of rajungan extract as a source of bioactive ingredients for nutraceutical and functional feed applications **(Zhang *et al*., 2013; Elshaarawy *et al*., 2023)**.

3. GC-MS analysis of whiteleg shrimp carapace extract (*Penaeus vannamei*)

GC-MS analysis of whiteleg shrimp carapace extract identified four main compounds with high antioxidant activity. Peak 30 (Area 1.94%) contains Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(2-methylpropyl)-, which is a cyclic peptide with high ability to neutralize free radicals, having an IC_{50} against DPPH of approximately ±15 µg/mL. This compound also exhibits antimicrobial activity and cytotoxic potential in cancer cells, making it relevant as an immunostimulant and enhancer of shrimp immune defense **(Gopi *et al*., 2014; Kiran *et al*., 2018; Priyanto *et al*., 2024)**.

Peak 41 (Area 5.93%) and Peak 44 (Area 2.57%) contain Linoleic Acid (omega-6 PUFA) and Conjugated Linoleic Acid (CLA), respectively. Both are known to function in enhancing the performance of antioxidant enzymes such as SOD and CAT, as well as reducing lipid oxidation levels. CLA also acts as an immunomodulator that reduces the expression of proinflammatory cytokines such as TNF-α and IL-6, strengthening the response of phagocytic cells and spleen cells **(Marianne *et al*., 2004; Ju *et al*., 2020)**. The combination of these effects is crucial for maintaining homeostasis and the immune resistance of shrimp to environmental stress and pathogens.

Peak 54 (Area 1.32%) contains Doconexent (methyl ester DHA), one of the omega-3 PUFAs that is important in the crustacean diet. DHA has been shown to enhance

survival, growth, and resistance to oxidative stress in the whiteleg shrimp (*Penaeus vannamei*) through antioxidant and immunostimulatory mechanisms **(Araujo *et al*., 2019)**. DHA also contributes to the control of inflammatory responses through the modification of cellular signaling pathways, making it a prime choice in the formulation of functional feed to improve the overall performance of whiteleg shrimp.

4. Specific growth rate (SGR) of whiteleg shrimp

The results of statistical analysis (Fig. 4) showed that the addition of 2g/ kg of crustacean shell extract in the feed contributed significantly to increasing the specific growth rate (SGR) of the whiteleg shrimp ($P< 0.05$). Treatment with the whiteleg shrimp carapace extract (WSC) produced the highest SGR value of 5.10%, followed by crab shell extract (SCS) 5.00% and mud crab shell extract (MCS) 4.93%, all of which were significantly different compared to the control (Fig. 4). The superiority of the whiteleg shrimp carapace extract (WSC) is due to its higher astaxanthin content and optimal bioavailability, thus increasing cell protection from oxidative stress and supporting growth **(Zhao *et al*., 2022; Rahmalia *et al*., 2024)**.

Swimming crab shell extract (SCS) and mud crab shell extract (MCS) also showed significant increases in growth, although the values were slightly lower than those of the whiteleg shrimp carapace extract (WSC) treatment. This indicates that the carotenoid compounds in swimming crab shell (SCS) and mud crab shells (MCS) still function as effective antioxidants. The content of bioactive compounds such as omega-3 PUFAs (EPA and DHA), squalene, and unsaturated fatty acids (oleic and palmitoleic acids) plays a role in improving the metabolic efficiency and immune system of shrimp **(Ambati *et al*., 2014; Prasetyo *et al*., 2020; Maharani *et al*., 2023)**. Overall, the increase in specific growth rate (SGR) in all treatments with the addition of crustacean extract indicates that the combination of antioxidant compounds, derived from carotenoids, PUFAs, and triterpenoids, can support physiological functions, maintain redox balance, and improve nutritional efficiency in intensive aquaculture systems **(Calder, 2012; Saini *et al*., 2020; Hidayati *et al*., 2023)**.

5. Survival rate of whiteleg shrimp

Statistical analysis showed that supplementation of crustacean shell extract in feed significantly affected the survival rate of the whiteleg shrimp ($P< 0.05$). The whiteleg shrimp carapace (WSC) treatment at 2g/ kg of feed (Fig. 5), resulted in the highest survival rate of 96%, significantly different from other treatments, followed by the swimming crab shell (SCS) treatment at 94%, the mud crab shell (MCS) treatment at 92%, and the control (no extract) at 90%.

This significant increase in survival rate is closely associated with the presence of potent antioxidant bioactive compounds in each extract, such as astaxanthin, squalene, PUFA omega-3 (EPA and DHA), phenolic compounds, and unsaturated fatty acids. These compounds play a crucial role in enhancing shrimp resilience against oxidative stress, strengthening the immune system, and improving cell membrane stability,

collectively supporting survival **(Ambati *et al*., 2014; Zhao *et al*., 2022; Hidayati *et al*., 2023)**.

Treatment using whiteleg shrimp carapace extract (WSC) resulted in the highest survival rate, due to its high astaxanthin content and easy absorption, making it more effective in supporting the physiological stability of shrimp. Astaxanthin is a highly effective antioxidant in reducing oxidative stress, boosting the immune system, and protecting cells from damage caused by free radicals. The significant increase in whiteleg shrimp survival indicates that antioxidant compounds in crustacean extracts, particularly carotenoids, play a crucial role in enhancing the survival of farmed organisms. This finding is consistent with the research of **Zhang *et al*. (2013)** and **Mansour *et al*. (2022)**, which states that the addition of astaxanthin in shrimp feed improves immune response and survival rates. The higher survival patterns in all treatments indicate a dose-responsive protective effect, with the WSC treatment providing the best effect in reducing environmental stress during maintenance.

6. Phagocytic activity

Based on the results of the phagocytic activity test, it showed that treatment with swimming crab shell extract at a dose of 2g/ kg of feed produced the highest phagocytic activity (Fig. 6), the results of the study indicate that the ability of crab shell extract to activate hemocytes to respond to pathogen exposure is effective. Increased phagocytic activity by hemocytes reflects an improvement in the non-specific immune defense system in whiteleg shrimp (*Penaeus vannamei*). The process of phagocytosis is an initial immune response that occurs when pathogens or foreign particles enter the host body, which are then recognized and destroyed by phagocytic cells.

Phagocytic is an important element in the innate immune system of shrimp, performed by hemocytes to capture and destroy pathogens **(Kilawati & Islamy, 2021)**. The increase in phagocytic activity in the treatment of the whiteleg shrimp carapace extract (WSC) and swimming crab shell extract (SCS) at a concentration of 2g/ kg feed is due to the presence of bioactive compounds such as PUFA (EPA and DHA), squalene, and phenols, which act as immunostimulants and antioxidants. These compounds are known to increase ROS production, maintain the integrity of immune cell membranes, and activate the phagocytic pathway **(Saini *et al*., 2020)**. The increased phagocytic response after challenge testing with *Vibrio harveyi* indicates that the swimming crab shell extract (SCS) and the whiteleg shrimp carapace (WSC) have the potential to effectively enhance shrimp immune resistance **(Prasetyo *et al*., 2020)**.

Phagocytic activity in shrimp was measured by comparing the number of hemocytes actively ingesting foreign particles with the total number of cells observed. An increase in this ratio in the treatment group, along with an increase in the number of phagocytic cells observed, indicates that administration of crustacean shell extract can effectively stimulate the non-specific immune response of shrimp. This mechanism is a crucial element of the physiological defense of shrimp against pathogenic infections. In line with

these findings, several previous studies have noted that increased phagocytic activity after exposure to pathogens is positively associated with increased host resistance to infection **(Khimmakthong *et al*., 2013)**. Thus, crab shell extract has the potential as an effective natural immunostimulant to enhance the innate immune system of the whiteleg shrimp (*Penaeus vannamei*).

CONCLUSION

Crustacean shell extracts derived from mud crab shell (*Scylla serrata*), swimming crab (*Portunus pelagicus*), and whiteleg shrimp (*Penaeus vannamei*) carapace contain bioactive compounds, including astaxanthin, phenolic compounds, and unsaturated fatty acids, as identified by GC–MS analysis. These compounds possess potential antioxidant, anticancer, anti-inflammatory, antibacterial, and antiviral activities. Antioxidant activity, confirmed by DPPH free radical scavenging assays, indicates their role in reducing oxidative stress under intensive aquaculture conditions. Dietary supplementation with 2g/ kg of crustacean shell extracts, particularly from swimming crab shell and whiteleg shrimp carapace, significantly improved phagocytic activity, specific growth rate, and survival rate of the whiteleg shrimp (*Penaeus vannamei*) compared to the control. The results highlight the potential of crustacean shell extracts as functional feed additives to enhance growth performance, immune response, and survival in shrimp culture, thereby contributing to sustainable aquaculture practices.

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