

Article

Impact of Dietary Administration of Seaweed Polysaccharide on Growth, Microbial Abundance, and Growth and Immune-Related Genes Expression of The Pacific Whiteleg Shrimp (*Litopenaeus vannamei*)

Eman M. Abbas ¹, Ahmed Said Al-Souti ^{2,*} , Zaki Z. Sharawy ¹ , Ehab El-Haroun ^{3,*}  and Mohamed Ashour ¹ 

¹ National Institute of Oceanography and Fisheries (NIOF), Cairo 11516, Egypt

² Head AL Hail Aquaculture Unit, Department of Marine Science and Fisheries, College of Agriculture and Marine Science, Sultan Qaboos University, Muscat 123, Oman

³ Fish Nutrition Research Laboratory, Animal Production Department, Faculty of Agriculture, Cairo University, Cairo 11562, Egypt

* Correspondence: souti@squ.edu.om (A.S.A.-S.); elharoun@gmail.com (E.E.-H.)

Abstract: This work aims to determine the impact of dietary supplementation of polysaccharide, extracted from brown seaweeds *Sargassum dentifolium* on growth indices, feed utilization, biochemical compositions, microbial abundance, expressions of growth and immunity-related genes, and stress genes of the Pacific Whiteleg shrimp *Litopenaeus vannamei*. A total of 360 post-larvae of *L. vannamei* were randomly distributed into a 12-glass aquarium (40 L of each) at a stocking density of 30 shrimp with an initial weight of $(0.0017 \pm 0.001 \text{ g})$. During the 90-day experiment trial, all shrimp larvae were fed their respective diets at 10% of total body weight, three times a day. Three experimental diets were prepared with different seaweed polysaccharide (SWP) levels. The basal control diet had no polysaccharide level (SWP₀), while SWP₁, SWP₂, and SWP₃ contained polysaccharides at concentrations of 1, 2, and 3 g kg⁻¹ diet, respectively. Diets supplemented with polysaccharide levels showed significant improvements in weight gain and survival rate, compared to the control diet. Whole-body biochemical composition and the microbial abundance (the total count of heterotrophic bacteria and *Vibrio* spp.) of *L. vannamei* showed significant differences among polysaccharide-treated diets compared to the control. At the end of the feeding experiment, the dietary supplementation of polysaccharide levels enhanced the expression of growth-related genes (Insulin-like growth factors (IGF-I, IGF-II), immune-related genes (β -Glucan-binding protein (β -Bgp), Prophenoloxidase (ProPO), Lysozyme (Lys), and Crustin), and stress genes (Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) in the muscle tissue of *L. vannamei*. However, the current study concluded that the inclusion rate of 2 g kg⁻¹ of polysaccharide as a dietary additive administration enhanced both weight gain and survival rate of *L. vannamei*, while the incorporation level of 3 g kg⁻¹ reduces the abundance of pathogenic microbes and enhances the growth-, immunity- and stress-related gene expressions of *L. vannamei*.

Keywords: *Sargassum dentifolium*; feed additives; shrimp industry; growth performances; growth-related genes; immune-related genes; stress genes



Citation: Abbas, E.M.; Al-Souti, A.S.; Sharawy, Z.Z.; El-Haroun, E.; Ashour, M. Impact of Dietary Administration of Seaweed Polysaccharide on Growth, Microbial Abundance, and Growth and Immune-Related Genes Expression of The Pacific Whiteleg Shrimp (*Litopenaeus vannamei*). *Life* **2023**, *13*, 344. <https://doi.org/10.3390/life13020344>

Academic Editor: Alejandro Romero

Received: 8 December 2022

Revised: 16 January 2023

Accepted: 17 January 2023

Published: 27 January 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The shrimp aquaculture industry has experienced rapid growth and has become the most significant and leading aquaculture sector [1,2]. Although the shrimp industry has developed rapidly, the challenges faced by farmers are obtaining an increase in growth rate, low-price diets, and reducing disease outbreaks [3,4]. Furthermore, the world's shrimp consumption has risen over the previous ten years, forcing nutrition experts to incorporate a lot of substances derived from agriculture in aquatic animal diets [1,5,6]. The Pacific

Whiteleg shrimp (*Litopenaeus vannamei*) is most frequently grown worldwide, achieving more than 70% of all worldwide shrimp cultivation [7,8]. To sustain the shrimp industry worldwide, many issues must be resolved, such as poor water quality, low survivability, and diet industry improvement [9–14]. Moreover, climate change and the negative impact of environmental pollution are significant problems restricting the sustainability of aquaculture, fisheries, aquatic ecosystems, and aquatic animals so far [15–20].

Hence, shrimp diets have been expanded using several strategies to deal with such global development in the shrimp farming sector [10,21]. One of the most fundamental strategies is feed additive supplementation, which has grown to be very important for many shrimp species as a growth stimulus, immunological booster, and alternative disease resistance approach [22,23]. Aquatic plants (microalgae and seaweeds) are still widely employed in many important sectors, such as aqua-feed additives [24], plant growth enhancers [25], phytoremediation [26–30], human food supplement [31,32], pharmaceuticals [33,34], cosmetics substances [35,36], antimicrobial activities [37,38], and bioenergy [39,40]. As reported in 2018, the global production of seaweeds (wild captured and farmed) was about 34.4 Million Tonnes, with an industrial value of about USD 13.3 Billion [41]. This production comes from about 35 countries, while the largest producer, which produces more than 99%, is China [42]. Seaweeds have high levels of proteins, fibers, vitamins, fatty acids, minerals, pigments, and several bioactive compounds [43–46]. Among seaweed families, brown seaweeds are known as a high source of sugars, which can protect aquatic organisms from several harmful impacts while their polysaccharide has been successively used as a feed additive for Nile tilapia [47] and red tilapia [2]. The available literature has demonstrated that the polysaccharide extracted from seaweed could promote innate immunity, and enhance the resistance against pathogen infection of shrimp [48–50] due to its polysaccharide composition and structure (degree of branching, substituents, sulphation, and type of linkages) which are quite different from terrestrial plants [51,52]. *Sargassum dentifolium*, brown seaweed is found to contain abundant polysaccharide which is a rich resource in Egypt, has been confirmed to exert multiple pharmacological properties, such as antitumor, antioxidation, hematopoiesis, immunomodulation, and gastrointestinal protection, while the dietary administration was reported to improve the non-specific immune responses in fishes [2].

Despite the importance of feed additives applied for the Pacific whiteleg shrimp, little is known about the application of polysaccharides prepared from brown seaweeds in the shrimp feed additive industry [53,54]. Immunostimulants have importance as synthetic substances that boost the immune system's capacity to combat infections and diseases by stimulating immunological responses. Bacteria and bacterial products, complex carbohydrates, dietary factors, animal extracts, cytokines, lectins, plant extracts, and synthetic medications such as levamisole are all examples of immunostimulants that are presently available [55]. Antibiotics in the diets of cultured fish and crustaceans have been commonly used to control disease infection as well as to improve both survival and growth. However, it has been widely criticized due to the drug resistance and accumulation of chemicals in aquatic animal tissues, which can be possibly dangerous to public health. Alternatively, natural immune stimulants such as probiotics, and prebiotics are generally suggested to use in feeds to effectively promote growth and immune response, and control various diseases in aquatic animals [56].

The stimulatory effects of immunostimulants such as glucan, chitosan, nucleotides, lipopolysaccharide (LPS), sodium alginate, and other polysaccharides have been the subject of several works on fish and crustaceans [55,57].

Recently, special attention has been paid to the use of prebiotics as natural alternatives to antibiotics and immune stimulants in aquaculture. Functional polysaccharides are non-digestible ingredients because of their β -1, 3 or β -1, 4 linkages. Consumption of functional polysaccharides can reportedly improve growth performance and enhance the immune response and disease resistance of aquatic animals [58]. According to previous studies, diets containing certain polysaccharides, including medicinal plants and marine-

derived polysaccharides, may improve growth rate in respect of the immune system and gastrointestinal condition in fishes and shrimps [59–61].

Disease resistance has been linked to an increase in cellular and humoral responses, including phagocytosis, bactericidal activity, phenoloxidase (PO) activity, respiratory burst, superoxide dismutase (SOD), and lysozyme activities in crustaceans [62]. Essential information regarding immune system activation and regulation is revealed by the expression of immune-related genes in shrimp [63]. Pattern recognition proteins (PRPs), which attach to molecules on the microbial surface, mediate the detection of invading organisms as an important step in the shrimp immune response [64].

PRP recognition of invading pathogens is a crucial intermediate step in prophenoloxidase-activating system (ProPO) system activation [65]. Peptidoglycan recognition proteins [66], C-type lectins [67], β -glucan-binding proteins (β -Bgps) [68], and lipopolysaccharide (LPS) and 1,3-glucan binding proteins (1,3-Lgp) [69] have all been described as PRPs in the ProPO system. The Prophenoloxidase (ProPO)-activating mechanism, which is triggered by PRPs binding to a microorganism's cell wall components, is known to activate the host's immune system [70]. Stress activates the glycolytic reactions which in turn increases the consumption of O_2 , and enhances the release of reactive oxygen species (ROS) (as hydroxyl radical, hydrogen peroxide, and superoxide anions) [71]. However, the ROS can eliminate the stressor; the increase in the ROS will cause severe destruction. Therefore, the rapid removal of excessive ROS is critical for the appropriate function of the cell. This is achieved by increasing the expression of antioxidant enzymes [72]. Superoxide dismutase (SOD) are antioxidant enzyme that relies on superoxide anions. Superoxide radicals are detoxified by SOD by being transformed into oxygen and hydrogen peroxide, which are subsequently changed into H_2O and O_2 by catalase and supplied to the cell as safe composites [73,74].

The copper–zinc superoxide dismutase *CuZnSOD* gene and other immune genes are also implicated in the indirect immunity of shrimp-like *Crustin*, which is essential for immunity to infections [75,76]. In *L. vannamei*, the dietary *Panax ginseng* polysaccharide extract reduces inflammation, boosts immune enzyme activity, and modifies immune gene expression [77]. A large number of genes regulate development characteristics, including growth hormone (GH), and insulin-like growth factors (IGF-I and IGF-II) [78]. The fast growth of *L. vannamei* aquaculture demands the creation of rapid genetic lines [79]. To the best of our knowledge, little is known about the influences of dietary polysaccharides supplementation of *Sargassum dentifolium* on shrimp growth, immunity, and stress-related gene expressions. Therefore, this study was undertaken to evaluate the effect of dietary administration of polysaccharide derived from brown seaweed (*S. dentifolium*) on growth performances, feed utilization, body composition, microbial communities, and growth, immunity, and stress genes expressions of the Whiteleg shrimp *Litopenaeus vannamei*.

2. Materials and Methods

2.1. Brown Seaweed, *Sargassum dentifolium*

Brown seaweed, *S. dentifolium*, was collected from Abu-Qir Bay, Alexandria, Egypt (31.3000 N and 30.1667 E) [2]. The epiphytes were removed from the obtained samples, as previously described [80]. Before use, the samples were then washed, cleaned, air-dried, powdered, and stored in plastic bags at room temperature [29]. The procedures outlined by [81] were used to extract the polysaccharide from the brown seaweed *S. dentifolium*.

2.2. Investigation of Water Quality

Throughout the feeding experiment, we made sure that the levels of NH_3^- ($mg\ L^{-1}$), NO_2^- ($mg\ L^{-1}$), NO_3^- ($mg\ L^{-1}$), alkalinity ($mg\ L^{-1}$), and PO_4^- ($mg\ L^{-1}$) were within the ranges suggested for shrimp [82] and the guidelines of APHA [83]. In addition, daily measurements of temperature ($^{\circ}C$), salinity (ppt), and pH were taken at 1 p.m. A thermometer hung at a depth of 30 cm was used to get an accurate reading of the water's temperature, and a pH meter and a refractometer (Orion, Ipswich, MA, USA) were used to get accurate readings of the water's acidity and alkalinity daily at 9.00 h.

2.3. The Pacific Whiteleg Shrimp (*Litopenaeus vannamei*)

2.3.1. Animal Experiment

A private hatchery supplied post larvae (PLs) of Pacific Whiteleg shrimp *L. vannamei* to the Invertebrates Laboratory, Aquaculture Division, Suez-Branch of NIOF, Egypt. PLs were then acclimatized for 15 days in two 500-L fibreglass tanks under controlled conditions (28.0 ± 1.0 °C and salinity 29 ± 3.0 ppt) The Research Committee of the NIOF, Egypt, approved the experimental design and the adherence to ethical standards of shrimp handling.

2.3.2. Experimental Design and Facilities

The current feeding trial was conducted using a completely randomized design, with triplicates. A total of 360 PLs (with an initial weight of 0.0017 ± 0.001 g) were stocked at a density of 30 shrimp in 12 glass aquariums (each with a 40 L capacity). For the 90-day feeding trial, PLs were given 10% of the total shrimp body weight three times a day (at 6:00 a.m., 12:00 p.m., and 6:00 p.m.). Each aquarium was emptied of waste and uneaten food every morning and cleaned with a siphon and 10% of the water volume was replaced with fresh, oxygenated, and filtered seawater daily [82].

2.3.3. Experimental Diet

Four diets were provided to shrimp: SWP₀: commercial shrimp diet (Aller-Aqua, Egypt, as a control basal diet, crude protein of 40% and crude lipid of 9%). The remaining three experimental diets (SWP₁, SWP₂, and SWP₃) are commercial shrimp diets supplemented with 1, 2, and 3 g kg⁻¹ of *S. dentifolium* polysaccharide, respectively. The additions of polysaccharide levels were performed, as previously described by Abdelrhman et al. [2]. Briefly, the commercial shrimp diet was first milled and split into three equal portions. Each polysaccharide level (1, 2, and 3 g kg⁻¹) was dissolved in distilled water and then sprayed on the diet surface until complete absorption and the same adequate volume of distilled water was sprayed on the control diet (SWP₀) without polysaccharide [84]. The sunflower oil (5 mL kg⁻¹) was then sprayed over diets to cover the polysaccharide solution [85]. Finally, the diets were homogenized and re-pelletized into pellets, air-dried, placed in cellophane bags, and refrigerated at 4 °C until use.

2.4. Tested Parameters

2.4.1. Growth Performances

At the end of the trial, the number of shrimps and weights were recorded, after 24 h of fasting, to determine the different growth indices and feed utilization using the following formulas:

$$\text{Weight Gain (WG, g)} = \text{FW} - \text{IW} \quad (1)$$

where IW & FW are initial and final body weight (g), respectively.

$$\text{Specific growth rate (SGR, \%/day)} = 100 \times \left(\frac{\text{Ln FW} - \text{Ln IW}}{t} \right) \quad (2)$$

where Ln and t are t natural logarithmic and time in days, respectively.

$$\text{Survival Rate (\%)} = 100 \times \frac{\text{Final number of shrimp}}{\text{Initial number of shrimp}} \quad (3)$$

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Body weight gain (g)}} \quad (4)$$

2.4.2. Biochemical Composition Analysis

Both experimental diets and shrimp were subjected to proximate analysis for estimating their biochemical content according to AOAC [86] guidelines prepared as detailed in the prior article [87]. To estimate the whole-body constituent (dry matter, crude fat, crude

protein, and crude ash), 5 shrimp were obtained randomly from each replicate after the feeding session was completed. Shrimp were then pulverized, blended until smooth, and stored at $-20\text{ }^{\circ}\text{C}$ for further examination.

2.4.3. Microbial Communities

The APHA approach [83] was used to determine the richness of microbial communities. Water (1 mL) and intestine of shrimp (1 g) samples were taken from each replicate (3 shrimp per replicate, $n = 9$) once the experiment was completed. Each sample (intestine and water) was inoculated with 9 mL of sterile distilled water onto plates of Trypticase soy agar (TSA) and Thio-sulphate-Citrate-Bile salts (TCBS) [88]. Plates of TSA and TCBS were incubated at $37\text{ }^{\circ}\text{C}$, while TCBS plates were incubated at $28\text{ }^{\circ}\text{C}$. Colony-forming units per milliliter were used to determine the quantity of hetero-trophic bacteria and *Vibrio* colonies present after 24 h (CFU mL^{-1}) [89].

2.4.4. RNA Extraction and cDNA Synthesis for Genes Expression

Triplicate samples of the shrimp's abdominal muscles from each replicate were directly excised with fully sterile dissecting tools under cold conditions. Before performing the gene expression study, part of the muscles was frozen at $-80\text{ }^{\circ}\text{C}$. TRIzol reagent (easy-RED, iNtRON Biotechnology) was used to extract total RNA from the shrimp's abdomen region at the end of the experiment, as directed by the manufacturer. Using a NanoDrop system (Bio-Drop), the optical density (OD) ratio of RNA purity was determined, and 1 ng L^{-1} of RNA was used for cDNA synthesis in each reaction when the ratio was ideal ($A_{260}/A_{280} = 1.8$). To determine the quality of the RNA, the 260/280 nm OD ratio was used. Total RNA that had been processed with DNase I (NEB, USA) was utilized as a template in a reverse transcriptase kit (RT-PCR beads, Enzynomics, Daejeon, Korea) to generate first-strand cDNA. The reaction was performed using PCR amplification (using an American product, an Applied Biosystems Veriti 96-Well Thermal Cycler) and was carried out following the manufacturer's instructions. Real-Time PCR (Bico, Thermo-Fisher) was performed under the following cDNA conditions to detect unique and distinct products: After an initial denaturation at $95\text{ }^{\circ}\text{C}$ for 15 min, the protein was subjected to 40 cycles at the following conditions: $95\text{ }^{\circ}\text{C}$ for 10 s, $58\text{--}62\text{ }^{\circ}\text{C}$ for 20 s, and $72\text{ }^{\circ}\text{C}$ for 30 s; and finally, after the final cycle, the temperature was raised from $58\text{--}62\text{ }^{\circ}\text{C}$ to $95\text{ }^{\circ}\text{C}$ in increments of $0.5\text{ }^{\circ}\text{C}$. Primers used to probe similar genes are listed in Table 1.

The housekeeping gene (β -actin) was utilized to assess target gene expression or fold change [90]. When the $2^{-\Delta\Delta\text{Ct}}$ method is used to normalize the critical threshold (Ct) quantities of the target genes with quantities of β -actin, the values reveal an n -fold difference in comparison to the control [91].

2.5. Statistical Analysis

To evaluate water quality, growth performances feed utilization indices, body composition analysis, microbial communities, and immunity and growth-related gene expression, a one-way ANOVA was employed to identify significant differences ($p < 0.05$) in the means for each variable between the polysaccharide treatments (SWP_1 , SWP_2 and SWP_0) and the control (SWP_0). The statistical analysis was performed using GraphPad Prism version 9. To examine any correlation between the treatments, Tukey's tests were utilized. Before performing the statistical analysis, all data have been checked for the normality of distribution and homogeneity of variance. Before the analysis, all data (percentages) were arc-sin transformed [92]. However, to facilitate comparisons, the data were presented as untransformed.

Table 1. Oligonucleotide primer sequences applied in RT-PCR for immune-related, antioxidant genes and growth-related genes.

Genes	Sequences	Amplicon Size
<i>β-actin</i> (AF300705)	F: GCCCATCTACGAGGGATA R: GGTGGTCGTGAAGGTGTAA	121 bp
<i>Bgp</i> (AY249858)	F: ACGAGAACGGACAAGAAGTG R: TTCAGCATAGAAGCCATCAGG	137 bp
<i>ProPO</i> (AY723296)	F: CGGTGACAAAGTTCCTCTTC R: GCAGGTCGCCGTAGTAAG	122 bp
<i>Crustin</i> (AF430076)	F: ACGAGGCAACCATGAAGG R: AACCACCACCAACACCTAC	141
<i>Lys</i> (AY170126)	F: GGACTACGGCATCTTCCAGA R: ATCGGACATCAGATCGGAAC	97 bp
<i>IGF-I</i> (KP420228) *	F: GTGGGCAGGGACCAAATC R: TCAGTTACCACCAGCGATT	123 bp
<i>IGF-II</i> (XM02739466) *	F: CTCTGTACAGTCAGCCCAGC R: CACACCCAGTCAGTCCCAAG	220 bp
<i>SOD</i> (DQ005531)	F: AATTGGAGTGAAAGGCTCTGGCT R: ACGGAGGTTCTGTACTGAAGGT	153
<i>GPx</i> (AY973252)	F: AGG GACTTC CAC CAG ATG R: CAA CAACTC CCC TTC GGTA	117

* Designed by NCBI tool.

3. Results

3.1. Water Quality

Table 2 displays the water quality conditions recorded during feeding experiments. According to the supplied data (Table 2), the water quality was acceptable (falling under the permissible limits) for raising shrimp.

Table 2. Water quality parameters of experimental diets.

Tested Parameters	SWP ₀	SWP ₁	SWP ₂	SWP ₃
NH ₃ [−] (mg L ^{−1})	0.119 ± 0.001	0.106 ± 0.016	0.123 ± 0.015	0.125 ± 0.004
NO ₂ [−] (mg L ^{−1})	0.119 ± 0.016 ^{bc}	0.101 ± 0.009 ^c	0.140 ± 0.001 ^a	0.132 ± 0.003 ^{ab}
NO ₃ [−] (mg L ^{−1})	0.222 ± 0.028	0.217 ± 0.008	0.262 ± 0.004	0.257 ± 0.005
PO ₄ [−] (mg L ^{−1})	0.485 ± 0.010	0.495 ± 0.018	0.505 ± 0.007	0.485 ± 0.018
Alkalinity (mg L ^{−1})	7.700 ± 0.625 ^b	7.625 ± 0.050 ^b	8.563 ± 0.438 ^{ab}	9.038 ± 0.763 ^a
Temperature (°C)	26.84 ± 0.20 ^a	26.75 ± 0.07 ^a	26.46 ± 0.04 ^b	26.65 ± 0.15 ^{ab}
Salinity (ppt)	32.25 ± 0.09 ^b	32.35 ± 0.03 ^b	32.46 ± 0.02 ^a	32.32 ± 0.04 ^b
pH	7.79 ± 0.02 ^a	7.82 ± 0.01 ^a	7.78 ± 0.03 ^a	7.73 ± 0.08 ^b

SWP₀, SWP₁, SWP₂, and SWP₃: diets supplemented with 0, 1, 2, and 3 g of polysaccharide extracted from brown seaweed *S. dentifolium*. The presented data are Means ± SD (*n* = 3). Different letters in the same column are significantly different (*p* < 0.05). The absence of letters in the same row means that there are no significant differences.

3.2. Growth Performances and Nutrient Utilization Indices

Table 3 demonstrates the impact of polysaccharide dietary supplementation on shrimp growth, survival, and feed utilization. Compared to SWP₀, Table 3 showed that SWP₁, SWP₂, and SWP₃ demonstrated significant (*p* < 0.05) increases in WG. Moreover, SWP₁ and SWP₂ showed significant (*p* < 0.05) increases in SR, while SWP₃ showed significant (*p* < 0.05) decreases, compared to SWP₀. On the other hand, there were no significant

differences ($p < 0.05$) in SGR or FCR across the supplemented diets (SWP₀, SWP₁, SWP₂, and SWP₃), as presented in Table 3.

Table 3. Growth performance and feed utilization of shrimp *L. vannamei* fed on experimental diets.

Indicator	SWP ₀	SWP ₁	SWP ₂	SWP ₃
IW (g)	0.0017 ± 0.001	0.0017 ± 0.001	0.0017 ± 0.001	0.0017 ± 0.001
WG (g)	10.43 ± 1.15 ^c	12.75 ± 2.21 ^b	14.97 ± 1.26 ^a	15.06 ± 1.28 ^a
SR (%)	75.56 ± 2.94 ^b	77.78 ± 4.08 ^a	83.33 ± 3.74 ^a	60.00 ± 2.45 ^c
SGR	7.29 ± 0.55	7.45 ± 0.77	7.59 ± 0.33	7.59 ± 0.41
FCR	1.58 ± 0.05	1.59 ± 0.07	1.59 ± 0.09	1.58 ± 0.15

SWP₀, SWP₁, SWP₂, and SWP₃: diets supplemented with 0, 1, 2, and 3 g of polysaccharide extracted from brown seaweed *S. dentifolium*. The presented data are Means ± SD ($n = 3$). Different letters in the same column are significantly different ($p < 0.05$). The absence of letters in the same row means that there are no significant differences.

3.3. Shrimp Body Composition Analysis

The body composition analysis of the content (% of dry weight) of protein, fat, ash, and dry matter is presented in Table 4. The highest significant ($p < 0.05$) values of protein and dry matter were reported by SWP₀ followed by SWP₁, SWP₃, and SWP₂, while the highest significant ($p < 0.05$) values of fat and ash were reported by SWP₂ followed by SWP₃, SWP₁, and SWP₀ (Table 4).

Table 4. Composition analysis (%) of shrimp *L. vannamei* fed on different experimental diets.

Diets	Composition Analysis (% of Dry Weight)			
	Dry Matter	Protein	Fat	Ash
SWP ₀	26.53 ± 0.13 ^a	23.12 ± 0.03 ^a	7.79 ± 0.01 ^d	1.60 ± 0.01 ^d
SWP ₁	25.33 ± 0.04 ^b	22.32 ± 0.03 ^b	10.61 ± 0.02 ^c	1.89 ± 0.01 ^c
SWP ₂	24.60 ± 0.03 ^d	21.88 ± 0.02 ^d	11.00 ± 0.01 ^a	2.48 ± 0.02 ^a
SWP ₃	24.93 ± 0.04 ^c	22.10 ± 0.01 ^c	10.78 ± 0.03 ^b	2.19 ± 0.01 ^b

SWP₀, SWP₁, SWP₂, and SWP₃: diets supplemented with 0, 1, 2, and 3 g of polysaccharide extracted from brown seaweed *S. dentifolium*. The presented data are Means ± SD ($n = 3$). Different letters in the same column are significantly different ($p < 0.05$). The absence of letters in the same row means that there are no significant differences.

3.4. Microbial Communities

Table 5 shows the impact of experimental diets supplemented with different concentrations of the polysaccharide (SWP₁, SWP₂, and SWP₃), compared to SWP₀, on the total count of THB and TVC in both the water and intestine of shrimp. The data showed that the abundance of microbes (THB and TVC) was higher in the intestine than in water. Compared to SWP₀ both THB and TVC count in both water and intestine gradually decreased as polysaccharide levels increased (Table 5).

Table 5. Effect of brown seaweed polysaccharide on the bacterial abundance in water and intestine of *L. vannamei*, total heterotrophic bacteria (THB), total vibrio count (TVC), and TVC/THB ratio.

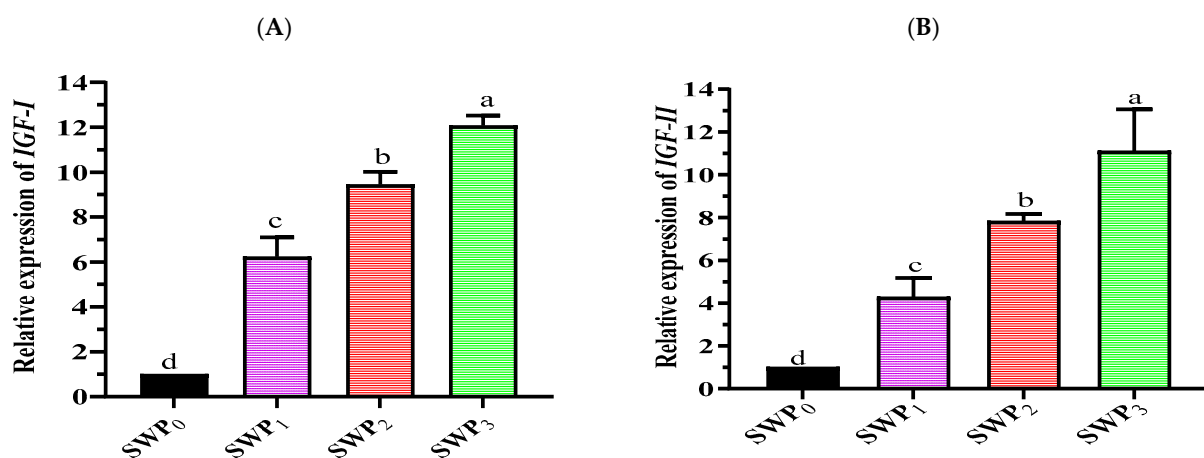
Bacterial Count (CFU mL ⁻¹ × 10 ⁵)	Experimental Diets			
	SWP ₀	SWP ₁	SWP ₂	SWP ₃
Water				
THB	7.251 ± 0.0033 ^d	4.200 ± 0.0030 ^c	2.651 ± 0.0063 ^b	0.119 ± 0.0066 ^a
TVC	0.114 ± 0.0005 ^d	0.068 ± 0.0002 ^c	0.045 ± 0.0003 ^b	0.005 ± 0.0004 ^a
Intestine				
THB	80.00 ± 0.0033 ^d	50.00 ± 0.0035 ^c	35.00 ± 0.0020 ^b	3.00 ± 0.0033 ^a
TVC	0.591 ± 0.4583 ^d	0.476 ± 0.4041 ^c	0.282 ± 0.5508 ^b	0.007 ± 0.0306 ^a

SWP₀, SWP₁, SWP₂, and SWP₃: diets supplemented with 0, 1, 2, and 3 g of polysaccharide extracted from brown seaweed *S. dentifolium*. The presented data are Means ± SD (*n* = 3). Different letters in the same column are significantly different (*p* < 0.05). The absence of letters in the same row means that there are no significant differences.

3.5. Growth, Immunity, and Stress-Related Genes Expressions

At the end of the experiment, the dietary supplementation of polysaccharides enhanced the expressions of immune-related, growth-related, and stress genes in the muscle tissue of *L. vannamei*. Regarding the expressions of growth-related genes (*IGF-I* and *IGF-II*), their expressions were considerably up-regulated (*p* < 0.05) in the treatments with the different polysaccharide concentrations compared to the control (SWP₀). The expression was increased in the SWP₃ and found to be higher than the SWP₀ with approximately 12 and 11-fold change, respectively (Figure 1A,B). The expressions of immune-related genes (*Bgp*, *ProPO*, *Crustin*, and *Lys*) were markedly up-regulated in the SWP₃ treatment where the fold changes were 9.3, 12.4, 10.5, and 8.8, respectively, which were higher than SWP₀ (Figure 1C–F).

Compared to the control group (SWP₀), the *ProPO* gene exhibits the highest expression levels across all treatment concentrations. For the *Crustin* gene, there is a significant difference between the SWP₃ treatment and the control, while there was no significant difference between the SWP₁ and SWP₂ and the control. Furthermore, the expression of stress genes (*SOD* and *GPx*) in SWP₃ were considerably increased by 5.2- and 6.9-folds, respectively, relative to the control (Figure 1G,H). However, there was a significant difference (*p* < 0.05) in *SOD* gene expression among all treatments compared to the control with more increase in the SWP₃ treatment. Meanwhile, there was a significant difference (*p* < 0.05) in the gene expression of *GPx* between SWP₃ and SWP₂ treatments relative to the control SWP₀, but no significant difference was observed between SWP₂ and SWP₁ treatments.

**Figure 1.** Cont.

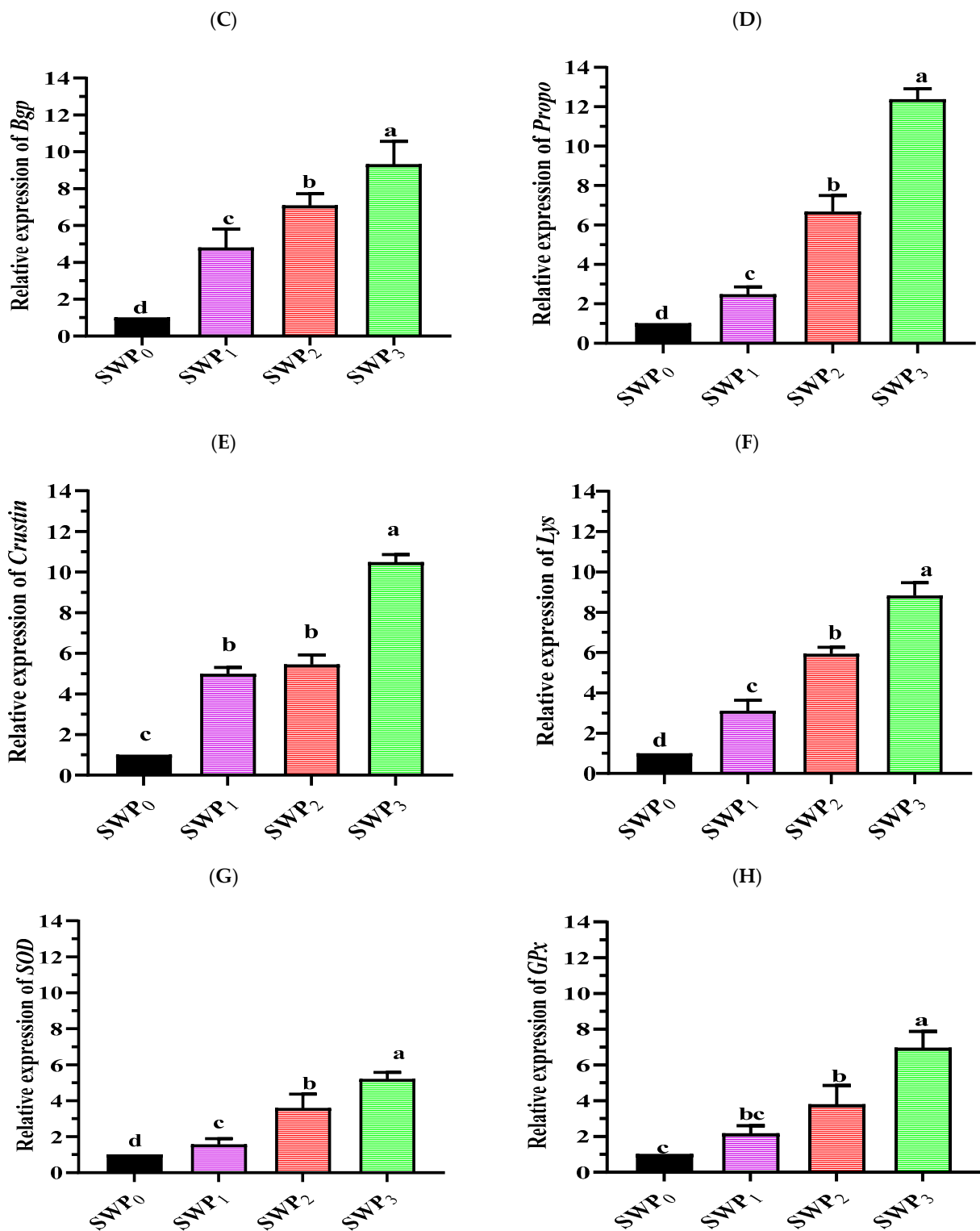


Figure 1. Analysis of gene expressions of growth-related genes [*IGF-I* (A) and *IGF-II* (B)], immune-related genes [*Bgp* (C), *ProPO* (D), *Crustin* (E), and *Lys* (F)], and stress genes [*SOD* (G) and *GPx* (H)], compared to the expression of a housekeeping gene (β -actin gene) in the different dietary supplementation of polysaccharide-extracted from brown seaweed, *S. dentifolium*. The presented data are Means \pm SD ($n = 3$). Different letters are significantly different ($p < 0.05$). In general, the commercial diet supplemented with 3 g kg⁻¹ of polysaccharide produced from brown seaweed *S. dentifolium* resulted in the highest expression of the eight genes ($p < 0.05$) compared to the other diets examined.

4. Discussion

Seaweed polysaccharides are recognized as high-value active molecules that improve growth performances, enhance the immune system response, and have many health benefits for aquaculture organisms [2,54,93–97]. In the present study, we hypothesized that the dietary administration of polysaccharide derived from brown seaweed (*Sargassum dentifolium*) ameliorates the growth performances, feed utilization, body composition, microbial communities, and growth, immunity, and stress genes expressions of the Whiteleg shrimp *L. vannamei*. The current feeding trial demonstrated that the weight gain of *L. vannamei* was improved significantly with increasing polysaccharide levels in the commercial diet compared to the control diet. The present findings are parallel to the previous studies conducted on different shrimp and fish species. For example, Lee et al. [98] reported that the hot-water extract of the brown seaweed *Sargassum horneri* significantly improves growth performances, stimulates innate immunities, and enhances immune gene expressions of shrimp *L. vannamei* and recommended that the ideal inclusion level is 5 g kg⁻¹. Additionally, the study by Liu et al. [99] investigated the impact of different inclusion levels (0, 1, 2, and 3 g kg⁻¹) of polysaccharides extracted from green seaweed (*Enteromorpha*) into the diet of banana shrimp *F. merguensis* and concluded that 1 g kg⁻¹ significantly enhances growth performance, improves nonspecific immunity, and modulates the intestinal function of *F. merguensis*, while Abdelrhman et al. [2] investigated the effect of different dietary inclusion rates (0, 10, 20, and 30 g kg⁻¹) of polysaccharides obtained from brown seaweed *S. dentifolium* on the hybrid red tilapia, and concluded that the 30 g kg⁻¹ level achieved the highest significant growth performance, FCR, and hematological indices. However, the inconsistency in the inclusion levels among these studies may be due to the different initial weight, seaweed species, species (fish and shrimp species), age, etc.

Gut microbiota abundance rapidly responds to variations in dietary intake, composition, and components. Therefore, it has a huge impact on the health benefits of all aquatic organisms such as food consumption, digestion, nutrient utilization, absorption, and immunity responses [22,87,100,101]. At present, the evaluation of disease resistance is important in the aquaculture industry, the blood antioxidant and immune factor activity is a good health status indicator for investigating the immune response and disease resistance in *L. vannamei* such as white spot syndrome virus (WSSV) [49] and *Vibrio alginolyticus* [102]. Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen, such as oxygen ions and peroxides. Excessive amounts of ROS can affect the structure and stability of functional proteins, unsaturated fatty acids, and nucleic acids, causing oxidative damage to the immune system of the organism and increasing the susceptibility to pathogens in shrimp [73]. Hence, the health of aquatic organisms depends on the balance between the production of ROS and antioxidant enzymes such as SOD and GPx which protect the animal cells against free radicals. The current findings showed that dietary polysaccharides derived from brown seaweed (*S. dentifolium*) effectively improved the activities of antioxidant enzymes, including SOD and GPx. Similarly, the SOD and GPx activities of different crustaceans were increased after feeding diets supplemented with *Angelica sinensis* polysaccharides in whiteleg shrimp [60] and β -glucan [103], and *Rhodiola rosea* polysaccharides in red swamp crayfish [104].

The current work reported that, compared to SWP₀, the THB and TVC counts were significantly ($p > 0.05$) decreased with the increase in the inclusion levels of polysaccharides (SWP₁, SWP₂, and SWP₃). These results are in agreement with those reported in the study by Mansour et al. [87] who found that the increasing levels of astaxanthin, extracted from the cyanobacterium strain, *Arthrospira platensis* NIOF17/003, in *L. vannamei* diet significantly ($p > 0.05$) decreased the counts of THB and TVC. However, the action mechanism of how seaweed-polysaccharide affected the abundance of microbiota is still not clear and requires further studies [87,101].

Several genes involved in immunological response were the focus of the current investigation. In SWP₃ treatment, the up-regulatory gene expression was noticeably higher. Results showed increased expression with the treatments compared to the control (SWP₀),

suggesting that the polysaccharide can improve the immune status of shrimp through microbial cell walls composed of peptidoglycans, lipopolysaccharides (LPS), and β -1, 3-glucans, which can activate the shrimp immune response by triggering the main non-specific defense mechanism [22,87,105,106].

Prophenoloxidase is a crucial enzyme in invertebrate humoral immunity that promotes melanization to get rid of invasive pathogens [107], and is linked to cuticle sclerotization and wound healing [108]. Invertebrates have a non-self-recognition system called the ProPO activation system, which may detect and react to intruders using peptidoglycan or lipopolysaccharides from bacteria and β -1, 3-glucans from fungi [109]. The mRNA expression of the *ProPO* gene was shown to be considerably higher across all treatments compared to the control group, and this expression was found to be the greatest among all the investigated genes as seaweed polysaccharide content was increased (3 g kg⁻¹ diet). Feeding *P. monodon* shrimp a diet that included the polysaccharide fucoidan from the brown seaweed *S. wightii* increased the expression of the *ProPO* gene [110]. Some other dietary supplements derived from microalgae and seaweeds raised the shrimp's *ProPO* system and improved the humoral immune response. Our findings are consistent with prior studies conducted on *L. vannamei* [22,87].

Crustin, defined as part of the innate immune system [111], is a protein found in the hemocyte granules of crustaceans and is effective against several microorganisms. In this study, supplemented diets of the extracted polysaccharide increased *Crustin* gene expression, and there was a clear difference between the three treatments. Significant elevation of *Crustin* mRNA levels in *Marsupenaeus japonicus* has been observed after the administration of peptidoglycan [112]. The *Crustin* gene was upregulated ($p < 0.05$) in Pacific white shrimp *L. vannamei* administered supplemental astaxanthin [87,113]. As a protein found in eukaryotes and prokaryotes, lysozyme has been around for quite some time and is considered to be one of the earliest known antibacterial proteins [114]. Non-specific innate immunity relies on its ability to break down the β -1,4 glycosidic link between N-acetylmuramic acid and N-acetylglucosamine in bacterial cell wall peptidoglycan [115].

In the current investigation, *Lys* gene expression was shown to be considerably greater in the treatment groups (SWP₁, SWP₂, and SWP₃) than in the control group (SWP₀). Another transcriptome investigation using species that face environmental challenges also produced similar findings [116,117]. These findings demonstrated that lysozyme is a crucial part of the shrimp's anti-bacterial defense mechanism and is evoked by a variety of immunostimulating substances. The antioxidant enzymes catalase and glutathione peroxidase convert hydrogen peroxide into oxygen and water, while *SOD*, one of the stress genes, is involved in the elimination of superoxide anions by converting them into hydrogen peroxide and water [118]. Consequently, these antioxidant enzymes give post-phagocytosis self-protection to the hemocytes of oxygen-respiring animals, hence preserving the organisms' health and viability [119,120]. Compared to the control, the expression of the *SOD* gene was elevated in the three experimental conditions, and previous research [22,87,113,116] indicated that the feeding additive increased the expression of the *SOD* gene, which is involved in the antioxidant enzyme system in *L. vannamei*.

In the glutathione defense system, *GPx* is responsible for the reduction of hydrogen peroxide to water [117,121]. In our investigation, the expression of *GPx* was found to be higher in the SWP₃ treatment where a higher concentration of seaweed polysaccharides was used. Thus, both stress genes in this study are significantly upregulated in comparison to the control group, and the activities of the *SOD* and *GPx* increase together with an increase in superoxide anion (O₂⁻) and hydrogen peroxide (H₂O₂), which may indicate increases in the activity of NADPH-oxidase and the production of a mass of reactive oxygen species (ROS) that can represent as a defense mechanism against microbial infection [73,122]. Recent research has evaluated the expression of genes involved in immunity in shrimp [123,124] and has concentrated on ways to boost their natural defenses.

There are two types of insulin-like growth factor (*IGF*) peptide hormones, *IGF-I* and *IGF-II*; there are also cell surface receptors and circulating binding proteins. *IGF-II*, like *IGF-*

I, has a role in protein metabolism, cellular differentiation, cell proliferation, and somatic growth. Based on the findings of the current study, it appears that seaweed polysaccharide extraction may increase the expression of growth-related genes at the mRNA level, hence boosting growth capacity indirectly. Other studies examining the impact of employing different carbon sources for boosting *IGF-I* and *IGF-II* gene expression revealed similar outcomes [123]. Furthermore, utilizing the green microalga, *T. suecica*, and *A. platensis* nanoparticles as the supplementary feeds for *L. vannamei* greatly increased the expression of both genes and improved growth [22,100].

5. Conclusions

Globally, shrimp diets have expanded by using several strategies to deal with the development in the farming of the Pacific whiteleg shrimp *L. vannamei*. Despite the importance of feed additives for *L. vannamei*, little is known about the application of polysaccharides prepared from brown seaweeds in the *L. vannamei* feed additive industry. In the current work, the inclusion rate of 2 g kg⁻¹ of polysaccharides, a high-value active molecule prepared from brown seaweed *Sargassum dentifolium*, as dietary additive administration enhances final weight gain and survival rate of the Pacific Whiteleg shrimp, *L. vannamei*, while incorporation level of 3 g kg⁻¹ reduces the abundance of pathogenic microbes, moreover, enhances the immunity and stress-related gene expressions of *L. vannamei*. However, further studies should be conducted to maximize the benefits of polysaccharides prepared from seaweed species as additive administrations to the Pacific whiteleg shrimp *L. vannamei*.

Author Contributions: Conceptualization, E.M.A., Z.Z.S. and M.A.; methodology, E.M.A., Z.Z.S. and M.A.; software, E.M.A., Z.Z.S. and M.A.; validation, E.M.A., Z.Z.S., M.A. and E.E.-H.; formal analysis, E.M.A., Z.Z.S. and M.A.; investigation, E.M.A., Z.Z.S., M.A. and E.E.-H.; resources, E.M.A., Z.Z.S. and M.A.; data curation, E.M.A., Z.Z.S. and M.A.; writing—original draft preparation, E.M.A., Z.Z.S. and M.A.; writing—review and editing, E.M.A., Z.Z.S., M.A., A.S.A.-S. and E.E.-H.; visualization, E.M.A., Z.Z.S., M.A. and E.E.-H.; supervision, Z.Z.S., M.A. and E.E.-H.; project administration, E.M.A.; funding acquisition, E.M.A., Z.Z.S., M.A. and A.S.A.-S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Compliance with ethical standards in the experimental setup and shrimp handling was approved by the Research Committee of the NIOF, Egypt, approval no.: NIOF/AQ3/1/22/R/012 on 22 January 2021.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The open access of this work was partially supported by AL Hail Aquaculture Unit, Department of Marine Science and Fisheries, College of Agriculture and Marine Science, Sultan Qaboos University, Oman.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Abbas, E.M.; Ali, F.S.; Desouky, M.G.; Ashour, M.; El-Shafei, A.; Maaty, M.M.; Sharawy, Z.Z. Novel Comprehensive Molecular and Ecological Study Introducing Coastal Mud Shrimp (*Solenocera crassicornis*) Recorded at the Gulf of Suez, Egypt. *J. Mar. Sci. Eng.* **2020**, *9*, 9. [CrossRef]
2. Abdelrhman, A.M.; Ashour, M.; Al-Zahaby, M.A.; Sharawy, Z.Z.; Nazmi, H.; Zaki, M.A.; Ahmed, N.H.; Ahmed, S.R.; El-Haroun, E.; Van Doan, H. Effect of polysaccharides derived from brown macroalgae *Sargassum dentifolium* on growth performance, serum biochemical, digestive histology and enzyme activity of hybrid red tilapia. *Aquac. Rep.* **2022**, *25*, 101212. [CrossRef]
3. Goda, A.; Saad, A.; Hanafy, M.; Sharawy, Z.; El-Haroun, E. Dietary effects of *Azolla pinnata* combined with exogenous digestive enzyme (Digestin™) on growth and nutrients utilization of freshwater prawn, *Macrobrachium rosenbergii* (de Man 1879). *J. Oceanol. Limnol.* **2018**, *36*, 1434–1441. [CrossRef]
4. Sharawy, Z.Z.; Abbas, E.M.; Abdelkhalek, N.K.; Ashry, O.A.; Abd El-Fattah, L.S.; El-Sawy, M.A.; Helal, M.F.; El-Haroun, E. Effect of organic carbon source and stocking densities on growth indices, water microflora, and immune-related genes expression of *Litopenaeus vannamei* Larvae in intensive culture. *Aquaculture* **2022**, *546*, 737397. [CrossRef]

5. Gillett, R. Global study of shrimp fisheries. *FAO Fish Tech. Pap.* **2008**, *475*, 25–29.
6. Ahmed, N.; Thompson, S.; Glaser, M. Global aquaculture productivity, environmental sustainability, and climate change adaptability. *Environ. Manag.* **2019**, *63*, 159–172. [[CrossRef](#)] [[PubMed](#)]
7. Lukwambe, B.; Nicholaus, R.; Zhang, D.; Yang, W.; Zhu, J.; Zheng, Z. Successional changes of microalgae community in response to commercial probiotics in the intensive shrimp (*Litopenaeus vannamei* Boone) culture systems. *Aquaculture* **2019**, *511*, 734257. [[CrossRef](#)]
8. Li, E.; Xu, C.; Wang, X.; Wang, S.; Zhao, Q.; Zhang, M.; Qin, J.G.; Chen, L. Gut microbiota and its modulation for healthy farming of Pacific white shrimp *Litopenaeus vannamei*. *Rev. Fish. Sci. Aquac.* **2018**, *26*, 381–399. [[CrossRef](#)]
9. Stevens, C.; Croft, D.; Paull, G.; Tyler, C. Stress and welfare in ornamental fishes: What can be learned from aquaculture? *J. Fish Biol.* **2017**, *91*, 409–428. [[CrossRef](#)]
10. El-Sayed, A.F.M. Use of biofloc technology in shrimp aquaculture: A comprehensive review, with emphasis on the last decade. *Rev. Aquac.* **2021**, *13*, 676–705. [[CrossRef](#)]
11. Anh, N.T.N.; Shayo, F.A.; Nevejan, N.; Van Hoa, N. Effects of stocking densities and feeding rates on water quality, feed efficiency, and performance of white leg shrimp *Litopenaeus vannamei* in an integrated system with sea grape *Caulerpa lentillifera*. *J. Appl. Phycol.* **2021**, *33*, 3331–3345. [[CrossRef](#)]
12. Emerenciano, M.G.; Rombenso, A.N.; Vieira, F.d.N.; Martins, M.A.; Coman, G.J.; Truong, H.H.; Noble, T.H.; Simon, C.J. Intensification of Penaeid Shrimp Culture: An Applied Review of Advances in Production Systems, Nutrition and Breeding. *Animals* **2022**, *12*, 236. [[CrossRef](#)] [[PubMed](#)]
13. Mansour, A.T.; Ashry, O.A.; Ashour, M.; Alsaqufi, A.S.; Ramadan, K.M.; Sharawy, Z.Z. The optimization of dietary protein level and carbon sources on biofloc nutritive values, bacterial abundance, and growth performances of whiteleg shrimp (*Litopenaeus vannamei*) juveniles. *Life* **2022**, *12*, 888. [[CrossRef](#)] [[PubMed](#)]
14. Mansour, A.T.; Ashry, O.A.; El-Neweshy, M.S.; Alsaqufi, A.S.; Dighiesh, H.S.; Ashour, M.; Kelany, M.S.; El-Sawy, M.A.; Mabrouk, M.M.; Abbas, E.M. Effect of Agricultural By-Products as a Carbon Source in a Biofloc-Based System on Growth Performance, Digestive Enzyme Activities, Hepatopancreas Histology, and Gut Bacterial Load of *Litopenaeus vannamei* Post Larvae. *J. Mar. Sci. Eng.* **2022**, *10*, 1333. [[CrossRef](#)]
15. Iber, B.T.; Kasan, N.A. Recent advances in Shrimp aquaculture wastewater management. *Heliyon* **2021**, *7*, e08283. [[CrossRef](#)] [[PubMed](#)]
16. Zaki, M.A.; Ashour, M.; Heneash, A.M.M.; Mabrouk, M.M.; Alprol, A.E.; Khairy, H.M.; Nour, A.M.; Mansour, A.T.; Hassanien, H.A.; Gaber, A.; et al. Potential Applications of Native Cyanobacterium Isolate (*Arthrospira platensis* NIOF17/003) for Biodiesel Production and Utilization of Its Byproduct in Marine Rotifer (*Brachionus plicatilis*) Production. *Sustainability* **2021**, *13*, 1769. [[CrossRef](#)]
17. Abisha, R.; Krishnani, K.K.; Sukhdhane, K.; Verma, A.; Brahmane, M.; Chadha, N. Sustainable development of climate-resilient aquaculture and culture-based fisheries through adaptation of abiotic stresses: A review. *J. Water Clim. Change* **2022**, *13*, 2671–2689. [[CrossRef](#)]
18. Alprol, A.E.; Ashour, M.; Mansour, A.T.; Alzahrani, O.M.; Mahmoud, S.F.; Gharib, S.M. Assessment of Water Quality and Phytoplankton Structure of Eight Alexandria Beaches, Southeastern Mediterranean Sea, Egypt. *J. Mar. Sci. Eng.* **2021**, *9*, 1328. [[CrossRef](#)]
19. Metwally, A.S.; El-Naggar, H.A.; El-Damhougy, K.A.; Bashir, M.A.E.; Ashour, M.; Abo-Taleb, H.A.H. GC-MS analysis of bioactive components in six different crude extracts from the Soft Coral (*Simularia maxim*) collected from Ras Mohamed, Aqaba Gulf, Red Sea, Egypt. *Egypt. J. Aquat. Biol. Fish.* **2020**, *24*, 425–434. [[CrossRef](#)]
20. Magouz, F.I.; Essa, M.A.; Matter, M.; Tageldein Mansour, A.; Alkafafy, M.; Ashour, M. Population Dynamics, Fecundity and Fatty Acid Composition of *Oithona nana* (Cyclopoida, Copepoda), Fed on Different Diets. *Animals* **2021**, *11*, 1188. [[CrossRef](#)]
21. Kesselring, J.; Gruber, C.; Standen, B.; Wein, S. Effect of a phytogetic feed additive on the growth performance and immunity of Pacific white leg shrimp, *Litopenaeus vannamei*, fed a low fishmeal diet. *J. World Aquac. Soc.* **2021**, *52*, 303–315. [[CrossRef](#)]
22. Sharawy, Z.Z.; Ashour, M.; Labena, A.; Alsaqufi, A.S.; Mensour, A.T.; Abbas, E. Effects of dietary *Arthrospira platensis* nanoparticles on growth performance, feed utilization, and growth-related gene expression of Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture* **2022**, *551*, 737905. [[CrossRef](#)]
23. Ceseña, C.E.; Jacinto, E.C.; González, A.L.; Villasante, F.V.; Castro, R.M.M.; Ochoa, N.; Montes, R.E.; Ramírez, D.T.; Ortiz, A.C.S.; Campa-Córdova, A.I. Dietary supplementation of *Debaryomyces hansenii* enhanced survival, antioxidant and immune response in juvenile shrimp penaeus vannamei challenged with *Vibrio Parahaemolyticus*. *Trop. Subtrop. Agroecosyst.* **2021**, *24*, 2. [[CrossRef](#)]
24. Mansour, A.T.; Ashour, M.; Alprol, A.E.; Alsaqufi, A.S. Aquatic Plants and Aquatic Animals in the Context of Sustainability: Cultivation Techniques, Integration, and Blue Revolution. *Sustainability* **2022**, *14*, 3257. [[CrossRef](#)]
25. Hassan, S.M.; Ashour, M.; Soliman, A.A.F.; Hassanien, H.A.; Alsanie, W.F.; Gaber, A.; Elshobary, M.E. The Potential of a New Commercial Seaweed Extract in Stimulating Morpho-Agronomic and Bioactive Properties of *Eruca vesicaria* (L.) Cav. *Sustainability* **2021**, *13*, 4485. [[CrossRef](#)]
26. Essa, D.; Abo-Shady, A.; Khairy, H.; Abomohra, A.E.-F.; Elshobary, M. Potential cultivation of halophilic oleaginous microalgae on industrial wastewater. *Egypt. J. Bot.* **2018**, *58*, 205–216. [[CrossRef](#)]
27. Mansour, A.T.; Alprol, A.E.; Abualnaja, K.M.; El-Beltagi, H.S.; Ramadan, K.M.A.; Ashour, M. Dried Brown Seaweed's Phytoremediation Potential for Methylene Blue Dye Removal from Aquatic Environments. *Polymers* **2022**, *14*, 1375. [[CrossRef](#)]

28. Mansour, A.T.; Alprol, A.E.; Abualnaja, K.M.; El-Beltagi, H.S.; Ramadan, K.M.A.; Ashour, M. The Using of Nanoparticles of Microalgae in Remediation of Toxic Dye from Industrial Wastewater: Kinetic and Isotherm Studies. *Materials* **2022**, *15*, 3922. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Mansour, A.T.; Alprol, A.E.; Ashour, M.; Ramadan, K.M.; Alhajji, A.H.; Abualnaja, K.M. Do Red Seaweed Nanoparticles Enhance Bioremediation Capacity of Toxic Dyes from Aqueous Solution? *Gels* **2022**, *8*, 310. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Abou-Shanab, R.A.I.; El-Dalatony, M.M.; El-Sheekh, M.M.; Ji, M.-K.; Salama, E.-S.; Kabra, A.N.; Jeon, B.-H. Cultivation of a new microalga, *Micractinium reisseri*, in municipal wastewater for nutrient removal, biomass, lipid, and fatty acid production. *Biotechnol. Bioprocess Eng.* **2014**, *19*, 510–518. [\[CrossRef\]](#)
31. Vieira, M.V.; Pastrana, L.M.; Fuciños, P. Microalgae encapsulation systems for food, pharmaceutical and cosmetics applications. *Mar. Drugs* **2020**, *18*, 644. [\[CrossRef\]](#)
32. Fais, G.; Manca, A.; Bolognesi, F.; Borselli, M.; Concas, A.; Busutti, M.; Broggi, G.; Sanna, P.; Castillo-Aleman, Y.M.; Rivero-Jiménez, R.A. Wide Range Applications of Spirulina: From Earth to Space Missions. *Mar. Drugs* **2022**, *20*, 299. [\[CrossRef\]](#)
33. Shao, W.; Ebaid, R.; El-Sheekh, M.; Abomohra, A.; Eladel, H. Pharmaceutical applications and consequent environmental impacts of *Spirulina* (*Arthrospira*): An overview. *Grasas Y Aceites* **2019**, *70*, e292. [\[CrossRef\]](#)
34. Ashour, M.; Omran, A.M.M.M. Recent Advances in Marine Microalgae Production: Highlighting Human Health Products from Microalgae in View of the Coronavirus Pandemic (COVID-19). *Fermentation* **2022**, *8*, 466. [\[CrossRef\]](#)
35. Mourelle, M.L.; Gómez, C.P.; Legido, J.L. The potential use of marine microalgae and cyanobacteria in cosmetics and thalassotherapy. *Cosmetics* **2017**, *4*, 46. [\[CrossRef\]](#)
36. Zhuang, D.; He, N.; Khoo, K.S.; Ng, E.-P.; Chew, K.W.; Ling, T.C. Application progress of bioactive compounds in microalgae on pharmaceutical and cosmetics. *Chemosphere* **2021**, *291*, 132932. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Osman, M.E.H.; Abo-shady, A.M.; Elshobary, M.E. In vitro screening of antimicrobial activity of extracts of some macroalgae collected from Abu-Qir bay Alexandria, Egypt. *Afr. J. Biotechnol.* **2010**, *9*, 7203–7208.
38. Osman, M.E.H.; Abo-Shady, A.M.; Elshobary, M.E.; Abd El-Ghafar, M.O.; Abomohra, A.E.-F. Screening of seaweeds for sustainable biofuel recovery through sequential biodiesel and bioethanol production. *Environ. Sci. Pollut. Res.* **2020**, *27*, 32481–32493. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Abomohra, A.E.-F.; Elshobary, M. Biodiesel, Bioethanol, and Biobutanol Production from Microalgae. In *Microalgae Biotechnology for Development of Biofuel and Wastewater Treatment*; Springer: Cham, Switzerland, 2019; pp. 293–321. [\[CrossRef\]](#)
40. Elshobary, M.E.; El-Shenody, R.A.; Abomohra, A.E.F. Sequential biofuel production from seaweeds enhances the energy recovery: A case study for biodiesel and bioethanol production. *Int. J. Energy Res.* **2021**, *45*, 6457–6467. [\[CrossRef\]](#)
41. Cai, J.; Lovatelli, A.; Aguilar-Manjarrez, J.; Cornish, L.; Dabbadie, L.; Desrochers, A.; Diffey, S.; Garrido Gamarro, E.; Geehan, J.; Hurtado, A. Seaweeds and Microalgae: An Overview for Unlocking Their Potential in Global Aquaculture Development. In *FAO Fisheries and Aquaculture Circular*; FAO: Rome, Italy, 2021. [\[CrossRef\]](#)
42. Chopin, T.; Tacon, A.G. Importance of seaweeds and extractive species in global aquaculture production. *Rev. Fish. Sci. Aquac.* **2021**, *29*, 139–148. [\[CrossRef\]](#)
43. Wan, A.H.; Davies, S.J.; Soler-Vila, A.; Fitzgerald, R.; Johnson, M.P. Macroalgae as a sustainable aquafeed ingredient. *Rev. Aquac.* **2019**, *11*, 458–492. [\[CrossRef\]](#)
44. Khalid, S.; Abbas, M.; Saeed, F.; Bader-Ul-Ain, H.; Suleria, H.A.R. *Therapeutic Potential of Seaweed Bioactive Compounds*; IntechOpen: London, UK, 2018.
45. Gamero-Vega, G.; Palacios-Palacios, M.; Quitral, V. Nutritional composition and bioactive compounds of red seaweed: A mini-review. *J. Food Nutr. Res.* **2020**, *8*, 431–440. [\[CrossRef\]](#)
46. Syakilla, N.; George, R.; Chye, F.Y.; Pindi, W.; Mantihal, S.; Wahab, N.A.; Fadzwi, F.M.; Gu, P.H.; Matanjun, P. A Review on Nutrients, Phytochemicals, and Health Benefits of Green Seaweed, *Litopenaeus vannamei*. *Foods* **2022**, *11*, 2832. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Pereira, V.A.; de Alencar, D.B.; de Araújo, I.W.F.; Rodrigues, J.A.G.; Lopes, J.T.; Nunes, L.T.; Ferreira, Y.M.; Lobato, J.S.; Montenegro, A.R.; Vanderley, C.S.B.S. Supplementation of cryodiluent media with seaweed or Nile tilapia skin sulfated polysaccharides for freezing of *Colossoma macropomum* (Characiformes: Serrasalminidae) semen. *Aquaculture* **2020**, *528*, 735553. [\[CrossRef\]](#)
48. Yudiati, E.; Isnansetyo, A.; Handayani, C.R. Innate immune-stimulating and immune genes up-regulating activities of three types of alginate from *Sargassum siliculosum* in Pacific white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol.* **2016**, *54*, 46–53. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Cantelli, L.; Goncalves, P.; Guertler, C.; Kayser, M.; Pilotto, M.R.; Barracco, M.A.; Perazzolo, L.M. Dietary supplementation with sulfated polysaccharides from *Gracilaria birdiae* promotes a delayed immunostimulation in marine shrimp challenged by the white spot syndrome virus. *Aquac. Int.* **2019**, *27*, 349–367. [\[CrossRef\]](#)
50. Setyawan, A.; Isnansetyo, A.; Murwantoko, M.; Indarjulianto, S.; Handayani, C. Comparative immune response of dietary fucoidan from three Indonesian brown algae in white shrimp *Litopenaeus vannamei*. *AACL Bioflux* **2018**, *11*, 1707–1723.
51. Yangthong, M.; Hutadilok-Towatana, N.; Thawonsuwan, J.; Phromkunthong, W. An aqueous extract from *Sargassum* sp. enhances the immune response and resistance against *Streptococcus iniae* in the Asian sea bass (*Lateolabrax niloticus* Bloch). *J. Appl. Phycol.* **2016**, *28*, 3587–3598.
52. Yuguchi, Y.; Bui, L.M.; Takebe, S.; Suzuki, S.; Nakajima, N.; Kitamura, S.; Thanh, T.T.T. Primary structure, conformation in aqueous solution, and intestinal immunomodulating activity of fucoidan from two brown seaweed species *Sargassum crassifolium* and *Padina australis*. *Carbohydr. Polym.* **2016**, *147*, 69–78. [\[CrossRef\]](#)

53. Øverland, M.; Mydland, L.T.; Skrede, A. Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. *J. Sci. Food Agric.* **2019**, *99*, 13–24. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Vidhya Hindu, S.; Chandrasekaran, N.; Mukherjee, A.; Thomas, J. A review on the impact of seaweed polysaccharide on the growth of probiotic bacteria and its application in aquaculture. *Aquac. Int.* **2019**, *27*, 227–238. [\[CrossRef\]](#)
55. Sakai, M. Current research status of fish immunostimulants. *Aquaculture* **1999**, *172*, 63–92. [\[CrossRef\]](#)
56. Yan, J.; Guo, C.; Dawood, M.; Gao, J. Effects of dietary chitosan on growth, lipid metabolism, immune response and antioxidant-related gene expression in *Misgurnus anguillicaudatus*. *Benef. Microbes* **2017**, *8*, 439–449. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Kumari, N.; Kumar, M.; Lorenzo, J.M.; Sharma, D.; Puri, S.; Pundir, A.; Dhupal, S.; Bhuyan, D.J.; Jayanthi, G.; Selim, S. Onion and garlic polysaccharides: A review on extraction, characterization, bioactivity, and modifications. *Int. J. Biol. Macromol.* **2022**, *219*, 1047–1061. [\[CrossRef\]](#)
58. Song, S.K.; Beck, B.R.; Kim, D.; Park, J.; Kim, J.; Kim, H.D.; Ringø, E. Prebiotics as immunostimulants in aquaculture: A review. *Fish Shellfish Immunol.* **2014**, *40*, 40–48. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Paiva, L.; Lima, E.; Neto, A.I.; Baptista, J. Seasonal variability of the biochemical composition and antioxidant properties of *Fucus spiralis* at two Azorean Islands. *Mar. Drugs* **2018**, *16*, 248. [\[CrossRef\]](#)
60. Pan, S.; Jiang, L.; Wu, S. Stimulating effects of polysaccharide from *Angelica sinensis* on the nonspecific immunity of white shrimps (*Litopenaeus vannamei*). *Fish Shellfish Immunol.* **2018**, *74*, 170–174. [\[CrossRef\]](#) [\[PubMed\]](#)
61. Eissa, H.; Hegazi, M.M.; Elmor, M.E.; Sharawy, Z.Z. Effects of partial substitution of fish meal with different levels of marine macroalgae species on growth indices and RNA/DNA ratio of hybrid red tilapia. *Egypt. J. Aquat. Biol. Fish.* **2021**, *25*, 395–410. [\[CrossRef\]](#)
62. Sritunyalucksana, K.; Söderhäll, K. The proPO and clotting system in crustaceans. *Aquaculture* **2000**, *191*, 53–70. [\[CrossRef\]](#)
63. Wang, Y.-B. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. *Aquaculture* **2007**, *269*, 259–264. [\[CrossRef\]](#)
64. Janeway, C.A. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb. Symp. Quant. Biol.* **1989**, *54*, 1–13. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Amparyup, P.; Sutthangkul, J.; Charoensapsri, W.; Tassanakajon, A. Pattern recognition protein binds to lipopolysaccharide and β -1, 3-glucan and activates shrimp prophenoloxidase system. *J. Biol. Chem.* **2012**, *287*, 10060–10069. [\[CrossRef\]](#)
66. Kanost, M.R.; Jiang, H.; Yu, X.Q. Innate immune responses of a lepidopteran insect, *Manduca sexta*. *Immunol. Rev.* **2004**, *198*, 97–105. [\[CrossRef\]](#)
67. Yu, X.-Q.; Kanost, M.R. Immulectin-2, a pattern recognition receptor that stimulates hemocyte encapsulation and melanization in the tobacco hornworm, *Manduca sexta*. *Dev. Comp. Immunol.* **2004**, *28*, 891–900. [\[CrossRef\]](#) [\[PubMed\]](#)
68. Romo-Figueroa, M.A.G.; Vargas-Requena, C.; Sotelo-Mundo, R.R.; Vargas-Albores, F.; Higuera-Ciapara, I.; Söderhäll, K.; Yepiz-Plascencia, G. Molecular cloning of a β -glucan pattern-recognition lipoprotein from the white shrimp *Penaeus (Litopenaeus) vannamei*: Correlations between the deduced amino acid sequence and the native protein structure. *Dev. Comp. Immunol.* **2004**, *28*, 713–726. [\[CrossRef\]](#)
69. Lee, S.Y.; Wang, R.; Söderhäll, K. A lipopolysaccharide- and β -1, 3-glucan-binding protein from hemocytes of the freshwater crayfish *Pacifastacus leniusculus*: Purification, characterization, and cDNA cloning. *J. Biol. Chem.* **2000**, *275*, 1337–1343. [\[CrossRef\]](#) [\[PubMed\]](#)
70. Lee, S.Y.; Söderhäll, K. Early events in crustacean innate immunity. *Fish Shellfish Immunol.* **2002**, *12*, 421–437. [\[PubMed\]](#)
71. Holmblad, T.; Söderhäll, K. Cell adhesion molecules and antioxidative enzymes in a crustacean, possible role in immunity. *Aquaculture* **1999**, *172*, 111–123. [\[CrossRef\]](#)
72. Campa-Córdova, A.; Hernández-Saavedra, N.; De Philippis, R.; Ascencio, F. 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 Shellfish Immunol.* **2002**, *12*, 353–366. [\[CrossRef\]](#) [\[PubMed\]](#)
73. Yu, B.P. Cellular defenses against damage from reactive oxygen species. *Physiol. Rev.* **1994**, *74*, 139–162. [\[CrossRef\]](#)
74. Zhang, Q.; Li, F.; Wang, B.; Zhang, J.; Liu, Y.; Zhou, Q.; Xiang, J. The mitochondrial manganese superoxide dismutase gene in Chinese shrimp *Fenneropenaeus chinensis*: Cloning, distribution and expression. *Dev. Comp. Immunol.* **2007**, *31*, 429–440. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Han, L.; Hole, J.A.; Stock, J.M.; Fuis, G.S.; Kell, A.; Driscoll, N.W.; Kent, G.M.; Harding, A.J.; Rymer, M.J.; González-Fernández, A. Continental rupture and the creation of new crust in the Salton Trough rift, Southern California and northern Mexico: Results from the Salton Seismic Imaging Project. *J. Geophys. Res. Solid Earth* **2016**, *121*, 7469–7489. [\[CrossRef\]](#)
76. Tassanakajon, A.; Rimphanitchayakit, V.; Visetnan, S.; Amparyup, P.; Somboonwiwat, K.; Charoensapsri, W.; Tang, S. Shrimp humoral responses against pathogens: Antimicrobial peptides and melanization. *Dev. Comp. Immunol.* **2018**, *80*, 81–93. [\[CrossRef\]](#) [\[PubMed\]](#)
77. Zhu, Z.-y.; Liu, R.-q.; Si, C.-l.; Zhou, F.; Wang, Y.-x.; Ding, L.-n.; Jing, C.; Liu, A.-j.; Zhang, Y.-m. Structural analysis and anti-tumor activity comparison of polysaccharides from *Astragalus*. *Carbohydr. Polym.* **2011**, *85*, 895–902. [\[CrossRef\]](#)
78. Picha, M.E.; Turano, M.J.; Tipsmark, C.K.; Borski, R.J. Regulation of endocrine and paracrine sources of Igfs and Gh receptor during compensatory growth in hybrid striped bass (*Morone chrysops* \times *Morone saxatilis*). *J. Endocrinol.* **2008**, *199*, 81. [\[CrossRef\]](#) [\[PubMed\]](#)

79. Castillo-Juárez, H.; Campos-Montes, G.R.; Caballero-Zamora, A.; Montaldo, H.H. Genetic improvement of Pacific white shrimp [*Penaeus (Litopenaeus) vannamei*]: Perspectives for genomic selection. *Front. Genet.* **2015**, *6*, 93. [\[CrossRef\]](#) [\[PubMed\]](#)
80. Ashour, M.; Abo-Taleb, H.A.; Hassan, A.-K.M.; Abdelzaher, O.F.; Mabrouk, M.M.; Elokaby, M.A.; Alzahrani, O.M.; Mahmoud, S.F.; El-feky, M.M.M.; Shaban, W.M.; et al. Valorization Use of Amphipod Meal, *Gammarus pulex*, as a Fishmeal Substitute on Growth Performance, Feed Utilization, Histological and Histometric Indices of the Gut, and Economic Revenue of Grey Mullet. *J. Mar. Sci. Eng.* **2021**, *9*, 1336. [\[CrossRef\]](#)
81. Tabarsa, M.; Shin, I.-S.; Lee, J.H.; Surayot, U.; Park, W.; You, S. An immune-enhancing water-soluble α -glucan from *Chlorella vulgaris* and structural characteristics. *Food Sci. Biotechnol.* **2015**, *24*, 1933–1941. [\[CrossRef\]](#)
82. Boyd, C.E.; Tucker, C.S. *Pond Aquaculture Water Quality Management*; Springer Science & Business Media: Berlin/Heidelberg, Germany, 2012.
83. APHA. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association (APHA): Washington, DC, USA, 2005.
84. Mehrabi, Z.; Firouzbakhsh, F.; Jafarpour, A. Effects of dietary supplementation of synbiotic on growth performance, serum biochemical parameters and carcass composition in rainbow trout (*Oncorhynchus mykiss*) fingerlings. *J. Anim. Physiol. Anim. Nutr.* **2012**, *96*, 474–481. [\[CrossRef\]](#)
85. Zeraatpisheh, F.; Firouzbakhsh, F.; Khalili, K.J. Effects of the macroalga *Sargassum angustifolium* hot water extract on hematological parameters and immune responses in rainbow trout (*Oncorhynchus mykiss*) infected with *Yersinia ruckeri*. *J. Appl. Phycol.* **2018**, *30*, 2029–2037. [\[CrossRef\]](#)
86. AOAC. *Official Methods of Analysis of the ASSOCIATION of Official Analytical Chemists*; The Association of Official Analytical Chemists: Washington DC, USA, 2003; Volume 2.
87. Mansour, A.T.; Ashour, M.; Abbas, E.M.; Alsaqufi, A.S.; Kelany, M.S.; El-Sawy, M.A.; Sharawy, Z.Z. Growth Performance, Immune-Related and Antioxidant Genes Expression, and Gut Bacterial Abundance of Pacific White Leg Shrimp, *Litopenaeus vannamei*, Dietary Supplemented with Natural Astaxanthin. *Front. Physiol.* **2022**, *13*, 874172. [\[CrossRef\]](#)
88. Draper, N.R.; Smith, H. *Applied Regression Analysis*; John Wiley & Sons: Hoboken, NJ, USA, 1998; Volume 326.
89. Ganesh, E.A.; Das, S.; Chandrasekar, K.; Arun, G.; Balamurugan, S. Monitoring of total heterotrophic bacteria and *Vibrio* spp. in an aquaculture pond. *Curr. Res. J. Biol. Sci.* **2010**, *2*, 48–52.
90. Xu, Z.; Regenstein, J.M.; Xie, D.; Lu, W.; Ren, X.; Yuan, J.; Mao, L. The oxidative stress and antioxidant responses of *Litopenaeus vannamei* to low temperature and air exposure. *Fish Shellfish Immunol.* **2018**, *72*, 564–571. [\[CrossRef\]](#) [\[PubMed\]](#)
91. Livak, K.J.; Schmittgen, T.D. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. *Methods* **2001**, *25*, 402–408. [\[CrossRef\]](#) [\[PubMed\]](#)
92. Zar, J. *Biostat—Stical Analysis*, 2nd ed.; Prentice-Hall Inc.: Englewood Cliffs, NJ, USA, 1984.
93. Abdel-Latif, H.M.; Dawood, M.A.; Alagawany, M.; Faggio, C.; Nowosad, J.; Kucharczyk, D. Health benefits and potential applications of fucoidan (FCD) extracted from brown seaweeds in aquaculture: An updated review. *Fish Shellfish Immunol.* **2022**, *122*, 115–130. [\[CrossRef\]](#) [\[PubMed\]](#)
94. Huang, X.; Zhou, H.; Zhang, H. The effect of *Sargassum fusiforme* polysaccharide extracts on vibriosis resistance and immune activity of the shrimp, *Fenneropenaeus chinensis*. *Fish Shellfish Immunol.* **2006**, *20*, 750–757. [\[CrossRef\]](#)
95. Milledge, J.J.; Nielsen, B.V.; Bailey, D. High-value products from macroalgae: The potential uses of the invasive brown seaweed, *Sargassum muticum*. *Rev. Environ. Sci. Bio/Technol.* **2016**, *15*, 67–88. [\[CrossRef\]](#)
96. Raguraman, V.; Ravindran, N.; Selvaraju, K.; Kasivelu, G. Seaweed polysaccharides as potential therapeutic agents against white spot syndrome virus (WSSV): A mini review. *Aquac. Int.* **2020**, *28*, 2333–2343. [\[CrossRef\]](#)
97. Thanigaivel, S.; Vickram, S.; Saranya, V.; Ali, H.; Alarifi, S.; Modigunta, J.K.R.; Anbarasu, K.; Lakshmipathy, R.; Rohini, K. Seaweed polysaccharide mediated synthesis of silver nanoparticles and its enhanced disease resistance in *Oreochromis mossambicus*. *J. King Saud Univ. Sci.* **2022**, *34*, 101771. [\[CrossRef\]](#)
98. Lee, P.-T.; Tran, H.T.Q.; Huang, H.-T.; Nan, F.-H.; Lee, M.-C. *Sargassum horneri* extracts stimulate innate immunity, enhance growth performance, and upregulate immune genes in the white shrimp *Litopenaeus vannamei*. *Fish Shellfish Immunol.* **2020**, *102*, 276–285. [\[CrossRef\]](#) [\[PubMed\]](#)
99. Liu, W.-C.; Zhou, S.-H.; Balasubramanian, B.; Zeng, F.-Y.; Sun, C.-B.; Pang, H.-Y. Dietary seaweed (Enteromorpha) polysaccharides improves growth performance involved in regulation of immune responses, intestinal morphology and microbial community in banana shrimp *Fenneropenaeus merguensis*. *Fish Shellfish Immunol.* **2020**, *104*, 202–212. [\[CrossRef\]](#)
100. Ashour, M.; Mabrouk, M.M.; Ayoub, H.F.; El-Feky, M.M.M.M.; Zaki, S.Z.; Hoseinifar, S.H.; Rossi, W.; Van Doan, H.; El-Haroun, E.; Goda, A.M.A.S. Effect of dietary seaweed extract supplementation on growth, feed utilization, hematological indices, and non-specific immunity of Nile Tilapia, *Oreochromis niloticus* challenged with *Aeromonas hydrophila*. *J. Appl. Phycol.* **2020**, *32*, 3467–3479. [\[CrossRef\]](#)
101. Ringø, E.; Zhou, Z.; Vecino, J.G.; Wadsworth, S.; Romero, J.; Kroghdahl, Å.; Olsen, R.E.; Dimitroglou, A.; Foey, A.; Davies, S. Effect of dietary components on the gut microbiota of aquatic animals. A never-ending story? *Aquac. Nutr.* **2016**, *22*, 219–282. [\[CrossRef\]](#)
102. Kitikiew, S.; Chen, J.-C.; Putra, D.F.; Lin, Y.-C.; Yeh, S.-T.; Liou, C.-H. Fucoidan effectively provokes the innate immunity of white shrimp *Litopenaeus vannamei* and its resistance against experimental *Vibrio alginolyticus* infection. *Fish Shellfish Immunol.* **2013**, *34*, 280–290. [\[CrossRef\]](#)

103. Wongsasak, U.; Chaijamrus, S.; Kumkhong, S.; Boonanuntanasarn, S. Effects of dietary supplementation with β -glucan and synbiotics on immune gene expression and immune parameters under ammonia stress in Pacific white shrimp. *Aquaculture* **2015**, *436*, 179–187. [\[CrossRef\]](#)
104. Cheng, Y. The growth performance and nonspecific immunity of red swamp crayfish *Procambarus clarkia* affected by dietary *Rhodiola rosea* polysaccharide. *Fish Shellfish Immunol.* **2019**, *93*, 796–800. [\[CrossRef\]](#) [\[PubMed\]](#)
105. Labbe, P.; Little, T.J. ProPhenolOxidase in *Daphnia magna*: cDNA sequencing and expression in relation to resistance to pathogens. *Dev. Comp. Immunol.* **2009**, *33*, 674–680. [\[CrossRef\]](#)
106. Panigrahi, A.; Saranya, C.; Sundaram, M.; Kannan, S.V.; Das, R.R.; Kumar, R.S.; Rajesh, P.; Otta, S. Carbon: Nitrogen (C: N) ratio level variation influences microbial community of the system and growth as well as immunity of shrimp (*Litopenaeus vannamei*) in biofloc based culture system. *Fish Shellfish Immunol.* **2018**, *81*, 329–337. [\[CrossRef\]](#)
107. Rowley, A.F.; Powell, A. Invertebrate immune systems—specific, quasi-specific, or nonspecific? *J. Immunol.* **2007**, *179*, 7209–7214. [\[CrossRef\]](#) [\[PubMed\]](#)
108. Ai-Aql, Z.; Alagl, A.S.; Graves, D.T.; Gerstenfeld, L.C.; Einhorn, T.A. Molecular mechanisms controlling bone formation during fracture healing and distraction osteogenesis. *J. Dent. Res.* **2008**, *87*, 107–118. [\[CrossRef\]](#)
109. Söderhäll, K.; Cerenius, L. Role of the prophenoloxidase-activating system in invertebrate immunity. *Curr. Opin. Immunol.* **1998**, *10*, 23–28. [\[CrossRef\]](#) [\[PubMed\]](#)
110. Sivagnanavelmurugan, M.; Thaddaeus, B.J.; Palavesam, A.; Immanuel, G. Dietary effect of *Sargassum wightii* fucoidan to enhance growth, prophenoloxidase gene expression of *Penaeus monodon* and immune resistance to *Vibrio parahaemolyticus*. *Fish Shellfish Immunol.* **2014**, *39*, 439–449. [\[CrossRef\]](#)
111. Vargas-Albores, F.; Martínez-Porchas, M. Crustins are distinctive members of the WAP-containing protein superfamily: An improved classification approach. *Dev. Comp. Immunol.* **2017**, *76*, 9–17. [\[CrossRef\]](#) [\[PubMed\]](#)
112. Rattanachai, A.; Hirono, I.; Ohira, T.; Takahashi, Y.; Aoki, T. Cloning of kuruma prawn *Marsupenaeus japonicus* crustin-like peptide cDNA and analysis of its expression. *Fish. Sci.* **2004**, *70*, 765–771. [\[CrossRef\]](#)
113. Wang, H.; Dai, A.; Liu, F.; Guan, Y. Effects of dietary astaxanthin on the immune response, resistance to white spot syndrome virus and transcription of antioxidant enzyme genes in Pacific white shrimp *Litopenaeus vannamei*. *Iran. J. Fish. Sci.* **2015**, *14*, 699–718.
114. Tyagi, V.V.; Buddhi, D. PCM thermal storage in buildings: A state of art. *Renew. Sustain. Energy Rev.* **2007**, *11*, 1146–1166. [\[CrossRef\]](#)
115. Ringø, E. Evaluation of probiotic strain *Bacillus subtilis* C-3102 as a feed supplement for koi carp (*Cyprinus carpio*). *J. Aquac. Res. Dev.* **2011**. [\[CrossRef\]](#)
116. Zhang, J.; Li, F.; Wang, Z.; Xiang, J. Cloning and recombinant expression of a crustin-like gene from Chinese shrimp, *Fenneropenaeus chinensis*. *J. Biotechnol.* **2007**, *127*, 605–614. [\[CrossRef\]](#)
117. Liu, W.-J.; Chang, Y.-S.; Wang, C.-H.; Kou, G.-H.; Lo, C.-F. Microarray and RT-PCR screening for white spot syndrome virus immediate-early genes in cycloheximide-treated shrimp. *Virology* **2005**, *334*, 327–341. [\[CrossRef\]](#) [\[PubMed\]](#)
118. Feng, K.; Yu, J.; Cheng, Y.; Ruan, M.; Wang, R.; Ye, Q.; Zhou, G.; Li, Z.; Yao, Z.; Yang, Y. The SOD gene family in tomato: Identification, phylogenetic relationships, and expression patterns. *Front. Plant Sci.* **2016**, *7*, 1279. [\[CrossRef\]](#) [\[PubMed\]](#)
119. Campa-Córdova, A.; Hernández-Saavedra, N.; Aguirre-Guzmán, G.; Ascencio, F. Respuesta inmunomoduladora de la superóxido dismutasa en juveniles de camarón blanco (*Litopenaeus vannamei*) expuestos a inmunoestimulantes. *Ciencias marinas* **2005**, *31*, 661–669. [\[CrossRef\]](#)
120. Wang, K.H.-C.; Tseng, C.-W.; Lin, H.-Y.; Chen, I.-T.; Chen, Y.-H.; Chen, Y.-M.; Chen, T.-Y.; Yang, H.-L. RNAi knock-down of the *Litopenaeus vannamei* Toll gene (*LvToll*) significantly increases mortality and reduces bacterial clearance after challenge with *Vibrio harveyi*. *Dev. Comp. Immunol.* **2010**, *34*, 49–58. [\[CrossRef\]](#) [\[PubMed\]](#)
121. Sharawy, Z.Z.; Thiele, R.; Abbas, E.M.; El-Magd, M.A.; Hassaan, M.S.; Peter, C.; Schmidt, J.; Saborowski, R.; Goda, A.M.; Slater, M.J. Antioxidant response and body composition of whiteleg shrimp co-cultured with Nile tilapia in recirculating aquaculture. *Aquac. Environ. Interact.* **2017**, *9*, 257–268. [\[CrossRef\]](#)
122. Roch, P. Defense mechanisms and disease prevention in farmed marine invertebrates. *Aquaculture* **1999**, *172*, 125–145. [\[CrossRef\]](#)
123. Hassan, S.A.; Sharawy, Z.Z.; El Nahas, A.F.; Hemeda, S.A.; El-Haroun, E.; Abbas, E.M. Carbon sources improve water quality, microbial community, immune-related and antioxidant genes expression and survival of challenged *Litopenaeus vannamei* Postlarvae in biofloc system. *Aquac. Res.* **2022**, *53*, 5902–5914. [\[CrossRef\]](#)
124. Hassan, S.A.; Sharawy, Z.Z.; El Nahas, A.F.; Hemeda, S.A.; El-Haroun, E.; Abbas, E.M. Modulatory effects of various carbon sources on growth indices, digestive enzymes activity and expression of growth-related genes in Whiteleg shrimp, *Litopenaeus vannamei* reared under an outdoor zero-exchange system. *Aquac. Res.* **2022**, *53*, 5594–5605. [\[CrossRef\]](#)

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.