fishes

MDPI

Article

Potential Symbiotic Effects of β-1,3 Glucan, and Fructooligosaccharides on the Growth Performance, Immune Response, Redox Status, and Resistance of Pacific White Shrimp, *Litopenaeus vannamei* to *Fusarium solani* Infection

El-Sayed Hemdan Eissa [1], Ragaa A. Ahmed [2], Nadia A. Abd Elghany [3], Amal Elfeky [4], Saadea Saadony [5], Norhan H. Ahmed [6], Salah El-Sayed Sakr [7], Geraldine B. Dayrit [8,9], Charlene Princess S. Tolenada [10], Adlene Anne C. Atienza [11], Mahmoud Mabrok [12] and Hala F. Ayoub [13,*]

1 Fish Research Centre, Arish University, Arish 45511, Egypt
2 Department of Aquaculture Nutrition, Faculty of Fish & Fisheries Technology, Aswan University, Aswan 81528, Egypt
3 Department of Fish Diseases, Animal Health Research Institute, ARC Dokki Giza, Cairo 12618, Egypt
4 Department of Animal Resources, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt
5 Department of Animal Production, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt
6 Department of Animal Production, Faculty of Agriculture, Zagazig University, AlSharqia 44519, Egypt
7 Fish Resources Department, Faculty of Agricultural and Environmental Sciences, Arish University, Arish 45511, Egypt
8 The Graduate School, University of Santo Tomas, Manila 1008, Philippines
9 Department of Medical Microbiology, College of Public Health, University of the Philippines Manila, Manila 1000, Philippines
10 Department of Medical Technology, Far Eastern University, Manila 1015, Philippines
11 School of Medical Laboratory Science, Far Eastern University-Dr. Nicanor Reyes Medical Foundation, West Fairview, Quezon City 1118, Philippines
12 Department of Fish Diseases and Management, Faculty of Veterinary Medicine, Suez Canal University, Ismailia 41522, Egypt
13 Department of Fish Health and Management, Central Laboratory for Aquaculture Research (CLAR), Agricultural Research Center (ARC), Abbassa 44662, Sharqia, Egypt
* Correspondence: DrHalaclar16@gmail.com

Abstract: The potential effects of dietary supplementation with β-1,3 glucan and fructooligosaccharides (β-1,3 GF) on antioxidant activities, immunological response, and growth performance of Pacific white shrimp (*Litopenaeus vannamei*) was investigated. Four diets (iso-energetic and iso-nitrogenous) with different levels of β-1,3 GF (0, 0.5, 1.0, and 1.5 g kg^{-1}) were fed to healthy shrimp juveniles weighing 3 ± 0.5 g for 75 days. Shrimps were randomly distributed into 12 net enclosures at a density of 30 shrimp/net, and the experiment was performed in triplicate. The results revealed that long-term supplementation with 1.5 g kg^{-1} β-1,3 GF significantly improved shrimp weight gain, feed conversion ratio, and digestive enzyme profiles compared to the control diet group. However, there were no substantial variations in the contents of moisture, crude protein, total lipids, and ash in the muscles of shrimp fed on different diets. Surprisingly, all antioxidants (superoxide dismutase, catalase, glutathione peroxidase) and immune biomarkers (lysozyme, total hemocyte count, phenol oxidase, and respiratory burst) activities were significantly elevated with increasing levels of β-1,3 GF in the shrimp diet, and the highest values were recorded in the 1.5 g kg^{-1} diet groups. Challenge test results revealed that *F. solani* could cause a high mortality rate (86.7%) in a group fed a normal basal diet within 14 days at a dose of 5×10^4 conidia mL^{-1}. Surprisingly, all dietary treated groups with different doses of β-1,3 GF showed high resistance against *F. solani*, represented by lower cumulative mortality rates (20–43.3%) compared to the control group. Moreover, most of the infected shrimp showed a typical black to brown gill lesion similar to that observed in the natural infection, where an identical fungus was successfully re-isolated from infected gills and muscles. Overall, this study recommends an appropriate incorporation level of β-1,3 GF that could enhance growth performance and improve the antioxidant activities, non-specific immunity, and disease resistance of *L. vannamei*, with an optimal level of 1.5 g kg^{-1}.

Citation: Eissa, E.-S.H.; Ahmed, R.A.; Abd Elghany, N.A.; Elfeky, A.; Saadony, S.; Ahmed, N.H.; Sakr, S.E.-S.; Dayrit, G.B.; Tolenada, C.P.S.; Atienza, A.A.C.; et al. Potential Symbiotic Effects of β-1,3 Glucan, and Fructooligosaccharides on the Growth Performance, Immune Response, Redox Status, and Resistance of Pacific White Shrimp, *Litopenaeus vannamei* to *Fusarium solani* Infection. *Fishes* **2023**, *8*, 105. https://doi.org/10.3390/fishes8020105

Academic Editors: Jiong Chen and Li Lin

Received: 31 December 2022
Revised: 26 January 2023
Accepted: 8 February 2023
Published: 10 February 2023

Keywords: growth performance; immunity; antioxidants; *Litopenaeus vannamei*; Beta-glucan; *Fusarium solani*

1. Introduction

Aquaculture is one of the most promising food sectors to provide the world's population with animal protein sources and tackle the problem of food scarcity that is caused by overpopulation [1,2]. Shrimp farming is one of the fastest-growing economic sectors in the Pacific Coast region, accounting for more than 58% of total shrimp production worldwide in 2016 [3]. One of the most economically significant penaeid shrimp currently being produced in several nations, including Egypt, is the Pacific white shrimp (*Litopenaeus vannamei*) [4]. Recently, numerous diseases have emerged in cultured *L. vannamei* due to further intensification and environmental water deterioration [5,6], resulting in a significant impact on the global shrimp farming business. Among these diseases, black spot disease (BSD) caused by *Fusarium* spp., is considered a major issue in shrimp-producing sectors [7]. *Fusarium* spp. belong to the family *Nectriaceae* and are widespread fungi commonly found on plants, in soil, freshwater, and brackish water [8,9]. The fungi have caused several diseases in plants and animals, resulting in decreased crop yields, lethal mycotoxins, and disease emergence [10,11]. *L. vannamei* is highly susceptible to BSD, with most naturally infected shrimp exhibiting black gill symptoms and serious tissue damage with high mortality [7]. *F. solani* was identified as the main causative organism [12], causing molting failure and rapid onset of BSD outbreaks among individuals [7]. The pathogen affects a wide range of cultured shrimp, including *Penaeus semisulcatus* in Israel [13], *Penaeus. japonicus* in Japan [14], and Pacific white shrimp in China [7].

Antibiotics, insecticides, synthetic herbal-based phenols, and other chemicals have been used extensively in shrimp culture systems to control pathogens and treat diseases [15–17]. However, overuse and indiscriminate use of these medicines could result in the emergence of drug resistant pathogens of public health significance [18–20]. Furthermore, these substances can negatively influence healthy bacteria or the host's normal flora and have direct impacts on consumer food safety [21,22]. Consequently, shrimp farming requires sustainable alternative non-classical strategies, including phage therapy, dietary plant-based medications, probiotics, prebiotics, and synbiotics administration [23,24]. Prebiotics are gaining popularity as an alternative to antibiotics and pesticides in aquaculture [25]. The term "prebiotic" was first defined as "non-digestible foodstuffs found in the digestive tract that stimulate growth or development of beneficial bacteria and thereby improving the intestinal balance of the organism" [26]. There are various prebiotics that have been evaluated in-depth in the aquaculture sector, such as fructooligosaccharides (FOS), short-chain FOS, galactooligosaccharides, inulin, mannan oligosaccharides, arabinoxylooligosaccharides, β-glucan xylooligosaccharides, and isomaltooligosaccharides [27,28]. Prebiotics are effective in preventing infectious infections from spreading during shrimp farming, as well as having the ability to increase growth efficiency, innate immunity, and disease resistance [19,29]. Prebiotics have also been demonstrated to improve the host's ability to absorb nutrients and to boost the performance of probiotics in the form of synbiotics during growth in the digestive system [30]. Among these, FOS and β-1,3 glucan are promising prebiotics that have been demonstrated to be immunomodulators in aquaculture [31]. Previous studies have assured these compounds are worthy and auspicious immunostimulant that support the growth of the gut microbiota, improve growth performance, boost immune responses, and monitor diseases in aquaculture [32,33]. However, there is no concrete evidence from the past about the use of these combination substances in shrimp. Therefore, the current study aimed to evaluate the potential use of dried β-1,3 glucan and fructooligosaccharide (β-1,3 GF) in shrimp diet, highlighting their main effects on Pacific white shrimp *L. vannamei* growth performance, immune response, antioxidants, and resistance to *F. solani* infection.

2. Materials and Methods

2.1. Preparation of Experimental Diets

Although dried β-1,3 GF is widely utilized in fish diets as synthetic commercial prebiotics, there were no intelligible databases to determine the required dosages in shrimp-producing sectors. The β-1,3 GF (Aquastem™ V) was purchased from Kemin Industries, Inc., USA, and was given at different concentrations of 0.0 (control), 0.5, 1.0, and 1.5 g kg^{-1} diet, corresponding to four dietary groups (C1–C4, respectively). AquastemTM V is a unique combination of an algal source of linear 1,3-beta glucan, enriched with vitamin C and fructooligosaccharides. Brief information about the main compositions, features, and instructions for use are available on the company website (www.kemin.com/content/dam/kemin/aquaculture/product/pdfs/Aquastem%20V_1pager_EN_v2020.pdf, accessed on 26 January 2023). The compound was diluted in 100 mL water and thoroughly mixed with the basic components of the control diet for 30 min. The feed is formulated from commercial ingredients (shrimp meal, fishmeal, rice bran, wheat flour, rice bran, soybean meal, fish oil, vitamin and mineral mixture). The composition and chemical analysis of the experimental diets are shown in Table 1. The dry ingredients were passed through a sieve (aperture of 1.5–2.0 mm in diameter) before being mixed into the diet. Emulsified oil was added with equal quantity of water with 0.7% phosphatidylcholine (lecithin) according to [34], to the experimental rations. Mixtures were homogenized in a model SNFGA forage mixer (St. Joseph Kitchen Assistant, M149085 USA). The boiling water was then mixed with the mixtures at a rate of 50% for pelleting. Diets were pelleted (1.5–2.0 mm in diameter) using Kitchen Assistant meat mincers and stored in a refrigerator (4 °C) until used.

Table 1. Ingredients and proximate chemical analysis of the control diet (g/kg on dry matter basis).

Ingredients (g/kg)	β-1,3 Glucan and Fructooligosaccharides Levels (g/kg Diet)			
	C1	C2	C3	C4
Wheat flour	120	120	120	120
Shrimp meal	250	250	250	250
Rice bran	70	69.5	69.0	68.5
Soybean meal	150	150	150	150
Fish meal	300	300	300	300
Fish oil	60	60	60	60
β-1,3 GF	0.0	0.5	1.0	1.5
* CMC	10	10	10	10
** Vit & Min Mix	40	40	40	40
Total	1000	1000	1000	1000
Proximate analysis (% dry weight)				
Dry mater	90.62	90.93	91.21	90.65
Moisture	9.38	9.07	8.79	9.35
Crude protein (N × 6.25)	38.82	38.77	38.76	38.77
Crude fat	10.96	10.84	10.83	10.77
Crude fiber	1.74	1.51	1.24	1.62
Ash	6.13	6.72	7.13	6.47
Carbohydrate (NFE)2	32.975	33.087	33.291	33.017
*** Gross energy (GE) kcal/100g3	459.467	458.56	459.22	457.61

Analysis was performed according to Official Methods of Analysis, Association of Official Analytical Chemists, Arlington [35]. * CMC, carboxymethylcellulose as a thickening agent. ** Vit & Min Mix, Vitamin and Mineral Mix; each 100 g contained minerals (Zn, 2.50 mg; Mn, 16.00 mg; Fe, 31.50 mg; Cu, 5.50; I, 0.55 mg; Ca, 1.15 gm and P, 450 mg). Vitamins (Vit A, 7,500,000 Iu; Bi, 100 mg; B3, 500 mg; B6, 150 mg; B12, 2.5 mg; E, 100 mg; K, 100 mg; pantothenic acid, 275 mg; folic acid, 100 mg; vit. D3, 7500 Iu). *** GE (kcal/100 g DM) = CP × 5.64 + EE × 9.44 + NFE × 4.11, calculated according to [36]. NFE = 100 − [% Ash + % lipid + % protein].

2.2. Shrimp Rearing and Feeding Trial

This study was carried out on a private shrimp farm in Damietta, Egypt, where *Litopenaeus vannamei* juveniles were reared at 22.36‰ salinity in a rectangular pond fitted with 12 identical hapas; each was built with green fine mesh and had a 175-L holding

capacity. Shrimp were fed with basal diet (~39% CP kg^{-1} diet) thrice daily until visual satiety and left to acclimatize for three weeks. Subsequently, 360 apparently healthy juveniles weighing 3 ± 0.5 g were randomly relocated into hapas in triplicate at a density of 30 shrimp/hapa, corresponding to the above four dietary treatments (C1–C4 groups). Shrimp were fed twice daily (9 a.m. and 3 p.m.) at a rate of 6% of their body weight, and the trial lasted for 75 days. The shrimps were weighed in bulk every two weeks, and the feeding amount was adopted accordingly. All water quality parameters were maintained close to the permissible levels for shrimp culture; meanwhile, the trial following [37] recommended levels as follows: water temperature (25.6 ± 1.3 °C), pH (7.6 ± 1.2), and dissolved oxygen (6.4 ± 1.4) mg L^{-1}. Water temperature, pH, and DO were measured using a handheld digital meter (YSI Pro1020), while salinity was measured by an ATC salinity refractometer. Ammonia and nitrite were evaluated twice per week using API test commercial kits, and never exceeded 0.05 and 0.25 mg L^{-1}, respectively. Periodic water exchange of 20% was performed every two days during the adaptation period to keep ammonia levels constant.

2.3. Growth Performance

All shrimp groups fasted for 24 h and were counted and weighed in bulk to determine the survival and growth rates at the onset and the end of the feeding trial. In order to determine the feed efficiency index, the consumed feed was also weighed and reco

The following equations were used:

$$\text{Weight gain (WG)} = \text{final weight (g)} - \text{initial weight (g)}.$$

$$\text{Survival rate (SR) \%} = (\text{Final No./Initial No.}) \times 100.$$

$$\text{Feed conversation ratio (FCR)} = \text{feed intake (g)/body weight gain (g)}.$$

$$\text{Specific growth rate (SGR) \%/day} = (\ln \text{final weight (g/shrimp)} - \ln \text{initial weight (g)})\ 100/n$$

Since [n: number of days).

$$\text{Weight gain rate (WGR \%)} = [\text{final weight (g)} - \text{initial weight (g)}] \times 100/\text{initial weight (g)}.$$

2.4. Sample Collection

At the end of the feeding trial, all groups were starved for 24 h prior to sampling. Six shrimp/net were randomly harvested and kept at −4 °C for carcass analysis. Muscles were collected after dissecting shells and shrimp heads. Samples of hemolymph were collected from the first abdominal segment in 1.5 Eppendorf tubes containing EDTA using a 1-mL sterile syringe. The collected samples were centrifuged at 3000× *g* for 10 min, and the supernatants were separated and kept at −80 °C for further analyses.

2.5. Carcass Chemical Analysis

At the start and the end of the experiment, the approximate chemical composition of six shrimp muscles and diets were determined following [38]. Moisture content was determined after drying to constant weight in an oven at 105 °C. The crude protein was assessed using a micro-Kjeldahl analyzer. Soxhlet apparatus was used with petroleum ether for 16 h to measure the total lipid content. Ash content was estimated using weight loss following 6 h burn at 550 °C in a muffle furnace. All the chemical analyses were performed in triplicate, and the data were expressed in a dry matter basis.

2.6. Digestive Enzymes Analysis

The intestinal samples (each was pooled from six biological replicates and weighing 1 g) were eviscerated and blended in a 10 mL Eppendorf tube with 4 mL phosphate buffer saline (PBS) solution following the method of [39]. Subsequently, the samples were emulsified using an electric homogenizer in an ice water bath for 15 s and centrifuged at 12,000 rpm at 4 °C for 20 min. The supernatant was then collected for digestive enzyme

analysis. The activities of trypsin and chymotrypsin were evaluated according to the protocol of [40] and calculated from the increase in absorbance at 410 nm for 10 min at 25 °C using p-nitro aniline as a standard. Amylase, protease and lipase activities were measured following the method of [41]. The weight (mg) of maltose released during the course of 10 min at 30 °C was used to compute one unit of amylase activity. Tyrosine released in 15 min under the experiment conditions was used to measure unit protease activity. The amount of 0.025 NaOH needed to neutralize the fatty acids released during 18-h incubation period at pH 6.9 and 30 °C was used to measure unit lipase activity. Digestive enzymes were calculated as enzyme unit per gram tissue.

2.7. Antioxidants and Immune Biomarkers

All antioxidant enzymes were measured from collected hemolymph samples. Superoxide dismutase (SOD) activity was assessed following the protocol of [42]. Catalase (CAT) activity was determined using H_2O_2 reaction according to [43]. Glutathione peroxidase (GPx) activity was measured using methyl catechol reaction according to [44]. Malondialdehyde (MDA), as a biomarker of lipid peroxidation, was detected using a thiobarbituric acid reaction following the protocol of [45]. Hemolymph lysozyme activity was assessed using the turbidimetric method described elsewhere by [46]. The respiratory burst activity was determined using nitro blue tetrazolium (NBT) following the method of [47]. Phenol oxidase activity (PO) was measured based on the method of [48]. Total hemocyte count (THC) was performed by adding diluted hemolymph samples to a Neubauer hemocytometer and calculating as follows:

$$\text{THC (cells mL}^{-1}\text{)} = \text{count No} \times 10^4 \times \text{dilution factor.}$$

2.8. Fusarium solani Challenge Trial

The tested strain of *F. solani* was kindly provided by the Microbiology Unit, Fish Diseases Department, Animal Health Institute, Dokki, Giza. The fungal strain was routinely cultured on Potato Dextrose Agar (PDA) at 25 °C for 7 days. To obtain the conidial mass, 20 mL of sterile distilled water was added to each culture plate, and the suspension was collected in 30 mL sterile tubes. The suspensions were purified using two layers of sterile medical dressing to ensure that the filtrate contained only fungal conidia. The fungal conidia concentration was determined by an erythrocyte-counting chamber and was finally adjusted to 5×10^4 conidia mL^{-1} in sterile distilled water.

At the end of the feeding trial, 30 shrimp per each treated group were randomly collected and reassigned in triplicate into 120-L aquariums (10 shrimp/aquarium). Shrimp were injected intramuscularly (IM) in the third abdominal segment with 0.1 mL of 5×10^4 conidia suspension according to [14]. Another group of shrimp was injected IM with 0.1 mL of PBS and served as a negative control group. Mortality rates were recorded daily for 14 days. Moribund and freshly dead shrimp were regularly harvested for clinical and mycological examinations to ascertain the cause of death. Mortality was considered only when the injected strain was retrieved from experimentally infected shrimp (Koch's postulates).

2.9. Statistical Analysis

All trials were performed in triplicate. Data were analyzed for both normality and homogeneity of variance using Kolmogorov–Smirnov and Levene's tests, respectively. When needed, they were transformed before being treated. Duncan's multiple range tests (SPSS, version 22.0) and one-way analysis of variance (ANOVA) were used to analyze the data. The resulting values were expressed as means ± standard error, and the value of $p < 0.05$ was considered significant.

3. Results

3.1. Growth Performance and Proximate Carcass Composition

All values of growth performance parameters are presented in Table 2. As indicated, The C4 group showed no significant difference from the C3 group in FW and WG. However, these groups showed higher values compared to C1 and C2. The feed conversion ratio (FCR) displayed lower values in all dietary-treated groups compared to the control ($p < 0.05$) and was more exceptional in the C4 group. The survival rate exhibited no notable differences ($p > 0.05$) among the tested groups during the feeding trial, and it ranged from 92.5 to 96.3 ± 0.1%. In addition, there were no significant variations ($p > 0.05$) in the contents of moisture, total lipids, crude protein, and ash in the muscles of tested shrimp following supplementation with different concentrations of β-1,3 GF (Table 3).

Table 2. Growth performance and survival rate of Pacific white shrimp, *Litopenaeus vannamei*, fed various concentrations of β-1,3 glucan and fructooligosaccharides for 75 days.

Parameters	β-1,3 G F Levels (g/kg Diet)			
	C1	C2	C3	C3
Initial weight (g)	3.0 ± 0.09	2.90 ± 0.26	3.03 ± 0.03	3.10 ± 0.06
Final weight (g)	13.1 ± 0.26 ^{c}	15.83 ± 0.32 ^{b}	17.93 ± 0.41 ^{a}	18.30 ± 0.26 ^{a}
Weight gain (g)	10.1 ± 0.21 ^{c}	12.93 ± 0.30 ^{b}	14.90 ± 0.42 ^{a}	15.20 ± 0.29 ^{a}
Weight gain rate (%)	336.67 ± 1.70 ^{c}	445.86 ± 11.20 ^{b}	491.74 ± 15.90 ^{a}	490.32 ± 16.20 ^{a}
SGR	1.97 ± 0.007 ^{c}	2.26 ± 0.026 ^{b}	2.369 ± 0.035 ^{a}	2.367 ± 0.036 ^{a}
FCR	2.02 ± 0.018 ^{a}	1.96 ± 0.015 ^{b}	1.57 ± 0.025 ^{c}	1.55 ± 0.02 ^{c}
Survival rate (SR%)	96.3 ± 0.10	92.5 ± 0.10	92.5 ± 0.10	93.8 ± 0.10

Data are presented as means ± SD ($n = 3$). Superscripted letters denote significant differences between experimental groups (ANOVA; $p < 0.05$).

Table 3. Proximate chemical composition (% on a dry matter basis) of muscles of Pacific white shrimp, *Litopenaeus vannamei*, fed various concentrations of β-1,3 glucan and Fructooligosaccharides for 75 days.

Parameter (%)	β-1,3 G F Levels (g/kg Diet)			
	C1	C2	C3	C4
Moisture	79.18 ± 0.23	78.85 ± 0.10	79.32 ± 0.33	79.30 ± 0.34
Protein	15.85 ± 0.19	16.59 ± 0.19	15.46 ± 0.05	16.32 ± 0.17
Lipid	1.98 ± 0.03	1.62 ± 0.37	1.99 ± 0.05	1.95 ± 0.04
Ash	3.06 ± 0.08	3.22 ± 0.18	2.96 ± 0.13	3.19 ± 0.04

Data are presented as means ± SD ($n = 3$). No significant difference was recorded.

3.2. Digestive Enzymes Analysis

The values of all digestive enzyme activities showed significant differences ($p < 0.05$) between the dietary treated groups and the control (Figure 1). Lipase activity was higher in shrimp fed the diet with a high dose of β-1,3 GF in C3 and C4 groups for 75 days than those fed only the lower dose in the C2 group and basal diet C1 group for the same period. Similarly, chymotrypsin, trypsin, amylase, and protease activities were significantly increased ($p < 0.05$) following dietary supplementation with a high dose of β-1,3 GF compared to other treatments.

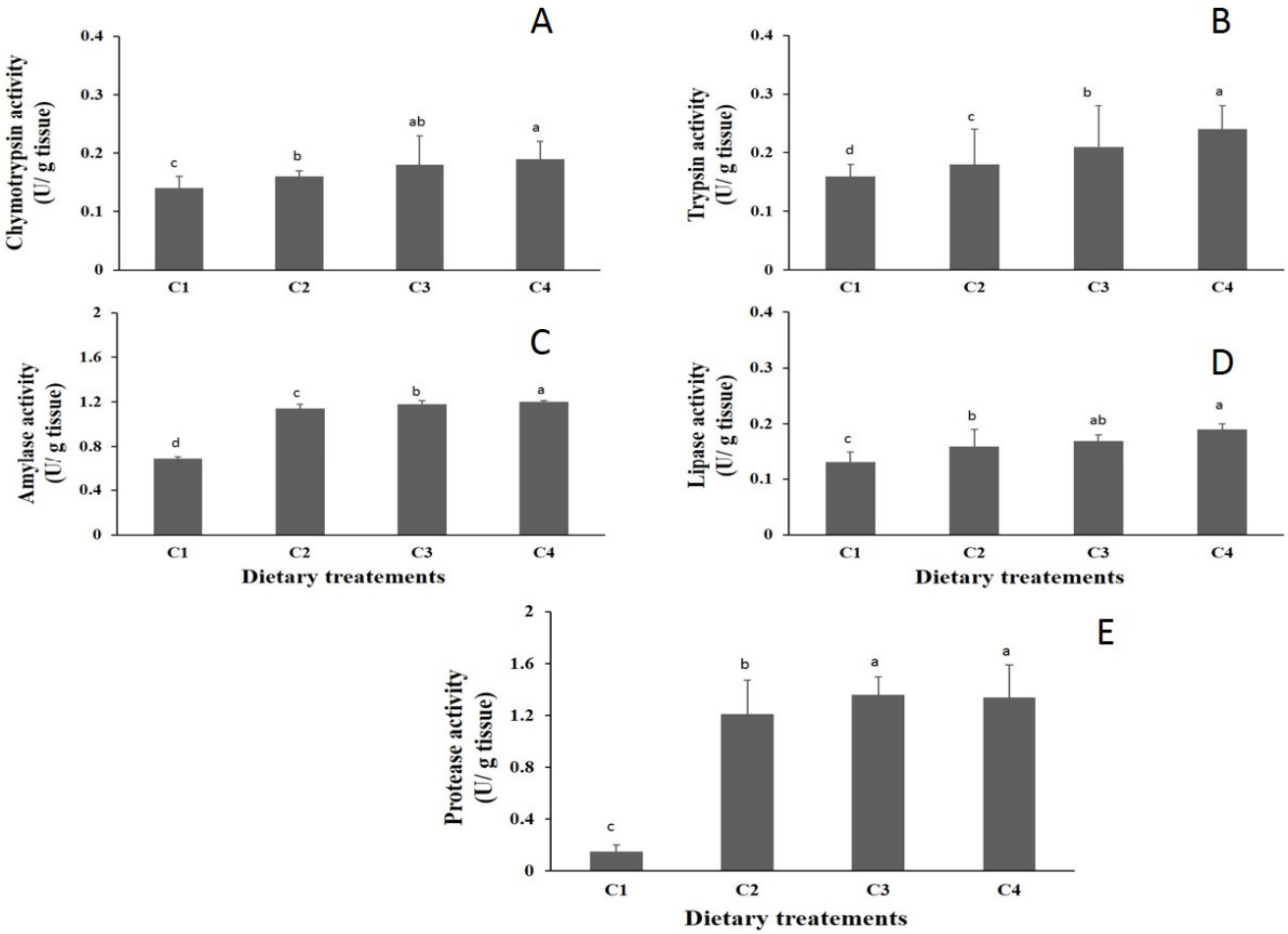

Figure 1. Digestive enzymes analysis of the intestine ((**A**), chymotrypsin; (**B**), trypsin; (**C**), amylase; (**D**), lipase; (**E**), protease (U/g tissue)) of Pacific white shrimp, *Litopenaeus vannamei*, fed various concentrations of β-1,3 glucan and Fructooligosaccharides for 75 days. Data are presented as means ± SD ($n = 3$). Superscripted letters denote significant differences between experimental groups (ANOVA; $p < 0.05$).

3.3. Antioxidants and Immunity Biomarkers

The kinetics of antioxidants and immunity biomarkers following supplementation with different concentrations of β-1,3 GF are presented in Table 4. SOD, CAT, and GPx activities increased with increasing β-1,3 GF levels, with the highest values recorded in the C4 group. Likewise, there was a remarkable improvement in lysozyme and respiratory burst activities in all treated groups in comparison to the control. In contrast, the level of MDA was significantly ($p < 0.05$) decreased in shrimp that received a higher dose of β-1,3 GF when compared to the control group. The total hemocyte count of the control group was consistently lower ($p < 0.05$) than the treated groups. However, the highest values were observed in the C4 group. Moreover, the PO activity of shrimp in the C4 group was notably higher compared to the other treatments.

Table 4. Antioxidants and non-specific immune response of white shrimp, *Litopenaeus vannamei*, fed different levels of β-1,3 glucan and fructooligosaccharides for 75 days.

Parameter	β-1,3 Glucan and Fructooligosaccharides Levels (g/kg Diet)			
	C1	C2	C3	C4
SOD (U/L)	21.01 ± 0.57 ^{d}	25.69 ± 0.25 ^{c}	28.31 ± 0.25 ^{b}	30.45 ± 0.57 ^{a}
GPX (U/L)	22.63 ± 0.38 ^{c}	26.44 ± 0.56 ^{b}	27.24 ±0.27 ^{ab}	28.50 ± 0.39 ^{a}
CAT (U/L)	3.11 ± 0.05 ^{c}	3.98 ± 0.26 ^{b}	4.82 ± 0.14 ^{a}	5.34 ± 0.25 ^{a}
MDA (nmol/mL)	18.13 ± 0.12 ^{c}	24.12 ± 0.03 ^{b}	25.21 ± 0.11 ^{ab}	26.03 ± 0.11 ^{a}
Lysozyme activity (μg/mL)	0.95 ± 0.02 ^{d}	1.24 ± 0.04 ^{c}	1.62 ± 0.03 ^{b}	1.85 ± 0.01 ^{a}
Respiratory burst activity (mg/mL)	0.23 ± 0.12 ^{d}	0.43 ± 0.12 ^{c}	0.61 ± 0.12 ^{b}	0.67 ± 0.12 ^{a}
THC ($\times 10^4$ cells/mL)	21.01 ± 0.57 ^{d}	25.69 ± 0.25 ^{c}	28.31 ± 0.25 ^{b}	30.45 ± 0.57 ^{a}
Phenol oxidase (U/min/mg)	18.13 ± 0.12 ^{c}	24.12 ± 0.03 ^{b}	25.21 ± 0.11 ^{ab}	26.03 ± 0.11 ^{a}

Data are presented as means ± SD (n = 3). Superscripted letters denote significant differences between experimental groups (ANOVA; $p < 0.05$).

3.4. Fungal Challenge Trial

Colonies of *Fusarium solani* grew well on PDA media, with dense aerial white to creamy mycelium, which were abundant and woolly, and the reversal color was brownish. After 3 days, microconidia in fresh isolates were abundant, ellipsoidal, fusiform, or kidney-shaped. Within 4–7 days, the macroconidia were abundantly developed and thick-walled, having three to four septa, straight and parallel-sided for most of the length. The apical cell blunt was end rounded.

At the end of the trial, no mortality was observed in the negative control group that was intramuscularly injected with PBS (C-ve), whereas different levels of mortality were observed in challenged shrimp groups (C1–C4) depending on the dosages of the experimental diet. Herein, the dietary control group exposed to *F. solani* (C1 group) started to die one day post-challenge (Figure 2). Shrimp-fed different levels of β-1,3 GF (C2–C4 groups) showed lower cumulative mortality (20–43.3%) than those fed a normal basal diet (86.7 %) (C1 group). SR% was clearly higher for shrimp fed the diet with 1.5 g kg^{-1} β-1,3 GF (C4 group). Most of the infected shrimp showed marked clinical signs similar to those of the natural infection. Within a few days of the challenge, the infected shrimp showed black to brown collapsed gills with severe necrosis and atrophy (Figure 3), whereas the negative control group displayed normal dusky white gills. Nonetheless, the fungal isolate was successfully recovered on PDA from the gills and muscles of infected shrimp and verified as described above. The fungal was also seen under a microscope in wet mount preparations from the same specimens.

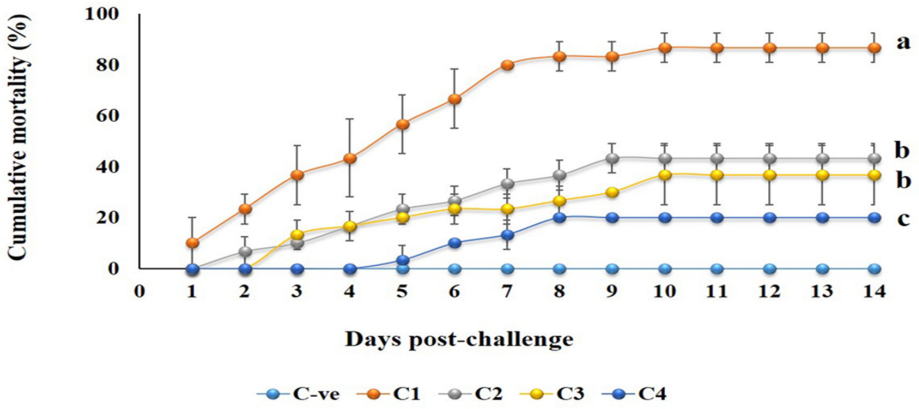

Figure 2. The cumulative mortality (%) over time (Days 0–14) for Pacific white shrimp, *Litopenaeus vannamei*, after challenge with *Fusarium solani*; shrimp were fed with β-1,3 glucan and fructooligosaccharides (β-1,3 GF) supplemented diets (0, 0.5, 1, 1.5 g/kg; C1–C4, respectively). C-ve, refer to shrimp groups that received a normal basal diet and were intramuscularly injected with PBS. Means (SD±) with different letters are significantly different ANOVA test, at $p \leq 0.05$.

Figure 3. Gills of experimentally infected *Litopenaeus vannamei* by *Fusarium solani* showing noticeable black to brown melanated gills (black arrows).

4. Discussion

Prebiotics are widely regarded as one of the most important and practical dietary supplements for sustainable shrimp farming [27]. Incorporating synthetic prebiotics into shrimp meals is now one of the most widely used alternative disease control and preventive strategies in aquaculture. However, their efficacy may vary depending on the species, dosage, and synergism of the combined compounds [49,50]. In the present study, β-1,3 GF enhanced the growth performance and feed utilization of white shrimp at an inclusion level of 1.5 g kg^{-1}. Additionally, it exhibited remarkably lower FCR and higher WG than the control. These findings were in accordance with earlier findings that revealed that the feeding of β-glucans augmented the growth and feed utilization of Pacific white shrimp [51,52], red seabream [53], and tilapia [54]. Similarly, *O. niloticus* fed a synbiotic containing high levels of β glucans and fructooligosaccharides showed a noticeable increase in weight gain and SGRs than non-treated fish [55]. Nevertheless, some studies did not elucidate the growth-promoting effect of β-glucans when administered to the white shrimp diet. It is still unclear how β-glucans work to improve this growth [56], but it is believed that the growth-enhancing impact of this compound could depend on its concentration, solubility, and structures, as well as the examined species.

In aquaculture, β glucans and fructooligosaccharides (FOS) are one of the main prebiotics mostly selected to be integrated with probiotics [57]. Several studies in the literature have elucidated the positive influence of both compounds on the gut microbiota and have definitively verified their roles as growth promoters in aquaculture sectors [58,59]. The principle of prebiotics is that these compounds are degraded to their respective sugars in the shrimp gut and used by beneficial bacteria as carbon sources [49]. Shrimp rely on the actions of digestive enzymes for nutrient breakdown and absorption. The intestines and the hepatopancreas are crucial organs for the secretion of digesting enzymes [60]. Shrimp growth is dependent on nutrient uptake, which is correlated with the activity of digestive enzymes in the hepatopancreas and intestine, including chymotrypsin, amylase, lipase, and trypsin [28]. According to the current study, feeding fish with 1.0 and 1.5 g kg^{-1} of β-1,3 GF considerably increased the digestive enzyme activity of shrimp. The outcomes were comparable to those of earlier research on Pacific white shrimp (*L. vannamei*) fed on different prebiotics such as β-glucan, dietary glycerol monolaurate, and microbial lysozyme [61], crude polysaccharides, arabinoxylan-oligosaccharide, inulin, and fructooligosaccharide [62]. Herein, the significant increase in the activities of digestive enzymes in *L. vannamei* fed on

β-1,3 GF suggests that the prebiotics may encourage the growth of endogenous probiotic bacteria, followed by an increase in the secretion of exogenous hydrolytic enzymes in the shrimp, which improved the digestion of ingested foods [62].

The most likely explanation may be the changes in the composition of the intestinal microflora increase the amount of bifidobacteria and lactobacilli while decreasing the growth of harmful bacteria such as *Escherichia coli* and *Clostridium difficile*. The intestinal microbiota is essential for many physiological and nutritional processes, because it lowers the loss of digesting enzymes, enhances their activity, and fosters growth [33]. Additionally, the digestive gland of shrimp has glucanases that may break down the glucose polymer-glucan to release energy and use the UDP-glucose route to turn glucose into glycogen [63]. Shrimp can obtain energy by digesting glucan through this mechanism, which enables quicker growth [32]. Contrarily, gut bacteria of the genera Bifidobacterium and Lactobacilli selectively digest the oligosaccharide FOS, which promotes the growth and activation of these bacteria's metabolic processes. This enhances the prebiotic property of FOS and benefits the host's health [58].

Regarding antioxidants and immunity biomarkers, it was known that SOD, CAT, and GPx are crucial enzymes that work through enzymatic pathways to reduce oxidative stress [64]. These enzymes can enhance the ROS imbalance and preserve the normal redox balance in biological systems [65]. In this study, the activities of SOD, CAT, and GPx were significantly increased; meanwhile, MDA levels were substantially lower in groups fed β-1,3 GF compared to the control group. These results reflect the main role of β-1,3 GF as a potential antioxidant due to its high phenolic compound content, which is likely responsible for its potent antioxidant activity [66]. Our results were in agreement with those reported by [67], who mentioned that dietary inclusion of β-glucans and other oligosaccharides could mitigate and improve shrimp antioxidant enzymes and innate immune parameters.

Lysozyme activity is one of the most essential indicators of the immune system and its action by the activation of the complement system and phagocytosis [68]. Further, it possesses bactericidal activity against a wide range of Gram-negative and other threatening pathogens [69]. The current study surmised that the dietary addition of β-1,3 GF to white shrimp significantly enhanced lysozyme activity compared to the control group. These results can be attributed to the complex structure of β-1,3 GF and its ability to activate the immunity of white shrimp [70].

Indeed, shrimp circulating hemocytes are the main sources of lysozyme [71]. Hence, the surge in hemocytes in this study was concomitant with elevated lysozyme activity. The results of the present investigation are consistent with the previous findings of [72,73]. The NBT assay primarily measures the production of free oxidative radicals produced by leukocytes in the defense in the face of fish pathogens [74,75]. Our results revealed that there was a remarkable improvement in lysozyme and respiratory burst activities of shrimp hemocytes following supplementation with β-1,3 GF in comparison with the control diet group. Similar findings were reported by [76], who observed an increase in respiratory burst and phenol oxidase activities in white shrimp hemocytes following in vitro culture with a hot water extract from the leaves and branches of the *Cinnamomum kanehirae*. In addition, [77] found that the hemocytes of prawns (*Macrobrachium rosenbergii*) displayed up-regulation of PO and RB activities after incubation of leaf extract of *Morinda citrifolia*.

Assessment of hematological profiles is an essential tool for shrimp health evaluation [78]. Hemocytes are critical components of cellular defense. In crustaceans, a lower number of circulating hemocytes corresponds well with decreased susceptibility to infections [79]. With regard to hematological profiles in this study, Total hemocyte values were proportionally increased as the level of β-1,3 GF increased; the highest values were recorded in the group fed a high dose (1.5 g Kg^{-1}). Similar enhancing patterns have been identified in *Fenneropenaeus indicus* shrimp fed on a diet supplemented with different levels of β-1,3 glucan [80]. Likewise, [81] observed an increase in total hemocyte count in *F. indicus* fed on a yeast-incorporated diet.

In this study, shrimp experimentally infected with *F. solani* showed remarkable clinical signs similare to those of natural infection, which appeared as black to brown-colored gills and collapsed and necrotized gill lamellae, consistent with the previous findings of [7]. The characteristic lesions may be related to granuloma formation or possibly due to mycotic enzymes and toxins causing severe degeneration of shrimp gills [82]. Following the challenge with *F. solani*, shrimp fed different levels of β-1,3 GF showed lower cumulative mortality than those fed a normal basal diet. The survival rate was clearly higher for shrimp fed the diet with 1.5 g kg^{-1} β-1,3 GF. Our results are consistent with previous studies, where herb administration enhanced the immunity of *L. vannamei* after one week of dietary inclusion [83,84]. Based on our findings, β-1,3 GF therapy may be a suitable alternative strategy for controlling fungal infection in the shrimp culture system.

5. Conclusions

Supplementation with β-1,3 GF has a pronounced effect on the immune response of Pacific white shrimp, *Litopenaeus vannamei*, through the enhancement of total hemocytes and immune-related enzymes. The favorable effect of these compounds depends mainly on their content, dosage, and days of supplementation. Lastly, dietary inclusion of β-1,3 GF at an optimum level (1.5 g/kg diet) in commercial shrimp farms is recommended as it enhances the growth parameters and immune responses, and increases shrimp resistance to invading fungi, especially *F. solani*. In this study, our results showed an improvement in the growth, immunity, and *Fusarium* resistance of shrimp following supplementation with β-1,3 glucan at concentrations of 1–1.5 g/kg diet. However, the impact of supplementing with β-1,3 glucan over 1.5 g/kg diet is still not fully elucidated, and requires further investigations.

Author Contributions: E.-S.H.E., conceptualization, methodology, data curation, and investigation; R.A.A., conceptualization, methodology, and investigation; N.A.A.E., conceptualization, methodology, and investigation; A.E., conceptualization, methodology, and investigation; S.S., conceptualization, methodology, and investigation; N.H.A., conceptualization, methodology, and investigation; S.E.-S.S., conceptualization, methodology, and investigation; G.B.D. data curation, review, and editing; C.P.S.T., data curation, review, and editing; A.A.C.A., data curation, review, and editing; M.M., data curation, writing—original draft, review, and editing; H.F.A., conceptualization, methodology, data curation, investigation, supervision, writing—original draft, review, and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Animal Ethics Review Committee of Suez Canal University (AERC-SCU), Egypt, with approved No. 2022037 on 9 November 2022).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon request.

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

References

1. Idenyi, J.N.; Eya, J.C.; Nwankwegu, A.S.; Nwoba, E.G. Aquaculture sustainability through alternative dietary ingredients: Microalgal value-added products. *Eng. Microbiol.* **2022**, *2*, 100049. [CrossRef]
2. Mohan, K.; Rajan, D.K.; Muralisankar, T.; Ganesan, A.R.; Sathishkumar, P.; Revathi, N. Use of black soldier fly (*Hermetia illucens* L.) larvae meal in aquafeeds for a sustainable aquaculture industry: A review of past and future needs. *Aquaculture* **2022**, *553*, 738095. [CrossRef]
3. Food and Agriculture Organization of the United Nations. *The State of World Fisheries and Aquaculture 2016*; Contributing to food security and nutrition for all; FAO: Rome, Italy, 2016.
4. Islam, T.; Hossain, M.I.; Alam, M.M.; Khalil, S.M.I.; Rahman, M.M.; Abdullah-Al-Mamun, M. Status of shrimp diseases and their management practices at Satkhira in Bangladesh. *J. Entomol. Zool. Stud.* **2020**, *8*, 1017–1026.

5. Luo, K.; Tian, X.; Wang, B.; Wei, C.; Wang, L.; Zhang, S.; Liu, Y.; Li, T.; Dong, S. Evaluation of paraprobiotic applicability of Clostridium butyricum CBG01 in improving the growth performance, immune responses and disease resistance in Pacific white shrimp, Penaeus vannamei. *Aquaculture* **2021**, *544*, 737041. [CrossRef]
6. Zou, Y.; Xie, G.; Jia, T.; Xu, T.; Wang, C.; Wan, X.; Li, Y.; Luo, K.; Bian, X.; Wang, X. Determination of the infectious agent of translucent post-larva disease (TPD) in Penaeus vannamei. *Pathogens* **2020**, *9*, 741. [CrossRef]
7. Yao, L.; Wang, C.; Li, G.; Xie, G.; Jia, Y.; Wang, W.; Liu, S.; Xu, T.; Luo, K.; Zhang, Q. Identification of Fusarium solani as a causal agent of black spot disease (BSD) of Pacific white shrimp, Penaeus vannamei. *Aquaculture* **2022**, *548*, 737602. [CrossRef]
8. Palmero, D.; Iglesias, C.; De Cara, M.; Lomas, T.; Santos, M.; Tello, J. Species of Fusarium isolated from river and sea water of southeastern Spain and pathogenicity on four plant species. *Plant Dis.* **2009**, *93*, 377–385. [CrossRef] [PubMed]
9. Ramesh, T.; Yamunadevi, R.; Sundaramanickam, A.; Thangaraj, M.; Kumaran, R.; Annadurai, D. Biodiversity of the fungi in extreme marine environments. In *Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nano-Technology*; Elsevier: Amsterdam, The Netherlands, 2021; pp. 75–100.
10. Figueroa, M.; Hammond-Kosack, K.E.; Solomon, P.S. A review of wheat diseases—A field perspective. *Mol. Plant Pathol.* **2018**, *19*, 1523–1536. [CrossRef] [PubMed]
11. Summerell, B.A. Resolving Fusarium: Current status of the genus. *Annu. Rev. Phytopathol.* **2019**, *57*, 323–339. [CrossRef]
12. Hatai, K.; Egusa, S. Studies on the Pathogenic Fungus Associated with Black Gill Disease of Kuruma Prawn, *Penaeus japonicus*-II Some of the Note on the BG-Fusarium. *Fish Pathol.* **1978**, *12*, 225–231. [CrossRef]
13. Colorni, A. Fusariosis in the shrimp Penaeus semisulcatus cultured in Israel. *Mycopathologia* **1989**, *108*, 145–147. [CrossRef] [PubMed]
14. Le, V.K.; Hatai, K.; Yuasa, A.; Sawada, K. Morphology and molecular phylogeny of Fusarium solani isolated from kuruma prawn *Penaeus japonicus* with black gills. *Fish Pathol.* **2005**, *40*, 103–109.
15. Rajeev, R.; Adithya, K.; Kiran, G.S.; Selvin, J. Healthy microbiome: A key to successful and sustainable shrimp aquaculture. *Rev. Aquac.* **2021**, *13*, 238–258. [CrossRef]
16. Braun, G.; Braun, M.; Kruse, J.; Amelung, W.; Renaud, F.G.; Khoi, C.M.; Duong, M.; Sebesvari, Z. Pesticides and antibiotics in permanent rice, alternating rice-shrimp and permanent shrimp systems of the coastal Mekong Delta, Vietnam. *Environ. Int.* **2019**, *127*, 442–451. [CrossRef] [PubMed]
17. Tinh, T.H.; Elayaraja, S.; Mabrok, M.; Gallantiswara, P.C.D.; Vuddhakul, V.; Rodkhum, C. Antibacterial spectrum of synthetic herbal-based polyphenols against Vibrio parahaemolyticus isolated from diseased Pacific whiteleg shrimp (*Penaeus vannamei*) in Thailand. *Aquaculture* **2021**, *533*, 736070. [CrossRef]
18. Algammal, A.M.; Mabrok, M.; Ezzat, M.; Alfifi, K.J.; Esawy, A.M.; Elmasry, N.; El-Tarabili, R.M. Prevalence, antimicrobial resistance (AMR) pattern, virulence determinant and AMR genes of emerging multi-drug resistant *Edwardsiella tarda* in Nile tilapia and African catfish. *Aquaculture* **2022**, *548*, 737643. [CrossRef]
19. Kewcharoen, W.; Srisapoome, P. Potential synbiotic effects of a Bacillus mixture and chitosan on growth, immune responses and VP (AHPND) resistance in Pacific white shrimp (Litopenaeus vannamei, Boone, 1931). *Fish Shellfish Immunol.* **2022**, *127*, 715–729. [CrossRef]
20. Thornber, K.; Verner-Jeffreys, D.; Hinchliffe, S.; Rahman, M.M.; Bass, D.; Tyler, C.R. Evaluating antimicrobial resistance in the global shrimp industry. *Rev. Aquac.* **2020**, *12*, 966–986. [CrossRef]
21. Okocha, R.C.; Olatoye, I.O.; Adedeji, O.B. Food safety impacts of antimicrobial use and their residues in aquaculture. *Public Health Rev.* **2018**, *39*, 21. [CrossRef]
22. Thitamadee, S.; Prachumwat, A.; Srisala, J.; Jaroenlak, P.; Salachan, P.V.; Sritunyalucksana, K.; Flegel, T.W.; Itsathitphaisarn, O. Review of current disease threats for cultivated penaeid shrimp in Asia. *Aquaculture* **2016**, *452*, 69–87. [CrossRef]
23. Ayoub, H.F.; Tohamy, E.Y.; Salama, H.M.; Mohamed, S.S. Citrullus colocynthis extract and synthesized Selenium nanoparticles enhance non-specific response and resistance against Aeromonas sobria in Nile tilapia (*Oreochromis niloticus*). *Aquac. Res.* **2021**, *52*, 4969–4982. [CrossRef]
24. Mabrok, M.A.E.; Wahdan, A. The immune modulatory effect of oregano (*Origanum vulgare* L.) essential oil on Tilapia zillii following intraperitoneal infection with Vibrio anguillarum. *Aquac. Int.* **2018**, *26*, 1147–1160. [CrossRef]
25. Vijayaram, S.; Sun, Y.-Z.; Zuorro, A.; Ghafarifarsani, H.; Van Doan, H.; Hoseinifar, S.H. Bioactive immunostimulants as health-promoting feed additives in aquaculture: A review. *Fish Shellfish Immunol.* **2022**, *130*, 294–308. [CrossRef]
26. Gibson, G.R.; Roberfroid, M.B. Dietary modulation of the human colonic microbiota: Introducing the concept of prebiotics. *J. Nutr.* **1995**, *125*, 1401–1412. [CrossRef] [PubMed]
27. Butt, U.D.; Lin, N.; Akhter, N.; Siddiqui, T.; Li, S.; Wu, B. Overview of the latest developments in the role of probiotics, prebiotics and synbiotics in shrimp aquaculture. *Fish Shellfish Immunol.* **2021**, *114*, 263–281. [CrossRef]
28. Ringø, E.; Olsen, R.; Gifstad, T.; Dalmo, R.; Amlund, H.; Hemre, G.I.; Bakke, A. Prebiotics in aquaculture: A review. *Aquac. Nutr.* **2010**, *16*, 117–136. [CrossRef]
29. Abdel-Latif, H.M.; Yilmaz, E.; Dawood, M.A.; Ringø, E.; Ahmadifar, E.; Yilmaz, S. Shrimp vibriosis and possible control measures using probiotics, postbiotics, prebiotics, and synbiotics: A review. *Aquaculture* **2022**, *551*, 737951. [CrossRef]
30. Chen, M.; Chen, X.-Q.; Tian, L.-X.; Liu, Y.-J.; Niu, J. Improvement of growth, intestinal short-chain fatty acids, non-specific immunity and ammonia resistance in Pacific white shrimp (*Litopenaeus vannamei*) fed dietary water-soluble chitosan and mixed probiotics. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2020**, *236*, 108791. [CrossRef] [PubMed]

31. Rodrigues, M.V.; Zanuzzo, F.S.; Koch, J.F.A.; de Oliveira, C.A.F.; Sima, P.; Vetvicka, V. Development of fish immunity and the role of β-glucan in immune responses. *Molecules* **2020**, *25*, 5378. [CrossRef]
32. Pooljun, C.; Jariyapong, P.; Wongtawan, T.; Hirono, I.; Wuthisuthimethavee, S. Effect of feeding different types of β-glucans derived from two marine diatoms (*Chaetoceros muelleri* and *Thalassiosira weissflogii*) on growth performance and immunity of banana shrimp (*Penaeus merguiensis*). *Fish Shellfish Immunol.* **2022**, *130*, 512–519. [CrossRef]
33. Yuan, X.; Xu, R.; Qi, Q.; Xu, M.; Li, B.; Wang, B.; Zhang, C. Dietary fructooligosaccharide and Bacillus licheniformis on growth performance, digestive enzyme, immune indices, and antioxidant capacity of common carp (*Cyprinus carpio*). *Reg. Stud. Mar. Sci.* **2022**, *56*, 102670. [CrossRef]
34. El-Dahhar, A.; El-Shazly, K. Effect of essential amino acids (methionine and lysine) and treated oil in fish diet on growth performance and feed utilization of Nile tilapia, *Tilapia nilotica* (L.). *Aquac. Res.* **1993**, *24*, 731–739. [CrossRef]
35. Association of Official Analytical Chemists. *Official Methods of Analysis: Changes in Official Methods of Analysis Made at the Annual Meeting*; AOAC: Rockville, MD, USA, 1990.
36. McDonald, P.; Edwards, R.A.; Greenhalgh, J.F.D. *Animal Nutrition*; CABI: Wallingford, UK, 1973.
37. Association, A.P.H.; Association, A.W.W. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 1995; p. 1000.
38. AOAC of Official Analytical Chemists. Coffee and tea. In *Official Methods of Analysis*, 17th ed.; AOAC: Gaithersburg, MD, USA, 2000.
39. Kiernan, J. *Histological and Histochemical Methods: Theory and Practice*, 4th ed.; Scion: Bloxham, UK, 2008.
40. Erlanger, B.F.; Kokowsky, N.; Cohen, W. The preparation and properties of two new chromogenic substrates of trypsin. *Arch. Biochem. Biophys.* **1961**, *95*, 271–278. [CrossRef]
41. Gamboa-Delgado, J.; Molina-Poveda, C.; Cahu, C. Digestive enzyme activity and food ingesta in juvenile shrimp *Litopenaeus vannamei* (Boone, 1931) as a function of body weight. *Aquac. Res.* **2003**, *34*, 1403–1411. [CrossRef]
42. McCord, J.M.; Fridovich, I. Superoxide dismutase: An enzymic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* **1969**, *244*, 6049–6055. [CrossRef]
43. Aebi, H. [13] Catalase in vitro. In *Methods in Enzymology*; Elsevier: Amsterdam, The Netherlands, 1984; Volume 105, pp. 121–126.
44. Paglia, D.E.; Valentine, W.N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* **1967**, *70*, 158–169. [PubMed]
45. Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* **1979**, *95*, 351–358. [CrossRef]
46. Engstad, R.E.; Robertsen, B.; Frivold, E. Yeast glucan induces increase in lysozyme and complement-mediated haemolytic activity in Atlantic salmon blood. *Fish Shellfish Immunol.* **1992**, *2*, 287–297. [CrossRef]
47. Rook, G.; Steele, J.; Umar, S.; Dockrell, H. A simple method for the solubilisation of reduced NBT, and its use as a colorimetric assay for activation of human macrophages by γ-interferon. *J. Immunol. Methods* **1985**, *82*, 161–167. [CrossRef]
48. Supamattaya, K.; Pongmaneerat, J.; Klowklieng, T. The effect of β–glucan (MacroGard®) on growth performance, immune response and disease resistance in black tiger shrimp, Penaeus monodon Fabricius. *Songklanakarin J. Sci. Technol.* **2000**, *22*, 677–688.
49. Goh, Y.J.; Klaenhammer, T.R. Genetic mechanisms of prebiotic oligosaccharide metabolism in probiotic microbes. *Annu. Rev. Food Sci. Technol.* **2015**, *6*, 137–156. [CrossRef]
50. Jana, U.K.; Suryawanshi, R.K.; Prajapati, B.P.; Kango, N. Prebiotic mannooligosaccharides: Synthesis, characterization and bioactive properties. *Food Chem.* **2021**, *342*, 128328. [CrossRef] [PubMed]
51. Boonanuntanasarn, S.; Wongsasak, U.; Pitaksong, T.; Chaijamrus, S. Effects of dietary supplementation with β-glucan and synbiotics on growth, haemolymph chemistry, and intestinal microbiota and morphology in the Pacific white shrimp. *Aquac. Nutr.* **2016**, *22*, 837–845. [CrossRef]
52. Li, H.; Xu, C.; Zhou, L.; Dong, Y.; Su, Y.; Wang, X.; Qin, J.G.; Chen, L.; Li, E. Beneficial effects of dietary β-glucan on growth and health status of Pacific white shrimp Litopenaeus vannamei at low salinity. *Fish Shellfish Immunol.* **2019**, *91*, 315–324. [CrossRef]
53. Dawood, M.A.; Koshio, S.; Ishikawa, M.; Yokoyama, S.; El Basuini, M.F.; Hossain, M.S.; Nhu, T.H.; Moss, A.S.; Dossou, S.; Wei, H. Dietary supplementation of β-glucan improves growth performance, the innate immune response and stress resistance of red sea bream, P agrus major. *Aquac. Nutr.* **2017**, *23*, 148–159. [CrossRef]
54. Pilarski, F.; de Oliveira, C.A.F.; de Souza, F.P.B.D.; Zanuzzo, F.S. Different β-glucans improve the growth performance and bacterial resistance in Nile tilapia. *Fish Shellfish Immunol.* **2017**, *70*, 25–29. [CrossRef]
55. Ismail, M.; Wahdan, A.; Yusuf, M.S.; Metwally, E.; Mabrok, M. Effect of dietary supplementation with a synbiotic (Lacto Forte) on growth performance, haematological and histological profiles, the innate immune response and resistance to bacterial disease in Oreochromis niloticus. *Aquac. Res.* **2019**, *50*, 2545–2562. [CrossRef]
56. Dalmo, R.A.; Bøgwald, J. ß-glucans as conductors of immune symphonies. *Fish Shellfish Immunol.* **2008**, *25*, 384–396. [CrossRef]
57. 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]
58. Huynh, T.-G.; Shiu, Y.-L.; Nguyen, T.-P.; Truong, Q.-P.; Chen, J.-C.; Liu, C.-H. Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: A review. *Fish Shellfish Immunol.* **2017**, *64*, 367–382. [CrossRef]

59. Mohammadian, T.; Nasirpour, M.; Tabandeh, M.R.; Mesbah, M. Synbiotic effects of β-glucan, mannan oligosaccharide and Lactobacillus casei on growth performance, intestine enzymes activities, immune-hematological parameters and immune-related gene expression in common carp, Cyprinus carpio: An experimental infection with Aeromonas hydrophila. *Aquaculture* **2019**, *511*, 634197.
60. Vogt, G. Synthesis of digestive enzymes, food processing, and nutrient absorption in decapod crustaceans: A comparison to the mammalian model of digestion. *Zoology* **2021**, *147*, 125945. [CrossRef]
61. Xu, B.; Zhang, G.; Wang, L.; Sagada, G.; Zhang, J.; Shao, Q. The influence of dietary β-1, 3-glucan on growth performance, feed utilization, antioxidative and immune status of Pacific white shrimp, Litopenaeus vannamei. *Aquac. Nutr.* **2021**, *27*, 1590–1601. [CrossRef]
62. Muttharasi, C.; Gayathri, V.; Muralisankar, T.; Mohan, K.; Uthayakumar, V.; Radhakrishnan, S.; Kumar, P.; Palanisamy, M. Growth performance, digestive enzymes and antioxidants activities in the shrimp Litopenaeus vannamei fed with Amphiroa fragilissima crude polysaccharides encapsulated Artemia nauplii. *Aquaculture* **2021**, *545*, 737263. [CrossRef]
63. Li, Y.; Liu, H.; Dai, X.; Li, J.; Ding, F. Effects of dietary inulin and mannan oligosaccharide on immune related genes expression and disease resistance of Pacific white shrimp, Litopenaeus vannamei. *Fish Shellfish Immunol.* **2018**, *76*, 78–92. [CrossRef] [PubMed]
64. Hoseinifar, S.H.; Yousefi, S.; Van Doan, H.; Ashouri, G.; Gioacchini, G.; Maradonna, F.; Carnevali, O. Oxidative stress and antioxidant defense in fish: The implications of probiotic, prebiotic, and synbiotics. *Rev. Fish. Sci. Aquac.* **2020**, *29*, 198–217. [CrossRef]
65. Livingstone, D. Contaminant-stimulated reactive oxygen species production and oxidative damage in aquatic organisms. *Mar. Pollut. Bull.* **2001**, *42*, 656–666. [CrossRef]
66. Kofuji, K.; Aoki, A.; Tsubaki, K.; Konishi, M.; Isobe, T.; Murata, Y. Antioxidant activity of β-glucan. *Int. Sch. Res. Not.* **2012**, *2012*, 125864. [CrossRef]
67. 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 merguiensis. *Fish Shellfish Immunol.* **2020**, *104*, 202–212. [CrossRef]
68. Magnadóttir, B. Innate immunity of fish (overview). *Fish Shellfish Immunol.* **2006**, *20*, 137–151. [CrossRef] [PubMed]
69. Saurabh, S.; Sahoo, P. Lysozyme: An important defence molecule of fish innate immune system. *Aquac. Res.* **2008**, *39*, 223–239. [CrossRef]
70. Miura, N.N.; Ohno, N.; Aketagawa, J.; Tamura, H.; Tanaka, S.; Yadomae, T. Blood clearance of (1→ 3)-β-D-glucan in MRL lpr/lpr mice. *FEMS Immunol. Med. Microbiol.* **1996**, *13*, 51–57. [CrossRef]
71. Burge, E.J.; Madigan, D.J.; Burnett, L.E.; Burnett, K.G. Lysozyme gene expression by hemocytes of Pacific white shrimp, Litopenaeus vannamei, after injection with Vibrio. *Fish Shellfish Immunol.* **2007**, *22*, 327–339. [CrossRef]
72. 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]
73. Wu, J.; Tian, S.; Luo, K.; Zhang, Y.; Pan, H.; Zhang, W.; Mai, K. Dietary recombinant human lysozyme improves the growth, intestinal health, immunity and disease resistance of Pacific white shrimp Litopenaeus vannamei. *Fish Shellfish Immunol.* **2022**, *121*, 39–52. [CrossRef] [PubMed]
74. Cook, M.T.; Hayball, P.J.; Hutchinson, W.; Nowak, B.F.; Hayball, J.D. Administration of a commercial immunostimulant preparation, EcoActiva™ as a feed supplement enhances macrophage respiratory burst and the growth rate of snapper (*Pagrus auratus*, Sparidae (Bloch and Schneider)) in winter. *Fish Shellfish Immunol.* **2003**, *14*, 333–345. [CrossRef]
75. Sahoo, P.; Kumari, J.; Mishra, B. Non-specific immune responses in juveniles of Indian major carps. *J. Appl. Ichthyol.* **2005**, *21*, 151–155. [CrossRef]
76. Yeh, R.-Y.; Shiu, Y.-L.; Shei, S.-C.; Cheng, S.-C.; Huang, S.-Y.; Lin, J.-C.; Liu, C.-H. Evaluation of the antibacterial activity of leaf and twig extracts of stout camphor tree, Cinnamomum kanehirae, and the effects on immunity and disease resistance of white shrimp, Litopenaeus vannamei. *Fish Shellfish Immunol.* **2009**, *27*, 26–32. [CrossRef] [PubMed]
77. Halim, A.M.; Lee, P.-P.; Chang, Z.-W.; Chang, C.-C. The hot-water extract of leaves of noni, Morinda citrifolia, promotes the immunocompetence of giant freshwater prawn, *Macrobrachium rosenbergii*. *Fish Shellfish Immunol.* **2017**, *64*, 457–468. [CrossRef]
78. Chang, C.-F.; Su, M.-S.; Chen, H.-Y. A rapid method to quantify total haemocyte count of Penaeus monodon using ATP analysis. *Fish Pathol.* **1999**, *34*, 211–212. [CrossRef]
79. Le Moullac, G.; Haffner, P. Environmental factors affecting immune responses in Crustacea. *Aquaculture* **2000**, *191*, 121–131. [CrossRef]
80. Subramanian, S.; Philip, R. Identification of haematological markers in shrimp health assessment from the immune profile of Fenneropenaeus indicus on β-1, 3-glucan administration and white spot syndrome virus challenge. *Aquac. Int.* **2013**, *21*, 1169–1184. [CrossRef]
81. Sajeevan, T.; Philip, R.; Singh, I.B. Immunostimulatory effect of a marine yeast Candida sake S165 in Fenneropenaeus indicus. *Aquaculture* **2006**, *257*, 150–155. [CrossRef]
82. Hose, J.; Lightner, D.; Redman, R.; Danald, D. Observations on the pathogenesis of the imperfect fungus, Fusarium solani, in the California brown shrimp, Penaeus californiensis. *J. Invertebr. Pathol.* **1984**, *44*, 292–303. [CrossRef]

83. Huang, H.-T.; Lee, P.-T.; Liao, Z.-H.; Chen, H.-Y.; Nan, F.-H. Effects of Phyllanthus amarus extract on nonspecific immune responses, growth, and resistance to Vibrio alginolyticus in white shrimp Litopenaeus vannamei. *Fish Shellfish Immunol.* **2020**, *107*, 1–8.
84. 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]