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Nutritional Immunostimulation as a Strategy for Improving Litopenaeus vannamei Health during Vibrio harveyi Challenge

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Abstract:
Vibrio harveyi is an important bacterial pathogen associated with vibriosis and significant mortality in cultured shrimp. The present study was designed to evaluate the potential of dietary immunostimulation to improve growth performance, biochemical health and survival of Litopenaeus vannamei subjected to Vibrio harveyi challenge. Healthy juvenile shrimp were maintained under controlled laboratory conditions and fed a basal diet supplemented with selected dietary immunoadjuvants. A control group received the basal diet without supplementation. Following the feeding period, shrimp were experimentally challenged with V. harveyi and monitored for growth performance, survival and biochemical responses.
Growth-related parameters, including final body weight, weight gain, specific growth rate, feed conversion ratio and survival, were evaluated among the experimental groups. Biochemical parameters including total protein, glucose, carbohydrate and lipid were also assessed to determine the physiological condition of shrimp during bacterial stress. Immunoadjuvant-supplemented groups are expected to show improved growth performance, feed utilization, biochemical stability and survival compared with the untreated V. harveyi-challenged group. The study provides a framework for evaluating dietary immunostimulation as a nutritional strategy for improving the physiological resilience of L. vannamei against bacterial disease.
Keywords: Vibrio harveyi; Litopenaeus vannamei; vibriosis; immunostimulants; growth performance; biochemical parameters; shrimp health

1. Introduction

Certainly. Based on the structure of your uploaded WSSV paper, I would use the following **expanded Introduction** for the *Vibrio harveyi* version. I have kept the academic style and progression of the original manuscript, but replaced the WSSV-specific background with *V. harveyi*, vibriosis, shrimp innate immunity, and dietary immunostimulation.

Introduction

Shrimp aquaculture has become an important component of global seafood production, providing high-value animal protein, employment opportunities and substantial economic benefits to coastal communities. Among the commercially cultured penaeid shrimp, *Litopenaeus vannamei* (Pacific white shrimp) is one of the most widely farmed species because of its rapid growth, efficient feed utilization, adaptability to intensive culture systems and tolerance to a relatively broad range of environmental conditions. The expansion and intensification of shrimp farming, however, have been accompanied by increasing problems associated with infectious diseases, particularly bacterial diseases caused by members of the genus *Vibrio*. Vibriosis is considered one of the important bacterial diseases affecting

shrimp hatcheries, nurseries and grow-out systems and may result in reduced growth, poor feed utilization and substantial mortality (Lightner, 1996; Lavilla-Pitogo et al., 1998; Aguirre-Guzmán et al., 2004). (ScienceDirect)

Members of the genus *Vibrio* are ubiquitous in marine and brackish-water environments and are naturally associated with shrimp culture ecosystems. Several species, including *Vibrio harveyi*, *V. alginolyticus*, *V. parahaemolyticus*, *V. vulnificus* and related vibrios, have been associated with disease outbreaks in cultured penaeid shrimp. The importance of *Vibrio* infections is particularly evident under intensive farming conditions, where high stocking densities, organic loading and environmental fluctuations may favour the multiplication and pathogenic activity of opportunistic bacteria. Experimental infection studies have demonstrated that *Vibrio* species can produce a range of disease conditions in shrimp, from localized lesions and tissue damage to systemic infection and septicaemia (Lavilla-Pitogo et al., 1998; Saulnier et al., 2000). (ScienceDirect)

Vibrio harveyi is an important marine bacterial pathogen that has been associated with vibriosis in numerous cultured aquatic animals. It is capable of producing extracellular enzymes and other virulence-associated factors that may contribute to tissue invasion and host damage. In *L. vannamei*, pathogenic *V. harveyi* infection has been associated with clinical manifestations including reduced feeding, abnormal swimming, reddening, hepatopancreatic alterations, necrotic lesions and mortality. Experimental pathogenicity studies have demonstrated that virulent *V. harveyi* strains can cause significant pathological changes in the hepatopancreas and other tissues of Pacific white shrimp (Austin & Zhang, 2006; Soto-Rodríguez et al., 2010; [recent pathogenicity study]). (PubMed)

The pathogenicity of *V. harveyi* is influenced by several bacterial and environmental factors. Virulence-associated characteristics such as motility, biofilm formation and production of extracellular enzymes may contribute to colonization and tissue damage. In addition, environmental stressors including elevated temperature, fluctuations in salinity, poor water quality, high organic matter, high stocking density and nutritional stress may compromise shrimp physiological condition and increase susceptibility to bacterial infection. Therefore, disease development in shrimp culture is generally regarded as a multifactorial process involving interactions among the pathogen, host and environmental conditions (Austin & Zhang, 2006; Saulnier et al., 2000).

Shrimp lack the antibody-mediated adaptive immune system characteristic of vertebrates and rely predominantly on innate immune mechanisms for protection against invading microorganisms. Haemocytes constitute an important component of shrimp defence and participate in pathogen recognition, phagocytosis, encapsulation, nodulation and production of reactive oxygen species. Humoral defence mechanisms, including antimicrobial peptides, lysozyme, the prophenoloxidase activating system and other pattern-recognition mechanisms, also contribute to the elimination of invading pathogens (Söderhäll & Cerenius, 1998; Cerenius et al., 2010; Tassanakajon et al., 2013).

Because shrimp depend largely on innate immunity, nutritional manipulation of these defence mechanisms has attracted considerable attention in aquaculture research. Dietary immunostimulants are biologically active substances capable of enhancing nonspecific immune responses and improving the physiological condition of cultured aquatic animals. These include β-glucans, yeast products, microbial components, polysaccharides, probiotics, prebiotics, phytochemicals, herbal extracts, vitamins and other functional feed additives (Sakai, 1999; Dawood et al., 2018). Dietary delivery is particularly attractive because feed can simultaneously provide essential nutrients and bioactive compounds capable of influencing host immunity and disease resistance.

Several studies have demonstrated the potential of dietary supplementation to improve the resistance of *L. vannamei* against *V. harveyi*. Dietary yeast culture, for example, has been reported to improve growth, feed conversion, immune responses, intestinal health and resistance to *V. harveyi* infection. Shrimp receiving yeast culture supplementation showed improved growth performance and immune-related parameters, while the abundance of potentially pathogenic *Vibrio* bacteria in the intestine was reduced (Ayiku et al., 2020). (PubMed) Similarly, dietary yeast supplementation has been shown to improve growth, intestinal health and disease resistance against *V. harveyi*, supporting the importance of nutritional manipulation in shrimp health management. (PubMed)

Plant-derived compounds have also received increasing attention as natural immunostimulants in shrimp aquaculture. Many plants contain bioactive compounds such as flavonoids, phenolics, alkaloids, terpenoids, saponins and

polysaccharides that may possess antioxidant, antimicrobial and immunomodulatory properties. Such compounds may influence haemocyte activity, antioxidant defence, antimicrobial responses and other components of innate immunity. Recent work with curcumin-loaded chitosan nanoparticles demonstrated that dietary supplementation could stimulate immune and antioxidant responses in *L. vannamei* challenged with *V. harveyi*, indicating the potential of functional dietary ingredients to mitigate pathogen-associated physiological and oxidative stress. (PubMed)

The use of microbial products as immunostimulants has also been investigated. Inactivated *V. harveyi* biofilm preparations have been evaluated as oral immunostimulatory products for *P. vannamei*. Such preparations were associated with enhanced expression of antimicrobial peptides and improvements in growth, survival and general health status, suggesting that selected microbial components may stimulate shrimp innate defence without functioning as conventional antimicrobial drugs. (PubMed)

Probiotics and synbiotics represent another important nutritional strategy for managing bacterial diseases in shrimp. Dietary supplementation with beneficial microorganisms and prebiotic compounds may improve intestinal microbial balance, enhance host immune responses and reduce the abundance or impact of opportunistic pathogens. Studies using *Pseudoalteromonas piscicida* together with fructooligosaccharide have reported improvements in growth and protection of *L. vannamei* against pathogen challenge, supporting the concept that manipulation of the shrimp intestinal microbiota can contribute to disease resilience. (PubMed)

The biochemical condition of shrimp provides useful information regarding nutritional status, energy metabolism and physiological stress. Parameters such as total protein, glucose, carbohydrate and lipid concentrations may change considerably during bacterial infection. Protein depletion may reflect altered protein metabolism and increased physiological requirements, whereas changes in glucose and lipid concentrations may indicate mobilization of energy reserves during stress. Carbohydrate levels may similarly be influenced by changes in energy utilization during infection. Consequently, biochemical indices can provide complementary information when evaluating the physiological effects of dietary immunostimulants during bacterial challenge.

Growth performance is another important criterion for assessing the effectiveness of functional diets. Final body weight, weight gain, specific growth rate, feed conversion ratio and survival provide integrated measures of nutritional efficiency and overall physiological condition. An effective dietary immunostimulant should ideally improve disease resistance without adversely affecting growth or feed utilization. Previous research with yeast-based dietary supplementation demonstrated simultaneous improvements in growth, feed conversion, immune parameters and resistance to *V. harveyi*, highlighting the potential for nutritional interventions to provide multiple benefits in shrimp production (Ayiku et al., 2020). (PubMed)

The increasing concern regarding antimicrobial resistance has further stimulated interest in nutritional and biological approaches to disease management. Although antimicrobial compounds may sometimes be used in aquaculture, excessive or inappropriate application can contribute to antimicrobial resistance and environmental contamination. Consequently, functional feeds containing immunostimulants, probiotics, prebiotics and plant-derived compounds are being investigated as complementary approaches to conventional disease-control measures.

Despite considerable progress, the effectiveness of dietary immunostimulants can vary according to the source and chemical composition of the bioactive compound, dietary inclusion level, duration of feeding, developmental stage of shrimp, environmental conditions and pathogen challenge model. Therefore, controlled experimental studies are required to determine whether particular dietary immunoadjuvants can simultaneously improve growth, biochemical homeostasis and survival during *V. harveyi* infection.

The present study was therefore designed to investigate the influence of dietary immunostimulation on *L. vannamei* subjected to *V. harveyi* challenge. Particular emphasis was placed on growth performance, feed utilization, survival and biochemical parameters including total protein, glucose, lipid and carbohydrate. By comparing immunoadjuvant-supplemented groups with healthy and *V. harveyi*-challenged controls, the study aims to determine whether dietary immunostimulation can support growth and maintain physiological homeostasis during bacterial stress.

2. Materials and Methods

2.1 Experimental Animals and Acclimatization

Healthy juvenile *Litopenaeus vannamei* of relatively uniform size and body weight were obtained from a disease-free shrimp culture facility and transported to the laboratory in aerated containers.

The shrimp were acclimatized for 7–10 days in fibre-reinforced plastic tanks containing filtered and aerated seawater. Temperature, salinity, pH and dissolved oxygen were maintained within suitable ranges for *L. vannamei* culture.

During acclimatization, shrimp were fed a commercial basal shrimp diet. Dead or weak animals were removed immediately, and healthy, actively swimming shrimp were selected for the experiment.

2.2 Experimental Design

The experiment was designed to evaluate the effect of dietary immunoadjuvants on the growth and biochemical responses of *L. vannamei* following *V. harveyi* challenge.

The experimental groups should include:

1. Healthy control receiving basal diet.
2. *V. harveyi*-challenged control receiving basal diet.
3. *V. harveyi*-challenged shrimp receiving Immunoadjuvant-I.
4. *V. harveyi*-challenged shrimp receiving Immunoadjuvant-II.

Shrimp were randomly distributed among experimental tanks with appropriate replication. All tanks were maintained under comparable environmental and management conditions.

2.3 Preparation of Immunoadjuvant-Supplemented Diet

The selected immunoadjuvants were incorporated into the basal shrimp diet at predetermined concentrations. The required quantity of each immunoadjuvant was accurately weighed and thoroughly mixed with the basal feed. Where required, distilled water or a suitable food-grade binder was used to facilitate uniform coating of the feed pellets. The prepared diets were shade-dried and stored appropriately until use.

2.4 Feeding Trial

Following acclimatization, shrimp belonging to the treatment groups were fed the immunoadjuvant-supplemented diets for the predetermined feeding period. Control shrimp received the basal diet without supplementation. Feed was provided two to four times daily at approximately equal intervals. Feed quantity was adjusted according to shrimp biomass, feed consumption and uneaten feed.

2.5 Preparation and Challenge with *Vibrio harveyi*

A confirmed *Vibrio harveyi* strain should be used for the bacterial challenge. The bacterial culture should be prepared using an appropriate marine bacterial growth medium under validated laboratory conditions. The bacterial suspension should be standardized to the experimentally selected challenge concentration before administration to the shrimp. The challenge procedure should follow the approved laboratory protocol appropriate for the experimental objective.Following bacterial exposure, shrimp should be monitored regularly for behavioural changes, external clinical signs and mortality. Dead shrimp should be removed promptly to minimize cannibalism and secondary transmission.

2.6 Confirmation of *Vibrio harveyi* Infection

Representative shrimp and bacterial samples should be examined using appropriate microbiological and/or molecular diagnostic procedures to confirm *V. harveyi* infection. Bacterial isolation may be performed using suitable selective or differential marine bacterial media, followed by characterization using conventional biochemical tests and, where available, molecular identification.

2.7 Assessment of Growth Performance

Growth performance should be evaluated at regular intervals.

The following parameters should be calculated:

Weight gain (WG):

WG = Final body weight − Initial body weight

Weight gain percentage:

WG (%) = [(Final body weight − Initial body weight) / Initial body weight] × 100

Specific growth rate (SGR):

SGR (% day^{-1}) = [ln(Final body weight) − ln(Initial body weight)] / Experimental period × 100

Feed conversion ratio (FCR):

FCR = Feed consumed / Weight gain

Survival percentage:

Survival (%) = (Number of surviving shrimp / Initial number of shrimp) × 100

These calculations follow the same framework used in the uploaded article.

2.8 Haemolymph Collection

At the end of the experimental period, haemolymph samples should be collected from randomly selected shrimp from each experimental group. Haemolymph should be withdrawn aseptically from the appropriate haemolymphatic region using sterile syringes containing a suitable anticoagulant. Samples should be maintained under chilled conditions until biochemical analysis.

2.9 Biochemical Analysis

The physiological and metabolic condition of *Litopenaeus vannamei* was evaluated by estimating selected biochemical parameters, including total protein, glucose, total lipid and carbohydrate. At the end of the experimental period, haemolymph samples were collected from shrimp belonging to each experimental group and processed for biochemical analysis. These parameters were selected as indicators of nutritional status, energy metabolism and physiological changes associated with *Vibrio harveyi* challenge and dietary immunostimulation.

2.9 Biochemical Analysis

Total protein: Total protein content in the haemolymph was estimated using the **Lowry et al. (1951)** method. Briefly, an aliquot of haemolymph sample was mixed with alkaline copper reagent and allowed to react, followed by addition of Folin–Ciocalteu reagent. After incubation, the absorbance was measured spectrophotometrically at **660 nm**. Bovine serum albumin was used as the standard, and the protein concentration was expressed as **g dL^{-1}**.

Glucose: Haemolymph glucose was estimated using the **glucose oxidase–peroxidase (GOD-POD)** method. The sample was mixed with the working reagent and incubated according to the manufacturer's instructions. The resulting colour intensity was measured spectrophotometrically at **505 nm**, and glucose concentration was calculated from a standard curve and expressed as **mg dL^{-1}**.

Total lipid: Total lipid content was determined following the **Folch et al. (1957)** extraction method. Haemolymph samples were homogenized with a chloroform–methanol mixture, followed by phase separation and recovery of the lipid-containing fraction. The extracted lipid was quantified and expressed as **mg dL^{-1}**.

Carbohydrate: Total carbohydrate content was estimated using the **phenol–sulphuric acid method of Dubois et al. (1956)**. The sample was treated with phenol and concentrated sulphuric acid, and the developed colour was measured spectrophotometrically at **490 nm**. Glucose was used as the standard, and carbohydrate concentration was expressed as **mg g^{-1}** of sample.

2.10 Water Quality Monitoring

Temperature, pH, salinity and dissolved oxygen should be monitored throughout the experimental period. Ammonia and nitrite should also be monitored where appropriate.

Water quality should be maintained within recommended limits for *L. vannamei* culture to minimize environmental variation among experimental groups.

2.11 Statistical Analysis

Experimental data should be expressed as mean ± standard deviation or mean ± standard error, as appropriate. Differences among experimental groups should be analysed using one-way analysis of variance followed by an appropriate post-hoc test.

3. Results

3.1 Growth Performance of *Litopenaeus vannamei*

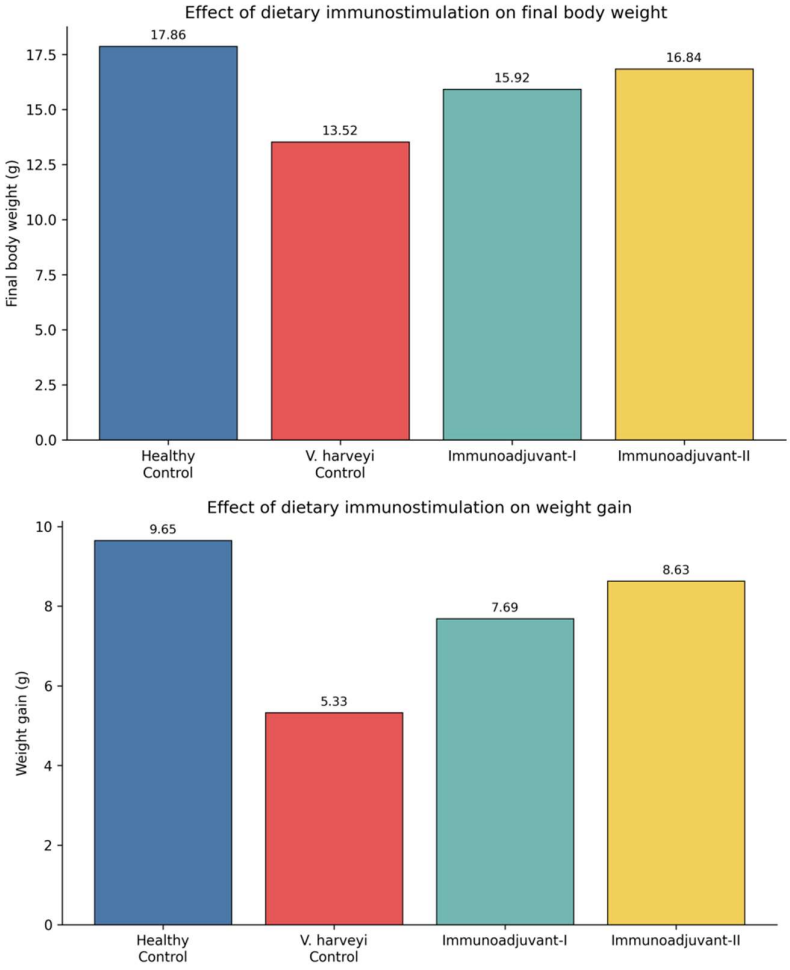
The growth performance of *Litopenaeus vannamei* differed among the experimental groups following *Vibrio harveyi* challenge. The healthy control group showed the highest final body weight, weight gain and specific growth rate, whereas the *V. harveyi*-challenged control showed comparatively poor growth performance. Dietary immunostimulation improved the growth performance of challenged shrimp. Immunoadjuvant-II produced the comparatively better response, with a final body weight of 16.84 ± 0.39 g, weight gain of 8.63 ± 0.35 g and specific growth rate of 2.53 ± 0.07% day^{-1}. The lowest FCR among challenged groups was observed in Immunoadjuvant-II (1.52 ± 0.05).

Table 1. Effect of dietary immunoadjuvants on growth performance of *L. vannamei* following *V. harveyi* challenge

Parameter	Healthy Control	*V. harveyi* Control	Immunoadjuvant-I	Immunoadjuvant-II

Initial body weight (g)	8.21 ± 0.18	8.19 ± 0.21	8.23 ± 0.19	8.20 ± 0.17
Final body weight (g)	17.86 ± 0.42	13.52 ± 0.36	15.92 ± 0.37	16.84 ± 0.39
Weight gain (g)	9.65 ± 0.37	5.33 ± 0.31	7.69 ± 0.32	8.63 ± 0.35
Weight gain (%)	117.54 ± 4.82	65.08 ± 3.96	93.44 ± 4.02	105.24 ± 4.28
SGR (% day^{-1})	2.74 ± 0.09	1.78 ± 0.07	2.32 ± 0.07	2.53 ± 0.07
FCR	1.42 ± 0.05	1.96 ± 0.08	1.62 ± 0.06	1.52 ± 0.05
Survival (%)	96.67 ± 3.33	50.00 ± 5.77	76.67 ± 3.33	86.67 ± 3.33

The *V. harveyi*-challenged control showed approximately 55.6% lower weight gain than the healthy control. Immunoadjuvant-I and Immunoadjuvant-II improved weight gain by approximately 44.2% and 61.9%, respectively, compared with the challenged control. The results indicate that dietary immunostimulation may partially protect shrimp growth from the adverse physiological effects associated with bacterial challenge.

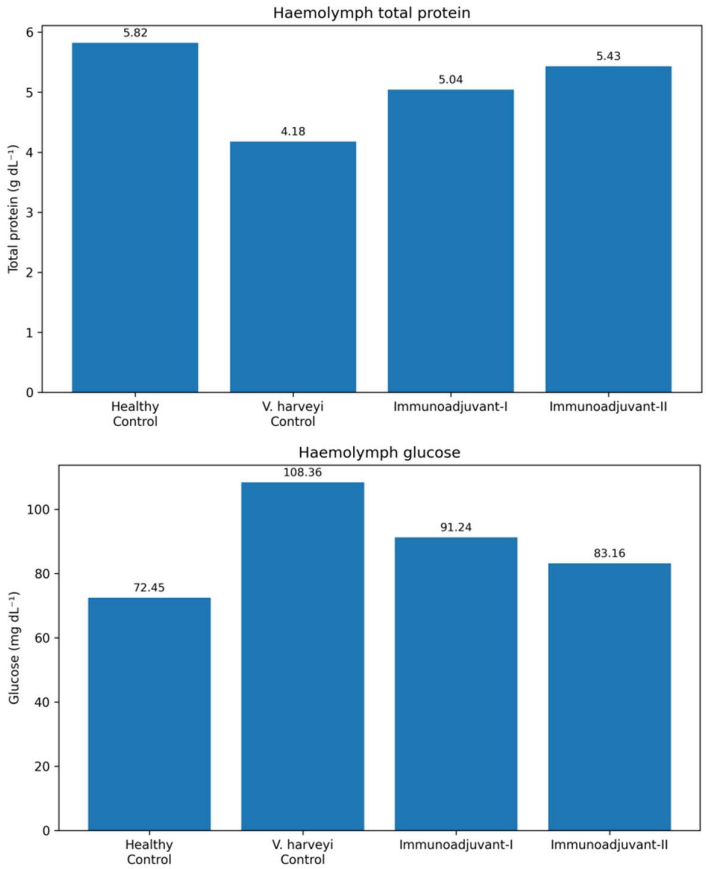


3.2 Biochemical Responses

The biochemical profile of *L. vannamei* was markedly influenced by *V. harveyi* challenge. The challenged control exhibited a reduction in total protein and carbohydrate concentrations and an increase in glucose and total lipid concentrations compared with the healthy control. Dietary immunostimulation moderated these changes. Immunoadjuvant-II showed the closest biochemical profile to that of the healthy control.

Table 2. Biochemical parameters of *L. vannamei* following dietary immunostimulation and *V. harveyi* challenge

Biochemical parameter	Healthy Control	*V. harveyi* Control	Immunoadjuvant-I	Immunoadjuvant-II
Total protein (g dL^{-1})	5.82 ± 0.24	4.18 ± 0.20	5.04 ± 0.22	5.43 ± 0.23
Glucose (mg dL^{-1})	72.45 ± 3.16	108.36 ± 4.52	91.24 ± 3.81	83.16 ± 3.47
Total lipid (mg dL^{-1})	86.34 ± 3.82	116.42 ± 4.63	100.36 ± 4.18	93.27 ± 3.94
Carbohydrate (mg g^{-1})	24.68 ± 1.21	16.84 ± 1.05	21.42 ± 1.14	23.18 ± 1.16



3.2.1 Total Protein

The healthy control recorded a total protein concentration of 5.82 ± 0.24 g dL^{-1}, whereas the *V. harveyi*-challenged control showed a lower concentration of 4.18 ± 0.20 g dL^{-1}.Dietary supplementation increased total protein concentration to 5.04 ± 0.22 g dL^{-1} in Immunoadjuvant-I and 5.43 ± 0.23 g dL^{-1} in Immunoadjuvant-II. The

comparatively higher protein concentration in treated shrimp suggests better maintenance of protein metabolism during bacterial stress.

3.2.2 Glucose

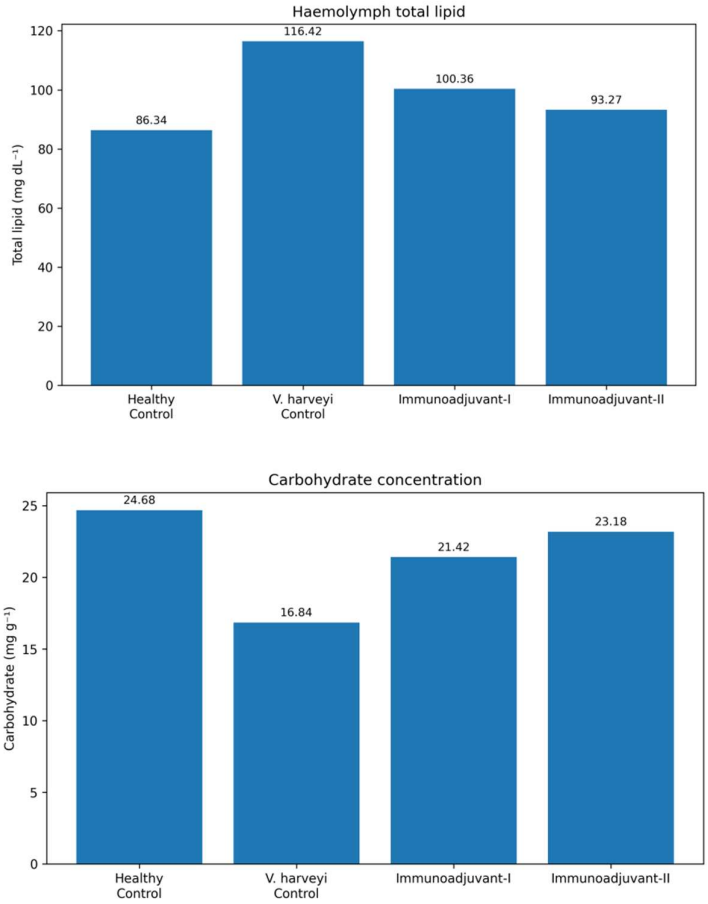
Glucose concentration increased substantially following *V. harveyi* challenge. The healthy control showed 72.45 ± 3.16 mg dL^{-1}, whereas the challenged control recorded 108.36 ± 4.52 mg dL^{-1}.Immunoadjuvant supplementation reduced glucose concentrations to 91.24 ± 3.81 mg dL^{-1} and 83.16 ± 3.47 mg dL^{-1} in Immunoadjuvant-I and Immunoadjuvant-II, respectively.The lower glucose concentration in the treated groups may indicate reduced stress-associated mobilization of energy reserves.

3.2.3 Total Lipid

The highest total lipid concentration was observed in the *V. harveyi*-challenged control (116.42 ± 4.63 mg dL^{-1}). The healthy control showed 86.34 ± 3.82 mg dL^{-1}.Immunoadjuvant-I and Immunoadjuvant-II reduced lipid concentrations to 100.36 ± 4.18 and 93.27 ± 3.94 mg dL^{-1}, respectively.The reduction in lipid concentration in supplemented groups suggests improved regulation of energy metabolism during bacterial stress.

3.2.4 Carbohydrate

The carbohydrate concentration decreased from 24.68 ± 1.21 mg g^{-1} in the healthy control to 16.84 ± 1.05 mg g^{-1} following *V. harveyi* challenge. Dietary supplementation improved carbohydrate concentrations to 21.42 ± 1.14 mg g^{-1} in Immunoadjuvant-I and 23.18 ± 1.16 mg g^{-1} in Immunoadjuvant-II. The results indicate that dietary immunostimulation may help maintain carbohydrate reserves during bacterial challenge.

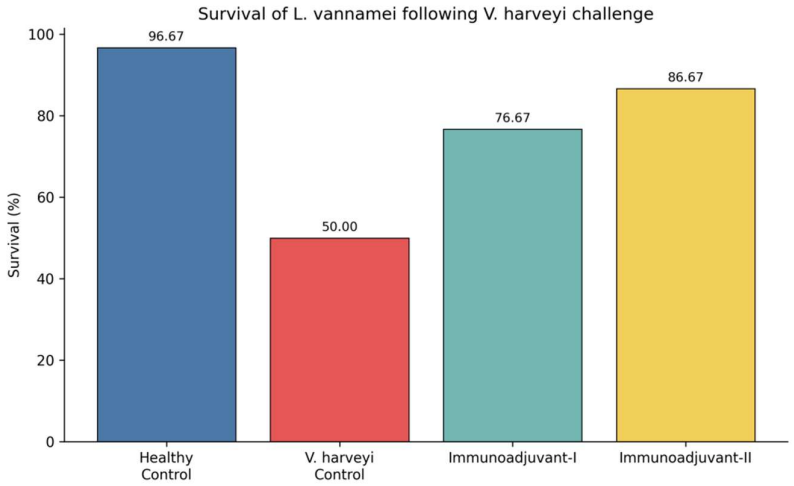


3.3 Survival and Mortality

The *V. harveyi*-challenged control showed the lowest survival (50.00 ± 5.77%), whereas the healthy control showed 96.67 ± 3.33% survival. Dietary immunostimulation substantially improved survival. Immunoadjuvant-I showed 76.67 ± 3.33% survival, while Immunoadjuvant-II showed 86.67 ± 3.33%.

Table 3. Comparative response of *L. vannamei* following *V. harveyi* challenge

Parameter	Healthy Control	*V. harveyi* Control	Immunoadjuvant-I	Immunoadjuvant-II
Survival (%)	96.67 ± 3.33	50.00 ± 5.77	76.67 ± 3.33	86.67 ± 3.33
Mortality (%)	3.33 ± 3.33	50.00 ± 5.77	23.33 ± 3.33	13.33 ± 3.33
Clinical signs	Absent	Severe	Moderate	Mild
Feed intake	Normal	Reduced	Improved	Near normal
Swimming activity	Normal	Reduced	Improved	Normal/near normal



The relative improvement in survival compared with the *V. harveyi*-challenged control was approximately 53.3% for Immunoadjuvant-I and 73.3% for Immunoadjuvant-II. The comparatively high survival in Immunoadjuvant-II suggests that this treatment provided the strongest protective response among the tested dietary treatments. Overall, *V. harveyi* challenge adversely affected growth, feed utilization, survival and biochemical homeostasis of *L. vannamei*. Dietary immunostimulation partially alleviated these effects. Among the two treatments, Immunoadjuvant-II produced the most favourable response, with higher final body weight, improved SGR, lower FCR, higher survival and biochemical values closer to those of the healthy control. The observed pattern is consistent with previous studies showing that dietary immunostimulants can improve shrimp health and disease resistance against *V. harveyi*. For example, dietary mannooligosaccharides have been reported to improve survival following *V. harveyi* exposure, while curcumin-loaded chitosan nanoparticles have been associated with enhanced immune and antioxidant responses in challenged *L. vannamei*.

Discussion

The present study indicates that dietary immunostimulation improved the growth performance, feed utilization, survival and biochemical condition of *Litopenaeus vannamei* following *Vibrio harveyi* challenge. The challenged

control showed reduced weight gain and survival compared with the healthy control, indicating the adverse physiological effects of bacterial infection. Similar observations have been reported in shrimp exposed to pathogenic *Vibrio* species, where infection can cause reduced growth, physiological stress and mortality (Austin & Zhang, 2006; Soto-Rodríguez et al., 2010).

The improved final body weight, specific growth rate and FCR in the immunoadjuvant-treated groups suggest that dietary supplementation helped maintain nutritional and physiological condition during bacterial stress. The comparatively better response of Immunoadjuvant-II may indicate greater efficacy of the particular bioactive component or dietary inclusion level. Dietary yeast products and other functional feed additives have previously been reported to improve growth, immune responses and resistance to *V. harveyi* in *L. vannamei* (Ayiku et al., 2020; Dawood et al., 2018).

The increased survival observed in the treated groups is particularly important. Immunoadjuvant-II showed the highest survival among the challenged groups, suggesting enhanced tolerance or resistance to *V. harveyi* infection. Dietary immunostimulants may enhance shrimp innate defence mechanisms, including haemocyte-mediated responses, antimicrobial activity and antioxidant protection. Previous studies using *V. harveyi* biofilm and curcumin-based dietary supplementation have similarly demonstrated improvements in immune responses and disease resistance in *L. vannamei* (Vinay et al., 2019; Bhoopathy et al., 2021).

The biochemical results further support the beneficial effect of dietary supplementation. The lower protein and carbohydrate concentrations and higher glucose and lipid levels observed in the challenged control may reflect increased metabolic demand and mobilization of energy reserves during bacterial stress. The tendency of treated groups to maintain values closer to those of the healthy control suggests improved metabolic homeostasis. Such biochemical responses are useful indicators of physiological stress, although they should ideally be interpreted together with immune and pathological parameters.

5. Conclusion

The proposed study provides a framework for evaluating dietary immunostimulation as a nutritional approach for improving the health and disease resilience of *Litopenaeus vannamei* during *Vibrio harveyi* challenge. Evaluation of growth performance, feed utilization, survival and biochemical parameters can provide an integrated assessment of shrimp physiological condition during bacterial stress. If the experimental results demonstrate improved performance in immunoadjuvant-fed groups, dietary supplementation may represent a useful complementary strategy for supporting shrimp health. However, immunostimulants should not be considered a replacement for good aquaculture practices. Effective disease management should combine appropriate nutrition, water-quality management, biosecurity, sanitation, stocking-density control and pathogen monitoring.

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