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Influence of Dietary Immunoadjuvants on Growth Performance and Haematological Parameters of Litopenaeus vannamei during WSSV Infection

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ABSTRACT

White spot syndrome virus (WSSV) is one of the most destructive viral pathogens affecting shrimp aquaculture and can cause severe mortality and considerable economic losses. Because effective therapeutic treatment for WSSV is unavailable, enhancement of the innate defence mechanisms of shrimp through dietary immunostimulants and immunoadjuvants represents an important alternative disease-management strategy. The present study evaluated the influence of dietary anti-WSSV egg-yolk immunoglobulin Y (IgY), produced with and without the herbal immunoadjuvant *Glycine max*, on growth performance and selected haematological parameters of the Pacific white shrimp *Litopenaeus vannamei* following WSSV challenge. Three herbal candidates, *Acalypha indica*, *Glycine max* and *Asparagus racemosus*, were initially screened, and *G. max* and *A. racemosus* showed better survival responses than *A. indica*. *G. max* was subsequently selected as the principal immunoadjuvant because of its phytochemical profile, particularly the presence of saponins. The experimental shrimp received diets containing control IgY, anti-WSSV IgY without immunoadjuvant (Ex-I), anti-WSSV IgY produced with 500 µg *G. max* (Ex-II), or anti-WSSV IgY produced with 1 ml *G. max* (Ex-III). After WSSV challenge, substantial improvements in weight gain and specific growth rate were observed in the experimental groups. At 25 days post-vaccination, weight gain increased from 1.6 g in the control IgY group to 2.4, 2.6 and 2.8 g in Ex-I, Ex-II and Ex-III, respectively. At 50 days, weight gain increased from 0.5 g in the control IgY group to 2.2, 2.9 and 4.7 g in the corresponding experimental groups. Haematological responses were also improved. Total haemocyte count, haemolymph coagulation and oxyhaemocyanin concentrations showed favourable changes in immunoadjuvant-treated shrimp. The Ex-III group exhibited the strongest overall response, with a total haemocyte count of 45.00 × 10^3 cells ml^{-1} at 25 days and 45.20 × 10^3 cells ml^{-1} at 50 days. Haemolymph coagulation time declined from 171.66 s in the control IgY group to 96.00 s in Ex-III at 25 days and from 167.00 to 89.66 s at 50 days. Oxyhaemocyanin also increased progressively in the experimental groups. The findings indicate that dietary anti-WSSV IgY combined with *G. max* immunoadjuvant can improve growth and strengthen important cellular and physiological defence parameters in WSSV-challenged *L. vannamei*. The results support the potential of herbal immunoadjuvant-based edible antibody diets as an alternative approach for sustainable shrimp health management.

Keywords: Litopenaeus vannamei; white spot syndrome virus; WSSV; immunoadjuvant; Glycine max; IgY; haemocytes; oxyhaemocyanin; growth performance; shrimp aquaculture

1. INTRODUCTION

Aquaculture has become an important component of global food production, employment generation and economic development. Shrimp farming occupies a particularly important position because penaeid shrimp are among the most valuable aquaculture commodities. The intensification of shrimp culture, however, has increased the risk of environmental stress and infectious disease. High stocking density, deterioration of water quality and frequent pathogen exposure can compromise shrimp health and facilitate disease outbreaks. The uploaded study notes that intensive aquaculture practices can favour pathogen transmission and mortality and that infectious diseases are major constraints to sustainable shrimp production.

Among shrimp pathogens, white spot syndrome virus is particularly important because of its high virulence and broad host range. WSSV has been reported from several cultured crustaceans, including *Penaeus monodon*, *Litopenaeus vannamei*, *L. stylirostris*, *Marsupenaeus japonicus*, *Fenneropenaeus chinensis*, *Macrobrachium rosenbergii* and *Procambarus clarkii*. The disease can produce extremely rapid mortality, with the uploaded source reporting that mortality may reach 100% within.

3–7 days after infection (Lightner, 1996). The economic consequences of WSSV outbreaks have historically been substantial in major shrimp-producing countries. The lack of a reliable therapeutic treatment for WSSV makes prevention and enhancement of host defence especially important. The source document states that there is no specific treatment for WSSV and identifies pathogen exclusion, biosecurity, PCR screening, controlled water exchange, disinfection and immunostimulant approaches as important preventive measures. Therefore, dietary approaches capable of strengthening shrimp innate immunity are increasingly attractive

Shrimp differ from vertebrates because they do not possess a classical adaptive immune system. Instead, their protection against invading microorganisms depends primarily on innate defence mechanisms involving circulating haemocytes and soluble haemolymph factors. Haemocytes participate in phagocytosis, encapsulation and nodulation, whereas humoral components include antimicrobial peptides, recognition molecules, clotting systems and the prophenoloxidase pathway. Consequently, total haemocyte count, haemolymph coagulation and oxyhaemocyanin are useful physiological indicators for assessing shrimp health and immune status. The use of medicinal plants as feed additives has attracted considerable interest in aquaculture. Plant-derived compounds such as saponins, flavonoids, tannins, alkaloids, terpenoids and polysaccharides have been associated with antimicrobial, antioxidant, immunostimulatory and growth-promoting effects. The uploaded source reports that medicinal plants can stimulate appetite, promote growth, enhance immune responses and provide antipathogen activity in fish and shrimp. Herbal immunostimulants are therefore considered potentially useful alternatives to excessive reliance on antibiotics and other chemotherapeutic agents.

Among plant-derived immunoadjuvants, *Glycine max* is of particular interest because soybean contains saponins, isoflavones, flavonoids, phenolic compounds, phytosterols and other biologically active constituents. The source identifies saponins and other phytochemicals in soybean and discusses their potential immunomodulatory properties. The source study detected saponins, tannins, alkaloids, glycosides and some flavonoid fractions in *G. max* extracts.

Egg-yolk IgY provides another promising approach for disease management in aquaculture. IgY can be obtained non-invasively from immunized laying hens and incorporated into edible preparations. The uploaded study describes the use of anti-WSSV IgY as an edible antibody and notes previous applications of IgY against aquatic pathogens. Earlier work cited in the source also reported that anti-*Vibrio harveyi* IgY-coated diets could reduce bacterial load and enhance immune responses in Indian white shrimp (*Fenneropenaeus indicus*) (Kumaran et al., 2018). The combination of an antigen-specific antibody and a plant-derived immunoadjuvant may provide complementary protection. The immunoadjuvant may enhance the production and effectiveness of antibody during immunization, whereas the resulting anti-WSSV IgY can be delivered orally to shrimp. The present study therefore examined whether dietary anti-WSSV IgY generated with *G. max* immunoadjuvant could improve growth and selected haematological parameters of *L. vannamei* following WSSV challenge.

2. MATERIALS AND METHODS

Experimental animals and acclimatization

Uniform-sized juvenile *L. vannamei* were selected from the available stock and transferred to experimental fibre tanks equipped with continuous flow-through water and aeration. Water temperature was maintained at approximately 26 ± 1°C and pH at 7.1 ± 0.2 during the culture period. Approximately 50% of the tank water was replaced daily with water of similar quality. The shrimp were acclimatized before experimental treatment. The experimental system consisted of four treatment conditions corresponding to control IgY, anti-WSSV IgY without immunoadjuvant, anti-WSSV IgY produced using 500 µg *G. max*, and anti-WSSV IgY produced using 1 ml *G. max*. The source describes distribution of shrimp in fibre-plastic tanks and maintenance of temperature and pH during the challenge experiment.

Selection of herbal immunoadjuvant

Three candidate medicinal plants, *Acalypha indica*, *Glycine max* and *Asparagus racemosus*, were initially screened. The preliminary screening showed survival of 12/20 shrimp for *A. indica*, corresponding to 40% mortality; *G. max* showed 20/20 survival with no mortality, whereas *A. racemosus* showed 18/20 survival with 10% mortality. Consequently, *G. max* and *A. racemosus* were selected for further consideration, with *G. max* subsequently used as the principal immunoadjuvant in the experimental protocol. The active constituents were extracted using hot water. Phytochemical screening of *G. max* revealed saponins, tannins, alkaloids, glycosides and flavonoid fractions. Thin-layer chromatographic analysis further detected saponin-related fractions in the soybean extract.

Production of anti-WSSV IgY

Laying hens were immunized using WSSV antigen with or without the selected herbal immunoadjuvant. Eggs were subsequently collected and egg-yolk antibodies were extracted. The experimental treatments were designated as follows:

- **Control IgY:** antibody obtained from non-immunized control egg yolk.
- **Ex-I:** anti-WSSV IgY produced without immunoadjuvant.
- **Ex-II:** anti-WSSV IgY produced with *G. max* at 500 µg.

- **Ex-III:** anti-WSSV IgY produced with *G. max* at 1 ml.

The treatment design is explicitly described in the source document. SDS-PAGE analysis demonstrated antibody-associated protein bands, with the source reporting an approximately 37-kDa band for IgY preparations.

Dietary administration

The shrimp were fed antibody-coated diets twice daily. The experimental animals received anti-WSSV IgY-coated feed, with the experimental groups receiving antibody produced without or with *G. max* immunoadjuvant. The source records feeding at 08:00 and 20:00 h at approximately 10% body weight, together with regular feed at midday, while control shrimp received uncoated feed according to the control schedule.

WSSV challenge

WSSV challenge was conducted at 25 and 50 days post-vaccination. The source reports intramuscular injection of 25 µl of semi-purified WSSV preparation containing approximately 300 µg protein. Blank control animals received phosphate-buffered saline. Mortality was monitored at four-hour intervals for 10 days after challenge, while additional animals were sampled for biochemical, haematological and immunological analyses.

Growth assessment

Growth performance was assessed by measuring initial and final body length and wet body weight. Weight gain was calculated from the difference between final and initial weight, while specific growth rate was calculated according to the experimental protocol. The source specifically reports weight gain and SGR at 25 and 50 days post-vaccination.

Haematological analysis

Haemolymph was collected from sampled shrimp after WSSV challenge. Three principal haematological parameters were evaluated:

1. Haemolymph coagulation time;
2. Total haemocyte count (THC);
3. Oxyhaemocyanin concentration.

These parameters were selected because haemocytes and haemolymph factors constitute major components of innate shrimp defence.

Statistical analysis

The source study used analysis of variance to determine differences among experimental treatments and sampling periods. Significant differences in growth and haematological parameters were reported at $P < 0.05$, while several survival and immunological measurements were analysed using one-way or two-way ANOVA with significance levels as indicated in the original experimental results.

3. RESULTS

Growth performance

Dietary anti-WSSV IgY supplementation produced a clear improvement in shrimp growth after WSSV challenge. At 25 days post-vaccination, the control IgY group showed a weight gain of 1.6 g. This increased significantly to 2.4 g in Ex-I, 2.6 g in Ex-II and 2.8 g in Ex-III ($P < 0.05$). The corresponding specific growth rates were 0.20%, 0.31%, 0.34% and 0.83% for the control, Ex-I, Ex-II and Ex-III groups, respectively.

At 50 days post-vaccination, the improvement was even more pronounced. Weight gain was only 0.5 g in the control IgY group but increased to 2.2, 2.9 and 4.7 g in Ex-I, Ex-II and Ex-III, respectively. The corresponding specific growth rates were 0.29%, 0.34%, 0.42% and 0.64%. The detailed growth table shows the progressive improvement associated with increasing immunoadjuvant treatment. The 50-day growth results demonstrate that the Ex-III diet containing anti-WSSV IgY produced with 1 ml *G. max* had the greatest weight gain. The final wet weight in Ex-III increased from 12.4 ± 0.12 g to 17.1 ± 0.57 g, corresponding to a weight gain of 4.7 ± 0.16 g.

Table 1. Growth performance of *Litopenaeus vannamei* fed anti-WSSV IgY-coated diets and challenged with WSSV at 25 days post-vaccination

Treatment	Initial length (cm)	Final length (cm)	Initial weight (g)	Final weight (g)	Weight gain (g)	Specific growth rate (%)
Control IgY	8.3 ± 0.18	10.4 ± 0.21	10.5 ± 0.24	12.1 ± 0.35	1.60 ± 0.12[a]	0.20 ± 0.00[a]
Ex-I	8.3 ± 0.12	10.7 ± 0.16	10.0 ± 0.21	12.4 ± 0.28	2.40 ± 0.08[b]	0.31 ± 0.12[b]

Ex-II	8.2 ± 0.29	11.6 ± 0.16	9.6 ± 0.14	12.2 ± 0.24	2.60 ± 0.16^{b}	0.34 ± 0.16^{b}
Ex-III	9.3 ± 0.47	11.0 ± 0.00	11.1 ± 0.23	13.9 ± 0.47	2.80 ± 0.40^{b}	0.83 ± 0.21^{c}

Table 2. Growth performance of *Litopenaeus vannamei* fed anti-WSSV IgY-coated diets and challenged with WSSV at 50 days post-vaccination

Treatment	Initial length (cm)	Final length (cm)	Initial weight (g)	Final weight (g)	Weight gain (g)	Specific growth rate (%)
Control IgY	12.8 ± 0.23	14.1 ± 0.62	14.3 ± 0.23	14.6 ± 0.23	0.50 ± 0.00^{a}	0.29 ± 0.009^{b}
Ex-I	12.4 ± 0.12	14.3 ± 0.12	12.2 ± 0.08	14.4 ± 0.16	2.20 ± 0.08^{a}	0.34 ± 0.08^{a}
Ex-II	11.5 ± 0.08	14.6 ± 0.16	12.4 ± 0.28	15.3 ± 0.216	2.90 ± 0.00^{b}	0.42 ± 0.16^{b}
Ex-III	12.4 ± 0.28	15.5 ± 0.29	12.4 ± 0.12	17.1 ± 0.57	4.70 ± 0.16^{c}	0.64 ± 0.08^{c}

Survival following WSSV challenge

The survival response also favoured the immunoadjuvant-containing diets. Following challenge at 25 days, Ex-I, Ex-II and Ex-III showed approximately 65%, 75% and 80% survival, respectively. The treatment and time effects were statistically significant.

Following the 50-day challenge, survival remained higher in the immunoadjuvant groups. By the seventh day after challenge, survival declined to approximately 60% in Ex-I, 75% in Ex-II and 85% in Ex-III. The control groups exhibited substantially greater mortality. These observations indicate that increasing the immunoadjuvant component was associated with improved protection following WSSV challenge.

Table 3. Survival of *Litopenaeus vannamei* following WSSV challenge

Treatment	Survival at 7 days after 25-dpv challenge (%)	Survival at 7 days after 50-dpv challenge (%)
Blank control	0	0
Control IgY	0	60
Ex-I	65	60
Ex-II	75	75
Ex-III	80	85

Haemolymph coagulation

Haemolymph coagulation time differed significantly among treatments. At 25 days post-vaccination, the blank control required 150.33 ± 3.39 s and the control IgY group required 171.66 ± 3.85 s for coagulation. Coagulation time decreased to 125.33 ± 3.09 s in Ex-I, 106.00 ± 1.68 s in Ex-II and 96.00 ± 0.81 s in Ex-III. At 50 days, the control IgY group showed a coagulation time of 167.00 ± 2.16 s, compared with 119.00 ± 0.81 s, 102.12 ± 1.14 s and 89.66 ± 1.69 s in Ex-I, Ex-II and Ex-III, respectively. Thus, the strongest reduction in clotting time was observed in Ex-III. The source interprets the faster coagulation as an indication of improved haemolymph defence and associates the response with reduced viral burden.

Table 4. Haemolymph coagulation time of *Litopenaeus vannamei* after WSSV challenge

Treatment	25 dpv challenge	50 dpv challenge
Blank control	150.33 ± 3.39^{a}	152.00 ± 4.32^{a}
Control IgY	171.66 ± 3.85^{b}	167.00 ± 2.16^{b}
Ex-I	125.33 ± 3.09^{c}	119.00 ± 0.81^{c}
Ex-II	106.00 ± 1.68^{d}	102.12 ± 1.14^{d}
Ex-III	96.00 ± 0.81^{e}	89.66 ± 1.69^{e}

Unit: seconds (s)

Total haemocyte count

Total haemocyte count showed a strong response to anti-WSSV IgY supplementation. At 25 days, the control group recorded 28.00 ± 0.81 × 10^3 cells ml^{-1}, whereas Ex-I, Ex-II and Ex-III recorded 35.66 ± 1.69, 42.12 ± 1.16 and 45.00 ± 1.44 × 10^3 cells ml^{-1}, respectively. At 50 days, the control group showed 28.33 ± 1.69 × 10^3 cells ml^{-1}, while Ex-I, Ex-II and Ex-III showed 38.66 ± 2.49, 43.16 ± 1.56 and 45.20 ± 0.81 × 10^3 cells ml^{-1}, respectively.

The increase in THC is biologically important because circulating haemocytes are central to shrimp cellular immunity. They participate in phagocytosis, encapsulation and nodular aggregation, and therefore increased haemocyte abundance may increase the capacity of the shrimp to respond to invading pathogens. The source also notes that haemocyte abundance can be affected by moulting, development, nutritional condition and disease.

Table 5. Total haemocyte count (THC) of *Litopenaeus vannamei* after WSSV challenge

Treatment	25 dpv challenge	50 dpv challenge
Control IgY	28.00 ± 0.81	28.33 ± 1.69
Ex-I	35.66 ± 1.69	38.66 ± 2.49
Ex-II	42.12 ± 1.16	43.16 ± 1.56
Ex-III	45.00 ± 1.44	45.20 ± 0.81

Unit: ×10^3 cells ml^{-1}

Oxyhaemocyanin

Oxyhaemocyanin concentration also increased following dietary treatment. At 25 days, the lowest value was observed in the control IgY group, whereas the Ex-I, Ex-II and Ex-III groups exhibited progressively higher values of approximately 0.91, 0.97 and 1.08 mmol l^{-1}. At 50 days, the control IgY group recorded approximately 0.86 mmol l^{-1}, while the experimental groups exhibited higher values. The source reports that oxyhaemocyanin is a useful physiological indicator of shrimp health and that higher oxyhaemocyanin is associated with improved oxygen transport.

Table 6. Oxyhaemocyanin concentration of *Litopenaeus vannamei* after WSSV challenge

Treatment	25 dpv challenge	50 dpv challenge
Blank control	0.91 ± 0.03	0.92 ± 0.04*
Control IgY	0.70 ± 0.03[b]	0.86 ± 0.03[b]
Ex-I	0.91 ± 0.03[c]	0.97 ± 0.04[c]
Ex-II	0.97 ± 0.04[c]	1.16 ± 0.04[c]
Ex-III	1.08 ± 0.04[d]	1.38 ± 0.05[d]

Unit: mmol l^{-1}

4. DISCUSSION

The present findings demonstrate that dietary anti-WSSV IgY, particularly when generated using *Glycine max* (*G. max*) as an immunoadjuvant, was associated with improved growth performance, survival, and haematological responses in WSSV-challenged *Litopenaeus vannamei*. Among the experimental treatments, Ex-III, which received anti-WSSV IgY produced with 1 ml *G. max* immunoadjuvant, generally produced the strongest response. The progressive improvement from Ex-I to Ex-III suggests that the immunoadjuvant component may have contributed to the biological effectiveness of the antibody preparation under the experimental conditions. However, because the present experiment evaluated a combined IgY–immunoadjuvant treatment, the individual contribution of IgY and *G. max* cannot be completely separated.

The improvement in growth performance may be associated with reduced physiological consequences of WSSV infection and improved ability of treated shrimp to maintain normal metabolic functions. Viral infection can impose considerable physiological stress on shrimp, potentially diverting energy from somatic growth toward maintenance and defence. In the present study, weight gain increased from 1.60 g in the Control IgY group to 2.80 g in Ex-III at 25 days post-vaccination, while at 50 days it increased from only 0.50 g in the control to 4.70 g in Ex-III. The source data therefore demonstrate a pronounced treatment-associated improvement in growth, particularly at the later sampling point. The approximately 9.4-fold difference between Ex-III and the control at 50 days should nevertheless be interpreted cautiously because it represents recorded weight gain between the specified measurement points rather than a direct measurement of viral suppression. The present growth response is consistent with the broader evidence that dietary biological interventions can improve growth and

disease resistance in cultured shrimp. Rengpipat et al. (1998) reported improved survival and growth of *Penaeus monodon* following dietary administration of *Bacillus* S11. Subsequently, Rengpipat et al. (2000) demonstrated that dietary *Bacillus* S11 enhanced several immune parameters and improved survival in *P. monodon*. These findings support the general principle that dietary modulation of shrimp health can influence both physiological performance and resistance to infectious challenge. However, probiotic supplementation and antibody-based dietary intervention are mechanistically different, and the present findings should therefore be considered complementary rather than directly equivalent to those studies.

A major finding of the present study was the increase in total haemocyte count (THC). Haemocytes are central components of the crustacean innate immune system and participate in phagocytosis, encapsulation, nodulation, coagulation and other defence processes (Rodríguez & Le Moullac, 2000). In the present experiment, THC increased from 28.00×10^3 cells ml^{-1} in the Control IgY group to 45.00×10^3 cells ml^{-1} in Ex-III at 25 days. At 50 days, the corresponding values were 28.33 and 45.20×10^3 cells ml^{-1}, respectively. This represents an increase of approximately 60% relative to the control at both sampling periods. The increase in circulating haemocytes may indicate improved cellular preparedness for recognition and elimination of pathogens. The present THC results are broadly consistent with previous observations that dietary immunostimulants can enhance cellular immune parameters in shrimp. Rengpipat et al. (2000) reported increased immune responses in *P. monodon* receiving *Bacillus* S11. Likewise, dietary immunostimulants have been investigated as means of enhancing innate immune mechanisms in aquaculture species (Chakraborty & Hancz, 2011). Nevertheless, THC alone should not be interpreted as definitive evidence of antiviral immunity. An increased haemocyte count may reflect enhanced immune readiness, but functional assays such as phagocytic activity, respiratory burst, phenoloxidase activity, antimicrobial peptide expression, or viral-load measurements would provide stronger mechanistic evidence.

The reduction in haemolymph coagulation time provides an additional indication of altered innate physiological defence. Crustacean haemolymph coagulation is an important protective mechanism involving haemocyte-derived components and clotting factors. Rodríguez and Le Moullac (2000) identified coagulation and related haemolymph responses as important components of crustacean health assessment. In the present study, coagulation time decreased progressively from 171.66 s in the Control IgY group to 125.33, 106.00 and 96.00 s in Ex-I, Ex-II and Ex-III, respectively, at 25 days. At 50 days, coagulation time decreased from 167.00 s in the control to 119.00, 102.12 and 89.66 s in the respective treatments. Thus, Ex-III produced the fastest coagulation response at both sampling periods. Faster haemolymph clotting may contribute to maintaining haemostasis and limiting the spread of invading microorganisms following tissue damage.

Oxyhaemocyanin concentration also showed a treatment-associated increase. Haemocyanin is the major respiratory pigment of crustaceans and is responsible for reversible oxygen binding and transport. In the present study, oxyhaemocyanin increased from 0.70 mmol l^{-1} in the Control IgY group to 0.91, 0.97 and 1.08 mmol l^{-1} in Ex-I, Ex-II and Ex-III, respectively, at 25 days. At 50 days, the corresponding values were 0.86, 0.97, 1.16 and 1.38 mmol l^{-1}. The higher oxyhaemocyanin concentration in treated shrimp may reflect improved physiological status during the post-challenge period. However, it is preferable to interpret this finding as an indication of altered respiratory physiology rather than directly equating increased oxyhaemocyanin with improved oxygen uptake, because oxygen consumption and respiratory-rate measurements were not reported in the present experiment.

The immunoadjuvant activity of *G. max* may be associated with its diverse phytochemical composition. The experimental source identified saponins, tannins, alkaloids and glycosides in the *G. max* extract. Saponins are of particular interest because several plant-derived saponins have recognized adjuvant properties and can enhance antigen-associated immune responses. More broadly, phytochemicals including saponins, flavonoids, alkaloids and phenolic compounds have been investigated for their immunostimulatory and antipathogenic potential in aquaculture (Chakraborty & Hancz, 2011). Therefore, the phytochemical constituents of *G. max* may have contributed to the enhanced biological response observed with Ex-II and Ex-III.

An important feature of the present approach is the combination of antigen-specific IgY with a plant-derived immunoadjuvant. IgY technology permits production of pathogen-specific antibodies in laying hens, followed by recovery of antibodies from egg yolk. The approach has attracted interest because eggs can be collected repeatedly without requiring invasive antibody recovery from the animal. In aquaculture, pathogen-specific IgY has been investigated as a potential prophylactic or dietary intervention against bacterial pathogens. Kumaran et al. (2018), for example, reported physicochemical and immunological characteristics of anti-*Vibrio harveyi* IgY and investigated its application in *Fenneropenaeus indicus*. Their findings provide support for the broader concept of incorporating pathogen-specific IgY into shrimp diets.

The present results extend this concept toward WSSV management. WSSV is a highly pathogenic virus responsible for substantial mortality in penaeid shrimp, and effective therapeutic options remain limited (Escobedo-Bonilla et al., 2008). Consequently, prevention and enhancement of host resistance are important components of WSSV management. Previous studies have demonstrated the potential of antigen-directed strategies against WSSV. Witteveldt et al. (2004) reported that oral vaccination with WSSV structural proteins could provide protection against WSSV in *P. monodon*, while VP28 has subsequently received considerable attention as a protective WSSV antigen. These findings provide biological support for

dietary delivery of WSSV-directed preparations, although the mechanism of protection in shrimp differs fundamentally from conventional antibody-mediated adaptive immunity in vertebrates.

Survival following WSSV challenge further supports the beneficial effect observed in the present experiment. At the 25-day challenge, survival at the seventh day after challenge was 65%, 75% and 80% in Ex-I, Ex-II and Ex-III, respectively, whereas the Control IgY group showed complete mortality by the corresponding endpoint. At the 50-day challenge, survival was 60%, 75% and 85% in Ex-I, Ex-II and Ex-III, respectively. The treatment and time effects were statistically significant in the original analysis. These findings suggest that the antibody–immunoadjuvant treatment may have improved the ability of shrimp to withstand WSSV challenge.

The survival results should nevertheless be interpreted together with the haematological findings. Ex-III showed the highest survival as well as the highest THC and oxyhaemocyanin concentration and the shortest coagulation time. The parallel changes suggest that the improved survival was accompanied by an enhanced physiological and cellular response. However, correlation between these variables does not establish a direct causal relationship. Future experiments incorporating quantitative WSSV copy-number analysis would be particularly valuable for determining whether the treatment actually reduces viral replication or instead improves host tolerance to infection.

The present findings are also relevant to the growing interest in reducing dependence on conventional chemotherapeutic agents in aquaculture. Excessive use of antimicrobial compounds can contribute to antimicrobial-resistance concerns and environmental contamination. Plant-derived immunostimulants have consequently attracted attention as potentially sustainable approaches to improving host health (Chakraborty & Hancz, 2011). The combination of a plant-derived immunoadjuvant with pathogen-specific IgY provides a potentially complementary strategy in which the plant component supports immunomodulation while the antibody preparation provides antigen-specific activity.

The results should, however, be interpreted within the limitations of the experimental design. The present study demonstrates an association between dietary treatment and improved growth, survival and haematological responses, but it does not establish the molecular mechanism of action of *G. max*. In addition, the design does not completely distinguish the independent effects of IgY from those of the herbal immunoadjuvant. The apparent dose-dependent improvement from Ex-I through Ex-III is suggestive, but a factorial design containing IgY-only, *G. max*-only, combined IgY + *G. max*, and untreated controls would provide stronger evidence of synergy. Similarly, quantitative WSSV viral-load measurements are necessary to determine whether the treatment has direct antiviral effects. The present findings indicate that dietary anti-WSSV IgY generated using *G. max* immunoadjuvant has considerable potential as an immunonutritional strategy for *L. vannamei*. The simultaneous improvement in growth, survival, THC, haemolymph coagulation and oxyhaemocyanin suggests that the treatment may improve the physiological resilience of shrimp during WSSV challenge. The strongest responses consistently observed in Ex-III provide a useful basis for subsequent dose optimization and mechanistic investigations.

5. CONCLUSION

The present investigation demonstrates that dietary anti-WSSV IgY combined with the herbal immunoadjuvant *Glycine max* positively influenced growth performance, survival and selected haematological parameters of WSSV-challenged *Litopenaeus vannamei*. The strongest overall response was observed in Ex-III, in which anti-WSSV IgY was generated using 1 ml *G. max* immunoadjuvant. Weight gain increased from 1.60 to 2.80 g at 25 days and from 0.50 to 4.70 g at 50 days in the Control IgY and Ex-III groups, respectively. THC increased from approximately 28×10^3 cells ml^{-1} in the control to approximately 45×10^3 cells ml^{-1} in Ex-III, while haemolymph coagulation time decreased markedly. Oxyhaemocyanin concentration also increased progressively with treatment. These responses were accompanied by improved survival following WSSV challenge. Taken together, the findings suggest that herbal immunoadjuvant-assisted IgY represents a promising dietary approach for supporting innate defence and physiological performance in WSSV-challenged shrimp. The strategy combines the potential immunomodulatory properties of *G. max* with pathogen-directed IgY and may therefore offer an alternative to conventional disease-management approaches. However, confirmation through quantitative viral-load analysis, mechanistic immunological studies, independent replication and field-scale validation is essential before recommending commercial application.

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