Effects of IgY Antibody on Growth, Survival, Immune Responses and Protection against *Vibrio parahaemolyticus* in Pacific White Shrimp

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ABSTRACT

Post larvae 12 (PL 12) of *Litopenaeus vannamei* (Pacific white shrimp) were subjected to feeding trials to decipher the effectiveness of IgY antibody as a feed supplement. This study aims to comprehend the growth performance, immune responses and survival rate of shrimps following an infection from *Vibrio parahaemolyticus*. This micro-organism was extracted from shrimps suffering from white feces syndrome (WFS). Post larvae (PL 12) were stocked in twenty 500-L fiberglass tanks at a density of 80 shrimps per tank (four replicate tanks per treatment) and salinity was maintained at 25 ‰. For analyses, shrimps were segregated into five categories based on the dosage of IgY antibody, given along with the standard pelleted shrimp feed (36 % protein) fed four times a day. This additive was fed at a dose escalation of 0, 0.2%, 0.3%, 0.4% and 0.5%. This 30-day trial demonstrated that the shrimps fed 0.5% of IgY additive had the highest average body weight. Besides this, shrimps nourished with 0.5% of IgY additive were found to have increases in growth and development. At the same time, a viable effect of antibody was also seen in the immunity of the shrimps, as evidenced by an escalation in immune responses such as total hemocyte count, phenoloxidase activity, phagocytosis and bactericidal activity of the shrimps. Along with this, a considerable mitigation of the total *Vibrio* spp. was found in the hepatopancreas of shrimps fed with IgY supplement. The shrimps infected with *V. parahaemolyticus* and furnished with IgY-feed were discovered to have a notable reduction in mortality rate (25-40 %) in contrast to the control group (60 %). This study demonstrates the positive effects of IgY on growth, immune responses and tolerance to pathogenic *V. parahaemolyticus* in the Pacific white shrimp.

Keywords: Antibody IgY, Pacific white shrimp, *Vibrio parahaemolyticus*, White feces syndrome

INTRODUCTION

At present, Pacific white shrimp (*Litopenaeus vannamei*) is the most extensively harvested and cultured species of shrimp worldwide (Chuchird *et al*., 2015). However, culturing and procuring the species requires tremendous care. This caution was heightened to a great extent in Thailand in 2011, when shrimp farmers discovered the occurrence of white feces syndrome (WFS), which has led to massive economic losses in shrimp cultivation areas throughout the country (Thitamadee *et al*., 2016). This disease is indicated by the presence white or yellow fecal strings floating on the water surface, and affected shrimps exhibit a whitish gut and loose shell (Sriurairatana *et al*., 2014). These signs and symptoms may become prominent within the time period from 30 days after stocking until harvest (Somboon *et al*., 2012). High loads of *Vibrio* spp. have been detected in

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WFS-affected shrimps. Hence, Somboon *et al*. (2012) and Supono *et al*. (2019) suggested that *Vibrio* spp. may contribute to WFS development. To hamper the spread of these bacteria, the affected shrimps need to be isolated along with promoting probiotics in the gut. Many researchers have described this scenario and employed techniques such as establishing probiotic bacteria or employing organic acids to reduce the number of *Vibrio* spp. in the gut of the shrimp (Walla *et al*., 2011; Nayak *et al*., 2012; Jueliang *et al*., 2013).

In disease prevention, chicken egg yolk immunoglobulin (IgY) acts as an inherited protective mechanism, where the natural antibody in chickens can be transferred to the egg yolk to protect the embryo from pathogens (Johnson *et al*., 1995). Several studies have applied the generation of a specific antibody against a specific antigen by employing chicken egg yolk (de Paula *et al*., 2011; Wu *et al*., 2011; Li *et al*., 2014). Prior studies have shown the potency of oral administration of IgY doses, which have escalated the immunity of organisms and kept pathogens at bay (Mine and Kovacs-Nolan, 2002; Xu *et al*., 2011; Li *et al*., 2016). According to Zhang *et al*. (2009), shrimps lack active immunity, and hence the alternative of egg yolk is used to provide passive immunity to protect them from potential infections (Lu *et al*., 2008). This study examines the effect of *Vibrio*-specific IgY supplementation on growth, immune parameters and survival after challenge with *Vibrio parahaemolyticus* isolated from WFS-infected shrimps under laboratory conditions.

MATERIALS AND METHODS

Experiment 1: The effect of IgY on the growth, survival, and immune response of Pacific white shrimp post larvae

Experimental diets

ADBIOTECH (South Korea) provided specific immunoglobulin Y (IgY) powders prepared from a hen which was immunized with *Vibrio parahaemolyticus*. Then, these powdered antibodies were mixed with distilled water to dilute them and formulate five different treatments with a dose escalation of 0%, 0.2%, 0.3%, 0.4% and 0.5%, and sprayed onto commercial pelleted feed for shrimps (36% crude protein, 6% lipid). Tuna oil was poured onto the pellets at approximately 1% W/W to reduce the leaching of IgY into the water before being consumed by the shrimps.

Shrimps and experimental protocol

The experiment consisted of five treatments according to the five concentrations of IgY described in the previous section. The experiment was carried out at the Aquaculture Business Research Center, Faculty of Fisheries, Kasetsart University, Thailand. Pacific white shrimp post larvae 12 (PL 12) were obtained from a hatchery in Chachoengsao Province, eastern Thailand. The shrimps were randomly stocked into twenty 500-L fiberglass tanks at a density of 80 shrimps per tank (four replicate tanks per treatment). Shrimps were fed four times a day along with the IgY supplement for a 30-day period. Various physical factors like salinity, pH and temperature were also maintained during the trial at optimum conditions of 25 ‰, pH 7.8-8.0, and 28 °C, respectively. The tanks were cleaned daily by siphoning off any remaining food or fecal matter. Water in each tank was changed twice a week with 20% replacement.

Growth and survival studies

The average body weight, survival rate, and feed conversion ratio (FCR) were calculated at the end of experiment.

Immune parameters study

After the completion of the 30-day trial, forty shrimps from each category were sampled and subjected to immunological tests. A volume of 250 µL of hemolymph from each shrimp was withdrawn using a syringe containing 750 µL of pre-cooled (4 °C) anticoagulant. The hemolymph-anticoagulant solution was later used to study total hemocyte count (THC), phenoloxidase activity, phagocytic activity and bactericidal activity following the methods described by Nonwachai *et al*. (2010).

Experiment 2: The effect of IgY on the survival of Pacific white shrimp challenged with Vibrio parahaemolyticus isolated from shrimps exhibiting white feces syndrome (WFS)

Experimental animals

The experimental shrimps from the control group of Experiment 1 were randomly stocked into eight new 500-L fiberglass tanks (30 shrimps each tank, 4 replicate tanks per treatment). The experimental shrimps from the groups fed 0.2% IgY, 0.3% IgY, 0.4% IgY and 0.5% IgY in Experiment 1 were randomly stocked into 16 new 500-L fiberglass tanks (4 replicate tanks per treatment) with the density of 30 shrimps per tank, with similar environmental conditions as in Experiment 1. The virulent strain of *Vibrio parahaemolyticus* isolated from WFS-infected shrimps was cultured in Tryptic Soy Broth with 1.5% NaCl. This strain was added to all tanks at a concentration of 10^5 CFU·mL^{-1} except for the negative control group. Each treatment group received the same diets as in Experiment 1. The survival rate of shrimp was recorded at day 7 post challenge.

Bacterial studies

Five shrimps from each replicate of the treatments were evaluated and analyzed after one week of the challenge. The hepatopancreas of each shrimp was removed, homogenized, spread on TCBS agar using the spread plate technique and then incubated at 37 ºC for 24 h. Finally, all colonies of bacteria were counted and calculated as CFU·g^{-1}.

Statistical analysis

The data were analyzed using one-way analysis of variance (ANOVA). Subsequently, Duncan's new multiple range test was used to evaluate differences between pairs of treatment means.

RESULTS

Effect of IgY on growth, survival and immune response of Pacific white shrimp post larvae

Average body weights of shrimps after the 30-day trail are shown in Table 1. Shrimps fed 0.5% IgY had the highest average body weight, followed by the groups fed 0.4% IgY, 0.3% IgY, 0.2% IgY and 0% IgY (the control group). The differences among the average body weights of the five experimental groups were significant ($p<0.05$). Shrimps fed 0.5% IgY had the highest average survival rate, and it was significantly higher than those of the other groups. No significant difference was found between the average survival rates of shrimps fed 0.4% IgY and 0.3% IgY. The average survival rates of shrimps from these two groups were significantly higher than those of the group fed 0.2% IgY and the control group. Shrimps from the group fed 0.2% IgY had a significantly higher ($p<0.05$) average survival rate than shrimps from the control group (Table 1). The lowest FCR was observed in shrimps fed 0.5% IgY, and this was significantly ($p<0.05$) lower than for the other groups. There were no significant difference in FCR of shrimps in the groups fed 0.2% IgY, 0.3% IgY and 0.4% IgY. The FCRs of shrimps from these

Table 1. Body weight, average survival rate, and average feed conversion ratio (FCR), expressed as mean±SD, of Pacific white shrimp fed with different diets for 30 days.

Treatment	Body weight (g)	Survival rate (%)	FCR
Control	2.03±0.03^{e}	86.88±1.25^{d}	1.42±0.04^{c}
IgY 0.2%	2.88±0.03^{d}	90.31±2.13^{c}	1.35±0.02^{b}
IgY 0.3%	2.94±0.02^{c}	92.50±1.02^{b}	1.33±0.01^{b}
IgY 0.4%	3.03±0.02^{b}	93.13±0.72^{b}	1.33±0.01^{b}
IgY 0.5%	3.46±0.05^{a}	95.63±1.25^{a}	1.21±0.03^{a}

Note: Means in the same column with different superscripts are significantly different ($p<0.05$).

three groups were significantly lower ($p<0.05$) than that of the control group (Table 1).

Similar trends were seen in the immune parameters after the trials, as displayed in Table 2. It was found that the total hemocyte count and phenoloxidase activity increased with increasing dosage of the supplement. Thereby, 0.5% IgY shrimps were found to have the highest values for these two immune response parameters, followed by 0.4% IgY and 0.3% IgY. Further, no considerable variation was found in the total hemocyte count or phenoloxidase activity of shrimps from these groups. However, the total hemocyte counts and phenoloxidase activities for these groups were significantly higher ($p<0.05$) than those of the control group. Meanwhile, phagocytic activity was highest in the 0.5% IgY shrimps followed by the groups that received 0.4% IgY, 0.3% IgY and 0.2% IgY. Among the latter three groups (excluding 0.5% IgY) minor differences were found among the percent phagocytosis values. However, shrimp in the control group had a significantly lower ($p<0.05$) percent phagocytosis than shrimps in the other groups. The bactericidal activity analysis revealed that the shrimps fed 0.3% IgY, 0.4% IgY and 0.5% IgY exhibited bactericidal activity at a serum dilution of 1:32 while shrimp fed 0.2% IgY and the control group exhibited bactericidal activity at serum dilutions of 1:16 and 1:8, respectively (Table 2).

Effect of IgY on the survival of Pacific white shrimp challenged with Vibrio parahaemolyticus isolated from shrimp exhibiting white feces syndrome (WFS)

The challenge test demonstrated a zero mortality rate in the negative control group. This rate was followed by those (from low to high) of the groups fed 0.5% IgY, 0.4% IgY, 0.3% IgY, 0.2% IgY and the positive control group. A remarkable difference was noted among the six categories (Table 3).

Table 2. Immune parameters (mean±SD) of Pacific white shrimp fed with different diets for 30 days.

Treatment	THC (10^7 cells·mL^{-1})	PO (units·min^{-1}·mg protein^{-1})	Phagocytosis (%)	Bactericidal activity
Control	3.16±0.06^{c}	223.51±1.37^{c}	72.00±0.94^{d}	1:8
IgY 0.2%	3.96±0.02^{b}	233.30±2.11^{b}	75.83±2.13^{c}	1:16
IgY 0.3%	4.03±0.07^{ab}	235.09±2.79^{ab}	76.83±1.99^{bc}	1:32
IgY 0.4%	4.15±0.14^{ab}	235.74±1.02^{ab}	78.50±1.14^{b}	1:32
IgY 0.5%	4.21±0.25^{a}	237.98±1.73^{a}	81.83±1.14^{a}	1:32

Note: Means in the same column with different superscripts are significantly different ($p<0.05$).

Table 3. Average mortality rate (%; mean±SD) and average total number of *Vibrio* spp. (CFU·mL^{-1}; mean±SD) in the hepatopancreas of Pacific white shrimp after being challenged with *Vibrio parahaemolyticus* isolated from WFS-infected shrimp for 7 days.

Treatment	Average mortality rate (%)	Total *Vibrio* spp. in hepatopancreas (×10^6 CFU·mL^{-1})
Negative control	0.00±0.00^{a}	0.04±0.00^{a}
Positive control	60.00±2.72^{f}	8.39±0.14^{d}
IgY 0.2%	45.00±1.92^{e}	3.97±0.10^{c}
IgY 0.3%	40.00±2.72^{d}	3.86±0.30^{bc}
IgY 0.4%	35.00±1.92^{c}	3.75±0.12^{bc}
IgY 0.5%	25.00±1.92^{b}	3.63±0.03^{b}

Note: Means in the same column with different superscripts are significantly different ($p<0.05$).

The average total number of *Vibrio* spp. in the hepatopancreas of shrimps in the negative control group was lowest among the treatment groups, while the infected shrimps in the positive control had the highest total number. Additionally, no considerable variation was found in the average total number of *Vibrio* spp. in the hepatopancreas of shrimps fed 0.3% IgY, 0.4% IgY and 0.5% IgY. However, shrimps from all groups fed IgY had a significantly lower total number of *Vibrio* spp. in the hepatopancreas than shrimps in the positive control group (Table 3).

DISCUSSION

It has been proposed that egg yolk immunoglobulin (IgY) imparts a specific role in boosting the immune systems of marine organisms by acting as a source of passive immunity (Arasteh *et al*., 2004; Lu *et al*., 2008; Wu *et al*., 2011). This technique is widely used in shrimp farming because this particular organism lacks any adaptive immunity and lacks a specific immune system. One can administer induced-IgY antibodies to battle a specific antigen (Baloch *et al*., 2015; Hu *et al*., 2018). This study demonstrated this by using an IgY antibody against *Vibrio* spp., which led to escalation of growth, survival and enhanced shrimp immune response, as well as offering resistance against *V. parahaemolyticus* isolated from WFS-infected shrimp. Recently, while trying to comprehend the underlying mechanism, Xu *et al*. (2011) suggested that specific antibodies attach to the surface of antigen bacteria, and such a bond changes the structure of the bacterial surface and leads to inhibition of bacterial growth. This hypothesis was supported by our previous study dealing with the oral administration of antibodies, which showed significant reduction of the total number of *Vibrio* spp. in the hepatopancreas of shrimps, and it is also consistent with previous reports by Gao *et al*. (2016) and Li *et al*. (2016). It was further found that by the action of IgY antibodies, the process of agglutination is seen among the invading bacteria; hence, destroying the pathogen as shown by a significant reduction in total number of *Vibrio* spp. (Zhang *et al*., 2015; Xu *et al*., 2019).

Shrimps are among the invertebrates considered to have the simplest immune system physiology. However, the oral dose administration for 30 days led to remarkable results by showing signs of stimulated immune responses, including significant increases in the total hemocyte count, phenoloxidase activity, phagocytosis and bactericidal activity compared to the control group. Similar results were observed in previous research, which showed improved immune response with the administration of IgY antibodies (Zhen *et al*., 2008; Xu *et al*., 2011; Kumaran *et al*., 2018). Besides reducing impacts of the disease, IgY was found to promote vigorous growth of shrimp. This might be due to the effects of egg yolk powder on weight gain, as it is rich in lipids, including egg lecithin and cholesterol, leading to a better FCR (Xu *et al*., 2019). Shrimps fed a higher concentration of IgY had a significantly higher average body weight than other groups. This result coincides with that reported by Kumaran *et al*. (2018), who stated that the action of antigen-specific IgY destroys *Vibrio* sp. and contributes to an improved immune response and weight gain as a growth enhancement. However, more comprehensive studies are needed to clarify the mechanisms by which IgY affects the growth performance and FCR of shrimp.

CONCLUSION

Our results indicated that supplementation with specific IgY against *Vibrio* sp. had an antibacterial effect against Vibrio spp. in the hepatopancreas of Pacific white shrimp. This additive at a dosage of 0.5% showed the most beneficial results by promoting better growth, immune responses, and resistance to *Vibrio parahaemolyticus* infection. These results indicate that the application of IgY is an effective tool to combat the prevailing bacterial disease in the shrimp farming industry.

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