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Effects of Ergosan and Vibromax to Prevent Vibriosis and WSSV in *Litopenaeus vannamei*

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Abstract: During the period of culture, immunostimulants and even vaccines may provide potential methods to protect shrimps from opportunistic and pathogenic agents. This study was designed to show the effects of Ergosan and Vibromax vaccine against WSSV and *Vibrio harveyi* in juvenile *Litopenaeus vannamei* and even growth and survival rate in PL stage. Among different treatments in PL stage, Ergosan significantly enhanced the body weight while in group treated with Ergosan and Vibromax the enhancement in survival rate was observed. Survival rate was also assessed by challenging with *Vibrio harveyi* and WSSV in juvenile stage. All treatments with Ergosan and Vibromax vaccine showed significant enhancement in survival rate compared to the control groups. This study indicates that Ergosan and Vibromax could be used to promote the health status of shrimp during the period of culture.

Key words: Immunostimulant, Ergosan, Vibromax, *Litopenaeus vannamei*, WSSV, *Vibrio harveyi*

INTRODUCTION

Outbreaks of infectious diseases are causing significant economic losses for the shrimp farming industry for example by the appearance of white spot syndrome in Asia from 1993, shrimp industry in this continent has encountered a severe damage (Lightner, 1996). Three to ten days after the onset of white spots in the exoskeleton and epidermis up to 100% mortality can occur (Cavalli *et al.*, 2008). Among bacterial agents in shrimp culture *Vibrio harveyi* has been recognized as significant bacterial agents and a cause of high rates of shrimp mortality in the shrimp culture industry worldwide (Chiari and Dubey, 2006). The approaches chosen for control of these pathogenic agents are improvement of environmental conditions, stocking of Specific Pathogen Free (SPF) postlarvae and use of vaccines and immunostimulants. There are a lot of studies on the effects of different immunostimulants on shrimps but few studies have focused on the vaccination in shrimps. Teunissen *et al.* (1998) was showed that vaccination significantly enhance the resistance of shrimp to vibriosis

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(Teunissen *et al.*, 1998; Adams, 1991; Itami *et al.*, 1989). Protective immune responses in *Procambarus clarkii* by BEI-inactivated WSSV was reported by Zhu *et al.* (2009), Brown (2002), Groseil *et al.* (1995), Wu *et al.* (2002) and Rajesh Kumar *et al.* (2008). Rajesh Kumar *et al.* (2008) reported protective efficacy of oral delivery of a DNA construct in shrimp. Ning *et al.* (2009) concluded protective immune responses in shrimp by DNA vaccine against WSSV with intramuscular injection. Therefore, the aim of this study was to test the effects of Vibromax vaccine and Ergosan on survival rate and body weight in PL stage and against WSSV and *Vibrio harveyi* in *Litopenaeus Vannamei* in juvenile level for possible use of them in shrimp rearing.

MATERIALS AND METHODS

Shrimp and their Maintenance

This study was done in the Shrimp Research Station of the Busher-Iran at 2005 (June to September). The 10300 SPF nauplii in stage 6 were used in this study. They were reared in the Shrimp Research Station of the Busher-Iran inside tanks with sand-filtration and flow-through seawater. The water quality parameters were as below: temperature ($27 \pm 1.0^\circ\text{C}$), salinity ($28 \pm 1.5\%$) and pH (8.2 ± 0.1). According to the stage of life they were fed Cetaceous and Skeletonema algae, Artemia and Crumble feed, respectively.

Artemia

Artemia Franciscana cyst (INVE, Belgium) was used for enrichment with polyvalent Vibromax vaccine (Schering Plough Aquaculture, UK) in PL stage.

WSSV Isolation and Preparation

Virus used in this study originated from an infected shrimp farm located near Busher, Iran. The presence of WSSV in infected shrimp was confirmed by PCR analysis of DNA isolated. The WSSV stock solution was prepared following the method of Tsai *et al.* (2000). Infected shrimp were homogenized in TNE and then centrifuged at $1700 \times g$ for 10 min at 4°C . The supernatant was filtered through a $0.45 \mu\text{m}$ membrane and was stored at -80°C until used for challenging.

Vibrio harveyi Isolation and Preparation

The *Vibrio harveyi* was isolated from the haemolymph of diseased juvenile shrimp (*P. indicus*) in a farm near Busher, Iran with signs of reddish coloration, anorexia and lethargy. *Vibrio harveyi* was inoculated in nutrient broth with 3% NaCl at 37°C and centrifuged at $10000 \times g$ for 10 min at 4°C (Hsu and Chen, 2007). The supernatant was removed and the bacterial pellet was resuspended in saline solution (0.85% NaCl) at $1.5 \times 10^8 \text{ cfu mL}^{-1}$ for the challenge test (Hettiarachchi *et al.*, 2005).

Experimental Design

Table 1 shows different treatment groups. In group 2 and 4 from Zoal till PL14 Ergosan, an alginate based immunostimulant (Schering Plough Aquaculture, UK), was used for 20 days

Table 1: Different treatment groups during PL stage

Groups	Component received in each group
1 (control)	Without any Ergosan or Vibromax
2	Ergosan and Vibromax
3	Vibromax vaccine
4	Ergosan

and in juvenile level shrimp were fed with Ergosan for 10 consecutive days. Doses of Ergosan were according to protocol mentioned in Schering Plough Aquaculture protocol. In group 2 and 3 Vibromax vaccine was used in 2 steps: primary vaccine was received in PL4 till PL14 enriched with Artemia and booster vaccine was used for 10 consecutive days in juvenile level. In control group shrimp were fed without using any Ergosan or Vibromax. At the end of PL14 some factors like survival rate and mean weight were measured.

Challenge Test

For challenging, the shrimp from group 1 and 2 were divided into four subgroups. All subgroups were divided into three replicate groups of 215 shrimp in each tank. In the first subgroup all juveniles were bathed with *Vibrio harveyi* suspension containing 1.5×10^8 cfu mL⁻¹. The second subgroup was bathed with hemolymph from healthy shrimp. Challenging in these two subgroups was performed 2 days before booster vaccination with Vibromax.

In the third subgroup, all juveniles were bathed with WSSV suspension as earlier described. The fourth subgroup was bathed with hemolymph from healthy shrimp as mentioned in the second subgroup. Challenging in these two subgroups was performed 2 days after booster vaccination with Vibromax. For 10 consecutive days after challenge all mortalities were recorded in all subgroups. The cause of death was confirmed by re-isolating the organisms from dead shrimp.

Statistical Analysis

All the data were statistically analyzed by one-way analysis of Variance and Duncan's multiple comparisons of the means using EXCEL version 16. The level of significances were $p < 0.05$ and $p < 0.01$.

RESULTS

Survival rate of different groups till PL14 is shown in Fig. 1. Among different groups, group 4 receiving Ergosan and Vibromax showed the maximum protection with survival rate of 47% compared to control group with 32% survival rate ($p < 0.05$). During the experiment a significant difference ($p < 0.05$) in body weight among the groups was observed. Group 2 treated with Ergosan showed higher increase in body weight (9 mg) with respect to control group (7.2 mg) (Fig. 2). While group 3 treated with Vibromax showed the least increase in body weight compared to control group ($p > 0.05$). Mortality rate was 100% in control

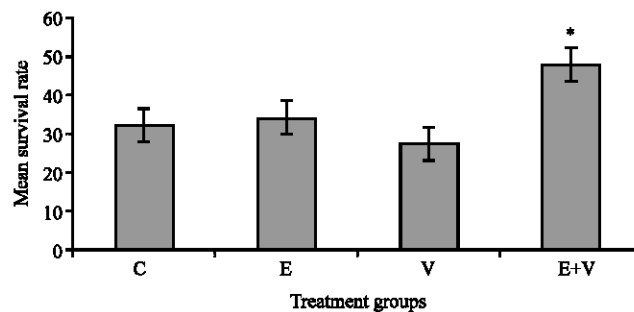


Fig. 1: Mean survival rate of *Litopenaeus vannamei* in different treatment groups during PL stage. Data represent the Mean $\pm$ SE. * $p < 0.05$

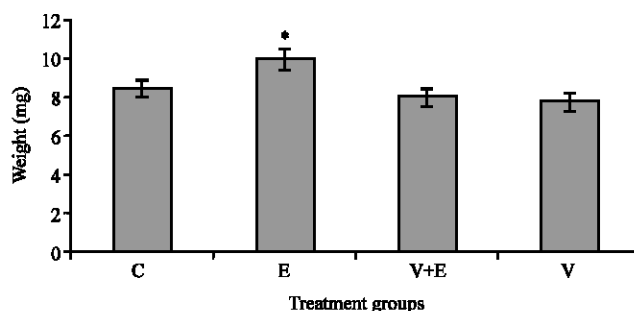


Fig. 2: Mean weight of *Litopenaeus vannamei* in different treatment groups during PL stage. Data represent the Mean $\pm$ SE. * $p < 0.05$

Table 2: Mortality rate in control and vaccinated groups for 10 days after challenging with *Vibrio harveyi*

Groups	Day										
	0	1	2	3	4	5	6	7	8	9	10
A ₁	0	4	8	18	26	26	26	26	26	26	26
A ₂	0	0	0	0	0	0	0	0	0	0	0
B ₁	0	2	5	5	6	6	6	6	6	6	6
B ₂	0	0	0	0	0	0	0	0	0	0	0

A₁: Control group challenged with *Vibrio harveyi* suspension, A₂: Control group challenged with hemolymph of healthy shrimp, B₁: Group 2 treated with Ergosan and Vibromax challenged with *Vibrio harveyi* suspension, B₂: Group 2 treated with Ergosan and Vibromax challenged with hemolymph of healthy shrimp

Table 3: Mortality rate in control and vaccinated groups for 10 days after challenging with WSSV

Groups	Day										
	0	1	2	3	4	5	6	7	8	9	10
A ₁	0	1	1	2	2	4	12	16	19	27	30
A ₂	0	0	0	0	0	0	0	0	0	0	0
B ₁	0	1	1	2	2	2	3	9	13	17	20
B ₂	0	0	0	0	0	0	0	0	0	0	0

A₁: Control group challenged with WSSV suspension, A₂: Control group challenged with hemolymph of healthy shrimp, B₁: Group 2 treated with Ergosan and Vibromax challenged with WSSV suspension, B₂: Group 2 treated with Ergosan and Vibromax challenged with hemolymph of healthy shrimp

subgroup challenged with *Vibrio harveyi* suspension while in subgroup treated with Ergosan and Vibromax it was significantly lower than the control subgroup ($p < 0.01$). The cause of death observed in the challenge trial was *Vibrio harveyi* as determined by bacterial isolation from dead shrimp. On the other hand, both subgroups of control and treated ones challenged with hemolymph of healthy shrimp did not show any signs of mortality with 100% survival rate (Table 2).

In the treated shrimp with Ergosan and Vibromax the mortality rate was significantly decreased ($p < 0.05$) when challenged with WSSV but control subgroup during the challenge showed 100% mortality during the period of observation. All dead shrimp showed the presence of WSSV in PCR technique. Both subgroups of control and treated ones challenged with hemolymph of healthy shrimp were with 100% survival rate too (Table 3).

DISCUSSION

Different approaches to prevent shrimp diseases have normally involved antibiotics, vaccination against specific pathogens and even different immunostimulants that protect

shrimps nonspecifically. Till some years ago scientists believed that shrimps do not possess acquired immunity but recent studies showed that shrimps have acquired immunity and even memory cells that make the vaccinations efficient in these groups of animals (Van Hulten *et al.*, 2009). According to our knowledge, there was just one report on application of Ergosan in preventing WSSV in *Litopenaeus Vannamei* during intermoult stage (Montero-Rocha *et al.*, 2006). In present study, we compared the effects of Ergosan and Vibromax vaccine against *Vibrio harveyi* and WSSV in *Litopenaeus Vannamei* in juvenile level. Meanwhile till PL14, which is time of transferring shrimps to rearing ponds, effects of Ergosan and Vibromax were studied on shrimp survival rate and weight. Highest survival rate was shown in group 4 that received Ergosan and Vibromax followed by group 2 that received Ergosan. Meanwhile the least survival rate was in group just received Vibromax. It seems that stress in this group was the highest and it can be assumed that during vaccination using a good immunostimulant can protect shrimps against stress. On the other hand, an increase in body weight was shown in group received Ergosan compared to control group ($p < 0.05$). This result is in accordance with study by Montero-Rocha *et al.* (2006) that showed an increase in body weight during 15 days of using Ergosan in intermoult stage shrimp.

In the group 4 that received Ergosan and Vibromax nearly the same situation as control group was observed while in the group 3 received Vibromax the least increase of body weight was shown. It again emphasizes on the use of a good immunostimulant during period of stress in rearing shrimps. During challenging with live *Vibrio harveyi* and WSSV, better protection was observed in the first challenging with *Vibrio* and WSSV even though after a longer lag phase in group received Ergosan and Vibromax mortality was also observed with WSSV but it was significantly lower than control group. According to previous studies PAP protein that has a role in phagocytosis can be induced by WSSV and formalin killed bacteria of *Vibrio* so in our study protection against WSSV by Vibromax vaccine was caused by this method (Chotigeat *et al.*, 2006). It is not worthy to mention that number of colonies of *Vibrio harveyi* separated from dead shrimp after 24 and 48 h were higher in control group than group treated with Ergosan and Vibromax demonstrating the lower severity of *Vibrio* bacteria after using Ergosan and Vibromax vaccine.

In conclusion, this study showed that Ergosan and Vibromax vaccine had the potential for protecting shrimp against some pathogenic diseases but further investigation is needed for applying different doses and durations of Ergosan during different stages of shrimps. Mean while studying different immunological parameters in shrimp during the use of Ergosan and Vibromax and even the exact place of effects in shrimp tissues are recommended.

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