



**PG & RESEARCH DEPARTMENT OF ZOOLOGY  
MUSLIM ARTS COLLEGE**

(Affiliated to Manonmaniam Sundaranar University)  
Thiruvithancode-629174, Kanyakumari District,  
Tamil Nadu, India



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**CURRENT ENVIRONMENTAL ISSUES  
AND MEASURES OF MITIGATION**

**CERTIFICATE**

This is to certify that Prof./Dr./Mr/Mrs./Ms.-*T. Kumaran, Asst. Professor, Dept. of Zoology, Muslim Arts College, Thiruvithancode* has participated / presented a research paper entitled *Growth and Survival Studies of Anti-wssv Fly. Supple mented diets in Shrimps*.....in the National Seminar on "Current Environmental Issues and Measures of Mitigation" organized by the PG & Research Department of Zoology, Muslim Arts College, Thiruvithancode- 629174, held on February 17, 2023.

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# CURRENT ENVIRONMENTAL ISSUES AND MEASURES OF MITIGATION

**PROCEEDINGS OF  
NATIONAL SEMINAR**



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**THIRUVITHANCODE - 629167,**

**KANYAKUAMRI DIST**

**TAMIL NADU - INDIA**

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## GROWTH AND SURVIVAL STUDIES OF ANTI WSSV IgY SUPPLEMENTED DIETS IN SHRIMPS

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**Abstract**

The production of IgY involves a non-invasive method that includes immunizing host with specific antigen. The antibody will be produced in the chicken mother will eventually transferred into egg. The Ex-I set up had 1ml of WSSV antigen mixed with 1ml of PBS immunized and produced the anti WSSV IgY (without immunoadjuvant). In Ex-II, the chick was immunized with 1 ml of WSSV antigen and 500 l of soya saponin (Glycine max adjuvant). Experiment III, feeding diet E-III (anti-WSSV IgY with adjuvant 1ml) to *L.vannamei* cultures. Eggs were collected after 24th and 50th dpv the treatment protocols was observed that shrimp showed increase range of survival and growth characteristics. It this treatment method proved the Anti WSSV IgY with adjuvant against the WSSV infection.

**Key words:** *Litopenaeus vannamei*, WSSV, Anti WSSV IgY

**Introduction**

Aquaculture, also known as aqua farming of aquatic organisms such as fish, crustaceans, molluscs and aquatic plants. It involves cultivating freshwater and saltwater populations under controlled conditions. White spot syndrome virus (WSSV) remains one of the most harmful pathogens because of its high virulence; shrimp mortality can reach 100 % within 3 to 7 days of infection, which causes great economic losses to the industry (Lightner, 1996). Some research has been performed to understand the effects of WSSV on white shrimp exposed to low-salinity water and seawater never the less, no studies have been conducted to test high salinity conditions. One of the major advantages of using IgY over other antibodies is their non-invasive way of production and purification. A chicken produces 260-300 eggs per year. From each egg 100-200 mg of antibody can be produced with 2-10 % of with Ag specific antibody (Schade *et al.*, 1994; Carlander, 2002).

**Current treatment methods in WSSV infection**

There is no treatment for WSSV and prevention is the best way to avoid outbreaks (Menasveta, 2002). Several studies have investigated the effect of disinfectants on WSSV (Chang *et al.*, 1998; Maeda *et al.*, 1998; Balasubramanian *et al.*, 2006). The use of vaccines and immunostimulants to control WSSV has also been explored (Citarasu *et al.*, 2006). Biosecurity measures to exclude the pathogen or reduce its risk have been suggested (Mohan *et al.*, 2005). Measures adopted by the shrimp industry include the stocking of shrimp larvae confirmed WSSV negative with a polymerase chain reaction

(PCR), use of disinfectants, closed culture systems to reduce water exchange, bird scares, crab fence, foot/tire bath, and limited access to the farm.

**Antiviral IgY against WSSV infection**

Hen egg yolk contains IgG (IgM and IgA absent), derived from hen serum and transferred into the egg follicle (Rose *et al.*, 1974). Egg yolk from immunized chickens are an important source of immunoglobulins, yielding large amounts of high affinity antibodies, easier to obtain than mammal sera. Furthermore, the IgY yield is up to 18 times greater than that of IgG from sera (Liu *et al.*, 2002). Some researchers have demonstrated passive immunization by anti-viral egg yolk antibodies (IgY) (Kweon *et al.*, 2000).

**Materials and Methods****Antibody coated feed preparation**

The IgY degradation prevented in the gastrointestinal tract of shrimp, oil coated diets were produced. Freeze dried pellet feeds were coated with 2 % WSSV IgY using cod liver oil. Feeds coated cod liver oil was prevented the leaching of egg yolk into water.

**Culture maintenance:**

Healthy shrimp, *L. vannamei* weighing approximately  $9.5 \pm 0.5$  g were purchased from shrimp farm at Orathanadu at Thanjavoor District, Tamil Nadu, India. They were stocked in a glass tank (1000 l capacity) in the Laboratory. Shrimps were acclimatized to ambient laboratory condition. The tanks were disinfected with 10 ppm of Potassium permanganate, and the water chlorinated followed to dechlorinate by vigorous aeration. Before starting experiment, uniform size of *L. vannamei* were selected from the stock and transferred in individual experimental fibre tanks.

of two experimental groups (A-B) and a control (C) with continuous flow through water and constant aeration system. The water quality parameters such as temperature ( $26 \pm 1.0^\circ \text{C}$ ), pH ( $7.1 \pm 0.2$ ) were maintained every day. Fifty percentage of water removed and added pure water of the same quantity in the culture tank every day.

**WSSV Challenge tests**

The four groups were challenged with after 25<sup>th</sup> and 50<sup>th</sup> day of immunization. After 25<sup>th</sup> day, the shrimps were divided in to two groups of each. The first group (30 nos) was challenged with after 25<sup>th</sup> day by IM injection with 25  $\mu\text{l}$  of WSSV semi purified WSSV having the protein concentration of 300  $\mu\text{g}$ . The remaining shrimps were challenged after 50<sup>th</sup> day by the same protocol. Meanwhile, 10 juveniles from each replicate were similarly injected with 0.01  $\mu\text{l}$  Phosphate buffer saline (PBS) as the blank control group. After injection, the shrimps were subdivided randomly into two clusters (A and B). Ten animals are introduced in cluster 'A'

$$(\text{Ln } W_2 - \text{Ln } W_1)$$

$$\text{SGR (\%)} = \frac{\text{Ln } W_2 - \text{Ln } W_1}{(t_2 - t_1)} \times 100$$

Where, Ln =Logarithmic number,  $W_2$  = Final weight at time  $t_2$ ,  $W_1$  = Initial weight at time  $t_1$ .

**Cumulative mortality after 25<sup>th</sup> and 50<sup>th</sup> dpv challenging**

The cumulative mortality of the shrimp *L.vannamei* fed the IgY, anti WSSV produced by with and without immunoadjuvant coated diets were given in the figure 4.11a. There is only 15 % mortality were observed in the blank control. After 25<sup>th</sup> dpv, the WSSV challenged *L.vannamei* fed succumbed to death the mortality of cent percent with in 9 days. Further the cent percent cumulative mortality was prolonged to 15 days in the diet Ex-I fed *L.vannamei* after

group separately in a fiber glass tank (1000L) with flow through water. In cluster 'A', the numbers of dead Juveniles were recorded once in every 4 hr for 10 days following the challenge.

**Treatments**

After the period of fifteen days acclimatization, different experimental treatments were given. In 4 fiber plastic of 100 l capacity 80 shrimps were distributed in triplicate, on each of the tank at temperature of  $28^\circ\text{C}$ , pH 7. semi purified viral protein 100 mg/ml challenge to each groups and afterwards the following treatments were carried out.

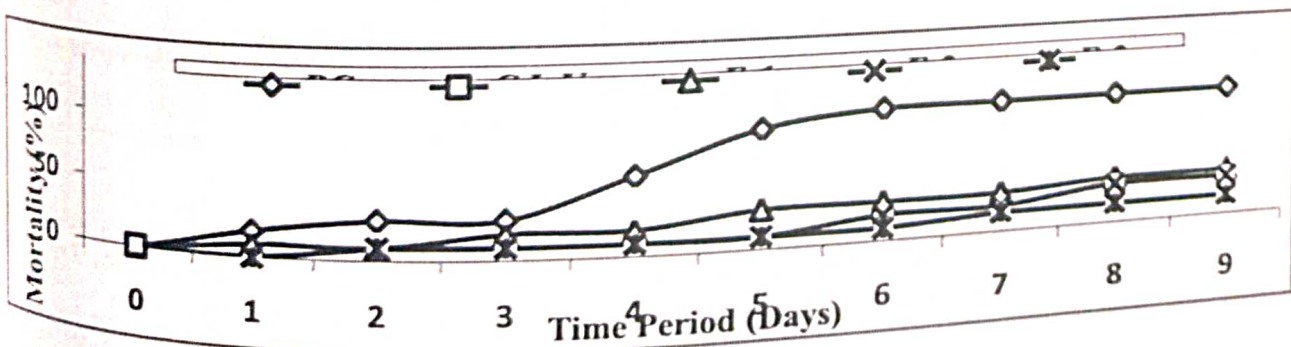
**Survival growth parameters**

The cumulative mortality survival and growth rate of *L.vannamei* after challenge with WSSV (100mg/ml) was assessed until 10 days. The weight (wet) gain was calculated by deducting the initial weight from final weight. The specific growth rate (SGR) was calculated by using the formula.

**Results**

challenge. The Ex-II group, the IgY produced with immunoadjuvant coated diets helped to decrease the cumulative mortality that was 20%. This group significantly increased ( $P \leq 0.001$ ) the survival of *L.vannamei* and with stand the resistance after the 25<sup>th</sup> dpv.

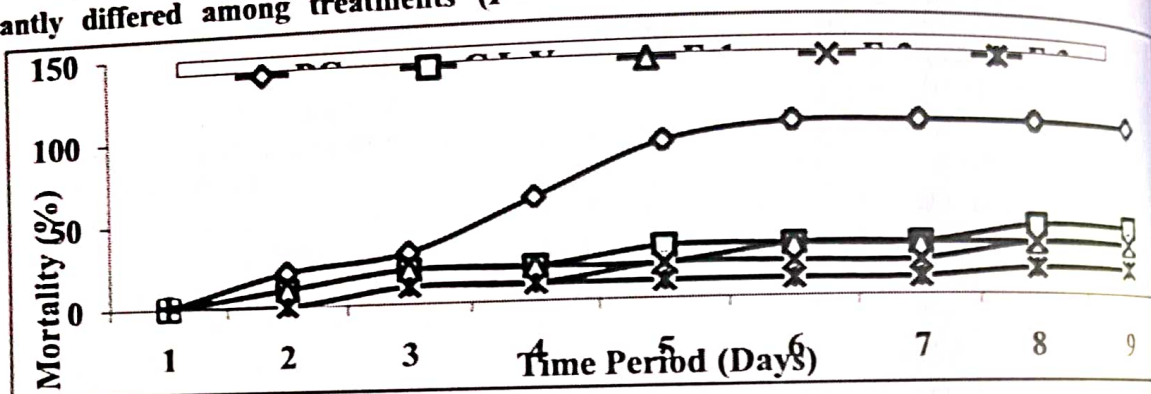
**Cumulative mortality of *L. vannamei* treated with anti-WSSV IgY and challenged with WSSV on 25<sup>th</sup> dpv.** The values were significantly differed among treatments ( $F = 82.37; P \leq 0.001$ ) and different time interval ( $F = 7.29; P \leq 0.001$ ) -Two way ANOVA



After, 50<sup>th</sup> dpv of challenging the different diets fed groups, the cumulative mortality was decreased with the influence of anti WSSV IgY. Like the 25<sup>th</sup> dpv all animals were succumbed to died within 9 days. The Ex-I have reduced the mortality rate of 50% and the Ex-II group, only 20 Cumulative mortality of *L. vannamei* treated with anti-WSSV IgY and challenged with WSSV on 50<sup>th</sup> dpv. The values were significantly differed among treatments ( $F=$

% of the mortality was observed. The other percent of the shrimps were survived against challenge. The data were statistically significant ( $P \leq 0.001$ ) in the anti WSSV antibody coated fed groups (Fig 4.9b).

81.56;  $P \leq 0.001$ ) and different time interval 8.16;  $P \leq 0.001$ ) -Two way ANOVA

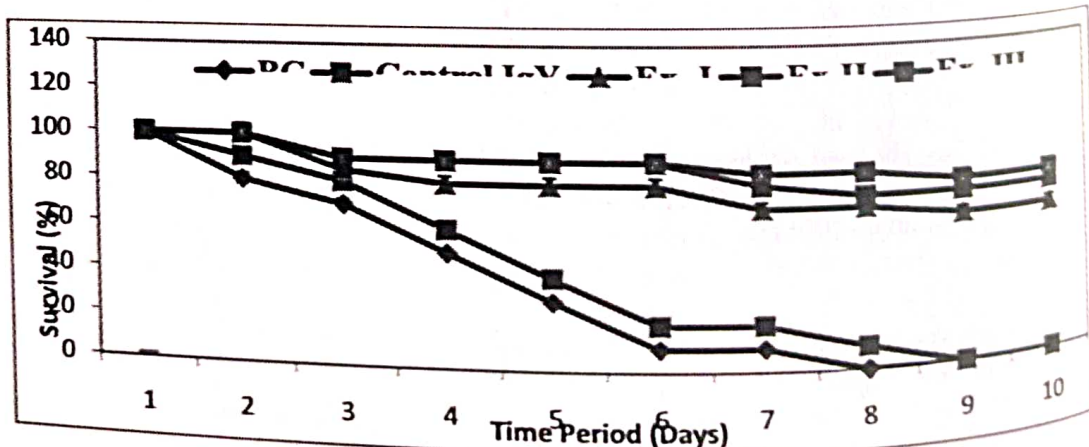


**Survival of *L.vannamei* after 25<sup>th</sup> and 50<sup>th</sup> dpv**

Survival (%) of *L.vannami* fed with anti WSSV IgY coated diets and challenged with WSSV on 25<sup>th</sup> day post-vaccination was described in the figure 4.12a 100% survival was observed in all diets including blank control for first two days. Then the survival percentage was gradually reduced in all the groups. Blank control and control IgY showed 0% survival after 7 days. Ex-I, Ex-II and Ex-III fed groups showed 65%, 75%

and 80 % of survival respectively. In Ex-II group the IgY produced with immunoadjuvant coated diet helped to improve the survival rate.

Mean Survival (%) of *L.vannamei* fed with anti WSSV IgY coated diets and challenged with WSSV on 25th day post-vaccination. The values were significantly differed among treatments ( $F= 25.83; P \leq 0.001$ ) and different time interval ( $F= 8.21; P \leq 0.001$ ) -Two way ANOVA

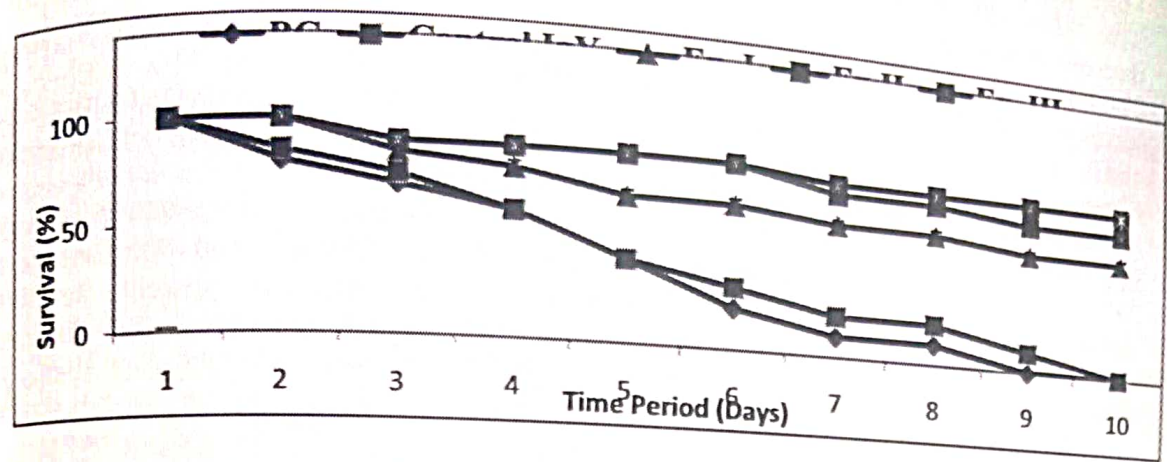


After 50<sup>th</sup> dpv the survival of *L.vannamei* fed with anti WSSV IgY coated diets and challenged with WSSV was shown in the figure 4.12b. The experimental groups showed 100% survival in first two days and then it was

decreased to 60%, 75% and 85% at Ex-I, Ex-II and Ex-III respectively after seventh day. After 7th day control group showed 0% survival in control and 60% in control IgY groups. In

group, the IgY produced with immunoadjuvant coated diet helped to improve the survival rate. Mean Survival (%) of *L.vannamie* fed with anti WSSV IgY coated diets and challenged with WSSV on 50th day post-vaccination. The

values were significantly differed among treatments ( $F= 26.67; P \leq 0.001$ ) and different time interval ( $F= 9.06; P \leq 0.001$ ) -Two way ANOVA



**Growth characteristics of *L. vannamei* of after 25<sup>th</sup> and 50<sup>th</sup> dpv**

The weight gain (g) and specific growth rate (%) of 25<sup>th</sup> dpv challenging were given in the table 4.14a. The weight gain was recorded in the control IgY antibody coated as 1.6g of the total period. This was significantly increased to ( $P < 0.05$ ) 2.4, 2.6 and 2.8 g of the total experimental

period of anti WSSV IgY coated diet fed groups. The specific growth rate (%) observed also reflected the same manner in the IgY, anti WSSV antibody coated diet fed groups. The SGR was observed in the control groups are 0.20 %. This was significantly increased to 0.31, 0.34 and 0.83 % respectively in the Ex-I , Ex-II and Ex-III groups.

**Growth characteristics of the shrimp *L.vannamie* fed with anti WSSV IgY coated diets and challenged with WSSV on 50<sup>th</sup> day post-vaccination.**

Treatment	Length (cm)		WET weight(g)		Weight gain (g)	Specific growth rate (%)
	Initial	Final	Initial	Final		
Control Ig Y	12.8 ± 0.23	14.1 ± 0.62	14.3 ± 0.23	14.6 ± 0.23	0.5 <sup>a</sup> ± 0.00	0.29 <sup>b</sup> ± 0.009
Ex-I	12.4 ± 0.12	14.3 ± 0.12	12.2 ± 0.08	14.4 ± 0.16	2.2 <sup>a</sup> ± 0.08	0.34 <sup>a</sup> ± 0.08
Ex -II	11.5 ± 0.08	14.6 ± 0.16	12.4 ± 0.28	15.3 ± 0.216	2.9 <sup>b</sup> ± 0.00	0.42 <sup>b</sup> ± 0.16
Ex -III	12.4 ± 0.28	15.5 ± 0.29	12.4 ± 0.12	17.1 ± 0.57	4.7 <sup>c</sup> ± 0.16	0.64 <sup>c</sup> ± 0.08

Means with the same superscripts (a-e) do not differ from each other ( $P < 0.05$ )

After 50<sup>th</sup> dpv the weight gain of 0.5 g only observed in the IgY antibody coated diet fed

*L.vannamei*. This was significantly ( $P < 0.05$ ) increased to 2.2, 2.9 and 4.7 g of maximum in the IgY produced by adjuvant coated diet fed animals. More than one time increased the weight gain. Like the weight gain the SGR also increased more than one time in the Ex-I, Ex-II and Ex-III groups. The group Ex-I, EX-II and Ex-III are significantly increased the specific growth rate of 0.24, 0.42 and 0.64 % respectively .

**Conclusion**

Survival (%) of *L.vannami* fed with anti WSSV IgY coated diets and challenged with WSSV on 25<sup>th</sup> day post-vaccination was described. 100% survival was observed in all diets including blank control for first two days. Then the survival percentage was gradually reduced in all the groups. Blank control and control IgY showed 0% survival after 7 days. Ex-I, Ex-II and Ex-III fed groups showed 65%, 75% and 80 % of survival respectively. After 50<sup>th</sup> dpv the survival of *L.vannamei* fed with anti WSSV IgY coated diets and challenged with WSSV was showed 100% survival in first two days and then it was decreased to 60%, 75% and 85% at Ex-I, Ex-II, Ex-III respectively after seventh day. After sixth day control group showed 0% survival in blank control and 60% in control IgY groups. In Ex-II group, the IgY produced with immunoadjuvant coated diet helped to improve the survival rate.

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