# Shrimp Production Results in Experimental Ponds in Ecuador with Presence of WSSV: Three Lucky Strikes or Three Indications of Hope for the Shrimp Industry?

Mario Velasco, Gorki Freire, Arturo Arias and Miguel Quiroz

Empacadora Nacional C.A.
P.O.Box 09-01-4344, Guayaquil, Ecuador
Tel. (5934) 2430600, Fax (5934) 496656, mvelasco@enaca.com.ec

## ABSTRACT

Since the very first appearance of White Spot Syndrome Virus (WSSV) in April 1999 in Central and South America, massive mortalities of Pacific white shrimp, *Litopenaeus vannamei* (Boone), in semi-intensive ponds are occurring. In very few cases shrimp survival is above 25 %. Three experiments were conducted in Ecuador from March 2000 to May 2001 to compare production results of shrimp directly stocked in 100 $m^2$ ponds at 150000 PL-12 / Ha. Experiment 1 evaluated immunostimulants, spray-dried plasma, tolerins and antibiotics in the feed. Production results were not significantly different among treatments that ranged from 800 to 1200 kg / Ha. Experiment 2 evaluated the use of $Ca(OH)_2$ and water exchange in plastic-lined and earthened ponds. Production results were significantly higher in lined ponds and ranged from 400 to 850 kg / Ha. Experiment 3 evaluated the use of kelp meal, wheat gluten, and a synthetic binder in the feed. Production results were not significantly different among treatments that ranged from 1100 to 1400 kg / Ha. It is speculated that these good production results were mainly obtained due to complete experimental system dry-out between culture cycles and water filtration through 300 μm mesh screen to fill ponds that probably resulted in reduced WSSV load in the culture system.

## INTRODUCTION

White Spot Syndrome Virus (WSSV) is a virulent pathogen that threatens the shrimp farming industry in the Americas since April 1999. By June 1999 confirmed WSSV shrimp mass mortalities were reported from Honduras, Nicaragua, Guatemala, Panama, Colombia, Ecuador and Peru. During the following months several international, regional and national meetings were held to inform on techniques and strategies for improvement of shrimp farms and hatcheries to combat WSSV. Concomitantly, a myriad of products (insecticides, disinfectants, bactericides, plant and fruit extracts, enzyme preparations, bacterial amendments, immunostimulants, tolerins, etc.) appeared in the market to ameliorate the impact of WSSV on production results (Jory, 1999).

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The present study was carried out in order to test some recommended management strategies and products to combat WSSV in Ecuador. An experimental system that minimized variability among treatments and simulated commercial shrimp culture practices as much as possible was used to compare production results.

## MATERIALS AND METHODS

### *Experiment 1*

This experiment evaluated the inclusion of several commercially available products (immunostimulants, spray-dried plasma, tolerins and antibiotics) in the same base feed. The experiment took place from March 16$^{th}$ to June 01$^{st}$ 2000. The culture period was 77 days. Hatchery reared PL-12 *L. vannamei* from our selection program were counted and stocked at 15 shrimp / $m^2$ in 100 $m^2$ earthened ponds. Pelleted feed was prepared in a commercial feed mill and contained 27.0 % crude protein, 7.0 % ether extract, 9.0 % ash, 4.0 % crude fiber and 10.0 % moisture. The inmunostimulants treatment contained fucoidan, peptidoglycan and lipopolisacharide sources, included together in the feed at 1.0, 0.05 and 0.3 %, respectively. Spray-dried plasma was included at 8.0 %. The tolerine was included in the feed at 1.0 %, after the first 28 days of culture the tolerine-feed was fed every other day in alternation with feed without the tolerine. Two different antibiotics at 0.2 % inclusion level were used to prepare two different feeds, after the first 14 days feeding without antibiotics these were given weekly changing antibiotics every other week. The cost of these additives ranged from $320.00 to $1,250.00 / MT of feed. The base feed with nothing added to it served as control. Shrimp were fed equal amounts of feed twice per day at 09:00h and 17:00h. Average temperature and salinity were 28 ˚C (ranged 26 – 30 ˚C) and 5 ups (ranged 4 – 7 ups), respectively. Early morning water dissolved oxygen concentration occasionally reached 2.0 mg / L in some ponds towards the end of the culture cycle. Shrimp histological sections from each treatment were prepared weekly. PCR technology was used to determine the presence of WSSV and IHHNV. At harvest, shrimp from each pond were weighted and counted, and shrimp survival, final weight, biomass and feed conversion ratio calculated.

Data obtained from this completely randomized design with 6 replicates per treatment were analyzed using one-way analysis of variance to determine significant differences ($P < 0.05$) among treatment means.

### *Experiment 2*

This experiment evaluated the use of calcium hydroxide ($Ca(OH)_2$) added at 100 kg / Ha three times per week in plastic-lined ponds, and continuous water exchange in earthened ponds. The experiment took place from September 21$^{st}$ 2000 to January 08$^{th}$ 2001. The culture period was 108 days. Hatchery reared PL-12 *L. vannamei* spawned from wild broodstock were stocked at 15 shrimp / $m^2$ in 100 $m^2$ ponds. The same pelleted feed prepared in a commercial feed mill containing 27.0 % crude protein, 7.0 % ether extract,

9.0 % ash, 4.0 % crude fiber and 10.0 % moisture was used for all treatments. Shrimp were fed equal amounts of feed twice per day at 09:00h and 17:00h. Average temperature and salinity were 26 ˚C (ranged 23 – 29 ˚C) and 25 ups (ranged 21 – 29 ups), respectively. Early morning water dissolved oxygen concentration occasionally reached 2.0 mg / L in some ponds towards the end of the culture cycle. Shrimp histological sections from each treatment were prepared weekly. PCR technology was used to determine the presence of WSSV and IHHNV. At harvest, shrimp from each pond were weighted and counted, and shrimp survival, final weight, biomass and feed conversion ratio calculated.

Data obtained from this completely randomized design with 6 replicates per treatment were analyzed using one-way analysis of variance to determine significant differences ($P < 0.05$) among treatment means.

***Experiment 3***

This experiment evaluated the use of kelp meal, wheat gluten, and a synthetic binder in the same base feed. The experiment took place from February 14$^{th}$ to May 16$^{th}$ 2001. The culture period was 90 days. Hatchery reared PL-12 *L. vannamei* from our selection program were counted and stocked at 15 shrimp / $m^2$ in 100 $m^2$ earthened ponds. Pelleted feed was prepared in a commercial feed mill and contained 29.0 % crude protein, 10.0 % ether extract, 8.0 % ash, 4.0 % crude fiber and 10.0 % moisture. The kelp meal was included at 3.5 % in the feed. Wheat gluten was included at 0.3 %, and the synthetic binder was included at 0.6 % in the feed. The base feed without any of the three tested ingredients or any other binder served as control. Shrimp were fed equal amounts of feed twice per day at 09:00h and 17:00h. Average temperature and salinity were 28 ˚C (ranged 26 – 30 ˚C) and 5 ups (ranged 4 – 7 ups), respectively. Early morning water dissolved oxygen concentration occasionally reached 2.0 mg / L in some ponds towards the end of the culture cycle. Shrimp histological sections from each treatment were prepared weekly. PCR technology was used to determine the presence of WSSV and IHHNV. At harvest, shrimp from each pond were weighted and counted, and shrimp survival, final weight, biomass and feed conversion ratio calculated.

Data obtained from this completely randomized design with 4 replicates per treatment were analyzed using one-way analysis of variance to determine significant differences ($P < 0.05$) among treatment means.

## RESULTS

### *Experiment 1*

Shrimp survival was above 30 % for all ponds and not significantly different regardless of treatment (Table 1). Shrimp final weight fed on the different feeds was not significantly different (Table 1). Shrimp mean survival and final weight for the feed with immunostimulants were 77 % and 8.6 g, with spray-dried plasma 85 % and 9.7 g, with tolerins 80 % and 7.0 g, and with antibiotics 64 % and 10.7 g, respectively. Shrimp survival and final weight for the control feed was 68 % and 10.0 g, respectively. Mean feed conversion ratios were between 0.6 and 1.0 (Table 1). WSSV was positively identified by PCR on day 19 for treatments with immunostimulants, spray-dried plasma and tolerins. On day 47 all treatments resulted positive to WSSV and negative to IHHNV (Heres et al,. 2001). For all treatments, characteristic lesions of WSSV, TSV and IHHNV were not present in histological sections (stomach, epidermis and gill tissue) through out the entire culture period (Heres et al., 2001).

Table 1. Biological performance of *L. vannamei* fed commercial feeds with different additives incorporated. Entries are sample mean of 6 replicates per treatment. Statistical differences were not found among treatments.

| | Survival (%) | Final weight (g) | FCR | Biomass (kg/Ha) |
|---|---|---|---|---|
| Immunostimulants | 77 | 8.6 | 1.0 | 1018 |
| Spray-dried plasma | 85 | 9.7 | 0.6 | 1233 |
| Tolerins | 80 | 7.0 | 1.0 | 841 |
| Antibiotics | 64 | 10.7 | 0.9 | 903 |
| Control | 68 | 10.0 | 0.9 | 961 |

### *Experiment 2*

Shrimp survival was above 30 % for all ponds and significantly different among treatments (Table 2). Shrimp survival was significantly higher in plastic-lined ponds compared to earthened ponds regardless of treatment. Shrimp final weight was not significantly different (Table 2). Shrimp mean survival and final weight for plastic-lined ponds treated with calcium hydroxide were 80 % and 6.6 g, for plastic-lined ponds without calcium hydroxide 74 % and 7.6 g, for earthened ponds with continuous water exchange 37 % and 7.1 g, and for earthened ponds without continuous water exchange 43 % and 6.7 g, respectively. Mean feed conversion ratios were between 1.3 and 3.0 (Table 2). WSSV and IHHNV were positively identified by PCR on day 45 for all treatments. However, characteristic lesions of WSSV and TSV were not found in histological sections (stomach, epidermis and gill tissue) through out the entire culture period for any of the treatments. Typical signs of IHHNV presence were observed in the population as a high percentage of dwarf shrimp and shrimp with deformities (Figure 1).

Table 2. Biological performance of *L. vannamei* treated with different water management in plastic-lined and earthened ponds. Entries are sample mean of 6 replicates per treatment. Values with different letters indicate significant differences.

| | Survival (%) | Final weight (g) | FCR | Biomass (kg/Ha) |
|---|---|---|---|---|
| Lined ponds with calcium hydroxide | 80[a] | 6.6 | 1.4[a] | 889[a] |
| Lined ponds without calcium hydroxide | 74[a] | 7.6 | 1.3[a] | 841[a] |
| Earthened ponds with continuous water exchange | 37[b] | 7.1 | 3.0[b] | 402[b] |
| Earthened ponds without continuous water exchange | 43[b] | 6.7 | 2.5[b] | 436[b] |

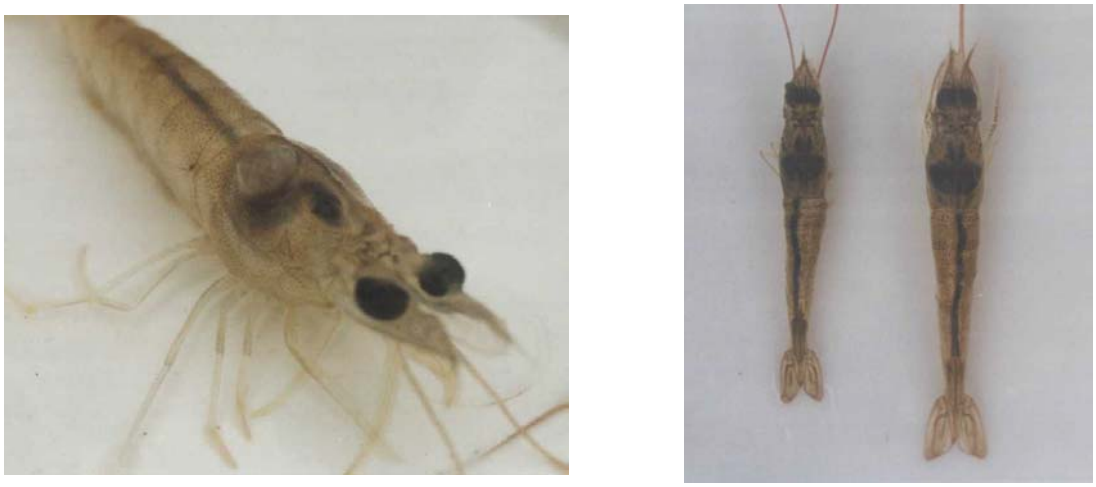

Figure 1. Dwarfed and deformed shrimp characteristic of IHHNV presence.

## *Experiment 3*

Shrimp survival was above 50 % for all ponds and not significantly different regardless of treatment (Table 3). Shrimp final weight fed on the different feeds was not significantly different (Table 3). Shrimp mean survival and final weight for feed containing kelp meal were 98 % and 8.6 g, with wheat gluten 91 % and 9.1 g, and with a synthetic binder 95 % and 10.6 g, respectively. Shrimp survival and final weight for the control feed was 94 % and 10.2 g, respectively. Mean feed conversion ratios were between 0.8 and 1.2 (Table 3). WSSV was not identified by PCR on days 27, 42, 56, 63 and 77 in any of the treatments. However, all treatments tested positive for IHHNV on day 42. For all treatments, characteristic lesions of WSSV, TSV and IHHNV were not present in histological sections (stomach, epidermis and gill tissue) through out the entire culture period.

Table 3. Biological performance of *L. vannamei* fed commercial feeds with different ingredients incorporated. Entries are sample mean of 4 replicates per treatment. Statistical differences were not found among treatments.

| | Survival (%) | Final weight (g) | FCR | Biomass (kg/Ha) |
|---|---|---|---|---|
| Kelp meal | 98 | 8.6 | 1.2 | 1270 |
| Wheat gluten | 91 | 9.1 | 0.9 | 1229 |
| Synthetic binder | 95 | 10.6 | 1.2 | 1159 |
| Control | 94 | 10.2 | 0.8 | 1443 |

## DISCUSSION

Overall, excellent survival and production results were obtained in all three experiments for all treatments during a time period (March 2000 to May 2001) in which the presence of WSSV was responsible for high shrimp mortalities (> 80 %) in commercial ponds in Ecuador. In fact, the same batch of postlarvae used in the experiments and stocked in the farm commercial ponds experienced mortalities higher than 90 %. WSSV was positively identified in the experimental shrimp population. However, clinical signs were not detected, possibly indicating that the viral load was low enough for the shrimp to defend itself and survive. Thus, regardless of tested treatment the characteristic high mortalities caused by WSSV did not develop during any of the experimental culture cycles.

Because of these excellent production results an evaluation of the management of the experimental system as a whole was conducted in an attempt to find out what was common to all three experiments that could have ameliorated the effects of WSSV. The first thing noticed was that water from the shrimp farm supply channel was been filtered through 300 µm mesh screen to fill the experimental water supply channel. Additionally, the water in the experimental water supply channel was again filtered through 300 µm mesh screen to fill the ponds. This water filtration probably retained WSSV vectors present in the water column minimizing their entrance in the ponds. The second observation made was that the experimental system, formed by the water supply channel and ponds, always was completely dried before filling the ponds with water for the next culture cycle. This dry-out time between cycles probably eliminated all vector populations in the experimental system that may have accumulated during the previous culture period. Thus, every time a shrimp culture cycle was initiated the total viral load in the system was very low and greatly reduced during the grow-out cycle.

Based on the harvest results obtained for all three experiments and the above observations, it is speculated that complete system dry-out between culture cycles and water filtration through 300 µm mesh screen to fill the water supply channel and ponds were the main factors that positively contributed to the excellent shrimp survivals with presence of WSSV. Due to the much larger scale and differences in design of shrimp farms compared to this experimental system set-up, implementation of this management would be difficult in semi-intensive farms. However, attempts to simulate this management in a larger scale are encouraged by the high survival results obtained in these experiments.

## REFERENCES

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