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Research Article

# EFFECT OF ASTAXANTHIN SUPPLEMENTATION ON THE INCIDENCE OF BLUE SHELL SYNDROME IN POST-LARVAL STAGES OF PACIFIC WHITE SHRIMP, *PENAEUS VANNAMEI,* DURING SEED PRODUCTION

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## ABSTRACT

Shrimp farming is among the key commercial marine crustaceans in aquaculture. Astaxanthin, a naturally occurring carotenoid, is frequently utilized as a dietary supplement in the culture industry of Pacific white shrimp, *Penaeus vannamei*. This research was carried out to examine the impact of Astaxanthin supplementation on the occurrence of Blue Shell Syndrome in the post-larval stages of Pacific White Shrimp, *Penaeus vannamei*, throughout seed production. The research was conducted at a private shrimp hatchery registered with the Coastal Aquaculture Authority situated in the suburban region of Chennai. The experiment was separated into four groups. Control "C" and experiments E1, E2, and E3 (in triplicate). Experimental animals received astaxanthin as a dietary supplement. The findings of this study indicate that the application of "Astaxanthin" 50 ppm as a feed additive for shrimp post larvae of *Penaeus vannamei* has a notable impact on treating or preventing the onset of "Blue Colour Syndrome" during the development of post larvae. Supplementation with "Astaxanthin" was discovered to improve survival rates, growth performance by facilitating appropriate rostral spine development, and also the ability to endure mechanical stress from the environment.

**Keywords:** Astaxanthin, Blue Shell Syndrome, Post-larva, *Penaeus vannamei.*

## INTRODUCTION

*Penaeus vannamei* is a significant and economically vital marine crustacean in aquaculture, known for its rapid growth, tender meat, strong tolerance to stress, excellent reproduction capabilities, and high nutritional quality. These exceptional characteristics result in an elevated market value and an increasing market demand (Liang *et al*., 2008; Pan *et al*., 2007). Nevertheless, shrimp are more vulnerable to multiple environmental influences due to intensive high-density farming aimed at boosting aquaculture output, particularly when the aquaculture system is inadequately designed or managed poorly. Astaxanthin (AX) is a naturally found carotenoid frequently utilized as a dietary supplement in the culture industry of Pacific white shrimp, *Penaeus vannamei*. Most Southeast Asian shrimp processors and importers agree that colour, among other factors, is one of the most crucial yet commonly overlooked quality criteria for penaeid shrimps. Today, inadequate general pigmentation and a type of blue discoloration, referred to as blue shrimp syndrome, are among the most concerning issues affecting the shrimp industry (Latscha, T. 1989). Niti Chuchird *et al*., (2015) disclosed that Thai shrimp farmers have experienced significant financial setbacks due to Early Mortality Syndrome. Impacted shrimp exhibit symptoms of a lighter hue due to pigment depletion, along with a reduced hepatopancreas. These indicators can manifest as soon as 4 days post-stocking (Munkongwongsiri *et al.,* 2013).

Astaxanthin (3, 3′-dihydroxy-β, β′-carotene-4,4′-dione) is a xanthophyll carotenoid found naturally in multiple microorganisms and marine life, such as the green microalga *Haematococcus pluvialis*, *Chlorella zofingiensis*, species of Chlorococcum and the yeast *Phaffia rhodozyma*. In *H. pluvialis*, astaxanthin levels can reach as high as 3.8% of the dry biomass. This pigment is soluble in fats and displays a distinctive red hue but does not have provitamin

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A activity in humans. Nonetheless, astaxanthin exhibits superior biological activity compared to other carotenoids, as shown in multiple studies (Higuera *et al*., 2006). The Food and Drug Administration of the United States (USFDA) has authorized the use of astaxanthin as a food colouring agent in animal and aquaculture feeds (Pashkow *et al*., 2008) and the European Commission similarly categorizes natural astaxanthin as an acceptable food dye. In *H. pluvialis*, the production of astaxanthin significantly increases under stress factors such as high salinity, lack of nitrogen, elevated temperatures and strong light exposure (Saratha *et al*., 2002; Ranga Rao, 2011; Saratha *et al*., 2012). Astaxanthin obtained from *H. pluvialis* is the main source for dietary supplements and consumption by humans (Kidd, 2011).

## MATERIALS AND METHODS

### Selection of Shrimp Post-larvae

The research was conducted at a private shrimp hatchery registered with the Coastal Aquaculture Authority in the suburban region of Chennai. The experimental subjects were newly metamorphosed post-larvae in the post-larvae 1 stage (PL1), which is identified as the day after metamorphosis from Mysis 3. The post-larvae's quality assurance was verified through polymerase chain reaction (PCR) diagnostics before the experimentation. The developmental stages of *P. vannamei* start with nauplii that emerge from eggs around 12 hours after laying. In this early nauplius phase, larvae depend on internal yolk sac supplies for nourishment. Following larval growth takes place through progressive metamorphic phases. The initial larval stage is protozoea, lasting around 3 days in ideal rearing conditions. After the protozoea phase, larvae progress to the mysis phase, which also lasts roughly 3 days under optimal environmental conditions. These phases represent the initial larval stages of development. Feeding ecology differs throughout these stages: protozoea mainly eat phytoplankton, predominantly unicellular algae, whereas mysis and early post-larvae transition to a diet of zooplankton, including rotifers, Artemia and copepods. Post-larvae subsequently move from early to late stages, where late post-larvae display morphological traits that closely resemble those of juvenile and adult shrimp (Bray *et al*., 1992; Wei *et al*., 2014; Hassan *et al*., 2021).

### Experimental Framework

This developmental framework establishes a foundation for comprehending the nutritional needs and physiological alterations affecting hatchery management and seed quality in *P. vannamei* aquaculture. The experiment was split into four groups - Control “C” and trails E1, E2 and E3 (in Triplicate). The experiment was conducted in a clear acrylic tank filled with sterilized seawater. The sterile seawater's quality was examined in a pathology lab located in Marakkanam, through microscopic examination and Polymerase Chain Reaction (PCR). The average length of post larvae was recorded. The colouration of animals and the hepatopancreas (HP) was noted. The control tank did not receive any additives. The experimental tanks received 50ppm of “Astaxanthin” (Niti Chuchird *et al*., 2015) as a feed supplement combined with artificial pelletized (non-encapsulated) feed (the absorption of the “Astaxanthin” colour will occur more quickly in non-encapsulated feed) and freshly hatched Artemia nauplii every day, administered at four-hour intervals. “Amaze-Asta” – a commercially available brand of Astaxanthin product (Figure 1) was used as feed supplement for shrimp larvae in this experiment and Stock solution of 1000 ppm Astaxanthin used for this study (Figure 2). Water changes were performed in all tanks at a rate of 20% daily and 2ppm of commercially available probiotics was added each day to sustain water quality. Daily observations were made of animal’s swimming and feeding behaviours. All animals display a greenish hue and their body colour is transparent in both control "C" and in the experiments "E1, E2 & E3" when the experiments commence. A stress test was performed across all groups utilizing 200 ppm formalin (active component 35%) and decreasing salinity by 50%. Each group had ten animals that participated in the experiment. Final survival rates were determined at the conclusion of the experiment (thirty minutes “prior to the end of the experiment). The average wet weight of the control “C” group and the experimental groups E1, E2, and E3 were computed. The survival rate was also determined at PL15 age across all tanks. The mean wet body weight of the animal was determined for both control and experimental groups.

### Statistical Analysis

Data collected and recorded for the average length of the post-larvae at each larval stage tested, wet weight of control and experimental groups and survival percentage of the control and experimental groups were analyzed using a one-way ANOVA table.

## RESULTS AND DISCUSSION

Initially before starting the experiment, the average length of the larva taken for the experiment, Hepato pancreas colour, body colour, survival rate was recorded (Table 1). The length of post-larvae in control group “C” at PL 5 age is shorter (5.6 ± 0.4) than the experimental groups E1 & E2, which showed 5.8 ± 0.8. The hepatopancreas of the control group animals were greenish in colour and these animals exhibited lower survival rates (78 ± 2%). The hepatopancreas of the animals in the experimental group appeared greyish in colour. The experimental groups (E1, E2, E3) that received "Astaxanthin" showed higher survival rates of 96%, 96% & 99% respectively, which is significantly higher than that of the control group animals (Table 2). At PL 10 age, the length of post-larvae is larger (9.5 ± 0.4) in the experimental groups E2 and E3 compared to the control “C” group (8.2 ± 0.2). The hepatopancreas of the control (C) group showed a greenish colour, while it exhibited a brownish tint in all experimental groups. Experimental groups E1, E2, and E3 that received the

"Astaxanthin" diet showed improved survival rates (E1: 81 ± 2; E2: 86 ± 4 and E3: 89 ± 2) compared to the control group, which had a lower survival rate of 62 ± 2% (Table 3). The length of post-larvae in control "C" at 15 days old measures less (10.3 ± 0.2). The length of post larvae was determined to be greater (12.5 ± 0.3 & 12.8 ± 0.4) in the experimental groups E1 and E2. The hepatopancreas appeared greenish in the control (C) group, while it was brownish in every experimental group. The control group exhibited a decreased survival rate (55 ± 2), while the experimental groups E1, E2, and E3 that received "Astaxanthin" demonstrated increased survival rates of 79 ± 2, 82 ± 2, and 82 ± 2, respectively. Survival percentage was determined on the initial day of the experiment (Day 1) and after larval ages 5, 10, and 15 days. Results of the stress test showed that, the control group (C) animals exposed to 200 ppm formalin for thirty minutes exhibited 78% stress, while those animals subjected to a lowered salinity of 50% expressed 82% stress (Table 4 and Figure 3).


**Figure 1**. Commercially available brand of Astaxanthin.

**Figure 2**. Stock solution of Astaxanthin (1000 ppm).

**Table 1.** Showing the physical parameters of Post larvae PL1.

| Exp | Age of PL | RSP | Average Length | HP Colour | Body Colour | % of Survival | Stress Test | | Dry Wt. (gm) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Formalin 200 ppm | Salinity 50% | |
| C | 1 | - | 5.2 ± 0.2 | Greenish | Clear | 100 | - | - | - |
| E1 | 1 | - | 5.0± 0.1 | Greenish | Clear | 100 | - | - | - |
| E2 | 1 | - | 5.2± 0.2 | Greenish | Clear | 100 | - | - | - |
| E3 | 1 | - | 5.2 ± 0.1 | Greenish | Clear | 100 | - | - | - |

**Table 2.** Comparative performances of Length, Number of rostral spines Body colour, survival percentage, stress test and Average wet weight of *P. vannamei* at PL 5.

| Exp | Age of PL | RSP | Average Length | HP Colour | Body Colour | % of Survival | Stress Test | | Dry Wt. (gm) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Formalin 200 ppm | Salinity 50% | |
| C | 5 | 3 | 5.6 ± 0.4 | Greenish | Clear | 78 ± 2 | - | - | - |
| E1 | 5 | 3 | 5.8 ± 0.8 | Greyish | Greyish | 96 ± 1 | - | - | - |
| E2 | 5 | 3 | 5.8 ± 0.8 | Greyish | Greyish | 96 ± 2 | - | - | - |
| E3 | 5 | 3 | 5.6 ± 0.6 | Greyish | Greyish | 99 ± 2 | - | - | - |

**Table 3.** Comparative performances of Length, Number of rostral spines Body colour, survival percentage, stress test and Average wet weight of *P. vannamei* at PL 10.

| Exp | Age of PL | RSP | Average Length | HP Colour | Body Colour | % of Survival | Stress Test | | Dry Wt. (gm) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Formalin 200 ppm | Salinity 50% | |
| C | 10 | 4 | 8.2± 0.2 | Greenish | Greenish | 62 ± 2 | - | - | - |
| E1 | 10 | 5 | 9.5 ± 0.3 | Pinkish | Brownish | 81 ± 2 | - | - | - |
| E2 | 10 | 5 | 9.5± 0.4 | Pinkish | Brownish | 86 ± 4 | - | - | - |
| E3 | 10 | 5 | 9.4 ± 0.4 | Pinkish | Brownish | 89 ± 2 | - | - | - |


**Figure 3.** Plate showing the Control "C" and Experimental (E1, E2&E3) groups exposed to Reduced salinity of 50% stress test.


**Figure 4.** Plate showing the Control "C" and Experimental E1, E2 & E3 groups exposed to Formalin 200 ppm stress test.

**Table 4.** Comparative performances of Length, Number of rostral spines, Body colour, survival percentage, stress test and Average wet weight of *P. vannamei* at PL 15.

| Exp | Age of PL | RSP | Average Length | HP Colour | Body Colour | % of Survival | Stress Test | | Avg.wet. Wt. (gm) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Formalin 200 ppm | Salinity 50% | |
| C | 15 | 5 | 10.3± 0.2 | Greenish | Bluish | 55 ± 2 | 78 | 82 | 0.17 ± 0.4 |
| E1 | 15 | 6 | 12.5± 0.3 | Pinkish | Brownish | 79 ± 2 | 100 | 100 | 0.29± 0.2 |
| E2 | 15 | 7 | 12.8± 0.4 | Pinkish | Brownish | 82 ± 2 | 100 | 100 | 0.27± 0.2 |
| E3 | 15 | 6 | 12.5± 0.4 | Pinkish | Brownish | 82 ± 2 | 99 | 100 | 0.25 ± 0.2 |

The outcomes of stress assessments revealed elevated rates of 100%, 100% and 99% accordingly in the experimental groups E1, E2, and E3, where the subjects were subjected to 200 ppm formalin for approximately thirty minutes (Table 4 & Figure 4). The same animal groups exposed to lower salinity instantly displayed 100% across all groups where "Astaxanthin" was utilized as a feed additive. The mean wet weight of the control group "C" is 0.17 ± 0.4, while the average weights for the experimental groups "E1, E2 & E3" are 0.29 ± 0.2, 0.27 ± 0.2, and 0.25 ± 0.2, respectively. The experimental groups with Astaxanthin as a feed supplement exhibited greater wet biomass (0.27 ± 0.2) compared to the control "C" group (0.17 ± 0.4) (Figure 5). At the conclusion of the experiment, the final survival rate of post larvae in the control group "C" was 55 ± 2%, while the experimental groups (E2 & E3) exhibited a survival rate of 82 ± 2% (Figure 6).

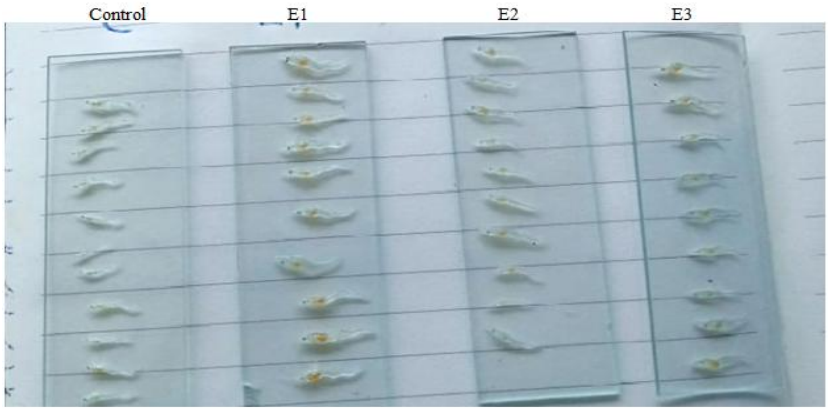


**Figure 5.** Plate showing the final survival rate of post-larvae of *P. vannamei* in control against Experimental groups.

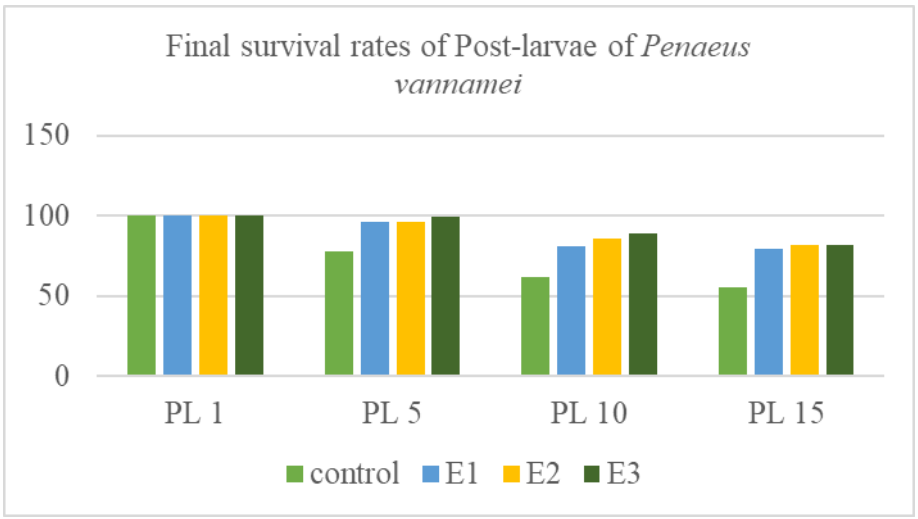


**Figure 6.** Graph showing Final survival rates of post-larvae of *P. vannamei* in Control and Experimental groups.

Previous research by Yingying Yu *et al*., (2020) demonstrated the impact of dietary astaxanthin (AX) on growth performance, antioxidant measures, and the repair of hepatopancreas damage in Pacific white shrimp (*Litopenaeus vannamei*). Howell & Mathew (1991) indicated that "Blue Color Syndrome" was reinstated in *Penaeus monodon* through the use of 50 ppm of astaxanthin as a feed additive for approximately four weeks. Findings from this study indicated that animals given "Astaxanthin" as a dietary supplement did not exhibit bluish coloration on their body surfaces, and the wet weight of the shrimp post-larvae along with other quality parameters were superior to the control group by the end of the experiment (15 days), aligning with earlier research outcomes.

## CONCLUSION

The findings of this research indicate that the application of “Astaxanthin” at 50 ppm as a feed supplement for *Penaeus vannamei* post larvae demonstrates a potential effect in treating or hindering the onset of “Blue Colour Syndrome” during their development. Additionally, the application of “Astaxanthin” improves the survival rate, boosts growth performances by properly developing rostral spines, and increases tolerance to environmental mechanical stress. In summary, Astaxanthin supplementation effectively manages Blue Shell Syndrome in the post-larval phases of *Penaeus vannamei* during seed production.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interest

## ETHICS APPROVAL

Not applicable

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## AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

## DATA AVAILABILITY

Data will be available on request

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