Effects of Vitamins A, C, E and their Combination on Growth and Survival of *Litopenaeus vannamei* Post Larvae

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Abstract

This study was carried out in order to determination of the effects of vitamins A, vitamin C, vitamin E and their combination on growth and survival rate of *Litopenaeus vannamei* post larvae (PLs). For this purpose, one control group (fed non-enriched Artemia) and 4 treatments (fed enriched Artemia) with three replications were considered. After 15 days, Special Growth Rate (SGR), Survival Rate (SR) after experimental period and salinity stress were measured. According to results, total length of post larvae were not different between treatments and control, although the highest and lowest values of SGR and total weight of PLs were observed in T3 (Vitamin E) (SGR: % 58) and T1 (Vitamin A), Control (SGR: % 30) respectively. Also, the highest and lowest values of SR after experimental period were found in T3 (SR: % 99) and T4 (A+E+C) (SR: % 86). After salinity stress, the SR was similar between experimental treatments, although these values were higher compared to control group. In conclusion, our results showed that vitamins especially vitamin E have key role in growth and survival of *Litopenaeus vannamei* PLs.
**Introduction**

Nowadays, the species diversity is an important issue for crescive development of aquaculture industry. Shrimps are one of the most important cultural species especially during two last decades. In this respect, three shrimp species including Asian tiger shrimp, *Penaeus monodon*, Chinese shrimp, *Penaeus chinensis* and *Litopenaeus vannamei* make most portion of aquaculture respectively [15]. After 2002 year, the vannamei shrimp become widespread and obtained the first place among cultural shrimps in Asia due to it's resistance against White spot disease and new intensive aquaculture techniques [4]. In Iran, with regard to fast growth of shrimp aquaculture, needs to suitable species and their healthy PLs is necessary. The production of PLs with suitable quality and resistant against diseases and environment stressors results finally marketable crop and brooders with high fecundity. Similar to fish, the nourishment of shrimp PLs is one of the most important parameter influencing PLs quality indices (i.e. SR, SGR, and resistance against diseases and environment stressor). In this respect, several studies showed that the vitamins improve immunity system, meat quality, survival, growth rates, resistance against diseases and stressors, fecundity and reproductive efficiency [3,5,12,14]. For example, vitamin C has good roles in compatibility with unsuitable environmental conditions, bony structure, improvement of larvae quality in stressful conditions and enhancement of immunity system against pathogens [5,12,14]. At now, vitamin C has been used successfully in diet of vannamei species. Vitamin A improves the growth, sexual maturation and immunity system. The vannamei species is one of the most important exotic shrimp species in Iran that is propagated and cultured in southern and northern coast of Iran. Therefore, studies for improvement of its quality and production are necessary. To this end, in this study, we investigated the effects of vitamins A, C, E and their combination on growth and survival of *Litopenaeus vannamei* PLs.

**Material and methods**

The present study was carried out in Chabahar bay, Iran for a period of two weeks. After propagation, a number of 500 PLs (mean total weight: 0.0013 g) was counted and then PLs were divided into small 20 liter tanks (each 100 PLs in one tank). For each tank, an air stone was used for aeration. During the experiment, the water temperature was 27–29 °C, salinity was 22-25 g.L⁻¹, dissolved oxygen was 5.2-6.5 mg.L⁻¹ and pH 8.2-8.3. Also, the experimental tanks were washed every two days to remove excretory and suspended materials. One control group (fed non-enriched Artemia) and 4 treatments (fed enriched Artemia) with three replications were considered as follow:
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Treatment 1 (T1): enriched artemia with vitamin A, T2: enriched artemia with vitamin C, T3: enriched artemia with vitamin E, T4: enriched artemia with combination of vitamins (A+C+E). To enrich artemia with experimental vitamins, the following protocols were used:

**a) Preparation of enrichment solution of vitamin C:**
Amount of 20 mg Ascorbyl palmitat was mixed with 50 ml cod oil (SeasSeven, England) and 50 ml lukewarm water in three steps and each step 3-5 min. After addition of 3-5 ml sodium polysorbate to this mixture, the mixture was mixed in three steps and each step 3-5 min until the small oil droplets be formed. Finally, this solution was diluted to 200 ppm with distilled water [10].

**b) Preparation of enrichment solution of vitamin A and E:**
Amount of 1 g Lecithin was added to 100 mL lukewarm water and then mixed by a mixer in three steps and each step 3-5 min. After that, a volume of 20 mL vitamin A or vitamin E in 100 mL Lecithin solution and mixed in three steps and each step 3-5 min until the small droplet be formed. The diameter of droplets should be less than 10 µm. Thus, the size of droplets investigated under a Stereomicroscope. Finally, this solution was diluted to 200 ppm with distilled water.

**c) Enrichment of artemia with vitamin:**
A density of 250000 artemia naplius per liter (A. Franciscana) were released to 2 liter Erlenmayer containing 200 ppm enrichment solution with 35 ppt salinity, temperature 28 °C and pH 8 [10]. During experimental period, the vannamei PLs were fed by enriched naplius and non-enriched naplius two times and four times per day and night with 4 h intervals respectively. The value of used naplius was calculated according to feeding chart and PLs needs.

**Measurement of special growth rate (SGR) and Survival Rate (SR)**
After 15 days, special growth rate (SGR), Survival Rate (SR) after experimental period and after salinity stress (decreasing of salinity from 25 ppt to 10 ppt) was measured. The SR was measured by counting of PLs. The SGR was calculated as follow:

\[ \% \text{ SGR} = 100 \times \frac{\ln W_2 - \ln W_1}{\text{days}} \]

Where \( W_1 \) = mean of primary weight, \( W_2 \) = mean of final weight

**Statistical analysis**
The SPSS software was used for data analysis. Because of percentage data (% SGR and % SR) did not have a normal distribution, proportional data were converted by angular transformation (arcsin \( \sqrt{p} \)). One-way analysis of variance (ANOVA) was employed to compare the means. When significant F-ratios were calculated by ANOVA, the Tukey test was applied to identify which means were different.
Results

After 15 days experiment period, the values of % SR and % SGR were compared between experimental treatments and control. Mean of assayed parameters (% SGR, % SR and Length (cm)) after the course of experiment has been shown in table 1. According to results, total length of PLs were not different between treatments and control (P>0.05), although the highest and lowest values of SGR and total weight of PLs were observed in T3 (SGR: % 58) and T1, Control (SGR: % 30) respectively (Figure 1,).

Also, the highest value of % SR after experimental period were found in T3 (SR: % 99) (Figure 2, P<0.05). Although, the values of % SR were not statistically different between experimental treatments and control (Figure 3, P>0.05) after salinity stress, nevertheless, the maximum values of was observed in T3.

Table 1. The Means of %SGR, %SR and Length (cm)) after the course of experiment. %SR1: the survival rate after the course of experiment and % SR2: the survival rate after salinity stress

<table>
<thead>
<tr>
<th>Treatments</th>
<th>% SGR</th>
<th>Total length (cm)</th>
<th>% SR1</th>
<th>% SR2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>0.3</td>
<td>1.1</td>
<td>0.283</td>
<td>0.9</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.45</td>
<td>1.1</td>
<td>0.141</td>
<td>0.97</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>0.58</td>
<td>1</td>
<td>0.141</td>
<td>0.99</td>
</tr>
<tr>
<td>Vitamin A+C+E</td>
<td>0.45</td>
<td>1.25</td>
<td>0.250</td>
<td>0.86</td>
</tr>
<tr>
<td>Control</td>
<td>0.3</td>
<td>1.05</td>
<td>0.071</td>
<td>0.93</td>
</tr>
</tbody>
</table>
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Figure 1. The mean values of %SGR in experimental treatments after the course of the experiment. The means with same letters on error bars are not statistically different (P>0.05).

Figure 2. The mean values of %SR in experimental treatments after the course of the experiment. The means with same letters on error bars are not statistically different (P>0.05).
Figure 3. The mean values of %SR in experimental treatments after salinity stress. The means with same letters on error bars are not statistically different (P>0.05).

**Discussion**

Our results demonstrated that vitamins especially vitamin E have enhancing effect on growth and survival rate of *Litopenaeus vannamei* PLs during the course of the experiment and after an acute stressor i.e. salinity falling. In this study, we used the Artemia as life food due to its simple propagation and efficient enrichment [1]. A few studies have described the useful impacts of vitamins (especially vitamin A, vitamin C and vitamin E) on growth, immunity and survival of penaeid shrimps. Lighter et al, 1977 [9] first demonstrated the essential role of dietary vitamin C in preventing the development of the Black Death syndrome in penaeid shrimp. Merchie et al. (1998) [11] showed that dietary vitamin C increases the survival rate of tiger shrimp, *Penaeus monodon* after an osmotic shock. … et al showed that the survival of *P. vannamei* decreases as dietary vitamin c decreases. Also, vitamin C enhanced the immune system of *P. vannamei* and *P. japonicus* against *Vibrio harveyi* and *Vibrio* Spp respectively [6,7]. Reddy et al., (1999) [13] showed that diet deficient in vitamin C and E resulted in poor feed conversion efficiency. The weight gains and total haemocyte count were higher in tiger shrimps fed diets supplemented with more dosage of vitamin E than in shrimp fed with less dosage [8]. Generally, vitamins play important roles in animal
health as antioxidants by inactivating damaging free radicals produced through normal cellular activity and from various stressors [2]. It has been suggested that the antioxidant function of these micronutrients could enhance immunity by preserving the functional and structural integrity of important immune cells. Vitamin C has good roles in compatibility with unsuitable environmental conditions, bony structure, improvement of larvae quality in stressful conditions and enhancement of immunity system against pathogens [5,12,14]. Also, vitamin A improves the growth, sexual maturation and immunity system. Due to the importance of polyunsaturated fatty acids in the diets of crustaceans, it assumed that vitamin E will be important as a metabolic antioxidant [3]. In conclusion, our study showed that the vitamins especially vitamin E improves growth, survival and resistance against stress in P. vannamei PLs. Therefore, adequate supply of these vitamins in diet o increases the commercial production of this species.

Acknowledgement

The authors express their sincere appreciation to Dr. Yahyavi, Dr. Mohamadizadeh for helpful comments during experiment and Mr. Madani (Manager of Shrimp Propagation Center, Chabahar) for kindly cooperation.

References


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Received: February, 2012