EFFECTS OF FEED ON THE SURVIVAL RATE, GROWTH RATE AND METAMORPHOSIS TIME OF THE WHITE LEG SHRIMP LARVAE (*Litopenaeus vannamei* Boone, 1931) AT ZOEA STAGE

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ABSTRACT

The larvae of white leg shrimp (*Litopenaeus vannamei* Boone, 1931) at phase nauplii 6 fed with a stocking density of 150 pcs per litre and 3 formula including formula 1 with dry microalgae *Spirulina + artemia* as main ingredients, formula 2 with microalgae *Thalasiosira weissflogii + artemia* as main ingredients and formula 3 with microalgae *Thalasiosira pseudonana + artemia* as main ingredients. Experiments were carried out in 18 tanks fifty-litre composite at Quang Binh branch of C.P Vietnam Corporation. The study results show that, with formula 3, white leg shrimps larvae at zoea stage have the highest growth and survival rates (86.57 %), followed by formula 2 (76.03 %) and finally formula 1 (67.90 %). White leg shrimps larvae at zoea stage in formula 3 have the shortest metamorphosis time (78.61 hours), followed by formula 2 (83.25 hours) and the longest in formula 1 (94.10 hours).

Keywords: Litopenaeus vannamei, feed, survival rate, metamorphosis time.

1. INTRODUCTION

With the developments of feeding technology and high-level management of breeding production technology, culturing white leg shrimps tends to accelerate in the future. There are many factors that affect the survival and growth rate during the period of shrimp breeding such as the environment [1], feeds [2, 3] and stocking density [4, 5]. According to Brown and his co-operators [6]: microalgae *Thalassiosira pseudonana* had high nutritional values from dry weight (g/cell), the mass of the components (for each g/cell) 28.4, chlorophyll a 0.27, protein 9.7, carbohydrate 2.5, and lipid 5.5; percentage of dry weight, respectively 28.4 %, chlorophyll a 0.95 %, protein 34%, carbohydrate 8.8 %, and lipid 19 %; high unsaturated fatty acid content (DHA + EPA 7.2 mg/ml cells) [6]. There have been a number of authors who published research results about the effects of feeds on survival rate, growth rate and metamorphosis time of white leg shrimps larvae such as Nguyen Trong Nho, Ta Khac Thuong, Luc Minh Diep [7], Dao Van Tri [8]. However, these studies have only solved the basic problems in the post-larval stage but

not paid adequate attention to the larvae. Besides, the experiments have not been controlled in optimum conditions for raising environmental factors. That's why we studied "Effects of diets on to the survival rate, growth rate and metamorphosis time of the white leg shrimps larvae (*Litopenaeus vannamei* Boone, 1931) at zoea stage".

2. MATERIALS AND METHODS

2.1. Materials

The larvae of white leg shrimps (*Litopenaeus vannamei* Boone, 1931) at phase nauplii 6, CPF-Turbo seed by brood stock production nauplii at Quang Binh branch of C.P Vietnam Corporation.

2.2. Conditions and experimental methods

Water quality is ensured the following factors: temperature 30 - 31 $^{\circ}$C, 29 – 32 ‰ salinity, pH 7.9 to 8.2, 160 - 180 ppm alkalinity.

The experiment was arranged with the formula: formula 1 (dry algae 50 % *Spirulina* + 30 % *artemia*); formula 2 (50 % microalgae *Thalassiosiraweissflogii* + 30 % *artemia*); formula 3 (50 % microalgae *Thalassiosirapseudonana* + 30% *artemia*). Density larvae in experimental: 150 pcs/liter [4, 5, 7, 8] and stocking culture in the volume of water 40 liter each tank. Feed used 20 % TNT (500 g/bag) with nutritional components of feed including 40 % protein, 9.5 % fat, 3 % fiber, 15 % ash, 10 % humidity. Microalgae are used from stage zoea 1 to stage mysis 1. Microalgae density is 25000 - 30000 cells/ml. Microalgae as feed is offered 4 times/day on 8, 14, 22 and 02 hours. At the stage zoea 2, provide *artemia*, from stage zoea 2 to stage mysis 3 *artemia* sources. Feed should be spread evenly throughout an experiment. The larvae are fed 8 times per day (alternating *artemia* and feed TNT) at 6, 9, 12, 15, 18, 21, 24 hours and according to the actual needs of the larvae in the experiments.

2.3. Methods of data collection

2.3.1. Subscribe to survival

The formula for calculating the survival rate in each stage (T_s):

$$T_s\,(\%) = \frac{T_2}{T_1} \times 100$$

in which: T_1 count is the number of larvae in the previous period (pcs); T_2 the numbers of larvae were counted at a later stage (pcs).

The formula for calculating the cumulative survival rate:

$$M\,(\%) = \frac{M_2}{M_1} \times 100$$

in which: M_1 the initial draft of larvae (pcs); M_2 count is the number of larvae at a later stage (pcs).

Quantitative of larvae:

$$A = \frac{m}{v} \times V$$

in which: A the total number of larvae in a tank (pcs); M the average number of larvae in samples v = 700 ml; V is the volume of water in the tank (40 liter).

2.3.2. Time monitoring of larval metamorphosis

The formula for calculating:

$$T_{bt} = T_2 - T_1$$

in which: T_{bt} duration of larval time metamorphosis (hours); T_1: Transfer time 50 % larval stage before (hours); T_2: Larvae time period after moving 50 % (hours).

2.3.3. Subscribe the length of the shrimp body

Collecting about 30 larvae/ experimental tank to test once.
Absolute length growth:

$$DG \text{ (mm/day)} = \frac{Ls - Lt}{\Delta t}$$

Length relative growth: S

$$GR \text{ (\%/day)} = \frac{\ln(Ls) - \ln(Lt)}{\Delta t} \times 100$$

in which: DG is the growth of the absolute length; SGR is the growth of the relative length; Ls is the index of average body length was measured at time t_2; Lt is the index of average body length was measured at time t_1; Δt is the period of time between two measurements.

2.4. Data processing methods

The data were statistically processed on IBM SPSS 22.0 software. Use $LSD_{0.05}$ Post Hoc, analysis of variance factor (ANOVA).

3. RESULTS AND DISCUSSION

3.1. Effects of formula feed on the survival of white leg shrimp larvae at stage zoea

The results obtained on the effect of formula feed on survival of white leg shrimp larvae at stage zoea are shown in Table 1.

Table 1. Survival of the larvae stage zoea 1-zoea 2; zoea 2-zoea 3; zoea 3-mysis 1 in the different formula.

Formula	Survival rate (%)		
	Zoea 1-Zoea 2	Zoea 2-Zoea 3	Zoea 3-Mysis 1
Formula 1	59.17 ± 2.84^a	64.60 ± 2.26^a	67.90 ± 0.87^a
Formula 2	68.87 ± 1.06^b	71.27 ± 1.21^b	76.03 ± 2.28^b
Formula 3	80.37 ± 0.72^c	84.20 ± 0.61^c	86.57 ± 1.16^c

Note: The data in the same column with different letters hat is significant difference (p < 0.05).

The data in Table 1 shows that, when using different types of feed, the survival rate of larvae is different. In stage zoea 1-zoea 2, the highest survival rate in formula 3 is 80.37 %, followed by the formula 2 reached 68.87 % and the lowest in formula 1 is 59.17 %, the difference between them has the statistical significance ($p < 0.05$).

Similarly zoea 2-zoea 3 stage, larval survival rate is relatively high and there is a clearer distinction with the previous period, the highest in the formula 3 is 84.20 %, followed in formula 2 reached 71.27 % and the lowest in the formula 1 is 64.60 %. $LSD_{0.05}$ inspection results showed that the difference between them was statistically significant ($p < 0.05$).

In stage zoea 3-mysis 1, the highest survival rate in formula 3 is 86.57 %, followed by the formula 2 reached 76.03 % and the lowest in formula 1 is 67.90 %, the difference between them has the statistical significance ($p < 0.05$). When stocking larvae in fresh algae *Chaetoceros sp, Skeletonema costatum*, *artemia* + feed Lansy and Frippak for survival rate 75.5 % to 76.8 % [8]. The results are: formula 3 for larval survival rate in all 3 phases the highest, followed by formula 2 and the lowest is formula 1, this demonstrates that feed has a clear impact on the survival rate of larvae white leg shrimps zoea stage. According to a study by Dao Van Tri and Nguyen Thi Thanh Hoa [9], at the period zoea the highest survival rate appears when using fresh algal live feed. Thus, the results of our experiments are fully consistent with the results of the authors.

3.2. Effects of formula feed to time metamorphosis of white leg shrimp larvae at stage zoea

Time metamorphosis is different in each stage, the results of monitoring the impact of feed rations to the time variation of white leg shrimps larvae at stage zoea shown in Table 2.

Table 2. Duration of larval metamorphosis at stages zoea 1-zoea 2; zoea 2-zoea 3; zoea 3-mysis 1 in the different formula.

Formula	Metamorphosis time (hours)			
	Zoea 1-Zoea 2	Zoea 2-Zoea 3	Zoea 3-Mysis 1	Total metamorphosis time (hours)
Formula 1	33.43 ± 0.96^c	31.10 ± 1.74^c	29.57 ± 0.59^c	94.10
Formula 2	27.45 ± 0.41^b	28.20 ± 0.52^b	27.60 ± 0.10^b	83.25
Formula 3	24.17 ± 0.29^a	27.49 ± 1.03^a	26.95 ± 0.43^a	78.61

Note: The data in the same column with different letters hat is significant difference ($p < 0.05$).

The results obtained in Table 2 show that the total duration of larval metamorphosis of white leg shrimps is shortest at period zoea with formula 3 is 78.61 hours, followed by formula 2 is 83.25 hours and was the longest in formula 1 is 94.10 hours. Accreditation $LSD_{0.05}$ shows the difference between them has the statistical significance ($p < 0.05$).

In stage zoea 1-zoea 2, formula 1 metamorphosis time is longest, followed by formula 2 and the shortest is formula 3, the differences between formula 1 with formula 2 and formula 3 have statistical significance ($p < 0.05$).

In the phase zoea 2-zoea 3, zoea 3-mysis 1 of white leg shrimps larvae metamorphosis time is shortest in formula 3, next is formula 2 and the longest is formula 1, the difference between them has the statistical significance ($p < 0.05$). In particular, stage zoea 3-mysis 1 metamorphosis time is longer than 2 phases, zoea 1-zoea 2 and zoea 2-zoea 3. According to Thai

Ba Ho and Ngo Trong Lu [10], metamorphosis time of the larval at stage zoea is 36 hours. Thus, the variation time of white leg shrimps larvae at stage zoea in our experiments in 3 formulas is shorter. This may be due to the effects of different formula on white leg shrimps larvae at stage zoea. Because the cells evenly, high dispersion, size 30-31μm fit mouth size of larvae, the larvae use more than the large-sized algae. Microalgae *Thalassiosira weissflogii* size is > 500 μm. Microalgae *Chaetoceros sp* and *Skeletonema costatum* are on the cell size of 4-6 μm.

3.3. Effects of formula feed on the growth of the total length of white leg shrimp larvae at stage zoea

3.3.1. Total length growth of white leg shrimp larvae at stage zoea

Results of monitoring growth of the white leg shrimps larval body length at stage zoea during the experiments were statistically analyzed in Table 3.

Table 3. The comparison of total length of the larval at stages zoea 1, zoea 2, zoea 3, mysis 1 in the different formula.

Formula	Total length (mm)			
	Zoea 1	Zoea 2	Zoea 3	Mysis 1
Formula 1	1.175 ± 0.047^a	1.296 ± 0.033^a	1.891 ± 0.240^a	2.255 ± 0.110^a
Formula 2	1.239 ± 0.037^b	1.420 ± 0.076^b	2.107 ± 0.119^b	2.565 ± 0.098^b
Formula 3	1.269 ± 0.017^c	1.582 ± 0.074^c	2.413 ± 0.092^c	2.980 ± 0.108^c

Note: The data in the same column with different letters hat is significant difference ($p < 0.05$).

Larvae at stage zoea 1 the full body length in formula 3 has the highest average (1.269 ± 0.017 mm), followed by the formula 2 (1.239 ± 0.037 mm) and the lowest is in formula 1 (1.175 ± 0.047 mm). Larvae at stage zoea 2 the total length in formula 3 has the highest average (1.582 ± 0.074 mm), followed by the formula 2 (1.420 ± 0.076 mm) and the lowest is in formula 1 (1.296 ± 0.033 mm), the difference between them is statistically significant difference ($p < 0.05$).

At stage zoea 3, the growth in average total length was highest in formula 3 (2.413 ± 0.092 mm), followed by the formula 2 (2.107 ± 0.119 mm) and the lowest was in formula 1 (1.891 ± 0.240 mm). At stage mysis 1, full body length in formula 3 has the highest average (2.980 ± 0.108 mm), followed by formula 2 ($2.56 5 \pm 0.098$ mm) and the lowest is formula 1 (2.255 ± 0.110 mm), the difference between them is obvious ($p < 0.05$).

3.3.2. Growth in absolute and relative total length of white leg shrimp larvae at stage zoea

The research results obtained showed that the different feed formulations can affect the length of the absolute growth of white leg shrimps larvae at stage zoea, the research results are presented in Table 4.

Table 4. The absolute and relative growth rate to body length of larvae zoea in formula.

Stage	The growth rate of total length					
	Formula 1		Formula 2		Formula 3	
	DG (mm/day)	SGR (%/day)	DG (mm/day)	SGR (%/day)	DG (mm/day)	SGR (%/day)
$Z_1 - Z_2$	0.121 ± 0.004^a	9.794 ± 0.328^a	0.180 ± 0.007^b	13.592 ± 0.566^b	0.313 ± 0.003^c	22.072 ± 0.164^c
$Z_2 - Z_3$	0.595 ± 0.003^a	37.794 ± 0.081^a	0.687 ± 0.006^b	39.484 ± 0.229^b	0.831 ± 0.007^c	42.204 ± 0.306^c
$Z_3 - M_1$	0.364 ± 0.007^a	17.591 ± 0.357^a	0.457 ± 0.011^b	19.645 ± 0.508^b	0.568 ± 0.001^c	21.127 ± 0.093^c
$Z_1 - M_1$	1.080 ± 0.005^a	65.178 ± 0.310^a	1.325 ± 0.004^b	72.721 ± 0.287^b	1.712 ± 0.006^c	85.403 ± 0.177^c

Note: The data in the same row with different letters hat is significant difference ($p < 0.05$).

The data in Table 4 shows that the day absolute growth rate of body length of shrimp larvae at stage zoea is different between different feed treatments. Absolute growth rate of body length of the shrimp zoea larvae is largest in stages zoea 2-zoea 3 (0.831 mm/day) in formula 3 and lowest in stage zoea 1-zoea 2 (0.121 mm/pcs/day) in formula 1.

In stage zoea 1-zoea 2, the day absolute growth rate of zoea larvae length is highest in formula 3 (0.313 mm/day), followed by the formula 2 (0.180 mm/day) and lowest in formula 1 (0.121 mm/day), the differences between them are statistically significant ($p < 0.05$). In stage zoea 2-zoea 3, the day absolute growth in length of zoea shrimp larvae is largest in formula 3 (0.831 mm/day) and lowest in formula 1 (0.595 mm/day).

In stage zoea 3-mysis 1, the absolute difference in the growth of the zoea larvae total length between feed formulations has statistical significance ($p < 0.05$). The day absolute growth rate has the highest total length in formula 3 (0.568 mm/day), followed by the formula 2 (0.457 mm/day) and lowest in the formula 1 (0.364 mm/day). When comparing both zoea 1-mysis 1 stage we can see, the day absolute growth rate of total length peaked in formula 3 (1.712 mm/day), followed by formula 2 (1.325 mm/day) and lowest in formula 1 (1.080 mm/day), the difference between them is significant ($p < 0.05$).

Relative growth rate of the total length of white leg shrimps larval stages zoea is different between feeding formula and decreases from zoea 1 to zoea 3: maximum relative growth rate in phase zoea 2-zoea 3 (42.204%/day) in formula 3 and lowest in stage zoea 1-zoea 2 (9.794%/day) in formula 1. Larvae in stages zoea 1-zoea 2 relative growth rate is highest in the formula 3 length (22.072%/day), followed by the formula 2 (13.592%/day) and lowest in the formula 1 (9.794%/day), with the difference between formula 1 with formula 2 and formula 3 is statistically significant ($p < 0.05$).

In stage zoea 2-zoea 3, the white leg shrimps larvae at stage zoea in formula 3 with relative growth rate of the total length is highest (42.204%/day) and lowest in formula 1 (37.794%/day). $LSD_{0.05}$ inspection results showed that the relative growth of the total length of the larval of white leg shrimps in formula 3 compared to formula 1 and formula 2 has differences and statistically significance ($p < 0.05$). At stage zoea 3-mysis 1, the relative growth rate of the total

length of the white leg shrimps larvae at stage zoea in formula 3 is highest (21.127%/day), followed by the formula 2 (19.645%/day) and lowest in formula 1 (17.591%/day). $LSD_{0.05}$ analysis results showed that the difference between them is statistically significant ($p < 0.05$).

Assess the day-average relative growth rate on the length of the white leg shrimps larvae in whole stage zoea 1-mysis 1 we see that the rate is highest in formula 3 (85.403%/day), followed by the formula 2 (72.721%/day) and lowest in formula 1 (65.178%/day). $LSD_{0.05}$ analysis results showed that the difference between them is significant ($p < 0.05$).

4. CONCLUSIONS

The formula including microalgae *Thalasiosira pseudonana + artemia* (formula 3) has helped white leg shrimps larvae growth at zoea phase with the highest survival rate, followed by the formula of microalgae *Thalassiosira weissflogii + artemia* (formula 2) and the lowest is the formula with dried *Spirulina* algae + *artemia* (formula 1). Formulas can affect the larval metamorphosis time. The formula with microalgae *Thalasiosira pseudonana + artemia* (formula 3) has the shortest metamorphosis time (78.61 hours), followed by the formula of *Thalassiosira weissflogii + artemia* (formula 2) is 83.25 hours and the longest metamorphosis time is 94.10 hours in the formula of dry feed algae *Spirulina + artemia* (formula 1).

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TÓM TẮT

ẢNH HƯỞNG CỦA THÀNH PHẦN THỨC ĂN ĐẾN TỈ LỆ SỐNG, SINH TRƯỞNG
VÀ THỜI GIAN BIẾN THÁI CỦA ẤU TRÙNG TÔM THẺ CHÂN TRẮNG
(*Litopenaeus vannamei* Boone, 1931) Ở GIAI ĐOẠN ZOEA

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Ấu trùng tôm thẻ chân trắng (*Litopenaeus vannamei* Boone, 1931) giai đoạn Nauplius 6 được nuôi ở mật độ 150 con/lít với 3 loại thức ăn, gồm công thức 1 có thành phần chính là tảo khô *Spirulina* + *artemia*; công thức 2 có thành phần chính là vi tảo *Thalassiosira weissflogii* + *artemia*; công thức 3 có thành phần chính là vi tảo *Thalassiosira pseudonana* + *artemia*. Các lô thí nghiệm được lặp lại 3 lần và 2 đợt thí nghiệm với tổng số 18 bể thí nghiệm, mỗi bể có thể tích 50 lít. Thí nghiệm được thực hiện tại Trại giống Quảng Bình, Công ti Cổ phần Chăn nuôi C.P Việt Nam. Kết quả thu được trong nghiên cứu cho thấy, công thức thức ăn có vi tảo *Thalasiosira pseudonana* + *artemia* (CT 3) đã giúp ấu trùng tôm thẻ chân trắng giai đoạn zoea có khả năng tăng trưởng, tỉ lệ sống cao nhất (86,57 %), tiếp đến công thức có vi tảo *Thalassiosira weissflogii* + *artemia* (CT 2) (76,03 %) và thấp nhất là công thức có tảo khô *Spirulina* + *artemia* (CT 1) (67,90 %). Ấu trùng tôm thẻ chân trắng khi kết thúc giai đoạn zoea có thời gian biến thái ngắn nhất (78,61 giờ) ở công thức 3, tiếp đến ở công thức 2 (83,25 giờ) và dài nhất là ở công thức 1 (94,10 giờ).

Từ khoá: Litopenaeus vannamei, thức ăn, tỉ lệ sống, thời gian biến thái.