

# Effect of Dietary Protein Levels on Growth Performance, Feed Utilization and Economical Evaluation of White Shrimp Larvae *Litopenaeus vannamei*

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**Abstract:** The present experiment was conducted to investigate the effects of five different protein levels (25%, 30%, 35%, 40% and 45%) on growth performance, feed utilization, survival and economic analysis of whiteleg shrimp, *Litopenaeus vannamei* larvae. The experiment was performed indoor at Invertebrate laboratory of National Institute Oceanography and Fisheries (NIOF), Suez governorate -Egypt. The experiment was carried out in 15 rectangular tanks (66\*47\*44 cm, 50 L) of reinforced with water salinity 20 ppt for 90-days. Triplicate groups of shrimp larvae with an initial weight 0.002g were fed twice a day at a feeding ratio of 14% from body weight (nit weight) and re-adjusted gradually to 5% at the end of the experiment. The results observed that the highest significant ( $p < 0.05$ ) weight gain (%), specific growth rate, best feed conversion ratio values were reported with the shrimp fed 35% protein group. The same trend was observed for protein utilizing efficiency. Protein efficiency ratio was significantly affected by dietary protein level. The present results revealed that dietary crude protein of 35% for *L. vannamei* larvae may optimum in terms of growth performance, feed utilization and economic evaluation under these experimental conditions.

**Keywords:** *Litopenaeus vannamei*, growth performance, feed utilization, survival (%), economic evaluation, white shrimp

## INTRODUCTION

The Pacific whiteleg shrimp, *Litopenaeus vannamei* (Boone, 1931), is the most important cultivated shrimp species and has presented the highest value of all traded crustacean products, consequently, the global production of white leg shrimp was 51.7 million tones 2020 (FAO, 2022). This species has several merits that make it more suitable for aquaculture than other penaeids shrimp species, such as high density tolerance, and adaptability to wide ranges of several environmental parameters, such as salinity and temperature (Rocha *et al.*, 2010).

Dietary protein is the most important factor affecting growth performance of shrimp and feed cost (Hu *et al.*, 2008). Protein is an essential and the most expensive nutrient in prepared feeds for shrimp and reducing its level will result in a drastic reduction in growth. Thus formulated diet should be carefully to meet the needs of the cultured shrimp (Jackson, 2003) especially for optimized dietary protein level, cost-effective growth, reduced environmental impact, and improved water quality (Güroy *et al.*, 2012).

Fish meal is an important protein source in shrimp feed, however, the shortage of fish resources and high price of fish meal restrict the sustainable development of feed industry and shrimp culture. Therefore, the use of alternative protein sources to reduce the inclusion of fish meal is an important trend in shrimp feed industry. Recently, the substitution of fish meal with animal and plant protein sources in shrimp feed have been reported in soybean protein concentrate (Chen *et al.*, 2017), fermented soybean meal (Yao *et al.*, 2020; Lin and Chen, 2022; Cai *et al.*, 2022). The optimal dietary protein requirement of *L. vannamei* has been reported from 20 to 45% depending on the shrimp size, water conditions, and dietary characteristics such as, protein quality, energy content, and palatability (Yun *et al.*, 2015; Yun *et al.*, 2016). Several studies have estimated the varying optimum dietary protein

requirements for *L. vannamei* under different conditions 32% (Kureshy and Davis, 2002), 36% (Smith *et al.*, 1985), 40% (Liu *et al.*, 2005) and 34% (Hu *et al.*, 2008). The end product of protein catabolism in shrimp aquaculture is ammonia; consequently, excess dietary protein will be used for energy and will lead to deterioration of water quality because of an increase in ammonia excretion. Most of the nitrogen input in shrimp culture systems enters the water column as ammonium (NH<sub>4</sub><sup>+</sup>) generated by feed and is not converted into shrimp tissue. Thakur and Lin (2003) reported that *Penaeus monodon* Fabricius, 1798, only assimilate 23-31% from dietary nitrogen when fed the diet contain (42% crude protein).

In this study, the effects of five dietary different protein levels (25%, 30%, 35%, 40% and 45%) of *L. vannamei* larvae on growth performance, feed utilization, survival and economic analysis.

## MATERIALS AND METHODS

### Experimental diets

Five isocaloric experimental diets with different crude protein (CP) levels at 25%, 30%, 35%, 40%, 45%, were formulated shown in Table (1). Protein sources for the experimental diets are fish meal and soybean meal. The Amino acid composition of experimental diets is shown in Table (2). The experimental diets were prepared individually weighting of each component and thoroughly mixing the mineral, vitamins and additives with corn. This mixture was added to the components together with oil. Water was added until the mixture became suitable for making granules. The wet mixture was passed through CBM granule machine with 2 mm diameter. The produced pellets were dried at room temperature and kept frozen until experiment start.

### Experimental Design

Shrimps larvae of *L. vannamei* were obtained from a commercial shrimp hatchery in Damietta - Egypt.

Shrimps were transported in oxygenated double-layered polythene bags. When the shrimp arrived at the laboratory, they were moved into the acclimation tanks filled with Seawater after filtered by plankton net (50  $\mu\text{m}$ ) to prevent the entry of unwanted materials and suspended particles into the tanks and was diluted with fresh water to achieve a salinity of (20 ppt). Prior to

start of experiment, shrimps were acclimated to laboratory condition for two weeks and fed twice daily with commercial diet (38%) crude protein. Initial samples were taken immediately after reaching larvae from hatchery, and final sample is taken from each tank at the end of study for the determination of chemical body composition.

**Table (1):** Formulation and proximate composition of the experimental diets (Dry matter base)

Ingredients	Experimental treatments				
	T <sub>1</sub> (25% CP)	T <sub>2</sub> (30% CP)	T <sub>3</sub> (35% CP)	T <sub>4</sub> (40% CP)	T <sub>5</sub> (45% CP)
<b>fish meal</b> <sup>(70%) protein</sup>	21.00	29.00	29.00	36.00	43.00
<b>Soybean meal</b> <sup>(44%) protein</sup>	23.00	23.00	34.00	34.00	34.00
<b>Yellow corn</b>	44.00	36.00	25.00	18.00	11.00
<b>Sunflower oil</b>	7.00	7.00	7.00	7.00	7.00
<b>Mineral mixture</b> <sup>1</sup>	2.00	2.00	2.00	2.00	2.00
<b>Vitamin mixture</b> <sup>2</sup>	1.00	1.00	1.00	1.00	1.00
<b>Molasses</b>	2.00	2.00	2.00	2.00	2.00
<b>Proximate analysis</b>					
<b>Moisture</b>	11.98	11.77	11.49	11.30	11.12
<b>Crude protein</b>	25.26	30.42	35.24	40.76	45.29
<b>Lipids</b>	13.34	14.08	13.96	13.61	14.26
<b>Ash</b>	6.72	7.61	7.16	6.95	7.73
<b>Fibers</b>	3.71	3.70	3.96	2.95	2.93
<b>NFE</b> <sup>3</sup>	50.97	44.19	39.68	35.73	29.79
<b>Gross energy ( Kcal /100g)</b> <sup>4</sup>	478.27	486.55	494.11	505.76	513.08
<b>P/E (%)</b> <sup>5</sup>	5.28	6.25	7.13	8.06	8.83

1- Each Kg mineral mixture premix contained Mn, 22 g; Zn, 22 g; Fe, 12 g; Cu, 4 g; I, 0.4 g, Selenium, 0.4 g and Co, 4.8 mg.

2- Each Kg vitamin contained Vitamin A, 4.8 million IU, D3, 0.8 million IU; E, 4 g; K, 0.8 g; B1, 0.4 g; Riboflavin, 1.6 g; B6, 0.6 g, B12, 4 mg; Pantothenic acid, 4 g; Nicotinic acid, 8 g; Folic acid, 0.4 g Biotin, 20 mg

3- Nitrogen Free Extract = 100 - (%Protein + %Fat + %Fiber + %Ash).

4- Gross Energy based on protein (5.65 Kcal/g), fat (9.45 Kcal/g) and carbohydrate (4.11Kcal/g). According to (NRC, 2011)

5- Protein energy ratio = Crude protein/Gross energy (Kcal /100g)<sup>4</sup>\*100

**Table (2):** Amino acid composition of experimental diets

Amino acid	Experimental treatments				
	T <sub>1</sub> (25% CP)	T <sub>2</sub> (30% CP)	T <sub>3</sub> (35% CP)	T <sub>4</sub> (40% CP)	T <sub>5</sub> (45% CP)
<b>Arginine %</b>	2.34	2.78	3.10	3.48	3.87
<b>Histidine %</b>	0.76	0.89	0.99	1.10	1.22
<b>Isoleucine %</b>	1.36	1.61	1.79	2.01	2.22
<b>Leucine %</b>	2.55	2.92	3.16	3.49	3.81
<b>Lysine %</b>	2.14	2.62	2.89	3.31	3.73
<b>Methionine %</b>	0.69	0.85	0.90	1.04	1.18
<b>Methionine Cysteine%</b>	1.13	1.35	1.44	1.63	1.81
<b>Phenylalanine%</b>	1.42	1.63	1.83	2.01	2.19
<b>Phenylalanine Tyrosine%</b>	2.48	2.85	3.19	3.51	3.83
<b>Threonine %</b>	1.37	1.64	1.79	2.03	2.26
<b>Tryptophan %</b>	0.32	0.38	0.42	0.47	0.52
<b>Valine %</b>	1.56	1.83	2.02	2.26	2.50

After two weeks of acclimatization, all tanks were stocked with shrimp larvae in triplicate. Before distributing on tanks, we weighed the shrimp, and the initial body weight (g). Shrimp were fed with experimental diets twice a day at 14% from body weight (initial weight) and re-adjusted gradually to 5% at the end of the experiment. The daily feeding rate (%) for each treatment was calculated and adjusted by estimating the biweekly sampled mean biomass.

To maintain water quality at optimum range for shrimps the following parameters were monitored during the experiment.

**Daily parameters:** Water temperature (°C) was measured daily at 13:00 h, salinity and pH was measured daily at 10:00 h using multiparameter analyzer.

**Biweekly parameters:** Water sample (100ml) were collected from each tank and filtered by filter papers to analyze total ammonium nitrogen (TAN), nitrite-N (NO<sub>2</sub>-N), nitrate-N (NO<sub>3</sub>-N) using spectrophotometer model (JENWAY 6100).

#### Growth performance parameters

Shrimp larvae weight (g) was measured at the initial of the experiment and biweekly by collected randomize number of shrimp from each tank and weighted in an analytical digital balance and then returned back to their tanks during the experiment. Shrimp weight gain (WG), specific growth rate (SGR) survival % (S) was calculated according to the following equations:

- Weight gain (WG) = Final body weight (g) - Initial body weight (g).
- Specific growth rate (SGR, %/days) =  $[(\ln \text{FBW} - \ln \text{IBW}) / \text{day of experiment}] \times 100$
- Survival (%) = (Final number of shrimp/Initial number of shrimp)  $\times 100$ .

#### Feed utilization parameters

Feed utilization parameters were calculated according to the following equations:

- Feed Conversion Ratio (FCR) = Total feed consumption (g)/ weight gain (g).
- Feed Efficiency (FE, %) = (Final weight - initial/ feed consumed)  $\times 100$ .
- Protein Efficiency Ratio (PER) = body weight gain (g)/ protein intake (g)

#### Economical Evaluation

The cost of feed to raise unit biomass of shrimp was estimated by a simple economic analysis. The estimation was based on local retail sale market price of all the dietary ingredients at the time of the study.

- Cost/kg diet (LE) = Cost per Kg diet L.E.
- Consumed feed to produce 1kg shrimp (kg) = Feed intake per shrimp per period/ final weight per shrimp Kg/Kg.
- Feed cost per kg fresh shrimp (LE) = Cost /kg diet (LE)  $\times$  Consumed feed to produce 1kg shrimp (kg).
- Relative % of feed cost/ kg shrimp = Feed cost per kg fresh shrimp (LE)/ highest figure in this step.
- Feed cost /1Kg gain (LE) = Feed intake per Kg gain  $\times$  Cost /kg diet (LE).
- Relative % of feed cost of Kg gain = Feed cost/1Kg gain (LE) / highest figure in this step.

All data were analyzed by two-way ANOVA. The ANOVA were performed by using the SAS v9.0.0 (2004) program. The ANOVA was followed by Duncan (1955) at  $P < 0.05$  level of significant.

## RESULTS AND DISCUSSION

The daily water quality parameters including temperature, dissolved oxygen, salinity and pH monitored during the experimental period are shown in Table (3). During the experimental period (90-days), temperature, dissolved oxygen, salinity and pH did not show any significant difference and they were at the optimum range for *L. vannamei* larvae cultured reported according to Da silva (2015).

The concentrations of nitrogen parameters measured during this experiment are presented in Table (3). The results showed that TAN, NO<sub>2</sub> and NO<sub>3</sub> concentrations were increased ( $P < 0.05$ ) by the increasing of protein level throughout the experiment. Nitrogen plays an important role in the aquaculture system due to its dual role, as a nutrient and toxicant (Burford and Lorenzen, 2004). Most of the nitrogen input in shrimp culture systems enters the water column as ammonia (Mishra *et al.*, 2008). Ammonia is the main end product of protein catabolism in crustaceans and is also generated in the aquatic system by the breakdown of uneaten feed and waste (Carbajal-Hernández *et al.*, 2012).

**Table (3):** Effects of dietary protein levels on tanks water quality (Mean $\pm$ SD) of *L. vannamei* larvae

Items	Dietary protein levels %				
	T <sub>1</sub> (25% CP)	T <sub>2</sub> (30% CP)	T <sub>3</sub> (35% CP)	T <sub>4</sub> (40% CP)	T <sub>5</sub> (45% CP)
Temperature °C	28.5 $\pm$ 0.53 <sup>a</sup>	28.4 $\pm$ 0.47 <sup>a</sup>	28.2 $\pm$ 0.58 <sup>a</sup>	28.3 $\pm$ 0.57 <sup>a</sup>	28.4 $\pm$ 0.43 <sup>a</sup>
Oxygen (mg/l)	5.20 $\pm$ 0.50 <sup>a</sup>	5.30 $\pm$ 0.53 <sup>a</sup>	5.20 $\pm$ 0.50 <sup>a</sup>	5.20 $\pm$ 0.52 <sup>a</sup>	5.20 $\pm$ 0.52 <sup>a</sup>
Salinity (ppt)	20.2 $\pm$ 0.51 <sup>a</sup>	20.2 $\pm$ 0.33 <sup>a</sup>	21.3 $\pm$ 0.32 <sup>a</sup>	20.2 $\pm$ 0.39 <sup>a</sup>	20.2 $\pm$ 0.58 <sup>a</sup>
pH	7.8 $\pm$ 0.33 <sup>a</sup>	7.9 $\pm$ 0.21 <sup>a</sup>	7.9 $\pm$ 0.30 <sup>a</sup>	7.9 $\pm$ 0.23 <sup>a</sup>	7.9 $\pm$ 0.31 <sup>a</sup>
TAN (mg/l)	0.61 $\pm$ 0.04	0.62 $\pm$ 0.04	0.66 $\pm$ 0.04	0.80 $\pm$ 0.04	0.10 $\pm$ 0.04
NO <sub>2</sub> (mg/l)	0.13 $\pm$ 0.01	0.12 $\pm$ 0.01	0.14 $\pm$ 0.01	0.16 $\pm$ 0.01	0.20 $\pm$ 0.01
NO <sub>3</sub> (mg/l)	0.32 $\pm$ 0.02	0.30 $\pm$ 0.02	0.32 $\pm$ 0.02	0.38 $\pm$ 0.02	0.40 $\pm$ 0.02

Data are presented as means  $\pm$ SD. Values in the same row with different superscript letters are significantly different ( $P < 0.05$ )

Therefore, throughout the study the TAN, NO<sub>2</sub> and NO<sub>3</sub> showed a slight increase up to optimum protein level in white leg shrimp fed up to 35% crude protein (CP) diet. Excessive dietary protein will increase excretion of nitrogenous waste (Boonyaratpalin, 1996). Therefore, the TAN, NO<sub>2</sub> and NO<sub>3</sub> significantly increased with a further increase in dietary protein levels from 35 to 45%. Nitrogen in the form of ammonia and nitrite are highly toxic to shrimp; however, the toxicity depends on various factors including species tolerance, water characteristics (pH, temperature, salinity, DO) and exposure duration (Barajas *et al.*, 2006). Nitrate, unlike ammonia and nitrite is less toxic to shrimp; however, a high concentration (100 mg/l) was reported to be lethal to shrimp (van Rijn *et al.*, 2006).

Dietary protein level has a significant effect on the growth of *L. vannamei*, as shown in Table (4). The group of white shrimp fed diet containing 35% CP had a significantly ( $P<0.05$ ) highest FBW, WG and SGR compared to other experimental groups. The group of white shrimp fed diet containing 45% crude protein had the lowest FBW, WG and SGR. Moreover, PER and FE was significantly ( $P<0.05$ ) highest in group of white shrimp fed diet containing 35% crude protein and the lowest value for group of white shrimp fed diet containing 45% crude protein. Proteins which have numerous structural and metabolic functions play an important role in growth. Protein is considered the major dietary nutrient affecting growth performance of aquatic animals, however the cost of proteins in feed is high and their inclusion in aquaculture diets has had a significant impact on overall feed costs (NRC, 2011). For these reasons, attempts to optimize the amount of

dietary protein in aquaculture feeds are necessary. Protein requirements for maximal growth of juvenile white shrimp have been reported to be between 30-36% in brackish or seawater (Kureshy and Davis, 2002). The optimum dietary protein level for maximum growth of *L. vannamei* can be affected by differences factor including shrimp size, stocking density, species of shrimp, culture system, and dietary protein sources. In the range of approximately 1 g sized shrimp, optimal growth was observed with 33 to 44% crude protein in diets when krill meal was used as a main protein source (Rosas *et al.*, 1995). Close results was obtained by Gong *et al.* (2004) reported that the optimum dietary protein level for *L. vannamei* (0.31–6.0 g size) was 34% when a semi-purified diet was used.

The PER values tended to decrease with increased dietary protein, which is consistent with results in shrimp (Shahkar *et al.*, 2014). The lowest PER values for larvae fed 45% CP diet indicates that the excessive protein was used for metabolic purposes other than growth. Usually, a low dietary protein is efficiently utilized for protein synthesis by shrimp (Hu *et al.*, 2008). Also, the differences in protein sources could result in different PER values (Hajra *et al.*, 1988). In the present study, fish meal was gradually increased to make the dietary protein level different.

During the 90 days of rearing, survival ranged between 90 and 94%, and averaged 92.2%. In this study, sometimes cannibalism was observed among shrimp fed all the experimental diets. The results of the experiment indicate that cannibalism might be an important source of mortality when the food supply is limited (Henderson and Holmes, 1989).

**Table (4):** Effects of dietary protein levels on growth performance and feed utilization (Mean±SD) of *L. vannamei* larvae

Items	Dietary protein levels %				
	T <sub>1</sub> (25% CP)	T <sub>2</sub> (30% CP)	T <sub>3</sub> (35% CP)	T <sub>4</sub> (40% CP)	T <sub>5</sub> (45% CP)
IBW (g)	0.002	0.002	0.002	0.002	0.002
FBW (g)	7.54±0.71 <sup>c</sup>	8.32±0.08 <sup>a</sup>	8.64±0.92 <sup>a</sup>	8.14±1.7 <sup>b</sup>	7.11±1.58 <sup>c</sup>
WG (g)	7.54 ±0.71 <sup>c</sup>	8.32±0.08 <sup>a</sup>	8.85±0.72 <sup>a</sup>	8.14±1.6 <sup>b</sup>	7.11±1.18 <sup>c</sup>
SGR (%/day)	9.19±0.67 <sup>b</sup>	9.26±0.69 <sup>a</sup>	9.30±0.67 <sup>a</sup>	9.23±0.69 <sup>b</sup>	9.08±0.41 <sup>b</sup>
FI (g feed/shrimp)	10.32±0.72 <sup>c</sup>	11.61±0.54 <sup>a</sup>	10.14±0.24 <sup>c</sup>	11.54±0.93 <sup>b</sup>	10.11±0.24 <sup>c</sup>
FCR	1.37±0.19 <sup>b</sup>	1.40±0.07 <sup>a</sup>	1.17±0.09 <sup>c</sup>	1.42±0.23 <sup>a</sup>	1.42±0.12 <sup>a</sup>
PER	2.58±0.21 <sup>b</sup>	2.15±0.35 <sup>b</sup>	2.34±0.23 <sup>a</sup>	1.69±0.24 <sup>b</sup>	1.53±0.12 <sup>b</sup>
FE (%)	73.04±2.3 <sup>b</sup>	71.65±2.51 <sup>b</sup>	85.19±1.89 <sup>a</sup>	70.52±1.35 <sup>c</sup>	70.31±1.23 <sup>c</sup>
S (%)	92.00±3.50 <sup>b</sup>	94.00±4.60 <sup>a</sup>	94.00±6.40 <sup>a</sup>	92.00±3.40 <sup>b</sup>	90.00±3.0 <sup>c</sup>

Data are presented as means±SD. Values in the same row with different superscript letters are significantly different ( $P<0.05$ )

### Economic analysis

Calculations of economic efficiency of the tested diets based on the cost of feed, costs of one kg gain in weight shown in Table (5). The diet contain 35% CP have the lowest feed cost per kg fresh shrimp (17.81

LE), Relative (%) of feed cost/kg shrimp recorded 66%), while feed cost/1 kg gain and relative (%) of feed cost of kg gain recorded 17.81 LE and 66%, respectively.

**Table (5):** Effects of dietary protein levels on economic analysis (Mean±SD) of *L. vannamei* larvae

Item	Dietary protein levels %				
	T <sub>1</sub> (25% CP)	T <sub>2</sub> (30% CP)	T <sub>3</sub> (35% CP)	T <sub>4</sub> (40% CP)	T <sub>5</sub> (45% CP)
Cost /kg diet (LE)	13.33	17.04	15.22	18.96	14.28
Consumed feed to produce 1kg shrimp (kg)	1.36	1.39	1.17	1.42	1.42
Feed cost per kg fresh shrimp (LE)	18.13	23.69	17.81	26.92	20.28
Relative % of feed cost/ g shrimp	67	88	66	100	75
Consumed feed to produce 1kg gain (kg)	1.37	1.40	1.17	1.42	1.42
Feed cost /1kg gain (LE)	18.26	23.86	17.81	26.92	20.28
Relative % of feed cost of kg gain	67	88	66	100	75

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## تأثير مستويات العليقة من البروتين على النمو والاستفادة الغذائية والتقييم الاقتصادي ليرقات الجمبري الفانمي

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أجريت هذه التجربة لدراسة تأثير محتوى خمس علائق مختلفة في مستوي بروتين (٢٥٪، ٣٥٪، ٤٠٪، ٤٥٪) في تانكات استزراع يرقات الجمبري الفانمي على أداء النمو وكفاءة الاستفادة من الغذاء ومعدلات الإعاشة والتحليل الاقتصادي للجمبري الفانمي. أجريت التجربة في معمل اللاقاريات التابع للمعهد القومي لعلوم البحار والمصايد (NIOF)، محافظة السويس - مصر لمدة ٩٠ يوم. نفذت التجربة فيخزان مستطيل (٦٦ × ٤٧ × ٤٤ سم، ٥٠ لتر لكل منها) ومملوء بماء ملوخته ٢٠ جزء في الألف، تم تغذية المجموعات المختبرة (ثلاث مكررات لكل مجموعة) من الجمبري (بمتوسط وزن ٠.٠٠٢ جم) مرتين يوميًا بمعدل تغذية يومية ١٤٪ من وزن الجسم الابتدائي وتم إعادة ضبطها تدريجيًا إلى ٥٪ في نهاية التجربة. سجلت النتائج زيادة في الوزن المكتسب ومعدل النمو النوعي مع أفضل معدل تحول غذائي في المعاملة ٣٥٪ وكان معدل كفاءة البروتين اعلي في هذه المعاملة. أظهرت النتائج الحالية أن مستوى بروتين العليقة الخامل يرقات الجمبري الفانمي ٣٥٪ قد يكون الأمثل من حيث أداء النمو واستخدام العلف والتقييم الاقتصادي في ظل هذه الظروف التجريبية.

**الكلمات الدالة:** الجمبري الفانمي، معدلات النمو، الاستفادة الغذائية، معدلات الإعاشة، والتحليل الاقتصادي