The Bioscan 9(3): 1093-1096, 2014
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECT OF DIFFERENT DIETARY PROTEIN LEVELS OF CYCLOP-EEZE MEAL ON GROWTH, SURVIVAL AND CARCASS COMPOSITION OF *LITOPENAEUS VANNAMEI* (BOONE, 1931)

BHANU PRAKASH. CH*[1], D. R. K. REDDY[2], T. K. GHOSH[3], ALOK RAJAK[4] AND D. RAMALINGAIAH[5]
[1]Department of Aquaculture, Faculty of Fishery Sciences, WBUAFS, 5-Budherhat Road, Panchasayar, Kolkata- 700 094
[2]Department of Aquaculture, College of Fishery Sciences, SVVU, Tirupati. Andhra Pradesh
[3]Department of Aquaculture, Faculty of Fishery Sciences, WBUAFS, 5-Budherhat Road, Panchasayar, Kolkata-700 094
[4]Department of Aquaculture, Faculty of Fishery Sciences, WBUAFS, 5-Budherhat Road, Panchasayar, Kolkata- 700 094
[5]Department of Fisheries Resource Management, College of Fishery Sciences, SVVU, Tirupati. Andhra Pradesh,
e-mail: chbhanu001@gmail.com

KEYWORDS
Cyclop-eeze meal
Litopenaeus vannamei
Growth
Survival
Carcass composition

Received on :
06.03.2014

Accepted on :
18.07.2014

***Corresponding author**

ABSTRACT
Present study was undertaken to study Cyclop-eeze (CE) meal of different protein levels (25%, 30% and 35%) were used in the experiment. The experiments were conducted for 56 days in aquaria. Triplicates were maintained for each of the treatment. Feed was given 3 times a day @ 5% of body weight. The growth performance of *L. vannamei* was found to be best when fed with diet CE30 (7.75gm). Specific growth rate was highest (1.863) in shrimps fed with diet CE30. Survival rates were best in shrimps fed on CE30, which showed 75% and FCR of 3.34. Carcass composition of *L. vannamei* showed the highest protein content in shrimps fed with CE30 diet (74.4%). Highest ether extract (2.5%) and crude fibre content (4.6%) was recorded. Ash content was high in shrimps fed with CE35 diet (9.3%). The present study suggests that, during the juvenile stages of *L. vannamei* culture use of Cyclop-eeze meal improved the survival rate, showing good growth, with less FCR.

INTRODUCTION
In India, the Shrimp culture received maximum importance in the human consumption due to its unique taste, high nutritive value and persistent demand in world market in general. Since feed constitutes 50 - 60% of the total variable costs of production in aquaculture, it would be highly desirable to develop an efficient and cost effective feed to improve the profitability. Undoubtedly, price is the key reason to look for alternatives. The supply and price of high quality fish meal can vary dramatically from year to year. There is also a general concern that fish meal production might have negative impact on natural fisheries (Nylor *et al.,* 2000). Protein is the primary and most expensive component of shrimp diets. *L. vannamei* has high growth rate, adoption to various culture systems, high market value and excellent response to compounded feed and high resistance against diseases. Sara Lindsay (2006) studied on the Cyclop-eeze diet in growth and survival of clown fish. Sulaiman and Charless (2007) studied the growth of silver pomfret under tank culture conditions by using salmon feed mixed with Cyclop-eeze. Woods (2003) studied that growth and survival of juvenile sea horse *Hippocampus abdominalis* reared on live, frozen and artificial foods. Mohankumar Nair (2007) reported the use of Cyclop-eeze as a substitute for Artemia nauplii in larval rearing of giant freshwater prawn *M. rosenbergii*. The data obtained on Growth, Survival and Food Conversion Ratio was statistically analyzed by applying Randomized Block Design (RBD) of two-way classification. The objective of choosing Cyclop-eeze (*Diaptomus connexus*) was not only for its nutritive value but its potential with highly unsaturated fatty acids to influence the farm pond survival of hatchery reared shrimp larvae (transition from controlled to farmers pond environment). Cyclop-eeze, the bioengineered micro crustacean Decapod widely used in aquaculture for its high nutrient profile including protein (60%), lipid (34%) and astaxanthin (3000 - 7500ppm) and total caroteinoid profile: 4452 - 9832ppm) . Mineral profile like Calcium (3.03 mg/L), Magnesium (4.28 mg/L), Sodium (12.72 mg/L) and Phosphorus (6.99 mg/L) etc., together with a significant amount of digestive enzymes like protease, amylase and lipase are available in Cyclop-eeze which helps in easy digestion by the larvae and post larvae of shrimp as well as fish. Compared with premium quality of freshly hatched *Artemia* nauplii, Cyclop-eeze is 40 times higher in nutritional quality particularly omega 3 fatty acid and astaxanthin. Cyclop-eeze has been used for penaeid larvae and post larvae for best conversion, moulting, feed consumption that enhances the growth and survival rate. Cyclop-eeze can be widely used to feed all kinds of aquatic animals, especially, shrimps, ornamental fish, etc., because, they are very rich in attractant (be-

taine) as well as other palatability factors. Hence an attempt was made to compare the growth, survival and carcass composition of *L. vannamei* by using Cyclop-eeze (CE) meal in different protein levels as an animal protein source by replacing fish meal totally.

MATERIALS AND METHODS

The study was conducted during 2010-2011 in the Department of Aquaculture, Sri Venkateswara Veterinary University, Tirupati, Andhra Pradesh. *Litopenaeus vannamei* (1500 numbers) were obtained from CP Hatchery, Nellore, and brought to the research centre in plastic bags having 5.00 ppt saline water and were acclimatized slowly to fresh water within a period of one week and kept in fresh water FRP tanks for 20 days till their use in the study. During this period the seed were fed with control diet.

Twelve aquariums tanks (60x30x40 cm) were stalked on iron racks and placed in a secured place to avoid direct sunlight. Tanks were covered all the sides with black paper to avoid algal growth in the tank. Water in the aquariums was aerated and filters were used for filtering the water. Bore well water was taken into FRP tank and allowed to aerate for 48 hours. Aerated water was used for filling aquariums and allowed 24 hours for filter before introducing the shrimps into aquariums.

Ten numbers of Shrimps (average weights 2.73-2.75gm; average lengths 6.0-6.5 cm) were introduced in each aquarium and triplicates were maintained for each treatment (CE25%, CE30%, CE35%) including control. Regular water exchange (50%) was done every day. Left over feed, excreta and other debris was siphoned off from the bottom of the tank without disturbing the shrimps.

In the experiment, formulated feed was used for feeding. The feeds for the experiment were formulated with different protein concentrations. Control feed having 30% protein was prepared using de-oiled rice bran and groundnut oil cake. Vitamin and mineral mixture @1% was added. Experimental feeds were prepared with Cyclop-eeze meal having 25%, 30%, 35% protein concentrations (Table 1). Other ingredients used in the experimental feeds were de-oiled rice bran and groundnut oil cake. Vitamin and mineral mixture @ 1% was also added to experimental feeds. All the ingredients used in feeds were obtained from local markets and were estimated for proximate composition (Table 2) (AOAC, 1995).

Crude protein

Nitrogen content of the sample was estimated by Kjeldahl method and the crude protein was estimated by multiplying nitrogen percentage by a constant factor 6.25.

Crude protein (%) = Nitrogen (%) x 6.25

Ether extract

Ether extract was estimated by soxhlet apparatus using petroleum ether as a solvent.

$$\text{Ether extract (\%)} = \frac{\text{Weight of initial sample} - \text{Weight after extraction}}{\text{Weight of initial sample}} \times 100$$

Ash

Ash content was estimated by taking a known weight of sample in silica crucible and placing it in a muffle furnace heated at 600ºC for 6 hours.

$$\text{Ash (\%)} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

Crude fibre

Crude fibre was estimated by treating the moisture and fat free sample successively with dilute acid (1.25%) and alkali.

$$\text{Crude fibre (\%)} = \frac{\text{Weight of crude fibre}}{\text{Original weight of sample}} \times 100$$

Weight increment: Weight increment (gm) = Final body weight (gm) - Initial body weight (gm).

Specific growth rate: $[(L_n \text{ FBW} - L_n \text{ IBW}) / \text{day}] \times 100$

FBW - Final body weight

IBW - Initial body weight

Ln - Logarithm

Survival Rate

$$\text{Survival (\%)} = \frac{\text{Total number of fish survived}}{\text{Total number of fish stocked}} \times 100$$

Feed Conversion Ratio (FCR)

$$\text{Feed Conversion Ratio} = \frac{\text{Feed given (dry weight)}}{\text{Body weight gain (wet weight)}}$$

RESULTS AND DISCUSSION

A large amount of experimental work has been performed on the requirements of protein to the L. vannamei. A wide range of practical formulations developed by feed manufacturers have been identified, emphasizing in the beginning, on water stability of pellets, based on farmer's recriminations. During the experimental period the dissolved oxygen values varied between 5.42-7.74ppm. The highest value of 7.74ppm was recorded in CE30 and the lowest value of 5.42ppm was recorded in Control (T1) during the entire experiment. The temperature value recorded on an average of 29.3-31.6. Water quality parameters remained stable throughout the experimental period (APHA 2005) (Table 3). The pH values observed were within the optimal range for shrimp culture. In the present experiment the variations in the pH are low and this can be attributed to higher total alkalinity observed during the experiment (Pandey and Shukla, 2005).

Growth performance of *Litopenaeus vannamei* (whiteleg

Table 1: Feed formulation of control diet and crude protein content (%) in feed ingredients

Sl.No	Ingredient	Protein (%)	ControlT1	CE 25T2	CE 30T3	CE 35T4
1	Cyclop-eeze	60.0	-	21.14	31.65	42.18
2	Groundnut oil cake	38.4	50.0	10	10	10
3	Deoiled rice bran	12.5	49.0	67.86	57.35	46.82
4	Vitamin and mineral Mixture	-	1	1	1	1

Table 2: Proximate composition of the various ingredients used in formulated feeds

Sl.No.	Ingredient	Moisture (%)	Crude protein (%)	Crudefat (%)	Crudefiber (%)	Ash(%)
1	Cyclop-eeze	9.4	60.0	34	8.5	3.5
2	Groundnut oil cake	8.8	38.4	5.6	7.3	7.6
3	Deoiled rice bran	7.7	12.5	3.9	22.5	15.8

Table 3: water quality parameters throughout the experimental period with survival

Treatment	Water quality parameters					Survival (%)
	D.O.(ppm)	pH	Alkalinity (mg/L)	Hardness (mg/L)	Temperature (ºC)	
Control (T1)	7.51-5.42	8.5-8.4	280-170	226-234	31.6-29.3	50
CE 25 (T2)	7.62-5.60	8.5-8.4	340-160	225-233	31.6-29.3	60
CE 30 (T3)	7.74-5.63	8.5-8.3	320-160	226-234	31.6-29.3	75
CE 35 (T4)	7.62-5.60	8.5-8.4	300-150	227-235	31.6-29.3	60

Table 4: Growth performance of *L. vannamei* (whiteleg shrimp) fed with different experimental diets

Treatment Control Period (days)	T1	Treatment CE CE 25T2	CE 30T3	CE 35T4
Initial	2.72 ± 0.19	2.73 ± 0.22	2.73 ± 0.12	2.75 ± 0.10
7	3.01 ± 0.10	3.15 ± 0.14	3.24 ± 0.11	3.23 ± 0.12
14	3.37 ± 0.42	3.51 ± 0.19	3.74 ± 0.23	3.71 ± 0.55
21	3.80 ± 0.10	3.85 ± 0.08	4.28 ± 0.31	4.24± 0.20
28	4.19 ± 0.01	4.30 ± 0.06	4.88 ± 0.21	4.82 ± 0.20
35	4.55 ± 0.10	4.78 ± 0.21	5.51 ± 0.30	5.44 ± 0.22
42	5.03 ± 0.13	5.29 ± 0.11	6.21 ± 0.23	6.12 ± 0.16
49	5.40 ± 0.17	5.84 ± 0.22	6.94 ± 0.13	6.82 ± 0.14
56	5.90± 0.10	6.44 ± 0.20	7.75 ± 0.65	7.59 ± 0.11

Table 5: Specific growth rates (%) in *L. vannamei* (whiteleg shrimp) fed on different experimental diets

Treatment Period (days)	Control T1	Treatment CE CE 25T2	CE 30T3	CE 35T4
Initial	2.72	2.73	2.73	2.75
Final	5.9	6.44	7.75	7.59
SGR	1.382	1.532	1.863	1.812

Table 6: Feed Conversion Ratio of *L. vannamei*(whiteleg shrimp) fed with different experimental diets

Treatment Period (days)	Control T1	Treatment CE CE 25T2	CE 30T3	CE 35T4
7	3.63	2.62	2.22	2.35
14	3.27	3.41	2.61	2.70
21	3.09	3.96	2.77	2.80
28	3.76	3.34	2.84	2.90
35	4.42	3.48	3.06	3.07
42	3.66	3.63	3.10	3.15
49	5.10	3.71	3.32	3.41
56	4.13	3.75	3.34	3.45

shrimp) fed on different experimental diets

Growth performance of *L. vannamei* fed with different experimental diets shows highest weight gain in the treatment of CE30 (Table 4). Protein will act according to their dietary level in a range from 10% to 50% and depending on the nature of protein (Cousin *et al.,* 1993; Durga Prasad Behera and Lakshman Nayak, 2011). A large amount of experimental work has been performed on the requirements of protein to the *L. vannamei* S. J. Meshram *et al.,* 2014. A wide range of practical formulations developed by feed manufacturers have been identified, emphasizing in the beginning, on water stability of pellets, based on farmer's recrimination. Results of the research conducted by (Green *et al*., 1997; Teichert-Coddington *et al.,* 1997) in Honduras indicate that 30% protein feed did not result in increased shrimp production compared to 20% protein feed. No significant difference in shrimp yield was reported when a 29 or 37% protein feed was offered to *L. vannamei* (Teichert-Coddington and Arrue, 1988). Feed conversion ratios were very high for that experiment because of over feeding. Smith *et al*. (1984) studied the response of three sizes of *L. vannamei* (4.0, 9.8 and 20.8gm) fed diets containing 22%, 29%, and 36% crude protein for period of 30 days. Dietary protein contain only affected weight gain for 4.0gm shrimps, with a significant increasing weight gain corresponding to the increasing dietary protein content. Davis *et al*. (2002) tested *L. vannamei* juveniles fed on different processed pea meals at 5%, 10%, and 20% of inclusion level, and found that when it was extruded or micronized, their protein and energy content were highly digestible without apparent adverse effects on growth or survival. Kureshy and Davis (2002) reported that juvenile shrimps fed with 32% protein diet had significantly higher weight gain compared to juveniles fed with 16% protein diet. Caldwell *et al*. (2010) developed a starter diet using cyclop-eeze to support rapid growth through the larval stage to the juvenile stage of Rio grande silvery minnow. Cyclop-eeze yielded highest survival (75%) with high growth (0.81gr) in 56 days experiment. In the present study also juveniles of *L. vannamei* fed with 30% protein showed highest weight gain than 25% and 35% of protein source. The survival percentage throughout the period of experiment was lowest for the control and highest for CE30. By the final sampling (56th day) the survival percentage was (highest) 75.0 - and (lowest) 50.0 (Table 3). An overall study indicated that the CE30 recorded total weight increment of 5.02gm in the 56 days experimental period. This was followed by the CE35 (4.84gm). The specific growth rates by end of the experimental period (56 days) were calculated for all treatments. Control group has the lowest Specific Growth Rate of 1.382%. The highest value was in CE30 with 1.863% (Table 5). The growth data was subjected to analysis of variance (ANOVA). The statistical analysis has shown that F- value is

Table 7: Proximate composition of *L. vannamei* carcass (whiteleg shrimp) fed with different experimental diets

Treatmentcomponents	Initial	Control	Treatment CE		
			CE 25	CE 30	CE 35
Moisture	96.2	95.8	97.3	98.9	98.1
Crude protein	69.8	70.2	74.2	74.4	74.3
Ether extract	1.8	2.0	2.4	2.5	2.4
Crudefibre	3.7	3.9	4.4	4.6	4.5
Ash	7.2	7.4	9.1	9.1	9.3

found to be significant among Treatments. Since F- value is found to be significant, the pair wise comparison of any two Treatments could be done by computing RBD two way classifications. The Treatment CE30 is found to be significantly superior when compare to other Treatments.

Feed Conversion Ratio in *L. vannamei* (whiteleg shrimp) fed different experimental diets

The Feed Conversion Ratio in different experiments of *L. vannamei* groups were calculated and presented (Table 6). Liberman (2001) reported that Cyclop-eeze is now being widely used in the larval rearing of *Penaeus monodon* hatcheries in USA as partial replacement of *Artemia* nauplii with apparently better results than the *Artemia*. Cyclop-eeze was used as a feed in the present study played a major role for the better growth of shrimps. It is observed that that growth rate of shrimps in the present study is increasing at 30% protein. The final day (56th day) Feed Conversion Ratio for the treatment of control was highest value 4.13 and lowest 3.34 for CE30. The lower weight gain, which resulted from feeding the diet containing 35% protein, is probably due to the low energy to protein ratio of the diet, which would cause shrimp to utilize protein as a source of energy. The 35% protein diet was probably due more to the protein to energy ratio of the diet. The protein requirement for maximum weight gain, 30% protein diet was found to induce superior growth in juvenile and sub adult *L. vannamei* as compared to 25% and 35% protein diets.

Proximate Composition of Carcass

The proximate composition of *L. vannamei* carcass was analyzed at the beginning of the experiment and also at the end of the experiment period (56th day) and presented (Table 7). Carcass analysis was given on shrimp receiving each diet at the beginning and at end or the experiment (Gauquelin *et al.,* 2007). The data on carcass composition in this study showed similar to the previously reported studies about fish and crustaceans that carcass lipid content increased with increasing dietary lipid.

REFERENCES

AOAC 1995. Official Methods of Analysis of the Association of Official Analytical Chemists. *Washington,* Dc. p.101.

APHA 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edition. American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF).

Caldwell, C. A., Barrows, T. F., Ulibarri, M. and Gould, W. R. 2010. Diet optimization of juvenile Rio Grnde Silvery Minnow. *North American J. Aquaculture.* **72:** 57-64.

Cousin, M., Cuzon, G., Blanchet, E. and Ruelle, F. 1993. Protein requirements following an optimum dietary energy to protein ration for *Penaeus vannamei* juveniles. In: Kaushik, Luquet, P. (Eds), 4. Int. Symp. *Fish Nutrition and Feeding*, Biarritz, (France), 24-27 Jun 1991 Colloq. INRA. **6:** 599-606.

Davis, D. A., Arnold, C. R and Mc Callum, I. 2002. Nutritional value of feed peas (Pisum sativam) in practical diet formulations for *Litopenaeus vannamei*. *Aquaculture Nutrition.* **8(2):** 87-94.

Durga Prasad, B. and Lakshman, N. 2011. Role of Probiotics In Sustainable Shrimp Farming Along Balasore Coast, Orissa. *The Bioscan.* **6(1):** 97-102.

Green, B. W., Teachert-Coddingaton, D. R., Boyd, C. E., Harvin, J. L., Corrales, H., Zelaya, R., Martinez, D. and Ramirez, E. 1997. Effect of diet protein on food conversion land nitrogen discharge during semi-intensive production of *Penaeus vannamei* dudring the dry season. In: Burke, D., Goetze, B., Clair, D. and Egna, H. (Editors), Fourteenth Annual Technical Report 1996. Pond Dynamics/ Aquaculture CRSP, Office of International Research and Development, Oregon State University, Corvallis, Oregon. pp. 77-86.

Gauquelin, F., Cuzon, G., Gaxiola, G., Rosas, C., Arena, l., Bureau, D. P. and Cochard, J. C. 2007. Effect of dietary protein level on growth and energy utilization by *Liptopenaeus stylirostris* under laboratory conditions. *Aquaculture.* **271:** 439-448.

Kureshy, N. and Davis, D. A. 2002. Protein requirement for maintenance and maximum weight gain for the white shrimp, *Litopenaeus vannamei*. *Aquaculture.* **204(1-2):** 125-143.

Lieberman, E. 2001. Cyclopeeze: a tiny crustacean with big aquaculture application. *Aquaculture,* Asia. **6:** 33-36.

Mohan Kumar Nair, C., Salin, K. R. and Ashok, K. 2007. Use of Cyclop-eeze as a substitute for *Artemia* Nauplii in larval rearing of giant freshwater prawn *Macrobrachium rosenbergii*, *Aquaculture Nutrition*. **13(2):** 88-93.

Nylor, R. L., Goldburg, R. J., Primavera, J. H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H. and Troell, M. 2000. Effect of aquaculture on world fish supplies. *Nature* (London), **405:** 1017-1024.

Pandey, K. and Shukla, J. P. 2005. Fish and Fisheries. *Raspogi Publications*. **275:** 265-266.

Sara, L. 2006. You are what you eat or how did the clown fish get brighter stripes. *School of marine sciences, University of Maine.*

Meshram, S. J., Shivananda Murthy, H., Swain H. S., Ali, H., Jagadeesh, T. D. and Dhamgaye, H. B. 2014. Effects of dietary beta-glucan on growth and body composition of *Macrobrachium rosenbergii, The Bioscan.* **9(2):** 543-546.

Smith, L. L., Lee, P. G., Lawrence, A. L. and Strawn, K. 1984. Growth and digestibility by three sizes of *Penaeus vannamei* Boone: effects of dietary protein level and protein source *Aquaculture.* **46:** 85-96.

Sulaiman, M. A. and Charles, M. J. 2007. Performance of different types of commercial feeds on the growth of juvenile silver pomfret, *Pampus argenteus*, under tank culture conditions. *J. World Aquaculture Society.* **38:** 4-14.

Teichert-Coddingaton, D. R., Green, B., Boyd, C. E., Harvin, J. L., Rodrigueaz, R., Martinez, D. and Ramirez, E. 1997. Effect of diet protein on feed conversion and nitrogen discharge during semi-intensive production of *Penaues vannamei* during the wet season. In: Burke, D., Goetze, B., Clair, D. and Egna, H. (Editors), Fourteenth Annual Technical Report 1996. Pond Dynamics/Aquaculture CRSP, Office of International Research and Development, Oregon State University, Corvallis, Oregon. pp. 71-76.

Teichert-Coddingaton, D. R and Arrue, M. 1988. Effectos de diets proteinas y densiadades de siembra sobre la produccion de *penaeus vannmei* en estanques de tierrs. *Revista Latinoamericana de Acuicultura.* **35:** 29-33.

Woods, C. M. C. 2003. Growth and survival of juvenile seahorse *Hippocampus abdominalis* reared on live, frozen and artificial foods. *Aquaculturte.* **220:** 287-298.