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PROOF

SHORT PAPER

A Novel Food Formulation for the Juveniles of *Penaeus monodon* (Fabricius)

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Abstract

The efficiency of carbohydrate rich food was studied and compared with four different feeds on the juveniles of *Penaeus monodon* (Fabricius). The average food consumption of formulated feed rich in carbohydrates (Feed 2) showed minimum consumption (0.479 g dry weight), faecal production (0.061 g dry weight) and Food Conversion Ratio (0.471) as compared to protein rich Feed-1 where these values were maximum. The assimilation efficiency (86.78%) and average gross efficiency (198.47%) was higher in Feed-2 as compared to these values obtained for other feeds. Mean relative growth (14.29%) and asymptotic weight (300 g) was calculated maximum for Feed -2 whereas, it was minimum for Feed-1. The von Bertalanffy's growth equation fitted well to describe the growth pattern of the juveniles and represented as: $W_t=180[1-e^{-0016(t+3.75)}]$ for Feed-1; $W_t=300[1-e^{-0.0013(t+3.00)}]$ for Feed-2; $W_t=220[1-e^{-0.0015(t+3.33)}]$ for Feed-3; $W_t=240[1-e^{-0.0016(t+3.18)}]$ for Feed-4 and $W_t=200[1-e^{-0.0015(t+3.60)}]$ for Feed-5

Keywords: Food Conversion Ratio; assimilation efficiency; shrimp feed.

Introduction

In shrimp farming, either it is a semi-intensive, intensive or extensive cultures, artificial feed is the main nutrient source and contributed about 60% of the total production cost (Akiyama *et al.*, 1992, Sarac *et al.*, 1993). Artificial feeds are nutritionally balanced, economical and growth efficient feeds (Sudaryano *et al.*, 1995). Supplementation of appropriate feed with respect to particular stage of shrimp results in better growth and survival of the organisms. Several studies have been conducted where the protein level in the artificial feed was reduced and diet was supplemented by non-protein elements to maintain total energy level (Myrna, 1986). The replacement of protein from 40 to 30% and increase in the percentage of carbohydrate to 30% in the diet has not been reported to affect the growth of the shrimp (Shiau and Peng, 1992).

A study has been done on the growth of *Penaeus monodon* (Fabricius) under different photoperiods (Chatterji *et al.*, 2015) but the requirements of carbohydrates in the diet for the juvenile shrimp especially *Penaeus monodon* (Fabricius) has not been given much attention except the work of Shiau and Peng (1992) and Cruz-Suares *et al.* (1994). Due to shortage in the supply of the food items, their high cost and deficiency of important nutrients in the feeds, an attempt was made to develop a suitable artificial, cost effective and growth efficient feed for the juveniles of *P. monodon*. A comparative study was also carried out between commercially available pelleted feeds and feed developed by us with high protein ingredients to show the efficacy of the carbohydrate rich formulated feed, its nutritional value and effect on the growth of the juveniles of *P. monodon* under laboratory conditions.

Materials and Methods

Juveniles (PL20) of *Penaeus monodon* (Fabricius) were brought to the laboratory from a commercial hatchery for the present study. The juveniles at first were kept for acclimatization in the laboratory for a week in 500 l FRP circular tanks. The juveniles were not given any feed for a day prior to the commencement of the feeding experiment. In the present investigation, 10 number of glass jars of 4 l capacity were used. In each glass jar, filtered seawater (< 1 micron) treated with UV and twenty juveniles of *P. monodon* of same size (weight 1.2-1.5 g) were transferred. The salinity (30±1 psu), pH (7.7±1) and temperature (29±1°C) was maintained constantly throughout the experiment. Initial weight of the juveniles was recorded up to mg on a single pan Electronic Digital Balance (Essae Digi Model DC-80). The temperature of the glass jars was recorded every day by a mercury thermometer, salinity by a salinity refractometer (Atago: S/Mill, Japan), pH by a portable pH meter (Philips PP 9046) and dissolved oxygen by a hand operated oxygen probe (YSI-60).

The juveniles of *P. monodon* were fed at the rate of 10% of the body weight as commercial feeds and 20% of the body weight with formulated feeds everyday, considering the consumption of the feed by the juveniles in each set of experiment. The feeding schedule arrived at after several feeding trails by observing the consumption rate of the juveniles. The uneaten food along with the moult, if any, was removed every morning without disturbing the water of the experimental jars. Faecal matters in the form of fine strings were siphoned out carefully on a bolting silk (60 μm) and then washed with distilled water several times to remove salt. The faecal matters and uneaten food were transferred to a glass vial and dried in an oven at 60º C. Total faecal matters and uneaten food were pooled for a week and weighed up to mg. Wet weight of the juvenile was taken once in a week after blotting the excess water, carefully. The water (75%) in each glass jar was exchanged at weekly intervals. The present experiment was conducted for a period of 7 weeks and data were analyzed statistically by applying Student **'t'** test.

In the present experiment, 5 different types of feeds were used:

Formulated Feed-1

Green Mussel meat (10%), clam meat (10%), oyster meat (10%), squid (10%), hen eggs (10%) and trash fish (30%) were mixed thoroughly in a kitchen mixer. After mixing these ingredients, wheat flour (10%) and yeast (10%) were added to the mixture. The ingredients were kneaded for further 5 minutes until tough dough was formed. The pellets (2 mm size) were prepared with the help of hand operated pellatizer. Pellets were kept for drying at 60° C for about 6 hrs in an oven. Dried pellets were grounded into small pellets and packed in air tight plastic containers. The plastic containers were kept in a refrigerator for further use.

Formulated Feed-2

Seaweed (*Ulva fasciata*) was selected as one of the important ingredients for this feed. The seaweeds was collected from the wild during the low tide and brought to the laboratory for further processing. Seaweed, at first was washed several times with freshwater to remove sand particles and other unwanted materials. The filaments of the seaweed were spread on a blotting paper under the sunlight for drying. Further drying of the seaweed was completed at 60º C for 48 hours in an oven. The soyabean seeds were soaked in water for 24 hours and then boiled for 15 minutes using a pressure cooker. The boiled soyabean seeds were dried and baked for 15 minutes at a temperature of 130° C whereas; the tapioca root was dried at 60° C for 3 days in an oven. Dried seaweed (10%), tapioca (20%), oil cake (5%), yeast (10%) and rice (15%) were grounded separately with the help of a kitchen mixer. The desired quantities of the ingredients were mixed with molasses (10%) and wheat flour (20%). The mixture was kneaded well until tough dough was obtained. A hand operated pelletizer was used to make pellets of 2 mm size. These pellets were dried at 60° C for 24 hours and stored in refrigerator for further use.

Feeds 3-5 were purchased from the local market. The Feed-3 was consisted of 41.49% proteins, 3% carbohydrates and 3% lipids whereas; 40.90% proteins, 3% carbohydrates and 6.50% lipids were in Feed-4. The composition of Feed-5 was having 40.77% proteins, 3% carbohydrates and 2.80% lipids.

Biochemical Analysis

In the present study the biochemical analysis of both the formulated feeds (dry weight) were performed. Protein content was estimated by the method of Lowry *et al.* (1951), carbohydrate by Dubais *et al.* (1956) and lipid by Bligh and Dyer (1959).

The data were analyzed for mean body weight (W), survival rate, assimilation efficiency (A/C), gross growth efficiency (K_1), net growth efficiency (K_2) , relative growth rate/day (P/W/49), consumption unit weight/day (C/W/49) and Food Conversion Ratio (FCR) (Weight gained/Food consumed).

The growth pattern of the juveniles of *P. monodon* was expressed by calculating the mean weekly weight, specific growth rate (G) and, von Bertalanffy's growth equation (Chatterji *et al.*, 1984) for each week using different feeds.

Results and Discussions

The efficiency of different feeds used in the present investigation is given in Table 1. The analysis of data on weekly basis showed that average food consumption for Feed-2 was minimum (0.479 g dry weight). A maximum consumption was recorded for Feed-1 with an average of 1.051 g (dry weight). However, data for the faecal production showed a minimum production (0.061 g dry weight) for Feed-2 (Table 1), whereas; maximum (0.248 g dry weight) for Feed-1 (Table 1). The faecal production for other feeds was closed to Feed-1. A significant difference was observed ($P<0.05$) after analyzing the data for assimilation which showed that for Feed-2, the assimilation efficiency was relatively higher (86.78%) as compared to other feed. However, minimum assimilation efficiency was observed for Feed-1 (74.59%). The average net growth efficiency for

Table 1. A summary of the efficiency of different diets. [Values are in dry wt (g) per animal and experimental period was 49 days

Type of feed	No of shrimp	Initial weight W1 (g)	Final weight W2 (g)	Mean weight W	Production P=W2-W1 (g)	Food consumed C	Faecal output F	Relative growth rate/day P/W/ 49	Assimilatio n A= C-F	Assimilation efficiency A/C (%)	Gross growth efficiency K_1=P/C (%)	Net growth efficiency K_2=P/A (%)	Food conversion ratio	Consumption /unit/wt/ day P/W/49
Feed-1	40	0.8	1.825	0.596 ±0.252	1.025	1.051 ±0.432	0.248 ±0.087	0.035	0.795 ±0.331	74.59 ±4.854	59.00 ±21.622	80.95 ±33.407	1.248 ±0.612	0.035
Feed-2	40	0.8	2.35	0.857 ±0.421	1.550	0.228 ±0.667	0.061 ±0.023	0.036	0.415 ±0.209	86.78 ±2.507	198.47 ± 9.039	231.54 ±108.19	0.471 ±0.396	0.011
Feed-3	40	0.8	2.125	0.839 ±0.367	1.325	0.667 ±0.208	0.121 ±0.036	0.032	0.542 ±0.168	81.84 ±1.219	121.82 ±35.31	150.19 ±44.590	0.588 ±0.232	0.016
Feed-4	40	0.8	2.25	0.792 ±0.438	1.450	0.690 ±0.226	0.147 ±0.034	0.037	0.540 ±0.200	77.60 ±4.202	108.74 ±45.80	138.81 ±52.980	0.675 ±0.286	0.017
Feed-5	40	0.8	1.875	0.750 ±0.236	1.075	0.750 ±0.236	0.161 ±0.041	0.033	0.599 ±0.185	77.64 ±3.617	85.96 ±12.788	107.97 ±17.044	0.877 ± 0.192	0.023

Feed-2 was 231.54% whereas a minimum (80.95%) for Feed-1 (Table 1).

The average gross growth efficiency value was maximum (198.47%) for Feed-2 whereas, minimum (59.00%) for Feed-1 (Table 1). For the other feeds, the average gross growth efficiency was between 85.96 and 121.82%.

The Food Conversion Ratio (FCR) values when compared for all the five feeds used in the present study showed a minimum FCR (0.471) for Feed-2 (Table 1). The highest FCR values (1.248) were calculated for Feed-1 whereas it was 0.877 for Feed-5.

The biochemical analysis for all the feeds used in the present investigation showed that carbohydrate was relatively higher in Feed-2 as compared to other feeds (Table 2).

Relative growths calculated in terms of percentage of weights for different feeds are presented in tables 3 to 7. The relative growths showed a

Table 2. Percent values of proteins, carbohydrates and lipids in different feeds (calculated as dry weight basis)

Feeds	Protein (%)	Carbohydrate (%)	Lipid (%)
Feed-1	47.87	16.86	11.80
Feed-2	19.93	25.78	5.40
Feed-3	41.49	3.00	3.00
Feed-4	40.90	3.00	3.00
Feed-5	40.77	3.00	2.80

Table 3. Average observed weight, theoretical weight, relative growth, growth increment and specific growth for Feed-1

Weeks	Average observed weight (g) ±SD	Theoretical weight (g)	Relative growth (%)	Growth increment (%)	Specific growth (%)
Initial	1.2±0.0	-	-	-	-
1	1.5±0.1	1.36	20.00	0.30	22.3
2	1.8±0.1	1.64	18.90	0.35	18.2
3	1.9±0.1	1.93	2.63	0.05	5.4
4	2.1±0.1	2.21	9.52	0.20	10.0
5	2.3±0.1	2.50	8.69	0.20	9.1
6	2.5±0.2	2.78	6.12	0.15	6.4
7	2.9±0.4	3.06	14.03	0.40	15.1
Mean	2.01	2.21	11.41	0.23	12.3

Table 4. Average observed weight, theoretical weight, relative growth, growth increment and specific growth for Feed-2

Weeks	Average observed weight (g) ±SD	Theoretical weight (g)	Relative growth (%)	Growth increment (%)	Specific growth (%)
Initial	1.2±0.0	-	-	-	-
1	1.7±0.2	1.55	27.27	0.45	31.8
2	1.9±0.1	1.94	13.15	0.25	14.1
3	2.0±0.1	2.33	5.00	0.10	5.2
4	2.4±0.3	2.71	14.89	0.35	16.1
5	2.8±0.3	3.10	14.54	0.40	15.7
6	3.2±0.4	3.48	12.69	0.40	13.6
7	3.6±0.3	3.87	12.50	0.45	13.3
Mean	2.32	2.71	14.29	0.34	15.6

Table 5. Average observed weight, theoretical weight, relative growth, growth increment and specific growth for Feed-3

Weeks	Average observed weight (g) ±SD	Theoretical weight (g)	Relative growth (%)	Growth increment (%)	Specific growth (%)
Initial	1.2±0.0	-	-	-	-
1	1.7±0.2	1.42	29.41	0.50	34.8
2	1.9±0.1	1.75	8.10	0.15	8.5
3	2.2±0.2	2.07	13.95	0.30	15.0
4	2.5±0.3	2.40	12.24	0.30	13.1
5	2.9±0.6	2.73	15.51	0.45	16.8
6	3.2±0.7	3.05	7.93	0.25	8.3
7	3.3±0.6	3.38	4.54	0.15	4.6
Mean	2.31	2.40	13.00	0.30	14.4

disorderly trend in all the cases. Maximum increment in growth was observed in the early phases as compared to the later phases for all feeds used in the present study. Mean relative growth value was maximum (14.29%) for Feed-2 whereas, minimum (11.41%) for Feed-1. The mean values of relative growth for other feeds were lower than the value calculated for Feed-2 (Table 3 to Table 7). The specific growths also showed a similar trend as observed in the case of relative growth (Tables 3 to 7). A maximum specific growth was observed for all feeds during the early phase of the experiments (Tables 3 to 7). The mean specific growth value was also maximum (15.6%) for Feed-2 and lowest (12.3%) for Feed-1 (Tables 3 to 7).

The observed weights obtained for different feeds were compared with the weights calculated by applying the von Bertalanffy's growth equation (Table 8). Maximum asymptotic weight calculated for Feed-2 was 300 g whereas it was minimum (180 g) for Feed-1 (Table 8). The $\log_e W\alpha + Kt_o$ values were calculated by plotting the graph for expressing the von Bertalanffy's growth equation where 't_o' value was calculated by the method given by Ricker (1958). The 't_o' values for feeds 1, 2, 3, 4 and 5 were (-) 3.75, (-)3.00, (-)3.33, (-)3.18 and (-)3.60 respectively (Table 8). The weight calculated following the von Bertalanffy's growth equation was very near to the values of observed weight for different feeds (Tables 3 to 7). This indicates that von Bertalanffy's growth equation fitted well in describing the growth pattern of the juveniles of *P. monodon.*

Much attention has been paid in recent years to find out various sources of readily available economical protein ingredients to keep the feed formulation easier and cost effective (Alava and Lim, 1983, Rajyalakshmi *et al.*, 1986, Sudaryano *et al.*, 1995). Formulation of a balanced feed containing low cost protein ingredients can bring the cost of supplementary feed for shrimp to a great extent. Carbohydrates show low amount of protein utilization for energy and are less expensive dietary energy source. As such carbohydrate not only substitute protein component to function as energy source but also provide feed stability and increase feed conversion through increase palatability. Carbohydrates are normally stored by the shrimp in the form of glycogen, glucosamine and trehalose in

Table 6. Average observed weight, theoretical weight, relative growth, growth increment and specific growth for Feed-4

Weeks	Average observed weight (g) ±SD	Theoretical weight (g)	Relative growth (%)	Growth increment (%)	Specific growth (%)
Initial	1.2±0.0	-	-	-	-
1	1.6±0.0	1.59	25.00	0.40	28.8
2	1.8±0.1	1.98	8.57	0.15	8.9
3	2.1±1.5	2.36	14.63	0.30	15.8
4	2.2±0.1	2.74	6.81	0.15	7.1
5	2.8±0.2	3.12	20.0	0.55	22.3
6	3.3±0.3	3.49	16.60	0.55	18.2
7	3.5±0.2	3.87	5.71	0.20	5.9
Mean	2.27	2.72	13.90	0.32	15.2

Table 7. Average observed weight, theoretical weight, relative growth, growth increment and specific growth for Feed-5

Weeks	Average observed weight (g) ±SD	Theoretical weight (g)	Relative growth (%)	Growth increment (%)	Specific growth (%)
Initial	1.2±0.0	-	-	-	-
1	1.7±0.2	1.37	27.27	0.45	31.8
2	1.7±0.1	1.67	2.94	0.05	3.0
3	1.9±0.2	1.97	8.10	0.15	8.5
4	2.1±0.2	2.26	11.90	0.25	12.6
5	2.2±0.2	2.56	4.54	0.10	4.7
6	2.7±0.4	2.85	18.51	0.50	20.5
7	2.9±0.4	3.15	6.89	0.20	7.1
Mean	2.03	2.26	11.45	0.24	12.6

Table 8. A comparison of asymptotic weight and von Bertalanffy's growth equation with different feeds.

Feeds	W_α (g)	K	t_0	$\log_e W_\alpha + Kt_o$	von Bertalanffy's growth equation
Feed-1	180	0.0016	-3.75	5.186	$W_t=180[1-e^{-0.0016(t+3.75)}]$
Feed-2	300	0.0013	-3.00	5.699	$W_t=300[1-e^{-0.0013(t+3.0)}]$
Feed-3	220	0.0015	-3.33	5.388	$W_t=220[1-e^{-0.0015(t+3.33)}]$
Feed-4	240	0.0016	-3.18	5.475	$W_t=240[1-e^{-0.0016(t+3.18)}]$
Feed-5	200	0.0015	-3.60	5.292	$W_t=200[1-e^{-0.0015(t+3.6)}]$

the hepatopancreas, cuticle and muscle respectively and considered as main source of energy (Kanazawa, 1981). Sugar is useful greatly for immediate energy needs (Capuzzo, 1981). Carbohydrates consist of mylose, a linear polymer and amyloprotein, a branched polymer. The amylase, amylopectin ration varies with the source for example wheat: 20/80, rice: 15/85 or even 65/35 (Cheftel and Cheftel, 1976).

In the present study Feed-2 showed maximum growth efficiency where the formulation of diet based on the combination of ingredients like molasses, soyabean, yeast, rice bran, tapioca and wheat flour. All these ingredients are rich source of carbohydrates contributing 25.78% of the feed composition. This formulated diet was also incorporated with fresh seaweed, a major resource of PUFA (Desvilettes *et al.*, 1997). In addition to this, *Ulva fasciata* contains 47.3% carbohydrates and 12.88% proteins (Dhargalkar *et al.*, 1980). Tapioca powder and wheat flour used in the Feed-2 have been reported to show a high binding capabilities (Goswami and Goswami, 1979).

Myrna (1986) also found higher efficiency of diet containing more carbohydrates (20%) and lesser proteins (30%) and lipids (15%) than feed containing 50% proteins, 10% carbohydrates and 5% lipids. However, the higher level of carbohydrates has been found to effect the growth of juveniles adversely (Myrna, 1986). An increase amount of carbohydrates in the diet imbalances the utilization of energy levels resulting in poor digestibility of the protein (Page and Andrews, 1973).

Results of the present study are with agreement with the findings of the Smith *et al.*, (1985) which showed that the optimal growth of shrimp is not significantly influenced by the dietary proteins level but also affected the dietary proteins source whereas, other studies also showed that proteins was the major factor effecting the growth rates of penaeids (Chen *et al.*, 1985, Rajyalakshmi *et al.*, 1986, Lim *et al.*, 1979). Essential fatty acids are also constituted an important part of the diet of penaeid. Similarly, vitamins like ascorbic acid, choline, inositol, riboflavin, thiamins and mineral are also important ingredients for the diet of juveniles (Trino and Sarroza, 1995).

The proteins requirements for *Penaeus* sps ranged between 30 and 50% (Laubier and Laubier, 1993). However, other investigations showed those protein requirements were ranged between 28 and 33% for *P. styliferus* (Andrews *et al.*, 1972), 43% for *P. monodon* (Colvin, 1976) and 34-43% for *P. merguiensis* (Sedgwick, 1979). In a study made by Alava and Lim (1983) showed that the feeds for juveniles of *P. monodon* consisting of 40% protein gave good results whereas, Lee (1971) found good results with 45.8% protein. However, Bages and Sloane (1981) reported that for the juveniles of *P. monodon*, 55% proteins showed better growth.

Food Conversion Ratio (0.471) obtained with the Feed-2 was the lowest value obtained in the present study. The juveniles of *P. monodon* have been reported to consume 40% protein with semi-purified diet that showed FCR value of 2.41 (Shiau and Chou, 1991). The FCR value obtained for Feed-1, Feeds-3, 4 and 5 were relatively higher than Feed-2. Low FCR value for Feed-2 (0.471) shows the efficiency of the nutrients used in the diet. These ingredients also represent great economic savings since less food is required to obtain greater biomass production (Crus-Suares *et al.*, 1994).

The faecal out, in all the feeds except Feed-2 was higher that showed less conversion efficiency of the feed used. The high assimilation efficiency (K_1 and K_2) and low faecal output for Feed-2 shows the high efficiency of Feed-2.

The growth observed using other feeds in the present study showed relatively a lower growth rates as compared to Feed-2. The lesser growth in the juveniles with other feeds used could be the differences in protein content and differences associated with processing techniques at the time of feed formulation. The Feed-1 consisted of combination of mussel, clams, oysters, fresh squid, yeast, egg, wheat flour and trash fish powder, showed a poorest growth performance though the ingredients used for this feed were rich in proteins. The chemical composition of other feeds showed a high protein value. However, the growth rate was relatively low in all the four feeds as compared to Feed-2. The average weight gained by the juveniles is directly related to the type of feed (Alava and Lim, 1983). The weight gain with Feed-2 was relatively higher ($P<0.05$) as compared to other feeds.

It is quite evident from the present study that the growth of penaeids shrimp is mainly dependent on the quality of feed and its protein level (Deshimaru and Shigeno, 1970, Colvin, 1976, Teshima *et al.*, 1986). A large number of shrimp feeds have been developed in the past 20 years which showed that the feed formulation involved mainly selection of suitable ingredients like protein source, binding agent, palatability of the feed and relative potential of the diet for better growth (Cuzon *et al.*, 1994). Palatability and physical studies of shrimp feed are interrelated and many substances have been tried to study the binding property of shrimp diets to prevent losing of the texture of the feed.

Author's Contribution

SC and AC designed the experiments whereas SP was responsible in conducting the experiments. All authors contributed to data analysis and writing of the article.

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