

**A STUDY ON THE BLACK CLAM VILLORITA CYPRINOIDES (GRAY)
AS PROTEIN SOURCE IN PRAWN DIET**

Dissertation submitted by Shri. C. REGUNATHAN in partial fulfilment for
the Degree of Master of Science (Mariculture) of the
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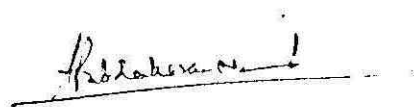
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Post-Graduate Programme in Mariculture
CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
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CERTIFICATE

This is to certify that this dissertation is a bonafide record of work carried out by **Shri. C. REGUNATHAN** under my supervision and that no part thereof has been presented before for any other degree.



**K. PRABHAKARAN NAIR
SCIENTIST-SG
CENTRAL MARINE FISHERIES
RESEARCH INSTITUTE
COCHIN.**

Countersigned by:



**DIRECTOR
CENTRAL MARINE FISHERIES
RESEARCH INSTITUTE
COCHIN.**

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P R E F A C E

With the situation that the production by capture fisheries has reached or is reaching a state of maximum exploitation, it is rightly presumed that there would be a decline in production. In context with this situation, aquaculture has been found to be a supplementary source to cater to the increasing world needs for fisheries products. This has led to the upsurge in aquaculture practices in many countries including India.

Apart from satisfying the national food needs, the fishery products have been contributing greatly to the foreign exchange earnings of many countries, with the shrimps taking the lion's share. Because of the increasing demand for shrimp in the world market and because of its very high export value, shrimp culture has advanced to a great extent, accounting for about 28% of world shrimp production (in 1991). India, with a culture area of 65,000 ha, accounts for about 5% of total production.

Proper formulation of nutritious feeds with high conversion rates is now one of the major priority areas in aquaculture research, as the feed takes up even 50% of the total cost. As the efficiency of the compounded feed depends to a great extent on its ingredients and as the cost of the feed plays a vital role in the overall economics of the culture operations, the search for more suitable and economically viable food source, is still continuing vigorously.

Amongst various sources of plant and animal proteins tested, those of the latter origin appear to have a significant effect on growth. With this aspect in view, the present study has been carried out using the black clam Villorita cyprinoides (Gray) as feed component for the Indian white prawn Penaeus indicus (H. Milne Edwards) which is one of the foremost cultivated species in India.

The objectives of the present investigation are the following:

- 1) To evaluate the proximate composition of clam meal with a view to use it as supplementary protein source in prawn feed;
- 2) To study the effect of different levels of clam meal in semi-purified diets on growth, protein efficiency ratio and food conversion ratio to determine its optimum inclusion level for Penaeus indicus,
- 3) To assess the overall biological value of clam protein through nitrogen balance studies.

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I N T R O D U C T I O N

Though primitive methods of prawn culture have been practised for centuries in India and other Asian countries, only after the Second World War and need for increased production of protein-food was felt and this prompted the revival and improvement of old culture practices, supplemented with new culture techniques. Among all the aquaculture products, prawn is the most lucrative commodity earning large amount of foreign exchange. India's prawn production by culture in 1991 was 35,000 metric tons (Rosenberry, 1992) and the money earned by exporting prawn in 1990-1991 was about Rs.68.33 crores (MPEDA, 1992).

In the high-density shrimp culture systems, complete or supplementary feeding becomes inevitable for better farm production. Feed constitutes one of the major fractions of the operational costs in aquaculture, accounting for 25-50% of the total production cost depending upon the type and efficiency of the culture operation. Considerable work has been done to understand the nutritional requirements of prawns, particularly in the Indian white prawn Penaeus indicus by several workers in recent years (Ali, 1982a, 1982b; Jyothy, 1983; Ali and Sivadas, 1983; Thomas, 1985; Gopal, 1986; Chandge, 1987).

As protein is the most important and expensive among all the feed components, greater emphasis has been given in recent years for the study of the protein requirements and to determine its optimum level in the diet for various species like Penaeus japonicus (Kanazawa et al, 1970; Deshimaru and Shigueno, 1972); P. monodon (Lee, 1971; Alava and Lim, 1983); P. indicus (Colvin, 1976; Ali, 1982); P. merguensis (Sedgwick, 1979; Aquacop, 1978) and P. aztecus (Venkataramiah et al, 1975). Besides these studies, evaluation of various plant and animal protein sources like soyabean meal (Sick and Andrews, 1973), mantis-shrimp (Ali et al, 1985), fish meal (Colvin, 1976) and shrimp meal (Balazs et al, 1973) in the compounded diets for prawn has also been carried out.

Molluscs like squid, clams, mussels and snails have been proved to be among the best diets for prawn. Molluscan meal is a major component of Taiwanese and French prawn diets (Maguire, 1987). In Japanese prawn farming the main diet consists of the short-necked clam Venerupis philipinarum (Deshimaru and Shigueno, 1972). In China the supplementary feed used for prawn includes fresh molluscs such as the blue clam Corbula sp, Brachidontes sp, Anatinella sp, Venerupis vareigata, fresh water snails and land snails (Wu Qin Se, 1987).

Molluscs like squid (Fenucci and Zein-Eldin, 1976; Shigueno and Deshimaru, 1972; Ali, 1982a) and the mussel Mytilus edulis (Sedgwick, 1979 Forster and Beard, 1973) have been tried as protein source in the prawn

diets. About 15% squid meal in the diet of P.aztecus resulted in high biomass increase, survival and increase in mean body weight (Fenucci et al, 1976).

Among molluscs, clams have been studied more deeply than others as protein source for prawn and they are now being widely used. Experiments with short-necked clam proved that it has a combination of aminoacids quite similar to that of the prawn meat (Deshimaru and Shigueno, 1972). Kanazawa et al (1970) prepared a purely chemical-based diet approaching the biochemical composition of the clam meat, and found the latter comparatively more effective. According to New (1976) diets with aminoacid profile closest to that of clam were most effective.

Shewbart' et al (1973) observed that clam solubles were good feeding attractants for Penaeus aztecus. Molluscs are found to be good source of essential aminoacids like eicosopentnoic acid (20;5w3), decosohexanoic acid (22;6w3) (Kanazawa et al, 1977) which are found to influence moulting (Guary et al, 1976). Addition of 1% lecithin from the short-necked clam (Tapes sp) lipids to a semi-purified diet significantly improved the growth of Penaeus japonicus (Kanazawa et al, 1979). Ali (1982a) evaluated the use of fresh meat of clam Villorita cyprinoides in the diets for Penaeus indicus and found that it has got moult-inducing effect. It was also reported that powdered meal of the marine clam Sunetta scripta gives higher growth rate in the same species of prawn, than fish meal and silk worm pupae (Ali, 1988).

The above works have shown that the molluscan meal contributes to

- (1) A correct aminoacid balance,
- (2) Feed attraction,
- (3) Unknown growth factor, and
- (4) Lipid requirement.

In India clams are widely distributed along the east and west coast and they form sustenance fishery especially in many estuaries of Manarashtra, Goa, Karnataka and Kerala. The black clam Villorita cyprinoides (Gray), selected for the present study, supports a regular fishery in many estuaries in Kerala, Karnataka and Goa providing cheap source of protein by way of meat. The annual production of black clam is about 29,077t (Narasimham, 1991).

In this study an attempt has been made to investigate the relative efficiency of clam meal as protein in the prawn diet, and to determine the optimum inclusion level by studying nutritional factors like food conversion ratio (FCR), protein efficiency ratio (PER), digestibility, net protein utilization (NPU), biological value (BV) and survival.

M A T E R I A L S A N D M E T H O D S

Diet formulation

The black clam Villorita cyprinoides was used as animal protein source for juvenile Penaeus indicus by making it one of the ingredients in the semi-purified diet. The feeds used in the experiment included one control feed which was a zero-clam diet, five experimental feeds and a zero-protein feed.

The control feed was a modified form of standard purified diet recommended by Kanazawa et al (1982). All the feeds had casein and gelatin as protein sources, the latter also serving as binder, while glucose, sucrose, starch and cellulose were the carbohydrate source; starch also served as binder. Cod liver oil formed the lipid source in the feeds.

The experimental feeds, numbered one to five (F_1 - F_5), included, in addition to casein and gelatin, clam as the protein source. Clam was used in the feeds as clam meal prepared from the meat of Villorita cyprinoides (Plate 1). The meat extracted from the animal was dried in an oven at 60°C for 15 hours. The dried meat was powdered and passed through a 250 μ sieve. This powder was used in various proportions as 10%, 20%, 30%, 40% and 50% in the experimental feeds F_1 , F_2 , F_3 , F_4 and

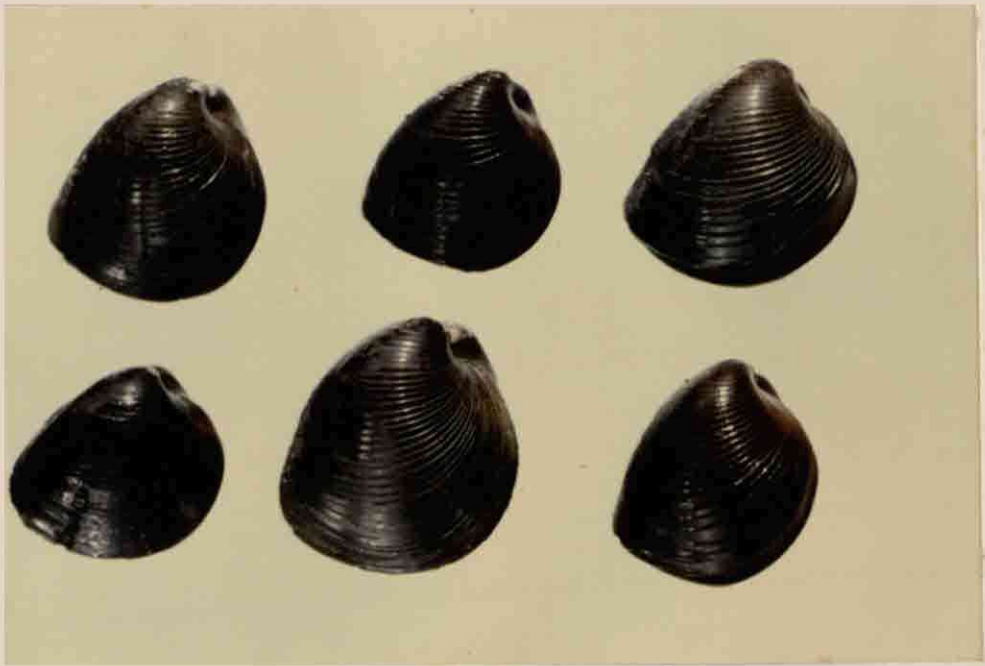


Plate 1. The black clam Villorita cyprinoides Gray.

F₅ respectively. The composition of control and experimental feeds are given in the Table 1a. Composition of mineral and vitamin mixture was the same in all six feeds (Table 1b, c). The zero-protein feed(F₀) contained all the other sources except protein sources.

Preparation of the feeds

Ingredients in the above proportions were individually powdered, weighed and mixed together. To this, water was added at the rate of 40ml per 100 g of feed. Gelatin was separately dissolved at 70°C in small quantity of water. To the dry ingredients, oil, gelatin, vitamin mixture and mineral mixture were added and thoroughly mixed. The dough was steamed for 15 minutes, and pelleted through a hand pelletiser with 1 mm diameter pored die. The pellets were then broken into pieces of 2-3 cm length and dried in an oven at 60°C for 6 hours. Dry feeds were stored in plastic containers during the experiment. The proximate composition of these feeds are given in the Table 2.

Experimental design

Experimental design followed was the completely randomized design (CRD), with three replicates for each treatment.

Table 1 a

Composition of control feed and experimental feeds (%)

Ingredients	Control feed	Experimental feeds				
		F ₁	F ₂	F ₃	F ₄	F ₅
Casein	55	45	35	25	15	5
Clam meal	0	10	20	30	40	50
Glucose	4.5	4.5	4.5	4.5	4.5	4.5
Sucrose	8	8	8	8	8	8
Starch	3.5	3.5	3.5	3.5	3.5	3.5
Sodium citrate	0.3	0.3	0.3	0.3	0.3	0.3
Sodium succinate	0.3	0.3	0.3	0.3	0.3	0.3
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5
Cod liver oil	10	10	10	10	10	10
Cellulose	1.7	1.7	1.7	1.7	1.7	1.7
Gelatin	4.0	4.0	4.0	4.0	4.0	4.0
Mineral mix *	8.5	8.5	8.5	8.5	8.5	8.5
Vitamin mix **	3.2	3.2	3.2	3.2	3.2	3.2
		100%	100%	100%	100%	100%

* composition as given in Table 1b.

** composition as given in Table 1c.

Table 1b**Composition of Mineral mixture**

Mineral	g/100g feed
Calcium lactate	2.720
Potassium dihydrogen orthophosphate	2.000
Sodium dihydrogen orthophosphate	0.790
Magnesium sulphate	3.020
Manganese chloride	0.004
Ferrous chloride	0.015

Table 1c

Composition of Vitamin mixture

	<u>mg/100g feed</u>
<u>Water soluble vitamins</u>	
Ascorbic acid (Sodium salt)	2.00
Choline chloride	0.60
Folic acid	0.30
Nicotinic acid	60.00
Pantothenic acid	60.00
Paraamino benzoic acid	10.00
Pyridoxine hydrochloride	12.00
Riboflavin	8.00
Thiamine hydrochloride	4.90
Cyanocobalamine	0.08
<u>Fat soluble vitamins</u>	
Biotin	0.40
β -carotene	9.60
Calcipherol	1.20
Inositol	400.00
Menadione	4.00
α -Tocopherol	20.00

Table 2

Proximate composition of feeds (%)

	Control feed	Experimental feeds				
		F ₁	F ₂	F ₃	F ₄	F ₅
Protein	46.20	43.10	44.60	44.60	44.60	44.60
Nitrogen-free extract	27.20	32.50	31.34	31.52	31.35	31.17
Fat	12.00	9.00	9.00	9.00	9.00	9.00
Moisture	8.00	8.50	8.20	8.00	8.10	8.20
Ash	6.50	6.60	6.50	6.50	6.50	6.50
Fibre	0.10	0.30	0.36	0.38	0.45	0.53

Experimental facilities

Circular plastic tubs of 54 cm x 30 cm size and 50 litre capacity were used to rear the prawn during the experiment (Plate 2). The tubs were arranged on wooden racks and the various treatments were randomly allotted. All the tanks were covered with velon screen to prevent the prawns jumping out.

Experimental animals

The clam used for the preparation of clam meal was collected from Nettur, situated about 13 km southeast of Cochin Harbour. They were brought from the field to the laboratory in plastic buckets containing water and stored at -8°C in a deep freezer.

Juveniles of the Indian white prawn Penaeus indicus (Plate 3) having an average weight of 1 ± 0.3 gram and average length of $55\pm 2\text{mm}$ were collected from backwater canals located in the Vypeen island near Cochin. Initially the juveniles were acclimatized to the experimental conditions for 5 days. During this transit phase they were not fed. After this phase the prawns were randomly selected and introduced in to the experimental tubs at the rate of 10 prawns/tub, and were fed with the respective feeds to acclimatize them to the artificial feeds. Feeding was suspended on the day prior to the start of the experiment.



Plate 2. Part of the experimental setup used for rearing the animals

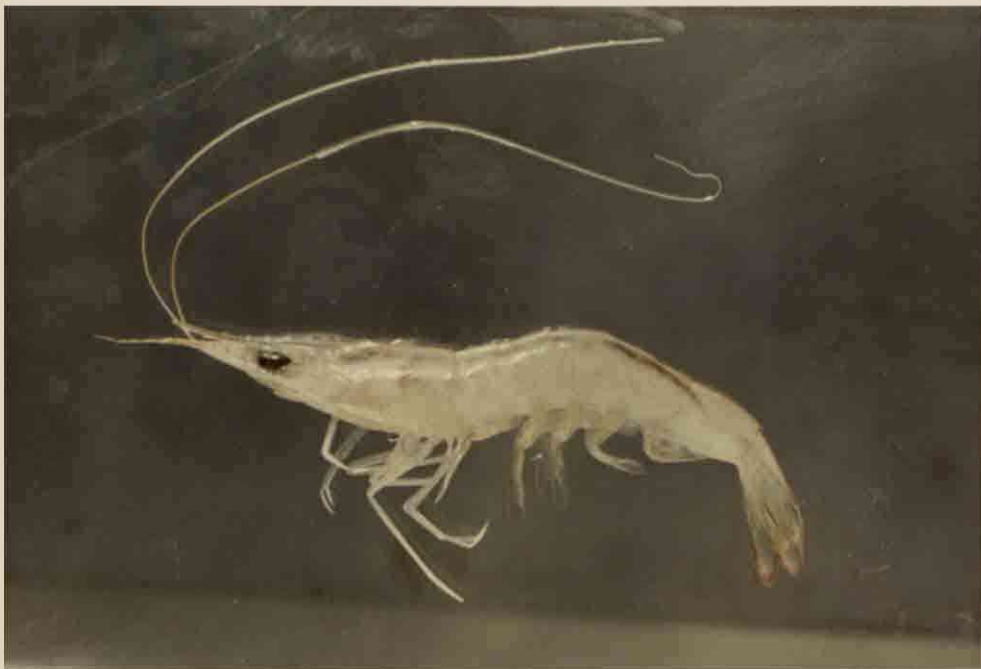


Plate 3. The Indian white prawn Penaeus indicus H. Milne Edwards

At the start of the experiment the length and weight (length measured to the nearest 1 mm from the tip of rostrum to the tip of telson and the weight to the nearest 0.01 g on a top loading balance) of the individual animals were recorded.

Water replacement

The plastic experimental tubs were filled with sediment-free sea water, diluted to the salinity of 15-20 ppt using fresh water. The quantity of water in the tub was maintained at the rate of 2 litres per prawn. One-third of the quantity of water was replaced every day with complete replacement once in four days. The aerator stones were cleaned with fresh water once in a week to prevent algal overgrowth.

Feeding strategy

The shrimps were fed twice daily, one-third ration between 0900 and 1000 hours and the rest between 1600 and 1700 hours at the rate of 10% (dry matter basis) of the body weight per day for the first week and 8% per day for the succeeding weeks. Care was taken to see that the feeding levels selected were in excess to animal's requirement. They were weighed individually every ten days to determine the weight gain and feed allowance.

Every morning, before feeding, feed remains and other detritus in each tub were siphoned out. Mortality, whenever occurred, was noted. When the shrimps were removed for weighing, the tubs were cleaned thoroughly to remove the algal growth on the inner surface.

The faecal matter from each experimental tub was collected with a wide mouthed pipette. This was immediately rinsed with distilled water to remove traces of salts, and then dried. The sample from replicates of each treatment were pooled and analysed.

The left-over feed was collected every morning on a bolting silk cloth by keeping it at one end of the siphoning tube when the water was siphoned out. The collected feed was washed with distilled water, and transferred to a pre-weighed (W) petridish, dried in an oven and then weighed (W_1). The weight of left-over feed ($W_1 - W$) aids in calculating the consumption rate.

Hydrological parameters (dissolved oxygen, salinity, temperature, pH and ammonia) were monitored regularly. Oxygen supply was ensured by uninterrupted aeration. The salinity was maintained at 15-20 ppt as suggested by Venkataranaiah et al (1975b). The hydrological data in respect of each experiment are given separately in Table 3.

The experiment was carried out for 45 days. At the termination of the experiment equal number of animals from all the treatments were sacrificed and kept in the deep freezer for carcass analysis.

Determination of metabolic faecal nitrogen (MFN)

For the determination of biological value of a protein, the true digestibility of protein is required, and this involves the determination of faecal nitrogen which contains not only the digested nitrogen from the diet but also the nitrogen excreted due to metabolic activity in the body.

Metabolic faecal nitrogen (MFN) was determined by feeding the animals with a known quantity of nitrogen-free diet, and the nitrogen appearing in the faeces is therefore considered as the metabolic faecal nitrogen (Mitchell and Bert, 1954; Forster and Gabbott, 1971).

For calculating MFN, animals were individually held separately in six rearing containers and fed with zero-protein diet or in otherwards, nitrogen-free diet (E) 'ad libitum' for 20 days. Faeces were collected every day, and the nitrogen and chromic oxide in the diet and faeces were determined. At the end of the experiment the carcasses of the animals were analysed for determining the net protein utilization.

$$\text{MFN excreted when 100g of feed consumed} = \frac{A \times B}{C}$$

A = Percentage nitrogen in faeces of animal fed with zero-protein diet

B = Percentage indicator in zero-protein diet,

C = Percentage indicator in faeces.

$$\text{MFN due to amount of test diet consumed} = \frac{E}{D} \times \frac{AB}{C}$$

D = Chromic oxide in test diet,

E = Chromic oxide in faeces of animals fed with test diet.

The value obtained is subtracted from the total faecal nitrogen of the test group animals to obtain the corrected faecal nitrogen of the test group.

Digestibility

Digestibility of protein in the feed was determined using the inert internal marker chromic oxide (Cr_2O_3) which has been successfully used to study digestibility of nutrients in prawns (Forster and Gabbott, 1971; Colvin, 1976; Ashmore et al., 1985 and Smith et al., 1985). The method consists of adding known amount of chromic oxide (0.5%) in the feed. The chromic oxide was excreted out by the animal undigested. The faeces was collected for a period of time and the protein and chromic oxide in the faeces and diet were determined. The apparent digestibility coefficient was calculated by the following formula:

$$\text{Apparent digestibility coefficient} = 100 - \frac{\% \text{ chromic oxide in the diet}}{\% \text{ chromic oxide in faeces}} \times \frac{\% \text{ nutrient in faeces}}{\% \text{ nutrient in diet}}$$

Using the corrected faecal nitrogen of the test group, the true digestibility of protein was calculated by the formula :

$$\text{True digestibility} = 100 \frac{\% \text{ chromic oxide in diet}}{\% \text{ chromic oxide in faeces}} \times \frac{\% \text{ corrected protein in faeces}}{\% \text{ protein in diet}} \times 100$$

Water stability pellets

Water stability of feed pellets was evaluated by employing the method described by Jayaram & Shetty (1991) with minor modifications. The loss of weight of pellets due to leaching when kept under water at specified time interval was determined. For this purpose cone-shaped pouches were made with bolting silk (1 mm mesh). These were thoroughly washed with water and dried at 60°C. The feed pellets were cut into pieces of approximately 5 mm length. These samples were weighed at the rate of 8 samples for each feed.

The pouches along with pellets were carefully lowered into the water and placed in petridishes kept at the bottom of a plastic container with water of 18 ppt salinity. At the end of 2 hours, one set of two pouches were carefully taken out of water. These were gently dipped in a container of fresh water for 3 minutes to remove the adhering salt. They were then transferred to the oven and dried at 60°C and weighed.

In the same manner, two pouches each were taken out at the end of 4, 6 and 8 hours and treated as mentioned above. The loss in the weight of pellets was calculated by the difference in the weight before and after the immersion of pellets. Experiment was repeated twice and average values were taken.

Analysis

The levels of crude protein in the feeds, faecal matter and the carcass were determined by micro-kjeldahi method (AOAC, 1975). Crude fat in the feed was estimated by soxhlet extraction method.

Ash content in the feeds was found out by keeping pre-weighed sample in muffle furnace at 600°C for 6 hours and Crude fibre by doing acid and alkali digestion followed by keeping in muffle furnace at 500°C for 3 hours (AOAC, 1975). The chromic oxide in the feed and faecal matter was estimated by the method suggested by McGinnis and Kasting (1964).

Water temperature was measured with an ordinary thermometer of 0-50°C range with 0.1 accuracy. Salinity was estimated by Mohr-Knudsen method, and dissolved oxygen using the modified Winkler method, as given by Strickland and Parsons (1968).

The pH of water was measured using a digital pH meter. Ammonia concentration in the water was determined by phenol hypochlorite method (Solarzano, 1969).

Parameters studied

- 1) % growth in length/weight : $\frac{\text{Final length/weight} - \text{Initial length/weight}}{\text{Initial length/weight}} \times 100$
- 2) Food conversion ratio(FCR): $\frac{\text{Average weight of food consumed(dry weight)}}{\text{Average live weight gain}}$
- 3) Protein efficiency ratio : $\frac{\text{Average live weight gain}}{\text{Average protein consumed}}$
- 4) Gross conversion efficiency ($K_1\%$) : $\frac{\text{Increase in average wet weight}}{\text{Consumption}} \times 100$
- 5) Net conversion efficiency ($K_2\%$) : $\frac{\text{Increase in average wet weight}}{\text{Assimilation}} \times 100$
- 6) Net protein utilization (NPU) : $\frac{\text{Body nitrogen of test group animals} - \text{Body nitrogen of animals receiving zero-protein feed}}{\text{Nitrogen consumed}}$
- 7) True digestibility : $100 - \frac{\% \text{ Indicator in diet}}{\% \text{ Indicator in faeces}} \times \frac{\% \text{ corrected protein in faeces}}{\% \text{ protein in diet}} \times 100$
- 8) Survival rate (%) : $\frac{\text{Initial number of animals} - \text{Final number of animals}}{\text{Initial number of animals}} \times 100$
- 9) Biological Value : $\frac{\text{Net protein utilization}}{\text{True digestibility of protein}}$

10) Moulting rate was calculated using the formula given by Petriella (1990)

$$\begin{aligned} \text{Moulting rate} & : \frac{\text{Moult percentage}}{\text{Mean life of the group}} \\ \text{Moult percentage} & : \frac{m}{n^1} \times 100 \\ m & : \text{Number of moults} \\ n^1 & : \text{Initial number of animals} \end{aligned}$$

Mean life of the group was calculated by adding the number of days each individual survived and then taking the mean.

Statistical Analysis

The data obtained with various parameters were subjected to Analysis of Variance (ANOVA) to find out the significance between treatments and Mean values were compared by least significant difference (LSD), in both cases following Snedecor and Cochran (1973).

Table 3

Hydrographic parameters observed during the experiment

Treatment	Salinity(ppt)	Oxygen(ml/l)	pH	Temperature(°C)
Control	15.2 ± 1	4 ± 0.3	8.06 ± 0.2	28.5 ± 0.5
F ₁	16.0 ± 1	4 ± 0.2	8.10 ± 0.1	27.8 ± 0.5
F ₂	16.8 ± 1	4 ± 0.3	8.10 ± 0.1	27.8 ± 0.5
F ₃	16.0 ± 1	3.7 ± 0.3	8.05 ± 0.2	28.0 ± 0.5
F ₄	16.8 ± 1	4 ± 0.3	8.2 ± 0.2	28.5 ± 0.5
F ₅	16.4 ± 1	4 ± 0.2	8.05 ± 0.1	28.0 ± 0.5

R E S U L T S

The results of the experiments conducted to evaluate the clam meal, its comparative efficiency at different inclusion levels as feed for Penaeus indicus are given in Tables 4 to 9.

Proximate composition of clam meal:

The result obtained in regard to proximate analysis of clam meal is given in Table 4, which showed a high protein (50.82%) as well as lipid content (8.5%) in the clam meal indicating that clam meal is suitable to the nutritional requirements of the prawn.

Increase in length, live weight and dry weight

In respect of six feeds (denoted as Control, F₁, F₂, F₃, F₄ and F₅) the prawns fed on the feed F₃ (30% clam meal) registered the highest growth of 30.22% in length, 52.50% in live weight and 96.13% in dry weight (Table 5). Animals fed with F₄ (40% clam meal) obtained the second highest growth of 28.34% in length, 44.48% in live weight and 92.66% in dry weight. Penaeus indicus fed on F₅ (50% clam meal) showed the third best growth rate with a growth of 25.77%, 39.50% 88.0% in length, live weight and dry weight respectively. F₂ (20% clam meal) provided a growth of 16.9% in length, 39.33% in live weight and 87.58% in dry weight. Among the experimental feeds, the feed with 10% clam meal (F₁) had shown the

Table 4**Proximate composition of the clam meal (%)**

Protein	50.82
Fat	8.50
Nitrogen free extract	20.63
Ash	10.50
Moisture	9.35
Fibre	0.20

Table 5

Estimated growth in length, live weight and dry weight of Penaeus indicus juveniles fed with different feeds

Parameters	Control feed	Experimental feeds				
		F ₁	F ₂	F ₃	F ₄	F ₅
Initial average length (mm)	55.5	55.7	55.5	55.6	55.4	55.5
Initial average weight (mg)	1022	1023	1022	1000	1023	1000
Initial average dry weight (mg)	151	156	153	155	150	150
Final average length (mm)	57.9	61.1	64.9	72.4	71.1	69.8
Final average weight (mg)	1064	1402	1424	1525	1478	1395
Final average dry weight (mg)	203	277	287	304	289	282
% increase in length	3.90	9.60	16.90	30.22	28.34	25.77
% increase in weight	4.11	37.05	39.33	52.50	44.48	39.50
% increase in dry weight	34.44	77.56	87.58	96.13	92.66	88.00
Food conversion ratio	10.09	2.11	2.10	1.60	1.70	1.83

lowest growth of 9.6% in length, 37.05% in live weight and 77.56% in dry weight. The lowest growth was recorded in the case of the control feed with a growth of 3.9% in length, 4.11% in live weight and 34.44% in dry weight.

Analysis of variance (ANOVA) showed that the growth in length, live weight and dry weight differ significantly between treatments at 1% level ($P < 0.01$) (Table 10, 11 and 12). Least significant difference (LSD) showed that in the case of increase in length all feeds differ significantly between them at 1% level. In the case of increase in live weight, the control feed differ significantly from all other feeds at 1% level ($P < 0.01$). The Feed F_1 did not show any significant difference with feeds F_2 , F_4 and F_5 . Similarly feeds F_2 , F_4 and F_5 did not differ significantly between them. Feeds F_3 and F_4 were significant at 5% level ($P < 0.05$). In the case of dry weight control feed showed significant difference at 1% level ($P < 0.01$) with all other feeds. Feeds F_1 and F_3 differed significantly at 5% level ($P < 0.05$), while other feeds did not differ significantly.

Food conversion ratio (FCR)

Fig. 4 shows the food conversion ratio of all the six feeds. Except the control feed, all other feeds gave good conversion rates. However, F_3 recorded the best FCR (1.6) followed by F_4 , F_5 , F_2 , F_1 with the ratio values of 1.70, 1.83, 2.10, 2.11 respectively. The control feed obtained a FCR of 10.09, thus showing that inclusion of clam meal helps in reducing the FCR at least by 5 times. Analysis of variance (Table 13)

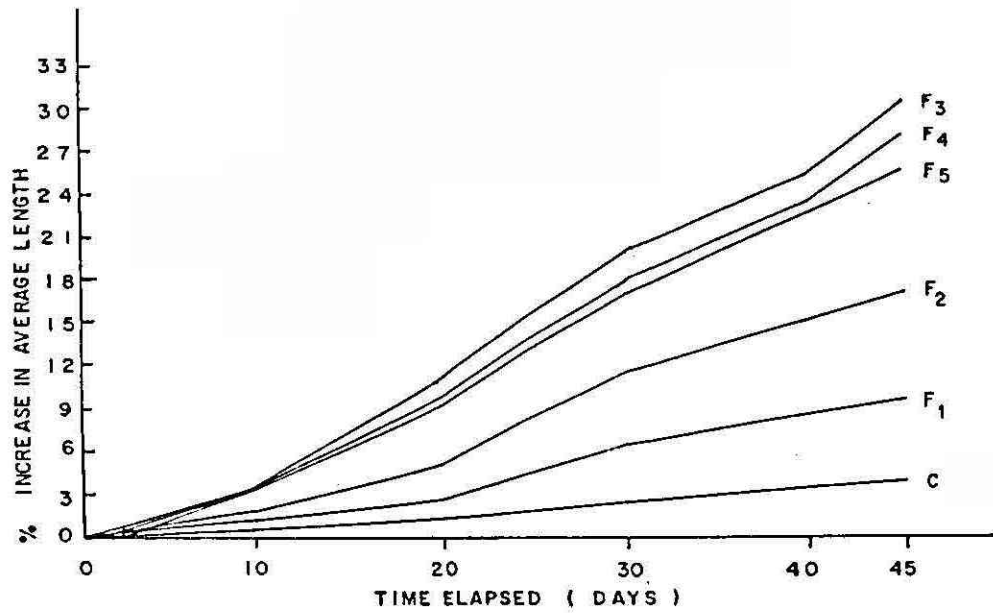


Fig. 1. Increase in length of *Penaeus indicus* fed with feeds having clam meal at various levels. (C-control; F₁=10% clam meal, F₂=20% clam meal; F₃=30% clam meal; F₄=40% clam meal and F₅=50% clam meal)

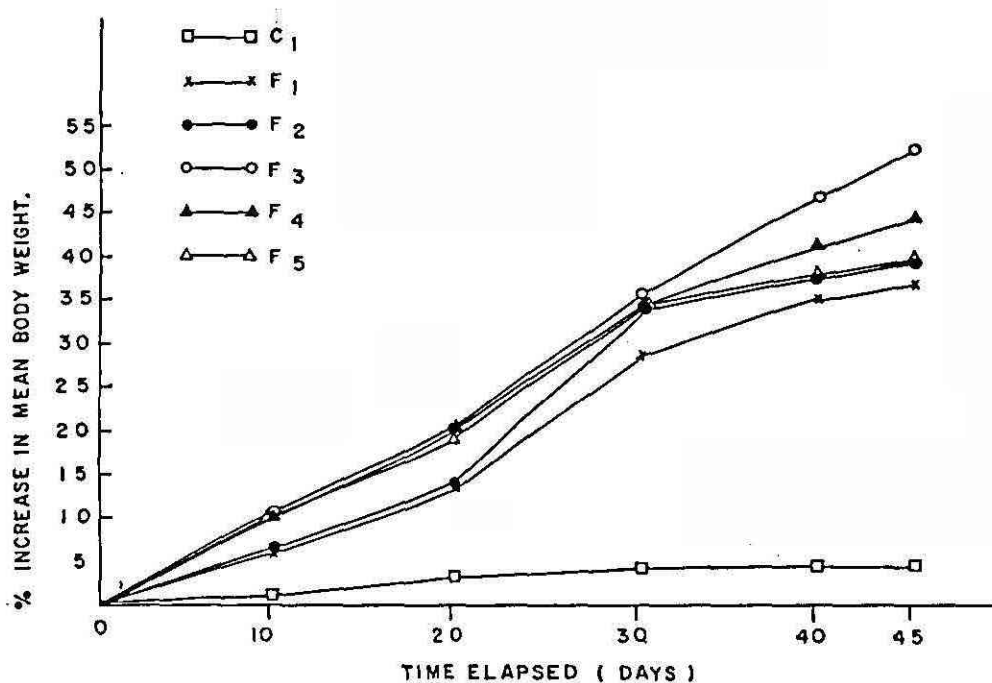


Fig. 2. Increase in body weight of *Penaeus indicus* fed with feeds having clam meal at various levels. (C-control; F₁ = 10% clam meal, F₂ = 20% clam meal; F₃ = 30% clam meal; F₄ = 40% clam meal and F₅ = 50% clam meal).

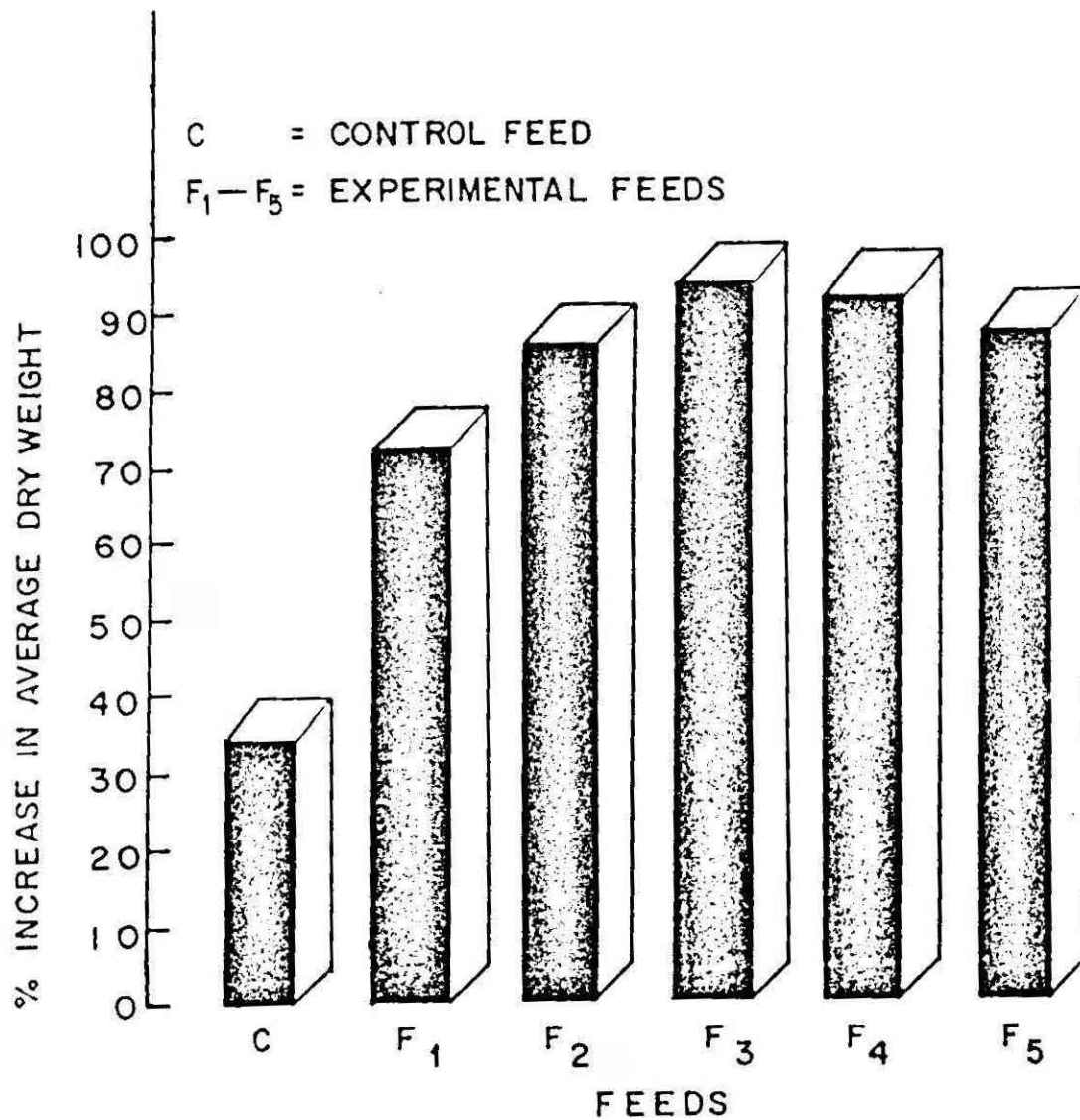


Fig. 3. Increase in dry weight of *Penaeus indicus* fed with feeds having clam meal at various levels. (C-control; F₁=10% clam meal, F₂=20% clam meal; F₃ = 30% clam meal, F₄ = 40% clam meal and F₅ = 50% clam meal)

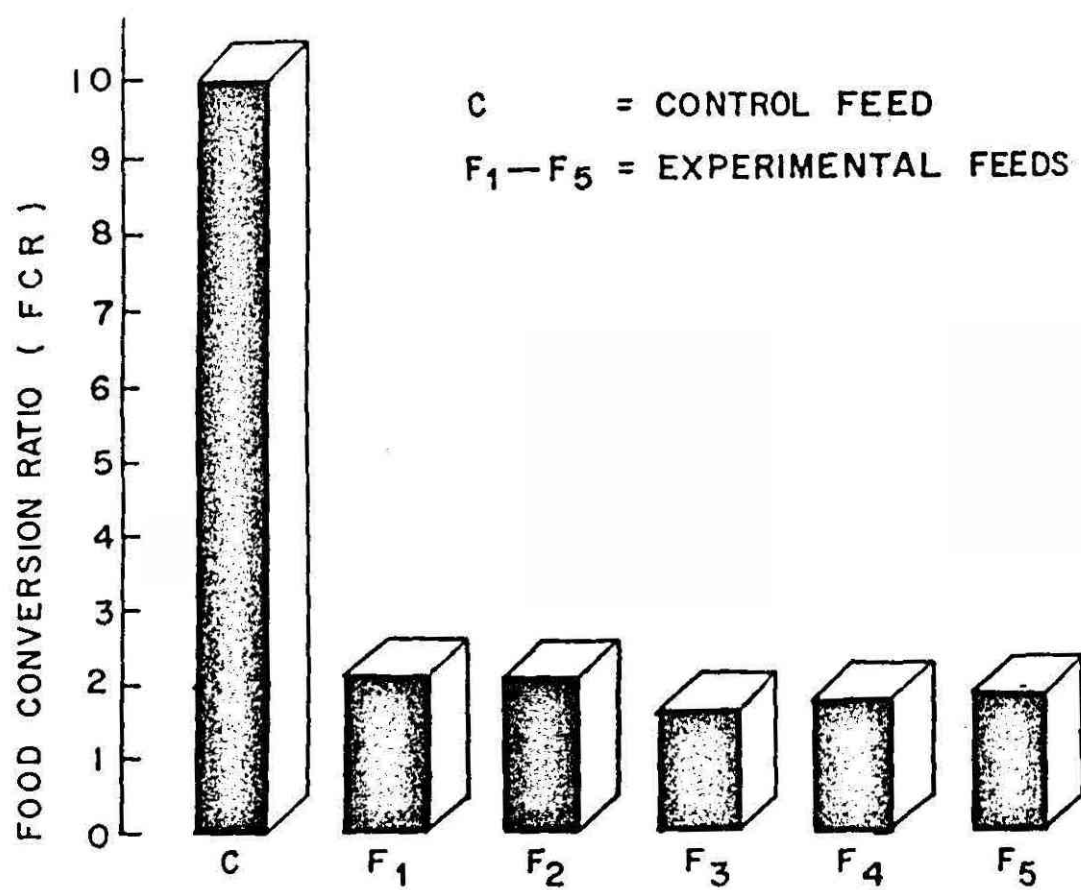


Fig. 4. The food conversion ratio (FCR) of different feeds fed to Penaeus indicus.

Table 10. Analysis of variance for increase in length.

Source	df	SS	MS	F	Remarks
Treatments	5	1746.65	349.33	2266.90	Hi.Sig. (1%)
Error	54	8.32	0.1541		
Total	59	1754.97			

Mean Comparisons

F ₁	**	C				
F ₂	**	**	F ₁			
F ₃	**	**	**	F ₂		
F ₄	**	**	**	**	F ₃	
F ₅	**	**	**	**	**	F ₄

** Significant at 1% level

* Significant at 5% level

ns not significant

Table 11. Analysis of variance for increase in weight.

Source	df	SS	MS	F	Remarks
Treatments	5	1.473	0.295		
Error	54	0.261	0.005	60.90	Hi.sig (1%)
Total	59	1.734			

Mean. Comparisons

F ₁	**	C			
F ₂	**	ns	F ₁		
F ₃	**	**	**	F ₂	
F ₄	**	ns	ns	*	F ₃
F ₅	**	ns	ns	**	ns

** Significant at 1% level

* Significant at 5% level

ns not significant

Table 12. Analysis of variance for increase in dry weight

Source	df	SS	MS	F	Remarks
Treatments	5	0.1081	0.0216		
Error	54	0.0285	0.0005	41.538	Hi.sig (1%)
Total	59	0.1366			

Mean. comparisons

F ₁	**	C				
F ₂	**	ns	F ₁			
F ₃	**	*	ns	F ₂		
F ₄	**	ns	ns	ns	F ₃	
F ₅	**	ns	ns	ns	ns	F ₄

** significant at 1% level

* significant at 5% level

ns not significant

Table 13. Analysis of variance for food conversion ratio (FCR)

Source	df	SS	MS	F	Remarks
Treatment	5	169.503	33.90		
Error	12	0.054	0.005	67.80	Hi.Sig (1%)
Total	17	169.557			

Mean comparisons

F ₁	**	C				
F ₂	**	ns	F ₁			
F ₃	**	**	**	F ₂		
F ₄	**	**	**	*	F ₃	
F ₅	**	**	**	**	**	F ₄

** Significant at 1% level

* Significant at 5% level

ns not significant

showed that treatments differ significantly at 1% level ($P < 0.01$). LSD showed that feeds F_1 and F_2 show no significance between them. Feed F_3 and F_4 differ significantly at 5% level ($P < 0.05$). All other feeds differed significantly at 1% level ($P < 0.01$)

Survival Rate

The survival rates of the prawns fed on control and experimental feeds (Fig. 5) were found the comparatively low, ranging from 40% to 60%. Among all the feeds F_2 recorded the maximum survival rate (60%) followed by F_5 (55%). Feeds F_4 and F_1 had a survival rate of 50%, control feed 45%, while F_3 recorded the lowest survival rate of 40%. The process of moulting was observed to be one of the major factors contributing to the mortality of shrimps. Those which were soft, probably just moulted within 24 hours, accounted for 36% of the total mortality.

Gross conversion efficiency ($K_1\%$) and Net conversion efficiency ($K_2\%$)

Table 6 shows the values of $K_1\%$ and $K_2\%$ obtained with all six feeds. Feed F_3 showed the highest value of K_1 as well as K_2 of 0.60 and 0.71 respectively. F_4 showed the second best K_1 and K_2 values (0.58 and 0.69 respectively). Though F_2 showed the next higher K_1 value (0.52) its K_2 value was found to be lower than that of F_5 . The other feeds F_5 , F_1 and control obtained K_1 value of 0.51, 0.48 and 0.40 respectively, their respective K_2 values being 0.57, 0.51 and 0.41.

Analysis of variance (ANOVA) (Table 14,15) showed that in the case of $K_1\%$ the treatments differ significantly at 5% level ($P < 0.05$), while $K_2\%$ treatments did not show any significant difference between them.

Moulting rate

It was found that the moulting rate values increased as the percentage of clam meal in the feed increased upto a certain level and after that it started decreasing (Table 7). The moulting rate was the maximum with F_3 (3.72) followed by F_4 (2.84), F_5 (2.50), F_2 (2.00), F_1 (1.79) and control (1.67). The moulting rate increases up to 30%, with higher percentages the value reduces gradually. Analysis of variance of the data showed that treatments differ significantly at 1% level ($P < 0.01$) (Table 16)

Protein efficiency ratio (PER)

The best value of PER was shown by F_3 (0.62) followed by F_4 (0.61), F_5 (0.55), F_1 (0.49) and F_2 (0.48) (Fig. 6). The control feed showed the lowest value (0.10) Analysis of variance showed that treatments differ significantly at 1% level ($P < 0.01$) (Table 17).

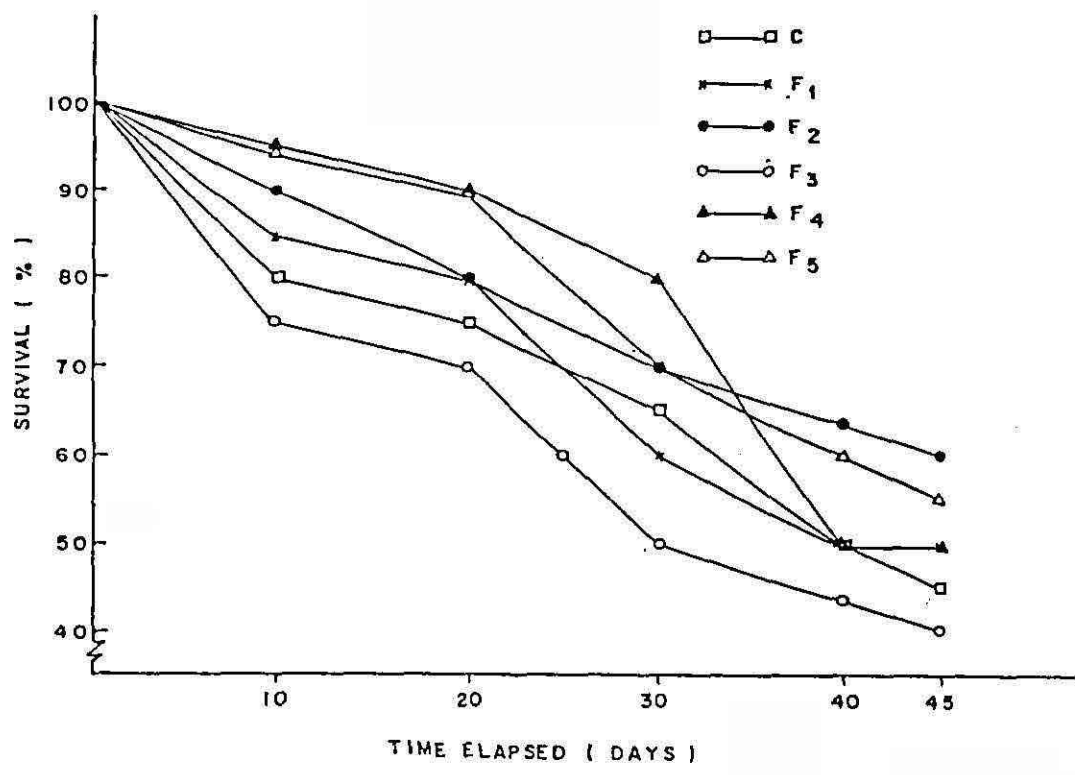


Fig. 5. Relationship of clam meal levels with Survival. (C-control; F₁ = 10% clam meal, F₂ = 20% clam meal; F₃ = 30% clam meal; F₄ = 40% clam meal and F₅ = 50% clam meal).

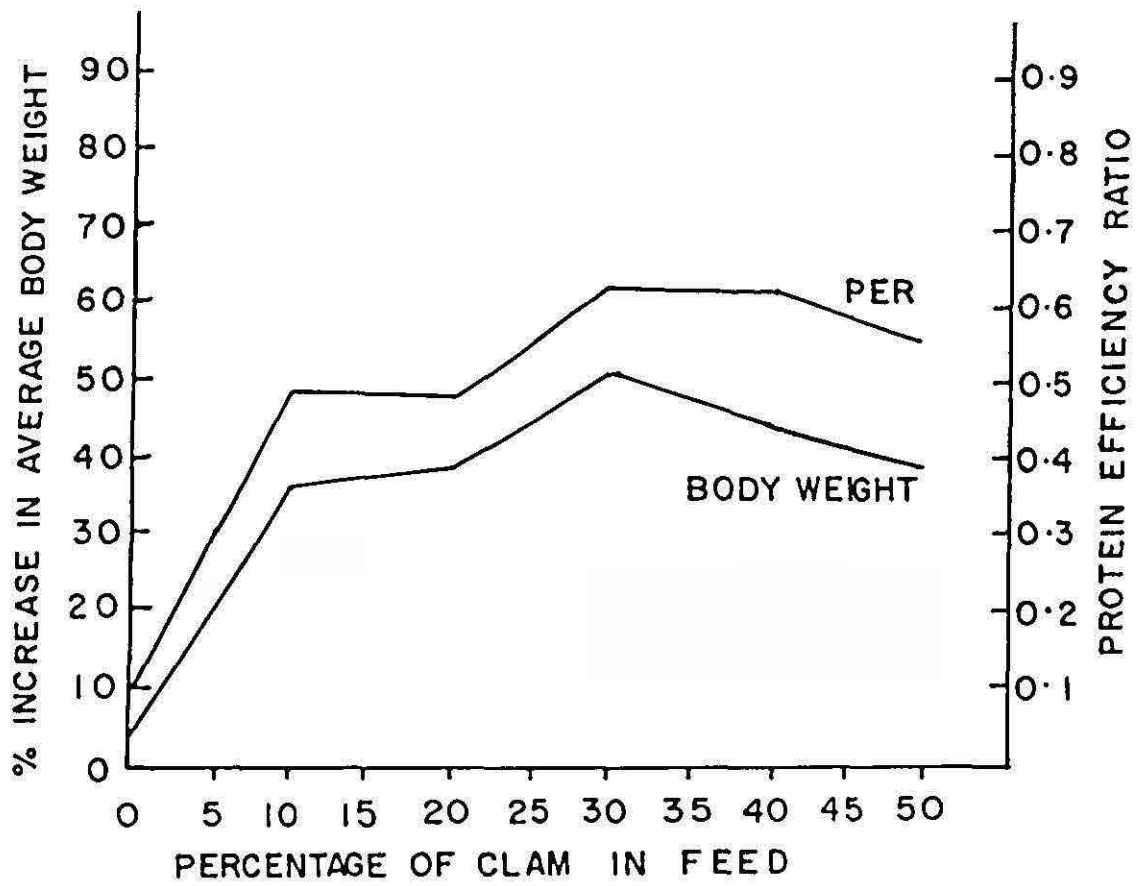


Fig. 6. Relationship of clam meal levels with dietary increase in body weight and with protein efficiency ratio (PER)

Table 6

Gross conversion efficiency ($K_1\%$) and Net Conversion efficiency ($K_2\%$) values
obtained for control and experimental feeds

Feeds	$K_1\%$	$K_2\%$
Control	0.40	0.41
F ₁	0.48	0.51
F ₂	0.52	0.56
F ₃	0.60	0.71
F ₄	0.58	0.69
F ₅	0.51	0.57

Table 7

Moulting rates obtained when fed with
control and experimental feeds

Feeds	Moulting rate
Control	1.67
F ₁	1.79
F ₂	2.00
F ₃	3.72
F ₄	2.84
F ₅	2.50

Table 14. Analysis of variance for Gross conversion efficiency ($K_1\%$).

Source	df	SS	MS	F	Remarks
Treatments	5	0.1899	0.03798		
Error	12	0.1157	0.0096	3.956	Sig.(5%)
Total	17	0.3056			

Table 15. Analysis of variance for Net conversion efficiency ($K_2\%$).

Source	df	SS	MS	F	Remarks
Treatments	5	1.9644	0.3029		
Error	12	1.7678	0.1473	2.6673	Not significant
Total	17	3.7322			

Table 16. Analysis of variance for moulting rate.

Source	df	SS	MS	F	Remarks
Treatments	5	9.15	1.5519	143.70	Hi.Sig. (1%)
Error	12	0.13	0.0108		
Total	17	9.28			

Mean. comparisons

F ₁	**	C				
F ₂	**	**	F ₁			
F ₃	**	**	**	F ₂		
F ₄	**	**	**	**	F ₃	
F ₅	**	**	**	**	ns	F ₄

** Significant at 1% level

* Significant at 5% level

ns not significant

Table 17. Analysis of variance for Protein efficiency ratio (PER)

Source	df	SS	MS	F	Remarks
Treatments	5	0.5712	0.1142		
Error	12	0.0507	0.004	28.55	Hi.Sig. (1%)
Total	17	0.6219			

Mean. comparisons

F ₁	**	C				
F ₂	**	ns	F ₁			
F ₃	**	**	**	F ₂		
F ₄	**	**	**	ns	F ₃	
F ₅	**	ns	ns	ns	ns	F ₄

** Significant at 1% level

* Significant at 5% level

ns not significant

Metabolic faecal nitrogen (MFN)

Table 8 shows the MFN values obtained with six different experiments. In the feeding experiments carried out, there was a loss in average body weight of animals fed with zero-protein feed (F_0). The animals gradually became less active and mortality occurred. The MFN values showed wide variation ranging from 287 mg N/100 g feed to 369.50 mg N/100 g feed, giving an average value of 344.20 mg N/100 g feed.

True Digestibility (TD)

The true digestibility value of control feed was found to be the best as it recovered the highest value of 96.52% (Table 9). Among the experimental feeds F_1 showed the best TD value (94.12%) followed by F_2 , F_3 , F_5 and F_4 with values 90.70%, 87.42%, 86.22% and 84.71% respectively.

It was seen that as the clam percentage in the feed goes up, the TD generally comes down, however, there was one exception, the TD of F_5 (86.22%) was found to be greater than that of F_4 (84.71%).

Anova (Table 18) showed that the treatments differ significantly at 1% level ($P < 0.01$). Least significant difference showed that feeds F_3 and F_4 did not differ significantly, while all the rest showed significant difference between them at 1% level.

Table 8

Estimated value of metabolic faecal nitrogen (MFN) in juvenile
Penaeus indicus using the zero protein diet.

(The value is expressed as milligram of nitrogen per 100g of
diet consumed)

Experiment No.	M F N
1	366.13
2	287.00
3	369.50
4	325.20
5	362.37
6	355.00
Average	344.20

Net protein utilization (NPU)

The NPU values exhibited no correlation with the increasing amount of clam meal in the diet (Table 9). It is important to note that all experiments had shown a NPU value greater than that of control diet. Among all feeds, F_4 showed the maximum value (68.44) followed by F_5 (57.98), F_3 (56.0), F_1 (39.47) and F_2 (37.50). F_3 showed a sudden increase in value when compared to F_1 and F_2 feeds. Analysis of variance (ANOVA) (Table 19) showed that all treatments differ significantly at 1% level ($P < 0.01$).

Biological value (BV)

Biological values of the feeds given to the prawn is shown in the Table 9. Maximum BV was shown by the feed F_4 (80.79), followed by F_5 (68.44), F_3 (64.41), F_1 (41.53), F_2 (41.34) and control (31.19). As in the case of NPU here also all experimental feeds showed a higher value than the control. Analysis of variance (Table 20) showed significant difference between treatments at 1% level. LSD showed that feeds F_1 and F_2 differ significantly at 5% level ($P < 0.05$), all the rest at 1% level ($P < 0.01$).

Table 9

Estimated value of true digestibility (TD), net protein utilization (NPU) and biological value (BV) in Penaeus indicus

Feed	Crude protein		Indicator		TD	NPU	BV	
	in faeces	in animal after feeding	In feed	In faeces				
Control	46.20	15.76	64.68	0.49	1.31	96.52	30.11	31.19
F ₁	43.12	15.20	66.22	0.52	3.17	94.12	39.47	41.93
F ₂	44.60	26.50	66.25	0.50	3.19	90.70	37.50	41.34
F ₃	44.60	31.45	69.30	0.48	2.72	87.42	56.00	64.41
F ₄	44.60	39.50	69.34	0.47	2.72	84.71	68.44	80.79
F ₅	44.60	30.49	67.76	0.53	2.63	86.22	57.98	68.44

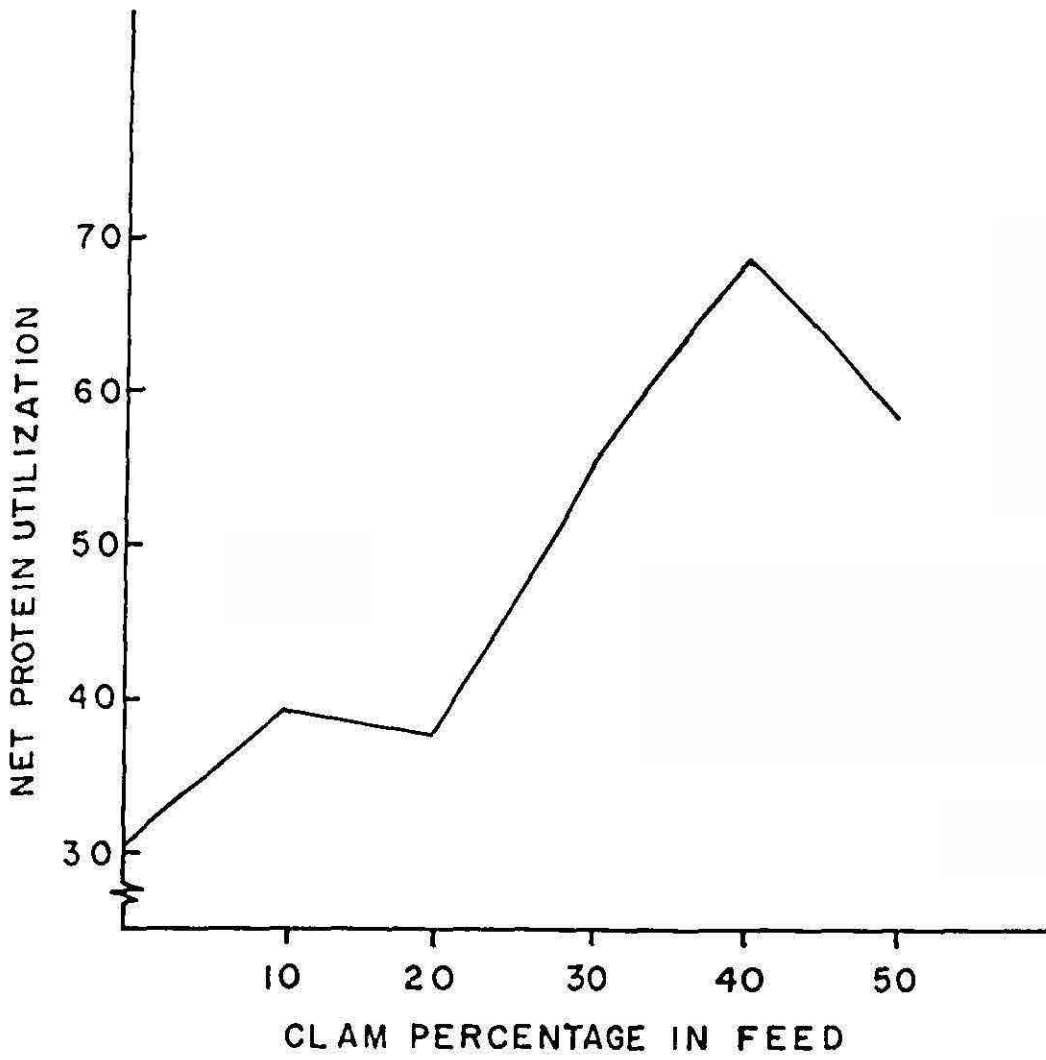


Fig. 7. Relationship of clam meal levels with net protein utilization (NPU)

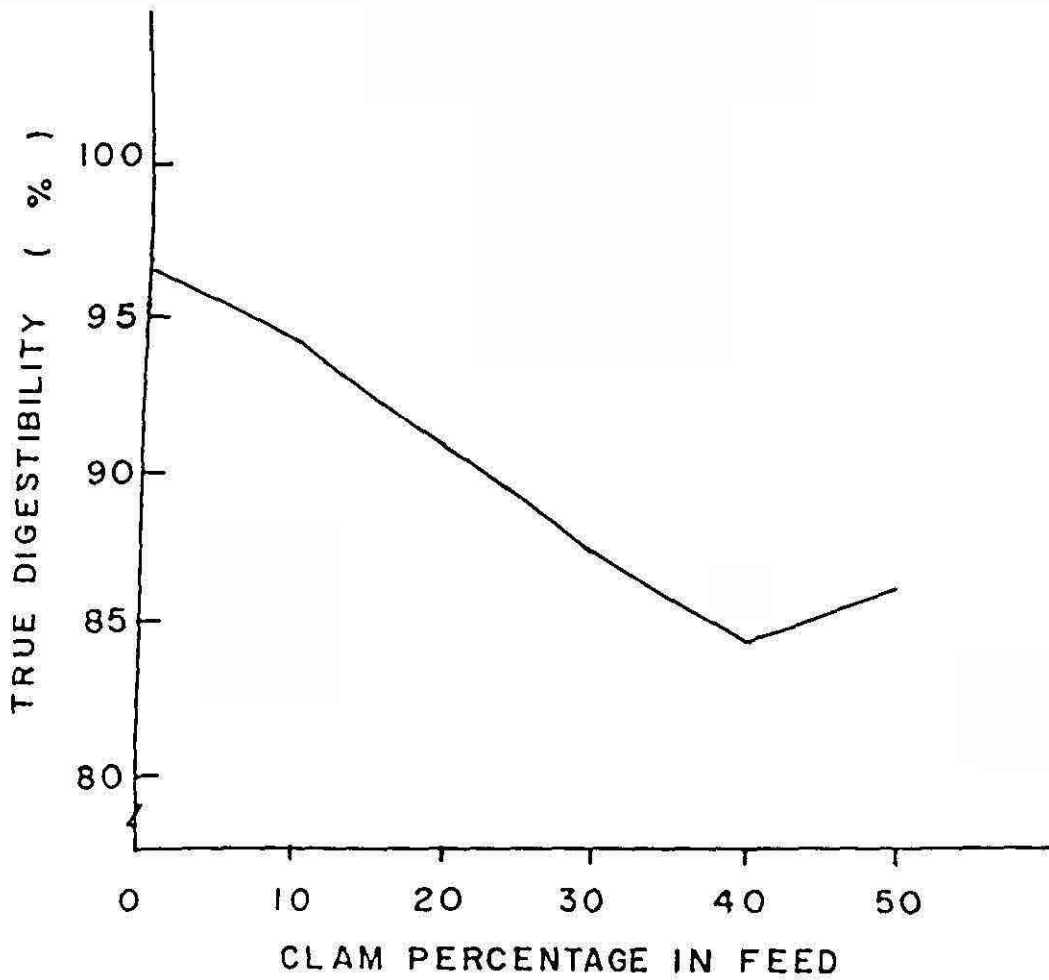


Fig. 8. Relationship of clam meal levels with True digestibility (TD)

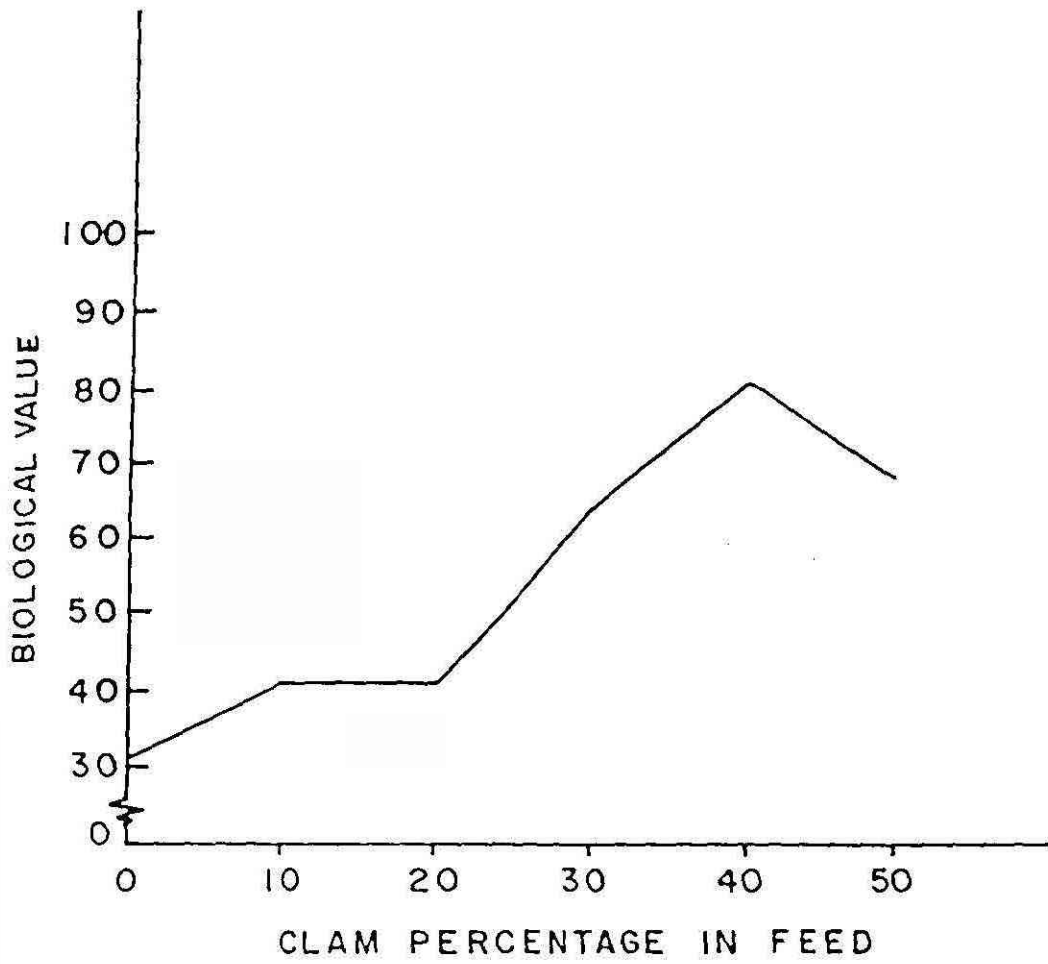


Fig. 9. Relationship of clam meal levels with Biological Value (BV)

Table 18. Analysis of variance for Digestibility.

Source	df	SS	MS	F	Remarks
Treatments	5	326.74	65.348	1423.08	Hi. Sig. (1%)
Error	12	5.51	0.4592		
Total	17	332.25			

Mean Comparisons

F ₁	**							
F ₂	**	**						
F ₃	**	**	**					
F ₄	**	**	**	ns				
F ₅	**	**	**	**	**			

** Significant at 1% level

* Significant at 5% level

ns not significant

Table 19. Analysis of variance for net protein utilization (NPU).

Source	df	SS	MS	F	Remarks
Treatments	5	3252.25	650.45	2329.70	Hi.sig (1%)
Error	12	3.35	0.2792		
Total	17	3255.70			

Mean Comparisons

F ₁	**	C				
F ₂	**	**	F ₁			
F ₃	**	**	**	F ₂		
F ₄	**	**	**	**	F ₃	
F ₅	**	**	**	**	**	F ₄

** Significant at 1% level

* Significant at 5% level

ns not significant

Table 20. Analysis of variance for biological value (BV).

Source	df	SS	MS	F	Remarks
Treatments	5	5574.12	1114.82	6789.40	Hi. Sig. (1%)
Error	12	1.97	0.1642		
Total	17	5576.09			

Mean Comparisons

F ₁	**								
F ₂	**	*							
F ₃	**	**	**						
F ₄	**	**	**	**					
F ₅	**	**	**	**	**				

** Significant at 1% level

* Significant at 5% level

ns not significant

Water stability of the feed

Fig. 10 (a,b,c,d) shows that the pellet stability was inversely related to the dietary level of clam meal. The percentage of dry matter remaining decreased with increasing clam meal level in the feeds.

After an exposure of 8 hours the feed remains were 77.5% of the feed before leaching for control, 75% for F_1 , 74% for F_2 , 73% for F_3 as well as F_4 and 72.5% for F_5 . In the case of all feeds the leaching rate was found to be higher in the initial stages (upto 4 hours), and then slowly coming down.

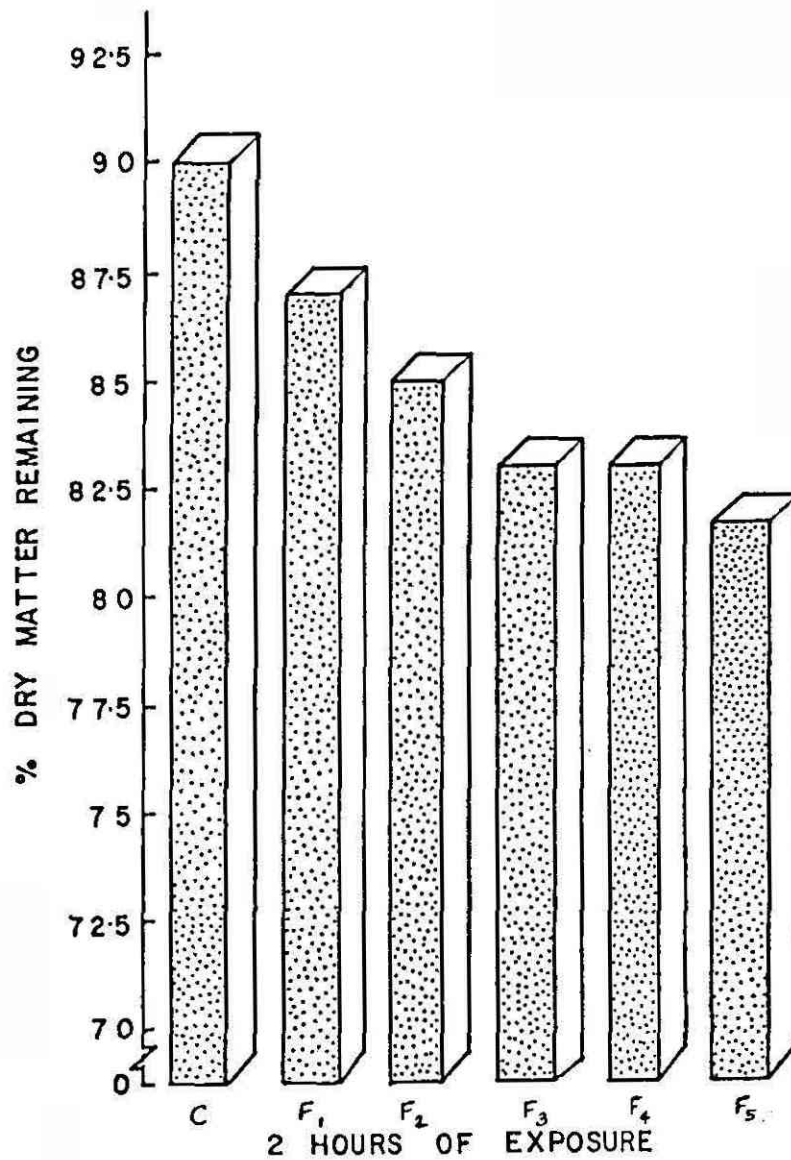


Fig. 10a. Relationship of clam meal levels with dry matter remaining after 2 hours exposure of feed.
(C-control; F₁= 10% clam meal, F₂= 20% clam meal; F₃= 30% clam meal; F₄= 40% clam meal and F₅= 50% clam meal).

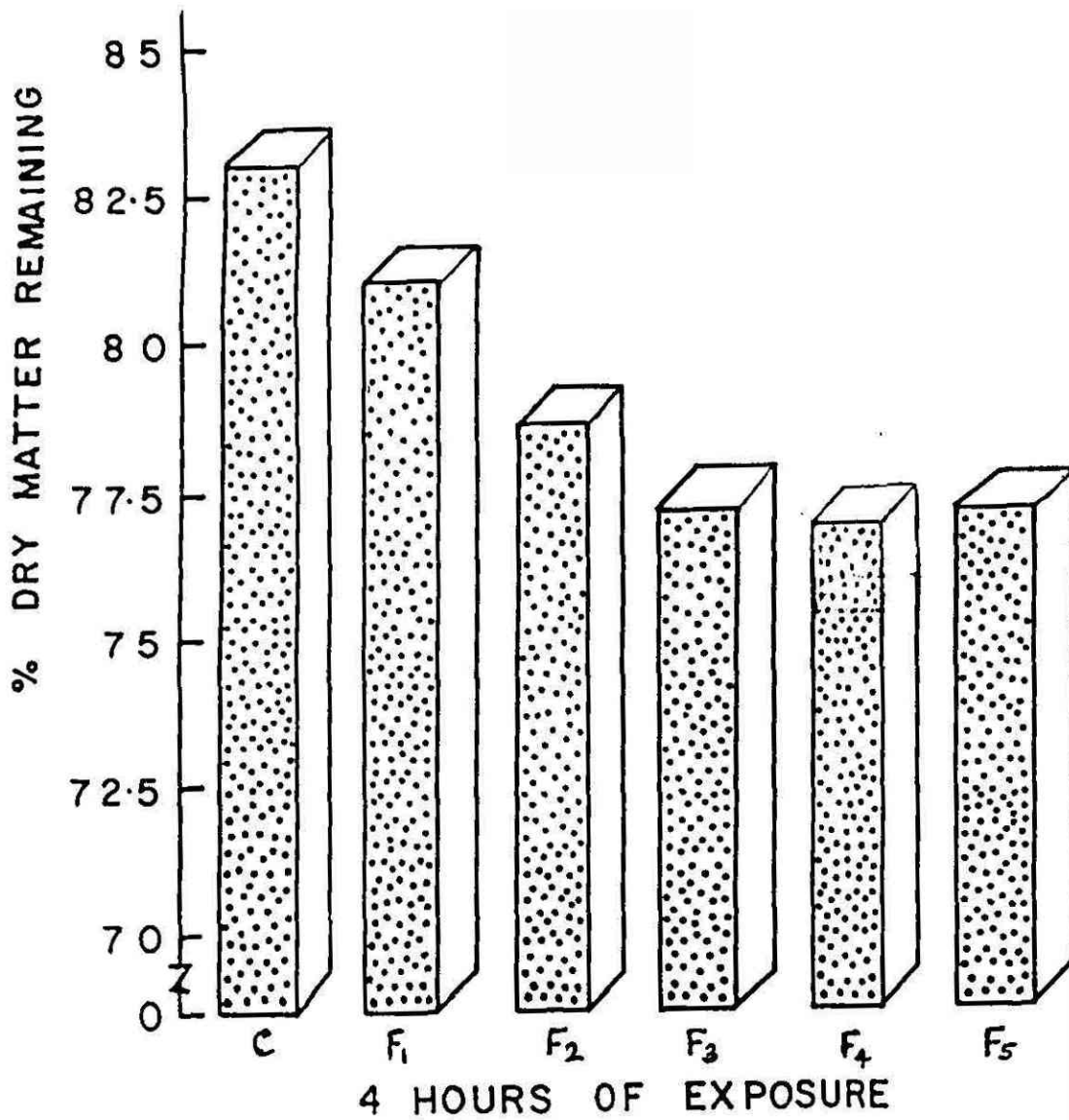


Fig. 10b. Relationship of clam meal levels with dry matter remaining after 4 hours exposure of feed.

(C-control; F₁= 10% clam meal, F₂= 20% clam meal; F₃= 30% clam meal; F₄= 40% clam meal and F₅= 50% clam meal).

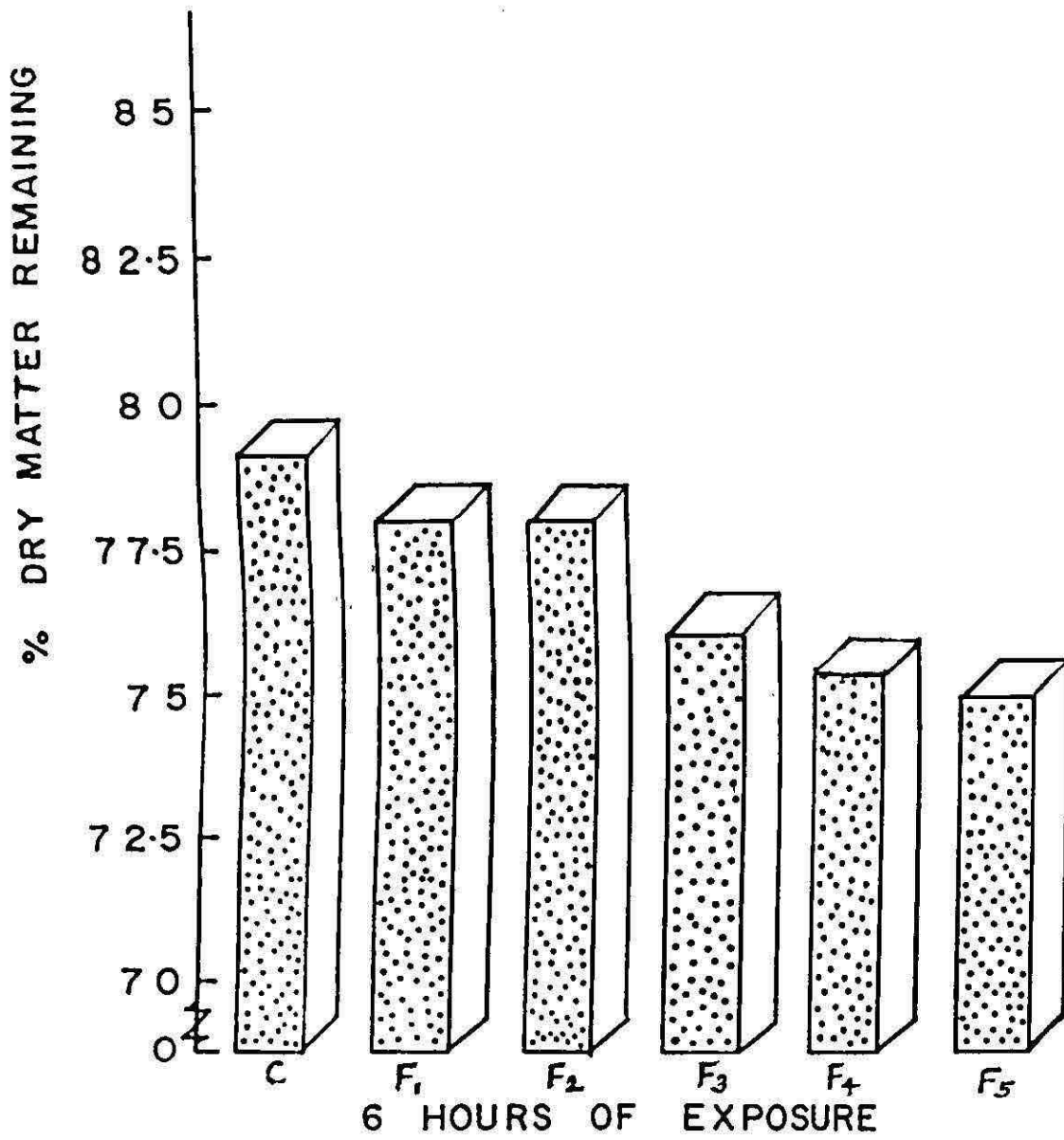


Fig. 10c. Relationship of clam meal levels with dry matter remaining after 6 hours exposure of feed. (C-control; F₁= 10% clam meal, F₂= 20% clam meal; F₃= 30% clam meal; F₄= 40% clam meal and F₅= 50% clam meal).

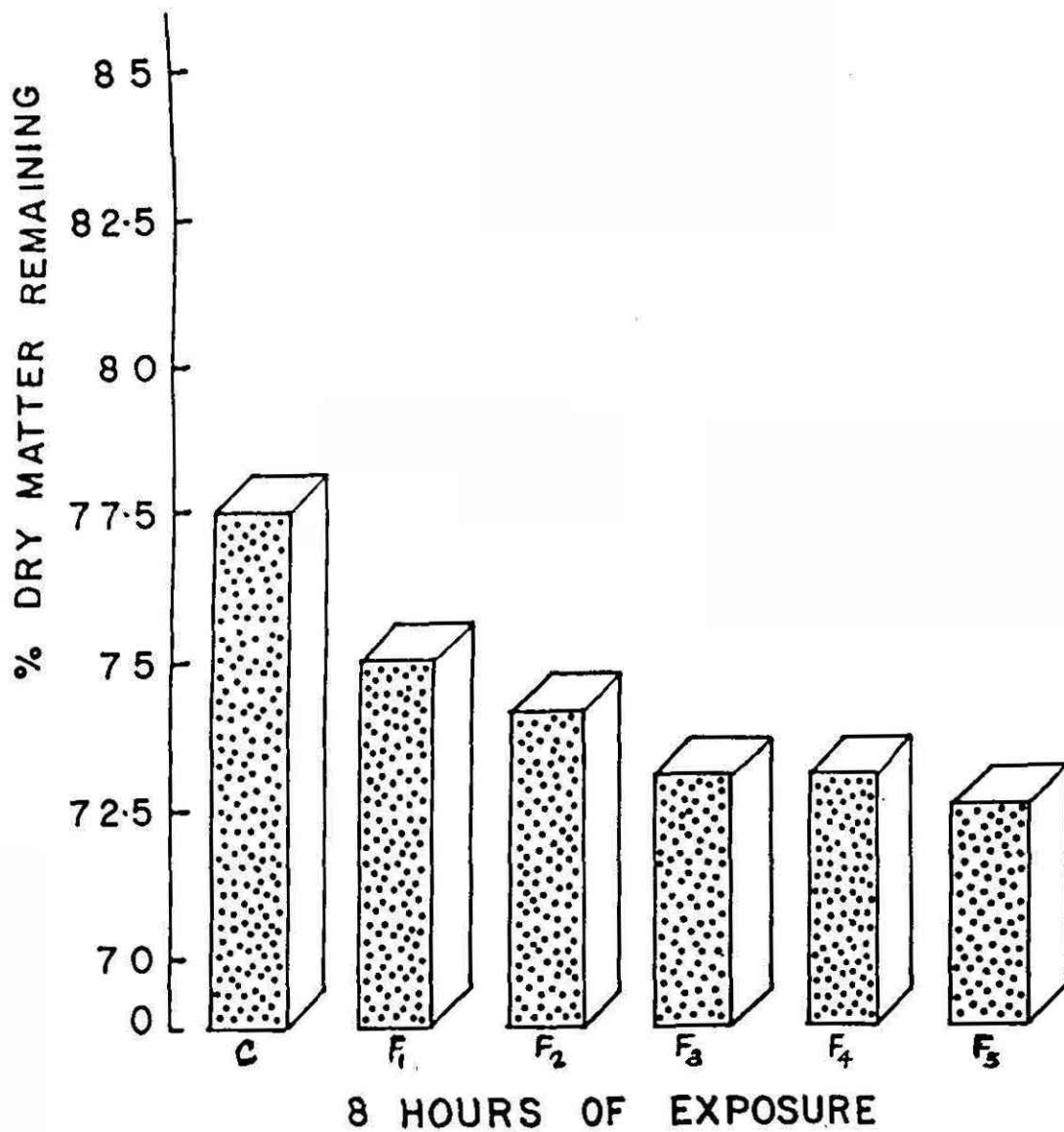


Fig. 10d. Relationship of clam meal levels with dry matter remaining after 8 hours exposure of feed. (C-control; F₁= 10% clam meal, F₂= 20% clam meal; F₃= 30% clam meal; F₄= 40% clam meal and F₅= 50% clam meal).

D I S C U S S I O N

What follows is a short discussion emanating from a careful perusal of the foregoing results.

The results obtained from the proximate analysis of clam meal in the present study are comparable to the values obtained by Ali (1988) for Sunetta scripta meal (protein 48.10%, lipid 13.55%, nitrogen-free extract 11.69% and ash 7.62%) and by Gopal (1986) for Meretrix casta meal (protein 56.6%, lipid 8.2%, nitrogen-free extract 20.8% and ash 10.50%).

Ali (1982 a) reported an average growth rate of 13.20 mg/day in Penaeus indicus of 0.1 g size when fed with 33.3% protein feed having 38% Villorita cyprinoides meat powder protein source and a growth rate of 10 mg/day for the same species with a stocking size of 0.2 g using fresh Sunetta scripta meat as feed.

Ali (1988) recorded an increase of 514%, 374.2% and 375.8% in terms of length, live weight and dry weight respectively in Penaeus indicus of average initial length of 10-20mm fed with 51% Sunetta scripta meal in 30 days. Gopal (1986) reported 575% gain in live weight in Penaeus indicus juveniles of 20±5 mm fed with diet containing 51.2% Meretrix casta meal. Fenucci et al (1976) observed 590.24% increase in live weight in 42 days with Penaeus aztecus.

Sedgwick (1979) studied the growth of Penaeus merguensis using 69% of freeze-dried mussel Mytilus edulis in diet in which the protein content was 39.5% and reported a weight gain of 57.14% in 8 weeks. Colvin (1976) observed a growth rate of 44 mg/day in P. merguensis of 0.95 g when fed with a combined meal of fresh mussel and fresh juvenile prawn in equal ratio.

Though the highest growth increase recorded in the present study was only 52.55%, is comparable to the result obtained by Sedgwick (1979). The higher growth rate obtained by Colvin (1976) may be due to the combination of two protein sources (with amino acid composition similar to prawn) since a mixture of two or more protein sources, invariably show better growth than single source (Deshimaru and Shigueno, 1972). The following reasons can be attributed to the low growth rate: variation in size of prawns, form of protein used and the protein content of the feed. Another reason that can also be attributed to lower growth rate obtained in the present study is that certain essential aminoacids are lost during drying because of the reactions with reducing sugars and carbonyl compounds present in the diet as suggested by Swaminathan (1967).

Ali (1988) has reported a Food conversion ratio (FCR) of 1.83 using a diet containing 51% Sunetta scripta meal in Penaeus indicus of 10-20 mm length. Gopal (1986) obtained a value of 0.92 with Penaeus indicus of 20±5 mm using 51.2% Meretrix casta meal and Ali (1982 b) a value of 1.46

with compounded diets having 38% Villorita meal, in Penaeus indicus juveniles. The highest FCR (1.6) obtained in the present study is comparable to these values, taking into account the variation in size, experimental duration and protein source. The water stability of the pellets are also said to influence the FCR value (Rani, 1984).

Protein efficiency ratio (PER) of 1.77 was obtained with a diet containing 51% Sunetta scripta meal in Penaeus indicus with 10-20 mm length (Ali, 1988). Alava and Lim (1983) using 40% protein diet with casein, fish meal, shrimp meal and squid meal as components reported a PER of 0.34 in Penaeus monodon juveniles. The highest PER of 0.62 obtained in the present study was for the group having 30% incorporation of clam meal in the diet, which show that clam protein is most efficiently utilized by the prawns at this level of inclusion.

Ali (1982 b) reported a survival of 70% in 30 days with 38% Villorita cyprinoides meal diet in Penaeus indicus juveniles (100 mg). Gopal (1986) using a diet with 50% clam meal (Meretrix casta) obtained a survival rate of 64% in 30 days. Ali (1982 b) using fresh Sunetta scripta as feed obtained 30% survival in 30 days, in Penaeus indicus of 100 mg initial weight. Villegas (1978) found that the growth and survival of Penaeus monodon larvae fed with Tapes clam was only next to compounded diets. All the afore mentioned studies agree with the present study in that the survival was found to be generally low when fed with clam included diets. Gopal (1986) reported 11 post moult deaths out of 27 moults observed during the 40 days of experiment. This is entirely in agreement with the present study where around 36% post moult deaths were observed.

The higher moult percentage values obtained with F_1 , F_2 and F_3 when compared to the control feed may be due to the moult inducing factor reported in fresh Sunetta scripta by Ali (1982 a). The same had found that the high moulting rate resulted in high mortality. Gopal (1986) reported that prawns fed on clam meal (Meretrix casta) had significantly lower calcium and phosphorous levels compared to those fed on diet with fish meal, crab meal and shrimp meal. This may indicate that lower calcium and phosphorous metabolism may perhaps affect these prawns resulting in comparatively high mortality rates, especially during post-moult stages (New, 1976).

Metabolic faecal nitrogen (MFN) in Penaeus indicus was determined for the first time by Ali (1988) and reported a value ranging from 248.5 mg to 351.6 mg N/100 g diet (average 326.4 mg N/100 g diet). Using zero-protein diet, Nose (1967) determined MFN in young rainbow trout and obtained varying values of 85.7, 139.7 and 151.0 mg N/100 g diet in 3 different experiments. Forster and Gabbott (1971) determined MFN in Palaemon serratus and obtained a value of 185.2 ± 27.9 mg/100 g diet.

The value obtained in the present study are comparable to the value obtained by Ali (1988). Here also varying values were obtained with 6 different experiments. The significantly different value with Palaemon serratus may be due to the difference in the nature and quantity of faecal membrane in the two types of prawns. In the case of finfish, the low value obtained when compared to prawn is because prawns are known to secrete a chitinous peritrophic membrane around the faecal pellets (Forster, 1953).

Ali (1988) recorded a true digestibility (TD) of 81.19 for Sunetta scripta meal fed to Penaeus indicus juveniles. Forster and Gabbott (1971) studying the assimilation of nitrogen in diets prepared with different ingredients for Palaemon serratus showed that the assimilation was 99.7% in casein based diet. Akiyama et al (1988) reported an apparent protein digestibility of 99.1% by Penaeus vannamei with casein based diet and 79.7% with squid meal. The values obtained in the present study are comparable to the above values. Among all the diets the highest TD was recorded for the control diet (96.52) which was a purified diet with casein as the main source. This value is very well comparable to the above observations, and the clam meal diets had shown a lesser TD values than the control diet, but all the values obtained were higher than the value reported for squid meal by Akiyama et al (1988).

Net protein utilization (NPU) of clam meal (Sunetta scripta) at 51% inclusion level was found to be 60.91, while a NPU of 28.68 was obtained with casein-based purified diet (Ali, 1988). Atack and Matty (1978) obtained the NPU of 49 and 40 for casein in carp and rainbow trout respectively. Teshima et al (1978) reported an NPU of 25.7 in Tilapia zilli using 35% casein-based diet. In the present study the value obtained with 50% clam meal diet was found to be closer to the value obtained by Ali (1988), and a value higher than that was obtained with 40% clam meal diet (Table 9). Agreeing with others results, the casein-based diet showed a lower value (30.11).

Ali (1988) recorded a biological value (BV) of 74.60 for Sunetta scripta meal when used at 51% level in Penaeus indicus, the same had obtained a value 61.93 and 53.10 for 86% prawn waste and 68.5% mantis shrimp meal respectively in the same species. This shows that clam meal used in the present study has got higher biological value than prawn waste and mantis shrimp meal. In the present study the value obtained with 40% clam meal diet was higher than the value reported by Ali for Sunetta scripta meal, and at 50% inclusion level the value obtained was found to be closer to the value reported by Ali.

When NPU, TD and BV are taken together, it was seen that 40% clam recorded the maximum value as far as NPU and BV are considered, but at this level, the TD was found to be the lowest. All the NPU, BV and TD values obtained show that 30% clam meal was the best giving a combination of BV and NPU values close to the maximum, and a good TD value. At lower levels of clam inclusion, though the TD was found to be good, the BV and NPU were found to be very low.

The inverse relationship between the clam percentage in the diet and pellet water stability, is totally agreeing with the study carried out by Lin et al (1987) where they found that percentage of dry matter remaining, after exposure to water decreased with increasing soyabean meal level in diets.

S U M M A R Y

Complete or supplementary feeding becomes inevitable in the high-density prawn culture systems for better farm production. For preparing nutritionally balanced, as well as low-cost feeds, a knowledge of the nutritional requirements of the species under farming and evaluation of locally available raw materials for feeding is essential. In this context, evaluation as well as optimum inclusion level of locally available black clam Villorita cyprinoides (Gray) in the diet of one of the foremost cultivated prawn penaeus indicus has been undertaken in the present study.

- 1) The proximate composition of clam meal shows that it has got high amount of protein and lipid indicating that the clam can be suitably used in prawn diet.
- 2) Seven different feeds were prepared using standard formula for purified diet. The various feeds were one control feed (zero clam feed) with casein as the main protein source and 5 experimental feeds (numbered F₁ to F₅ each having clam meal at 10%, 20%, 30%, 40% and 50% respectively). The 6th diet being zero-protein used to study the metabolic faecal nitrogen.
- 3) Evaluation of clam protein was carried out using standard methods of nutritional biochemistry, by measuring true digestibility, biological value (BV), net protein utilization (NPU), protein

efficiency ratio (PER) and growth in statistically designed feeding experiments carried out for 45 days. The other parameters studied were gross conversion efficiency ($K_1\%$), net conversion efficiency ($K_2\%$), food conversion ration (FCR), moulting rate and survival. True digestibility was studied using the internal marker chromic oxide (Cr_2O_3). Feed quality was assessed by examining the pellet water stability up to 8 hours.

- 4) The endogenous nitrogen excretion (metabolic faecal nitrogen) was determined for the penaeid prawn using zero-protein diet.
- 5) The results showed that the maximum growth, FCR, $K_1\%$, moulting rate and PER were obtained in prawns fed with 30% clam meal diet. The FCR values from the control and experimental feeds ($F_1 - F_5$) showed that the clam inclusion can increase the food conversion at least by 5 times. The survival rate ranged from 40 to 60 %. Pellet water stability showed inverse relationship with the clam percentage in the feed.
- 6) Moulting rate was found to be influenced by the clam meal level. As in the case of growth the values showed an increase up to 30% clam meal level and then started decreasing.
- 7) The estimated MFN ranged from 287 to 369.5mg N/100g with 6 different experiments, giving an average value of 344.2 mg N/100 g feed consumed.

- 8) True digestibility values were generally found to come down as the clam meal percentage in the feed went up. True digestibility as high as 94.12% was obtained with experimental feeds.
- 9) In the case of NPU, it was found that all experimental feeds showed NPU values higher than that of control feed. The NPU values of different experimental feeds did not show correlation relationship with the amount of clam meal in the diet.
- 10) Biological value as high as 80.79 was obtained.
- 11) Analysis of variance of data obtained with various parameters showed that in the case of growth, FCR, PER, Moulting rate, NPU, TD and BV the treatments were significant at 1% level ($P < 0.01$) K_1 treatments were significant at 5% level ($P < 0.05$) K_2 treatments were not significant.
- 12) When NPU, TD and BV which are the major parameters for evaluating the quality of protein are considered together, it was been that the feed containing 40% clam meal (F_4) recorded the maximum NPU and BV values, but at this level the TD was found to be the lowest.

- 13) The overall results obtained show that clam meal has got high amount of protein and lipid, thus can be suitably used in the prawn diets, the 30% clam meal inclusion level is the best, giving the maximum values of growth, FCR, PER, Moulting rate, K_1 %, NPU and BV and a good level of TD.

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