Use of Squilla (*Orato squilla nepa*), Squid (*Sepia pharonis*) and Clam (*Katelysia opima*) Meal Alone or in Combination as a Substitute for Fish Meal in the Postlarval Diet of *Macrobrachium rosenbergii*

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ABSTRACT : Two hundred and forty post-larvae (PL) of fresh water prawn, *Macrobrachium rosenbergii* were distributed in eight treatment groups with three replicates each. Eight experimental diets were prepared by using squilla *(Orato squilla nepa)*, squid (*Sepia pharonis*) and clam (*katelysia opima*) alone or in different combination as source of animal protein and compared to a control diet containing fish meal. Total crude protein content for all dietary treatments was around 32%. Total protein content of fish meal was replaced by an equal amount of protein from different animal protein sources on isonitrogenous basis. Diets were fed at 5% of the body weight of post-larvae twice daily. The experiment was conducted for a period of 60 days. It was found that all the above three protein sources could be used by completely replacing fishmeal except clam meal at higher level of inclusion (26%). A combination of squid and squilla meal at 14% each in the diet increased the growth performance of PL significantly ($p<0.05$) in terms of Specific growth rate (SGR) % (5.17), FCR (2.12) and PER (1.51). Squilla meal can be used to the maximum level of 38% without any growth depression. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 9 : 1272-1275)*

Key Words : *Macrobrachium rosenbergii*, Squid, Squilla, Clam Meal, Fish Meal, SGR, Growth

INTRODUCTION

Protein is the principal and costly component of aqua-feeds. Hence, studies have been directed to investigate alternative protein sources in the prawn diet. Generally, protein sources of animal origin are considered to be superior to the plant protein sources in prawn diets due to their better amino acid make-up. Animal protein sources of marine origin are superior to those from fresh water due to their more acceptable amino acid and unsaturated fatty acid composition (Cowey and Sergent, 1972).

Squilla (*Orato squilla nepa*), a stomatopod crustacean is abundantly available along the coast of India as well as China, Thailand, Malaysia, Philippines and tropical and subtropical Asian countries (Nair et al., 1987). The total catch of squilla by fishing trawlers along the east and west coast of India cannot be accurately estimated because most of the catch is thrown over board when caught or, when landed, mixed with trash fishes and prawns, is discarded as waste at the landing site (Mathew et al., 1982). Squilla meal was selected for its low cost, abundance and local availability. Until now the use of this abundant animal protein was restricted to manure use exclusively (Nandeesha et al., 1989).

Squid (*Sepia pharonis*) contain more than 80% crude protein and a good source of Ca, P, Fe and moderate amounts of B-complex vitamins (Pandit et al., 1972). Beside these, among the exploited bivalve resources, clam occupy the top position with an annual production of 50,000 tonnes in India, which is a cheaper source of marine animal protein containing around 70% crude protein and a relatively high level of glycogen similar to prawns and crabs (Narsimaham et al., 1993).

Considering the rapidly expanding freshwater prawn farming, the high requirement for animal protein, and the increasing cost of fish meal, the present experiment was designed to study the possibility of using abundantly available different marine protein sources as a substitute for scarce and costly fish meal in the postlarval diet of *Macrobrachium rosenbergii*.

MATERIALS AND METHODS

Experimental set-up

Postlarvae of *M.rosenbergii* were obtained from the *Macrobrachium* hatchery of the Central Institute of Fisheries Education, Versova, Mumbai. The animals were exposed to a formalin immersion at 25 ppm and kept in a FRP (Ferrocement Reinforced Plastic) tank (300 L capacity) for 24 h. Two hundred and forty post larvae (PL) having initial body weight of 0.011 to 0.013 g were equally distributed in 8 different treatments, 3 replicates each. Thus, all the 24 experimental containers containing 10 PL each were arranged in 3 rows following a completely randomized design (CRD). Total volume of water in each tub was maintained at 30 L throughout the experimental period. About 50% of water from each container was siphoned out along with the faecal matter and residual feed and replaced

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Table 1. Composition of the experimental diets, % DM basis

Ingredients	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8
Fish meal	30	-	-	-	-	-	-	-
Squid meal	-	22	-	-	12	14	-	9
Clam meal	-	-	26	-	12	-	15.5	9
Squilla meal	-	-	-	38	-	14	15.5	9
Prawn head meal	15	15	15	15	15	15	15	15
Ground nut oil cake	10	10	10	9	9	10	10	10
Soybean meal	10	7	9	13	8	8	10	9
Rice polish	14	20	17	5	20	18	13	18
Wheat flour	10	15	12	9	13	10	10	10
Brewer's yeast	1	1	1	1	1	1	1	1
Cod liver oil	3	3	3	3	3	3	3	3
Soybean oil	3	3	3	3	3	3	3	3
Guargum	1	1	1	1	1	1	1	1
Vitamin premix *	2	2	2	2	2	2	2	2
Mineral premix**	1	1	1	1	1	1	1	1
TOTAL	100	100	100	100	100	100	100	100
Chemical composition								
Crude protein	32.15	32.12	32.69	32.66	32.64	32.45	32.60	32.33
Lipid	11.26	11.72	9.35	8.15	10.36	9.84	8.60	9.23
Crude fibre	5.05	5.18	7.82	10.35	5.95	7.82	9.34	7.71
NFE	30.95	32.74	27.28	29.84	30.81	30.79	28.74	30.35
Crude Ash	20.59	18.24	22.86	19.00	20.24	19.10	20.72	20.38
Crude protein content of the animal protein sources								
	Squilla	Squid	Clam	Fish meal				
Crude protein (%)	48.12	83.12	70.00	61.25				

* -Composition (per kg): Vitamin A, 2,000,000 I.U.; Vit D3, 400,000 I.U.; Vit B2 800 mg; Vit E, 300 I.U.; Vit K, 400 mg; Cal. pantothenate, 1 g; Nicotinamide, 4 g;Vitamin B 12, 2.4 g; Choline chloride 60 g; VitC was supplemented separately in the form of Celein tablets, Glaxo Company, India at 300 mg·kg^{-1} diet.

** Composition (per kg) :calcium, 750 g; manganese, 27.5 g; iodine, 1 g; iron, 7.5 g; zinc 15 g; copper, 2 g; cobalt, 0.45 g.

with fresh borewell water (chlorine free) every day. Continuous aeration was provided using a 2 HP air blower. During the experimental period the physico-chemical parameters of water viz., temperature, pH, dissolved oxygen, free carbon-dioxide, total ammonia and total alkalinity were recorded every other day, as per APHA (1985), and are given in table 3.

Feed ingredients

Fresh squilla, squid and clams were collected from the fish landing center, Versova, Mumbai, washed thoroughly in tap water and sun dried. Clams were initially boiled in water for 15 minutes for easy removal of meat before drying. Other ingredients such as fish meal, prawn head waste, groundnut oil cake and rice bran etc. were collected from the local market of Versova, Mumbai. All ingredients were oven dried at 60 to 70°C until completely dried, and were finely ground through a pulverizer so as to pass through a 60-mesh size nylon netting.

Diet preparation

Different feed ingredients for all experimental diets were mixed as presented in table 1. Attempts were made to replace fish meal with squilla, squid and clam meat alone or in different combinations on an isoprotein basis. The CP content of the clam, squid and squilla and fish meal are presented in table 1. Total CP content of the diets was around 32%. All the ingredients except vitamins were mixed in water to form a dough which was steam cooked for 10 min. The mixture of vitamins was then mixed into the dough after cooling and passed through a hand pelletizer fixed with a die of 2 mm. The pellets were dried at 60°C until complete drying and stored in an air tight container for subsequent use.

Biochemical analysis

The moisture, ash and nitrogen free extract contents of the diets were determined using the method of AOAC (1980). CP content of the diets was determined by micro-Kjeldahl method whereas CF and EE levels in the diets were determined by using Fibertech System (Model M,1017 hot extractor, Tecator) and Soxtech System (Model ST2, 1045 extraction unit, Tecator), respectively.

The moisture and ash contents of the animal tissues were determined by the methods described previously. Total carbohydrate and protein levels were determined following

Table 2. Growth and survival of PL fed with different experimental diets

Parameters	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8
Initial body wt, g	0.012±0.009	0.012±0.008	0.012±0.011	0.011±0.017	0.013±0.005	0.013±0.010	0.012±0.006	0.012±0.006
Final body wt, g	0.229±0.023	0.234±0.019	0.187±0.018	0.191±0.009	0.252±0.014	0.290±0.036	0.220±0.019	0.225±0.027
Body wt gain, g	0.217	0.222	0.175	0.180	0.239	0.277	0.208	0.213
SGR, %	4.91^{b}±0.12	4.95^{b}±0.03	4.58^{c}±0.03	4.76^{b}±0.20	4.94^{b}±0.09	5.17^{a}±0.09	4.85^{b}±0.12	4.88^{b}±0.11
FCR	2.97^{b}±0.27	3.00^{b}±0.13	4.30^{c}±0.13	3.68^{b}±0.27	2.89^{b}±0.30	2.12^{a}±0.12	3.46^{b}±0.13	3.29^{b}±0.12
PER	1.01^{b}±0.12	1.07^{b}±0.13	0.71^{c}±0.03.	0.92^{b}±0.06	1.08^{b}±0.10	1.51^{a}±0.12	0.90^{b}±0.13	0.97^{b}±0.12
Survival, %	83.83±0.88	86.67±0.33	100.00±0.00	90.00±0.00	100.00±0.00	86.33±0.33	96.67±0.33	90.00±0.57

Figures bearing same superscript in a row do not differ significantly ($p>0.05$).

Body weight gain = Final body weight - Initial body weight, Daily weight gain = Weight gain / Total duration.

SGR % = ($\log_e$ Final body weight - $\log_e$ Initial body weight × 100) / Total duration.

FCR = Feed given (dry weight) / weight gain (wet weight), PER = Protein gain - protein fed.

Survival = PL harvested at the end of the experiment × 100 / PL stocked initially

Table 3. Water quality parameters during the experimental periods

Parameters	GROUPS							
	D_1	D_2	D_3	D_4	D_5	D_6	D_7	D_8
Water temp, °C	21-26	21-26	21-26	21.2-26.2	21-26	21-26	21-26	21-26
pH	7.6-8.2	7.6-8.2	7.6-8.0	7.6-8.2	7.4-8.0	7.6-8.2	7.4-8.2	7.6-8.0
DO, ppm	7.6-9.0	7.4-9.2	7.2-8.5	7.4-8.0	7.2-9.2	7.4-9.0	7.4-8.5	7.4-9.2
CO_2	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Total alkalinity, ppm	186-228	186-224	188-210	188-220	185-224	196-224	184-220	184-222
Ammonia nitrogen, ppm	0.10-0.16	0.12-0.16	0.13-0.17	0.09-0.14	0.12-0.17	0.13-0.19	0.11-0.15	0.13-0.18

the procedure of Plummer (1988) and Lowry et al. (1951), respectively.

Growth evaluation criteria

Body weights of the prawns were determined every 15 days. Accordingly body weight gain, specific growth rate (SGR) %, FCR, PER were calculated. Survival was recorded at the end of each sampling day and the termination of the experiment.

Statistical analysis

Data were statistically processed by one-way analysis of the variance (ANOVA) and significance of differences among the treatment means was tested as per methods of Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

The effects of squid, clam or squilla alone or in combination as a complete substitute of fish meal was tested by measuring average individual weight gain and calculating the SGR%, FCR and survival of PL fed each diet.

At the beginning of the experiment the initial weight of the PL was 0.011 to 0.013 g. The mean weight gain at the end of 60 days varied between 0.175 to 0.277 g. The SGR % of the different experimental groups varied significantly ($p<0.05$). Highest (5.17) and lowest (4.58) SGR % was recorded in D6 and D3 groups, respectively. Only D3 group registered significantly lower ($p<0.05$) SGR than the control and other groups (table 2). Similarly D6 group registered significantly higher ($p<0.05$) SGR than all other groups, but variations in rest of the groups were not significantly different ($p>0.05$). Similar trends were also recorded for FCR and PER.

From the above findings it appears that almost all diets could replace fish meal completely, except D3 diet which contained only clam meal to the maximum level of 26%. However, growth rates of other groups (D5, D7 and D8) fed with less than 26% clam meal compared well ($p>0.05$) with the control group. A gradual-declining trend of SGR was noted in D8, D7 and D3 group as the clam meal level increased in the diet with little variation in D5 group, which may be due the presence of squid meal. So, it appears that there might be accumulation of some growth inhibiting factor at higher level of feeding of clam. However, Wheaton and Lawson (1985) reported that molluscs fed on dinoflagellates which are toxic unicellular organisms, concentrate the toxins produced by the organisms in their own bodies without themselves being affected. This might be the reason for lower SGR but needs further investigation.

A trend of higher growth rate was noticed in those groups fed with squid based diets (D2, D5, D6 and D8) irrespective of its level of inclusion. A growth promoting effect of squid meal based diet may be due to its better quality protein as evident from the PER values. Growth

promoting effect of squid meal was also reported by Shigueno et al. (1972). Cruz-Ricque et al. (1987) reported that better growth rate obtained by feeding squid meal may be due to an unknown growth factor present in squid. This unknown growth factor believed to be a small peptide, increases the digestive efficiency of shrimp and enhances the growth rate (Akiyama et al., 1991). Beside these, squid meal also act as attractant in the diet of freshwater prawns (Mendoza et al., 1997). This is in agreement with the present findings for better FCR in squid based diets.

Highest weight gain was achieved in the D6 group where squid meal was supplemented with squilla meal. It seems addition of squilla promoted the growth rate which may be due to the supplementation of chitin from squilla. Ali and Mohammed (1983) also reported that squilla meal proved to be a very effective feed ingredient for prawns. Further, in prawns, glucosamine, the breakdown product of chitin is known to be a growth promoter (Kitabhyashi et al., 1971). In the present findings, encouraging results were found when squilla meal was added at 38% in the diet by completely replacing fish meal.

Mortality recorded in the different groups was only due to accidental causes, indicating no adverse effect on survival of PL due to diet. It is assumed that presence of some toxin in clam meal is responsible for reducing the PL growth rate, but it is not fatal to PL as no mortality was recorded in D3 group containing the maximum level of clam meal.

CONCLUSION

From the above findings it is suggested that squid, clam and squilla can satisfactorily replace fish meal in the post larval diet of fresh water prawns. However, clam meal alone at 26% in the diet was found unsuitable. Presence of some antinutritional factor in it needs further investigation. Squilla meal to a extent of 38% can be used as an alternative to fish meal in the diet of *Macrobrachim rosenbergii* PL. A combination of squid and squilla at 14% each in the post larval diet accelerated the growth rate.

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