Indian J. Fish., 58(2) : 67-73, 2011

Presentation and evaluation of formulated feed for mud crab *Scylla serrata*

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ABSTRACT

A formulated pellet and ball form feed with 41.6% crude protein, consisting of fish meal, squid meal, shrimp meal, mantis shrimp meal, soyabean meal, bread flour and other additives was evaluated for mud crab, *Scylla serrata*. Fish meat (tilapia), which is conventionally used for feeding mud crab by farmers was used as control. Crabs with average weight of 151.7 g were stocked in 500 l oval shaped FRP tanks containing seawater @ 2 crabs per tank with six replicates per treatment. The crabs were fed daily with dry pellets @ 10% of their body weight in a 30-day feeding trial. The crabs showed excellent acceptability of the compounded feed from the start and had shown a weight gain of 36.7% and a feed gain ratio (FGR) of 2.94, compared to 33.5% weight gain and 5.56 FGR shown by the crabs fed with fish meat. The amino acid profiles of the feed, fish meat and the crabs before and after the feeding trial showed that the ratio of indispensable amino acids (IAA) and the dispensable amino acids (DAA) is same (0.8) for both feeds. Performance of dry as well as semimoist form of pelleted feeds also studied. In a 30-day feeding trial, the weight gain of crabs fed dry feeds was significantly higher ($p<0.05$) compared to moist feeds. It was due to the higher wastage of feed (30.4%) in semi-moist feeds compared to the dry feeds (17.2%). There was no difference in the FGR shown by different feeds. The proximate composition of crabs fed with the different forms of feed also did not show any variation. Dry pellet feed is preferred over the semi-moist form of feed for feeding mud crab.

Keywords: Essential amino acids, Formulated feed, Growth, Mud crab, *Scylla serrata*

Introduction

Disease problems in shrimp aquaculture posed a serious threat to the growth of brackishwater aquaculture throughout Asia in the 1990s (Boyd and Clay, 1998) which necessitated the diversification of brackishwater aquaculture. Marichamy and Rajapackiam (2001) suggested mud crabs as a potential alternative crustacean species to shrimps. The mud crab, *Scylla serrata* is identified as a potential candidate species for commercial culture (Williams and Field, 1999) because of its larger size, capacity to live out of water for a long period, compatibility with other species like shrimp, mullets and milk fish, high meat quality and nutritive value. The potential for commercial aquaculture production of mudcrab in the Indo-Pacific region was recognized at the International Scientific Forum held in Darwin, Australia during 21-24 April 1997 (Keenan and Blackshaw, 1999). In India, mud crab culture is presently carried out in traditional brackishwater tide-fed fish farming systems (Kathirvel, 2003). Fattening and grow-out culture of mud crab is becoming popular due to better economic returns.

At present, mudcrabs are fed with low price feeds such as trash fish, molluscs and fresh or boiled slaughter-house waste. Use of these feeds requires proper management to avoid deterioration of water quality. It is necessary to freeze these feeds for storage. In addition, the problem of availability of fresh feeds restricts their usefulness as a regular diet. Availability of ready to use off-the shelf feeds are necessary for the development of mud crab culture. No significant differences ($p>0.05$) were observed in growth, feed conversion, survival and production among the mud crab *S. serrata* fed with salted fish bycatch or a mixed diet of 75% salted brown mussel flesh and 25% fish bycatch during pen culture (Trino and Rodriguez, 2002).

Nutrient requirement data of any candidate species is crucial for the development of nutritionally balanced, cost-effective commercial feeds (Tacon, 2000). The crude protein requirement in mud crabs were first reported to be 35-40% in ~ 600 g size group (Chin *et al*., 1992) and 45% in 0.25 g size juveniles (Unnikrishnan and Paulraj, 2010). Sheen and Wu (1999) reported lipid levels ranging from 5.3 to 13.8% and Sheen (2000) reported 0.5-0.79% of dietary cholesterol in *S. serrata* for the best growth performance. Catacutan (2002) reported a dietary protein level ranging from 32 to 40% with a 6-12% lipid level for best growth response. A formulated feed was tested for mud crab fattening in Thialand with 21% reduction in cost of production compared to fresh fish (Wilson, 2005). New (1976) stressed the importance of palatability and physical structure of diets for prawns and shrimp. It was concluded that moist diets were in general preferable to dry pellets. Ali *et al.* (2008) successfully tested for both fattening and culture in the farms at CIBA and at Central Institute of Fisheries Education, Kakinada. The present study evaluated a compounded feed for its acceptance and attempted to understand the preferred physical form of formulated feed by mud crab for better feed utilization. Testing of semi-moist form of feed was aimed at exploring the possibility of using feeds prepared by farmers at farm site. It was also aimed at finding out performance of semi-moist feed over the dry form of feed.

Materials and methods

Feed preparation

A test feed was formulated consisting of marine and plant ingredients (Table 1), such as dry fish (*Anchoviella* sp.), squid (*Loligo* sp.), mantis shrimps (*Oratosquilla* sp.), *Acetes* sp. and soybean meal (defatted). These were powdered in a micropulveriser and passed through a 0.5 mm mesh. The ingredients including binder were mixed according to the formula in an electrical blender. Water was then added (300 ml kg^{-1} feed to the diet mix) and kneaded into a dough. It was steamed at atmospheric pressure for 5 min, made into spherical balls and dried. The dried balls were stored in desiccator until use. Fresh chopped trash fish (*Tilapia* sp.) was used as control diet in the first experiment.

In the second experiment, the moist dough was prepared as described above. This was divided into four parts to prepare balls (moist, dry) and pellets (moist, dry) for four treatments. In the first treatment, moist spherical balls (approximately 1 cm diameter) were made by hand. In the second treatment, dried spherical balls were prepared by drying moist balls in an electrical oven at 70 ^{0}C. In the third and fourth treatments, moist pellets and dry pellets, respectively were used. Pellets were prepared using a bench top pelletizer (3 mm dia). Wet balls and pellets were kept in the refrigerator. The dried balls and pellets were stored in desiccator until use.

Feeding trials

Live crabs (*S. serrata*) were collected from Lake Pulicat and individually weighed. In the first experiment, 24 crabs with an average initial weight of 151.7 ± 5.38 g were randomly distributed in twelve 500 l capacity oval shaped FRP tanks (0.82 m^2 area) for two treatments with six replicates for each treatment. In the second experiment, 72 crabs with an average initial weight of 148.9 ± 2.48 g were randomly distributed in twelve 1000 l capacity oval FRP tanks (1.64 m^2 area) for four treatments with three replicates for each treatment. The water was aerated through a porous stone. Eighty percent of the seawater in experimental tanks was replaced daily with fresh seawater.

Table 1. Ingredient and proximate composition of formulated test feeds used for *Scylla serrata*

Ingredients	Feed (%)	
Fish meal	35	
Squid	5	
Squilla	10	
Acetes sp.	5	
Soya cake	15	
Wheat flour	15	
Starch	6.5	
Cholesterol	0.5	
Binder*	1.0	
Fish oil	2.0	
Lecithin	2.0	
Vitamin mixture[1]	1.0	
Mineral mixture[2]	2.0	
Proximate composition (%)	**Feed**	**Fish meat**
Moisture	9.80	70.21
Crude protein	41.57	12.30
Ether extract	6.81	1.10
Crude fibre	3.77	0.29
Nitrogen free extract[3]	24.94	8.10
Ash	13.11	8.00

* Poly Methylol Carbamide [1] Vitamin mixture: (mg/100 g) Vitamin A 2.0, Vitamin D 0.4, Vitamin E 12.0, Vitamin K 6.0, Choline Chloride 600.0, Thiamine 18.0, Riboflavin 24.0, Pyridoxine 18.0, Niacin 108.0, Pantothenic acid 72.0, Biotin 0.2, Folic acid 3.0, Vitamin B_{12} 0.015, Inositol 150.0, Vitamin C 900.0. [2] Mineral mixture: (g/kg) $CaCO_3$ 28.0, $NaHPO_4$ 22.0, K_2SO_4 10.0, Mg SO_4 12.5, Cu SO_4 0.2, $FeCl_3$ 0.5, $MnSO_4$ 0.5, KI 0.01, $ZnSO_4$ 1.0, $CoSO_4$ 0.01, Cr_2 SO_4 0.05, Bread flour 7.14. [3] NFE calculated by difference = 100 - (moisture % + Crude protein % + Crude fibre % + Ether extract % + Ash %)

PVC pipes of 10 cm diameter and 30 cm length were placed as shelters in each tank. During the experiment, the water temperature, salinity and dissolved oxygen were measured by standard methods (APHA, 1998). Crabs were fed on a daily ration equal to 10% of body weight. Feeding was done twice daily in equally divided doses, at 11.00 AM and 4.00 PM. The left-over feed was colleted after two hours of feeding, dried and weighed to determine the feed intake. Duration of each experiment was 30 days. The average percentage weight gain, feed gain ratio (FGR) and moulting frequency (%) were calculated as:

Weight gain (%) = $[w_1 - w_0] \times 100 / w_0$

FGR = Feed consumed (g) / wet weight gain (g)

Moulting frequency = 100 x (No. of crabs moulted/ total no. of crabs)

where W_0 = initial wet weight (g), W_1 = final wet weight (g)

Analysis of samples

The proximate composition of the experimental diets and samples of pooled whole crabs was analysed as per the standard AOAC (1990) methods. Amino acids were analysed by hydrolysis with 6N HCl for 22 h at 110 ^{0}C in sealed tube as described by Spackman *et al*. (1958) and Finlayson (1964). After hydrolysis, the acid was evaporated under vacuum and the samples were kept in NaOH desiccator to remove traces of acid. The residue is brought in to 1 ml of sodium citrate-perchloric acid sample diluent (pH 2.20) and filtered through 0.2 µm membrane filter. Amino acids were analyzed using HPLC (Shimadzu HPLC model LC-10A, Shimadzu Corp., Japan). Separation of amino acids was done in a column (Shimpack ISC-07/ S1504 Na) packed with a strongly acidic Na^+ type cation exchange resin (styrene-divinyl benzene copolymer with sulfinic group) under gradient elution at a flow rate of 0.3 ml^{-1} min^{-1}. The amino acids were detected and quantified using a fluorescent detector (FLD-6A) after post column derivitization with O-pthaladehyde. Amino acid standard solution (Sigma-Aldrich Inc., USA) for fluorescent detection was used as external standard.

Statistical analysis

The data on the feeding trials were statistically analyzed using one-way analysis of variance (Gomez and Gomez, 1984). Duncan's multiple range test was applied to identify significant differences between individual treatments.

Results

Test feed contained crude protein, ether extract, crude fibre, nitrogen free extract and ash at 41.57%, 6.81%, 3.77%, 24.94% and 13.11%, respectively (Table 1). In the first experiment, the mud crabs fed on test feed exhibited excellent acceptance of the feed and showed better growth (36.73%) and FGR (2.94) when compared to the animals fed trash fish meat (growth 33.54% and FGR 5.65). The survival of crabs at the end of the experiment was 83.3%. Six crabs moulted in the treatment fed with fish meat (Fig. 1) whereas seven crabs moulted in formulated feed fed group. During the experiment, the water temperature, salinity and dissolved oxygen were at 30 ± 1° C, 31$^0/_{00}$ and 6 mg l^{-1} respectively. The proximate composition of

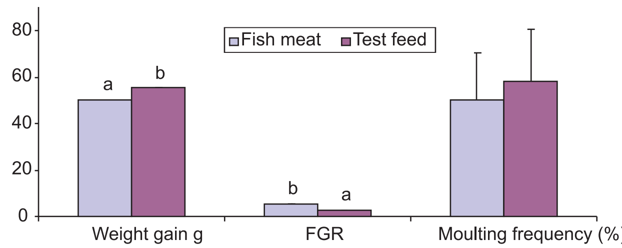

Fig. 1. Growth, FGR and moulting frequency of *Scylla serrata* fed with fish meat (control) and test feeds. Bars in the same parameter showing different superscripts differ significantly ($p<0.05$)

experimental animals (Table 3) revealed that there was significant ($p<0.05$) increase in crude protein (13.04%) and ether extract (2.11%) in crabs fed with formulated diet compared to the initial crabs (10.52 and 0.93%, respectively). The dry matter content of whole crabs was in the range of 24.5 to 36.5%. The amino acid composition of test feed, fish meat and experimental animals showed that glutamic acid, arginine and glycine are the three predominant amino acids. In the fish meat, arginine, valine and threonine are the three significant indispensable amino acids whereas in the feed, arginine, leucine and phenylalanine are predominant amino acids. The ratio of indispensable amino acids/ dispensable amino acids (IAA/ DAA) in test feed and fish meat was almost similar (0.8). The total indispensable amino acids of post-fed crabs (Table 4) in both the treatments (5.1% and 5.3%) were higher than the corresponding initial samples (4.48%). The E/A ratios (essential amino acid/ total amino acids x 100) of crabs were 9.02, 5.95, 5.90, 5.40, 4.94 and 4.80 for arginine, isoleucine, valine, threonine, lysine and phenyl alanine, respectively for initial crabs (Fig. 2). Similar trend was observed in crabs fed on both formulated feed and fish meat. The essential amino acid index (EAAI) of both control as well as test feed were 0.92 and 0.86, respectively.

The results of the second experiment are shown in Table 2. Dry pellet feed resulted in significantly ($p<0.05$) higher weight gain (40.5 ± 2.40%) followed by dry balls (35.9 ± 3.82%), semi-moist pellets (29.6 ± 0.64%) and

Table 2. Results of 30-day feeding trial of mud crab (*Scylla serrata*) fed on different formulated feeds

Parameters	Balls		Pellets	
	Dry	Semi-moist	Dry	Semi-moist
Initial weight (g)	150.3± 7.09	143.5± 6.72	152.0± 3.65	149.7± 2.38
Final weight (g)	203.8^{bc}± 5.05	178.7^{a}± 7.24	213.3^{c}± 1.49	194.3^{ab}± 3.88
Weight gain (%)	35.9^{bc}± 3.82	24.6^{a}± 1.51	40.5^{c}± 2.40	29.6^{ab}± 0.64
FGR	3.1± 0.11	3.1± 0.08	3.0± 0.05	3.2± 0.12
Survival (%)	94.4^{b}± 5.55	83.3^{ab}± 9.62	94.4^{b}± 5.55	66.7^{a}± 9.62
Wastage (%)	19.4^{a}± 1.10	30.4^{b}± 1.40	17.2^{a}± 1.08	26.7^{b}± 1.07
Moulting frequency (%)	61.1± 5.55	50.0± 9.62	66.7± 9.62	50.0± 9.62

Means in the same row showing different superscripts differ significantly ($p<0.05$)

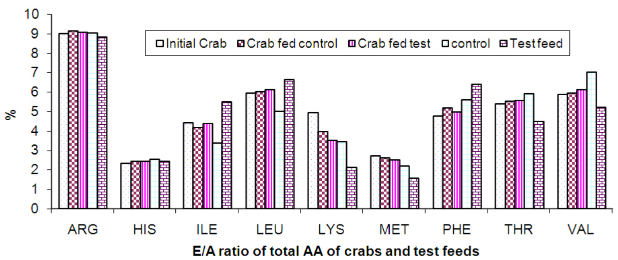

Fig. 2. Essential amino acid ratio of total amino acids in the mud crab *Scylla serrata* and test feeds

semi-moist balls (24.6 ± 1.51%) in the decreasing order. In the case of feed gain ratio (FGR), no significant difference (p>0.05) was observed among the different treatments. It was observed during feeding trial that when crabs actively feed, the semi-moist diets fell apart rather quickly. This led to wastage of feed in the moist diets. Wastage was low (17.2 ± 1.08%) in the case of dry pellets compared to the semi-moist pellets (26.7 ± 1.07%). The trend was similar in the case of ball feeds also. Survival was higher among the crabs fed with dry feeds (94.4%) compared to those fed with semi-moist feeds (83.3% and 66.7%, for semi-moist balls and pellets, respectively). No significant difference (p>0.05) was observed in moulting frequency among different treatments (Table 2) but better moulting frequency was observed in crabs fed on dry feeds. The proximate composition of experimental animals (Table 3) revealed that crude protein (13.37%) and ether extract (2.15%) increased in crabs fed on dry pellet diet when compared to the initial samples of crabs (9.93% and 0.82%, respectively). However, no significant difference was observed in the proximate profiles of final samples of crabs fed with different test feeds.

Discussion

Mud crabs are regarded as omnivorous scavengers that feed on a variety of benthic organisms, but they cannot be placed in any one trophic level because they are generally opportunistic feeders. Hill (1976) concluded that mud crabs are not well adapted to capturing moving prey. Their natural food consists mainly of molluscs, followed by crustaceans. Crude protein and lipid requirements were in the range of 32-45% and 6-12%, respectively in mud crab *S. serrata* (Chin *et al.,* 1992; Sheen and Wu, 1999; Catacutan, 2002; Unnikrishnan and Paulraj, 2010). Hence in the present study, formulated feed was prepared with 41.57% of crude protein and 6.81% of lipid (Table 1). Moist compounded feed was used for maturation of shrimp by Marseden *et al*. (1997). In the present study, the presentation of both moist and dry formulated feeds were studied for better feed management and utility of farm made feeds in mud crab aquaculture. The results of the experiments have shown that formulated feed is readily accepted by the crab and consumed well. The feed resulted in better weight gain of 36.7% and FGR of 2.94 compared to the weight gain of 33.5% and FGR of 5.56 in crabs fed with fish meat, which is conventionally used for feeding mud crabs by the farmers. Trino and Rodriguez (2002) reported an FCR of 5.30 by using fish meat in a pen culture of *S. serrata*.

Results of the feeding trials with different types of feed have indicated that crabs fed with dry form, either as balls or pellets showed better performance over those fed with the corresponding semi-moist feed either in ball form or pellet form. There is no significant difference in feed gain ratio in respect of different types of feed. The survival of crabs fed with dry form of feed is higher (94%) compared to those fed with the semi-moist feeds (83.3% for wet balls and 66.7% for wet pellets). The difference in performance of these two types of feeds is due to the difference in the physical properties of the feeds. As the crabs approach and grab the feeds with their chelae, the dry pellet or ball feeds, being more compact and hard, withstand the handling and do not fall apart. The semi-moist feeds, being soft fall apart more easily during handling by crabs leading to disintegration. This is reflected in the feed wastage computed, which is higher in moist ball and pellet feeds

Table 3. Proximate composition of test animals fed with different feeds

Treatment	Moisture	Crude protein	Ether extract	Crude fibre	Nitrogen free extract	Total ash
Experiment 1						
Initial crab	71.53	10.52	0.93	0.41	5.34	11.27
Crab fed on fish meat	67.42	12.10	1.83	0.50	6.24	11.91
Crab fed on test feed	66.13	13.04	2.11	0.46	6.54	11.72
Experiment 2						
Initial crab	75.50	9.93	0.82	0.43	5.25	8.07
Dry balls fed crab	64.30	13.27	2.15	0.63	6.66	12.99
Semi-moist balls fed crab	65.21	13.05	2.05	0.67	6.67	12.36
Dry pellets fed crab	63.50	13.64	2.20	0.58	7.31	12.77
Semi-moist pellets fed crab	66.50	12.51	1.95	0.68	7.01	11.35

(30.4% and 26.7%, respectively) compared to that of the dry form of feeds (19.4% and 17.2%, respectively). This might have lead to faster leaching of nutrients and more suspended feed particles in the water. Both these factors might have influenced the growth performance and survival of crabs fed with semi-moist forms of the feed. Trends in the results suggest that feeding dry form of compact feed is preferable for crabs. Between the dry ball and pellet form, the latter has shown better performance over the former.

The proximate composition of crabs fed with formulated feed reflected better body protein (Table 3) than those fed with fresh fish meat. Besides being readily acceptable, formulated feed seems to perform better for crabs. However, no significant differences were found in the proximate composition of crabs fed with different forms of formulated feed.

In the absence of precise data on amino acid requirements, the alternative approach is to provide a balanced dietary amino acid profile that matches practically with the amino acid profile of the muscle protein of the recipient animal (Dishimaru and Shigeno, 1972). In the present study, the amino acid profiles of the crabs fed with formulated feed and the control feed vis-à-vis the amino acid profiles of the test diets did not show any perceptible difference between the treatments. Both the essential and non-essential amino acid composition (Table 4) of the crabs fed with different diets is practically matching with each other. In crustaceans such as penaeid shrimps, glutamic acid is the predominant amino acid (Sarac *et al*., 1994; Dayal *et al*., 2005), followed by aspartic acid and leucine. In crabs also glutamic acid has emerged as the predominant amino acid, however it is followed by arginine and aspartic acid as seen in the present study. Among the essential amino acids (EAA), arginine, isoleucine, valine, threonine and phenylalanine emerged in significant levels in decreasing order in mud crabs, by virtue of E/A ratio. The same essential amino acids are observed to be predominant amino acids in prawns *Macrobrachium rosenbergii* (Farmanfarmaian and Lauterio, 1980), and penaeid shrimp *Penaeus japonicus* (Deshimaru and Shigeno, 1972), *Penaeus monodon* (Penaflorida, 1989; Sarac *et al*., 1994) and *Fenneropenaeus indicus* (Dayal *et al*., 2005). The trend in the EAA is maintained in crabs before and after the feeding trial with test feed and the control diet in the present study. The essential amino acid indices of control and test diets are 0.92 and 0.86, respectively and according to Mente *et al.* (2002), EAAI with more than 0.80 is useful feed and

Table 4. Amino acid profiles of test animals and feeds used for feeding mud crab, *Scylla serrata* (%)

Amino acids	Initial crab	Final crab fed on fish meat	Final crab fed on feed	Fish meat	Feed
Indispensable amino acids (IAA)					
ARG	0.888	1.036	1.080	1.031	3.346
HIS	0.231	0.277	0.291	0.289	0.929
ILE	0.438	0.472	0.525	0.387	2.084
LEU	0.586	0.684	0.732	0.572	2.508
LYS	0.487	0.450	0.420	0.393	0.812
MET	0.268	0.296	0.301	0.253	0.595
PHE	0.473	0.590	0.593	0.638	2.426
THR	0.532	0.629	0.664	0.673	1.705
VAL	0.581	0.674	0.732	0.801	1.975
Total IAA	4.484	5.108	5.338	5.037	16.38
Dispensable amino acids (DAA)					
ALA	0.578	0.671	0.704	1.016	2.192
ASP	0.857	1.043	1.067	1.150	4.609
CYS	0.145	0.160	0.180	0.134	0.541
GLU	1.170	1.346	1.467	1.239	6.179
GLY	0.877	1.013	1.084	1.013	2.625
PRO	0.769	0.818	0.833	0.688	1.615
SER	0.510	0.590	0.640	0.551	1.813
TYR	0.464	0.560	0.576	0.539	1.777
Total DAA	5.37	6.201	6.551	6.33	21.351
Ratio of IAA/DAA	0.835	0.824	0.815	0.796	0.767
EAAI[$]				0.92	0.86

[$]EAAI: Essential Amino Acid Index: Calculated based on initial crab amino acids and aa/AA ratios are set at 0.01 minimum and 1 maximum (Penaflorida, 1989).

if it is less than 0.70, the feed is inadequate. Similarly, EAAI of shrimp feeds ranged between 0.88 - 0.92 (Dayal *et al.*, 2010; Rajaram *et al*., 2010). The results of the study indicate that the use of dry feed pellets is preferable to the semi-moist pellets or ball forms.

Acknowledgements

Authors express their sincere thanks to Dr. G. R. M. Rao, former Director, CIBA, Chennai for providing facilities and support to carry out this work and Dr. A.G. Ponniah, Director, CIBA, Chennai for his encouragement.

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Date of Receipt : 23.11.2006
Date of Acceptance : 27.04.2011