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Evaluation of Fish Meals as Natural Feed Stimulants on the Feeding Behaviour of Fry and Juveniles of *Lates calcarifer* (Bloch)

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Abstract

Six purified diets were prepared for this experiment. Five of these six diets contained fish meals of five different fishes namely Bombay duck, Anchovy, Lesser sardine, Ribbon fish and shrimp head as natural stimulants for sea bass fry and juveniles. These five diets were prepared by adding 10% of each stimulant by weight in the purified diet. The sixth diet (F) was without stimulant and considered as control. The experiment was conducted on sea bass fry, 20 (±0.02) mm total length and juvenile, 217 (±0.15) mm total length by using one fish and two fish per tank respectively. The results indicated that Bombay duck meal containing diet i.e. diet 'A' has significant stimulating effect on the feeding behaviour of fry and also in juveniles of sea bass although the positive responses were higher in the case of juveniles (30 in case of fry and 34 in case of juveniles). Further, there were 34 positive responses in the case of two juvenile sea bass per tank experiment where as it was 30 in the case of two fry per tank experiment. The feeding responses were significantly different with two fish per tank as compared to one fish per tank showing that the number of fish had an influence on the feeding behaviour of both fry and juveniles. However, no feeding response was observed in the control diet. The study also revealed that Bombay duck meal is better and superior as feeding stimulant over the other fish meals for the Sea bass fry and juveniles.

Introduction

Sea bass (*Lates calcarifer*, Centropomidae) is an esteemed fish inhabiting the estuarine and fresh water zones of the coastal regions. It tolerates wide salinity changes and fetches higher market price in market due to its good taste (Singh 2000). The twin qualities of fast growth and good taste tempt aquaculturist to undertake the pond culture of this fish. The euryhaline character make it an ideal fish for coastal aquaculture since the fluctuation of salinity is very frequent specially in rainy season. The fry of sea bass are collected during the monsoon period from June to September in India (Singh and Mehta 1997). The fry and juveniles are carnivorous and prefer trash fishes and normally don't accept formulated feed when raised in ponds. The sea bass is also cultured with Indian major carps in India due to its wide range salinity tolerance nature (Singh et al. 2001). However, it is not always possible and economical to provide the trash fish to a desired quantity. The poor acceptance of formulated

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feed and requirement of large quantity of trash fish deters the farmers from undertaking culture of sea bass despite the fact that the good quality seed is available from natural habitats and also from hatcheries to meet the seed requirement of the farmers (Singh et al. 2005). However in view of the aquaculture potentials of this species, the role of feeding stimulants is crucial to lure the fish to feed. Barlow (2002) and Ghiselin (2002) reported the behavioural data of some species but little is known about the feeding behaviour of sea bass.

The feeding stimulants are supposed to promote quicker food intake without disturbing the water quality and help the fishes at several feeding steps like initiation at longer distance ('attractant' effect), help the catching of the prey at shorter distances ('arrestant' effect), initiate the feeding via tasting ('incitant' effect), or promote continuation of the feeding ('stimulant' effect), (Mackie 1982). The free amino acids are reported to have attractant and arrestant effects as they diffuse in the water effectively and stimulate olfactory bulb (Hara 1973). However, the high cost of these chemicals again renders the sea bass culture uneconomical (Singh et al. 2005). The role of stimulants in the feeding behaviour of fry and juveniles of sea bass has not yet been studied. The diet costs up to 60% of total farm production cost for shrimp (Tan and Dominy 1997) and little less in culture of fin fishes. Therefore, it is absolutely important that feed is utilized fully and quickly to avoid the wastage. The stimulant effect of fish meal of certain fishes was observed to be superior than the Glycine, Proline and L-lysine for fry of *Lates calcarifer* (Singh et al. 2005). However, more meals of fishes needs screening for better selection and wider availability at affordable price. Hence, it was proposed to investigate the stimulant effects of meals of Bombay duck (*Harpadon nehereus,* Harpadontidae), Anchovy (*Coilia dussumieri,* Coilinae), Lesser sardine (*Sardinella fimbriata,* Clupeidae), Ribbon fish (*Trichiurus lepturus,* Trichiuridae) and shrimp (*Penaeus monodon,* Penaeidae) head meal on the feeding behaviour of sea bass fry and juveniles. All above fishes and shrimp species are marine and abundantly available at low cost in various countries.

Materials and Methods

Experiment I. The effect of stimulants on the feeding behaviour of sea bass fry

Sea bass fry, 20 (± 0.02) mm total length, 300 (± 0.01) mg in weight were obtained from Pancham Aquaculture, Safale, District Thane, Maharashtra, India. They were held for acclimation in 3m (Diameter) X 2m (Height) circular plastic pool at the laboratory of Taraporevala Marine Biological Research Station, Mumbai, India. The sea bass fry were gradually acclimated to freshwater in one week from the date of procurement. During the acclimatation period, the fry were fed with tubifex worms. This experiment was conducted by using one fry per tank and two fry per tank respectively.

Experiment II. The effect of stimulants on the feeding behaviour of sea bass juveniles

Sea bass juveniles, 217 (± 0.15) mm total length, 138 (± 0.20) g in weight were collected from coastal waters in the district Thane, Maharashtra, India. They were held for acclimation in 6m (Diameter) X 2m (Height) circular plastic pool at the laboratory of Taraporevala Marine Biological Research Station, Mumbai, India. The sea bass juveniles were gradually acclimated to freshwater in one week period and were fed with red worms (*Tubifex tubifex,* Tubificidae). This experiment was also conducted by using one juvenile per tank and two juveniles per tank respectively as above experiment.

The plastic pool was provided with continuous aeration and 50% of the water exchanged daily in both the experiment. The water temperature was in the range of 29 to 30 ºC with natural photoperiod. Water quality parameters i.e. pH (7.1 to 7.3) and dissolved oxygen, (5.5 to 6.1 mg.l^{-1}) were recorded on alternate day and were found to be within the optimum range.

Preparation of experimental diet

Six purified diets (A, B, C, D, E and F) were prepared with a protein level of 46% in the diet as worked out by Cuzon and Fuchs (1988) by using casein as a protein source. The composition and the ingredients included in the diet are mentioned in Table 1. This experiment is designed to study the stimulant effect of fish meals that's why pure ingredient like casein, soyabean meal were used. One can also use the cheap alternative for casein and soyabean meal. The meal of fishes viz Bombay duck, Anchovy meal, Lesser sardine meal, Ribbon fish meal and shrimp head meal are prepared by drying the fish and grounding the same separately in the mixer. The meal of each species was added to each purified diet @ 10% (Table 1). The inclusion level of Bombay duck meal has determined in the earlier experiment of Singh et al. (2005), hence the level of inclusion was mentioned uniformly in all the diets. The diet 'F' had no meal or feeding stimulants and used as a control diet. The moist pellets of uniform size 2 to 3 mm were used for the experiment.

Table 1. Composition of the purified diets (%)

Ingredients	Diets					
	A	B	C	D	E	F
Casein*	28	28	28	28	28	28
Soyabean meal*	28	28	28	28	28	28
Rice bran¶	9.00	9.00	9.00	9.00	9.00	9.00
Gelatin*	5.86	4.86	6.00	6.86	6.50	10.50
CMC*	5.00	5.00	5.00	5.00	5.00	5.00
Vitamin mix.[a]	2.00	2.00	2.00	2.00	2.00	2.00
Cellulose*	6.74	7.74	6.60	5.74	6.10	12.10
Soyabean oil†	5.40	5.40	5.40	5.40	5.40	5.40
Bombay duck meal‡	10	-	-	-	-	-
Anchovy meal‡	-	10	-	-	-	-
Lesser sardine meal‡	-	-	10	-	-	-
Ribbon fish meal‡	-	-	-	10	-	-
Shrimp head meal‡	-	-	-	-	10	-
Control	-	-	-	-	-	-
Proximate composition						
Protein	46.00	46.00	46.00	46.00	46.02	46.00
Fat	8.00	8.31	8.40	8.25	8.30	8.28
Moisture	14.00	14.20	14.05	14.30	13.90	14.25
Ash	4.00	4.11	4.05	4.15	4.20	4.09

[a] Vitamin premix contained the following vitamins per kilogram feed: vitamin A, 5,500 IU; vitamin D_3, 1000 IU; vitamin E, 50 IU; vitamin K_3 10 IU; choline chloride, 550 mg; niacin, 100 mg; riboflavin, 20 mg; thiamin, 20 mg; pantothenic acid, 50 mg; biotin, 0.1 mg; folacin, 5 mg; cyanocobalmin (B_{12}), 20 mg; vitamin C, 100 mg; inositol, 100 mg.
* Thomas Baker (Chemicals), INDIA
¶ Rice bran obtained from private rice mill
† Soyabean oil obtained from Classico Oils, Thane, INDIA
‡ Fish and shrimp heads obtained from landing centre at Mumbai, INDIA, sun dried (8% moisture) and grounded to make fine powder.

Experimental design and treatments

Five rectangular all glass tanks (1.2 X 0.45 X 0.45 m) with the water capacity of 243 liter were used for the sea bass juvenile experiment and the separate five rectangular all glass

tanks (0.6 X 0.3 X 0.3 m) with the water capacity of 54 liter were used for the sea bass fry experiment. The water temperature was maintained between 29 to 30 ^{0}C by a 300W thermostatic heater (Rena, Cal Basic, France). Feed was introduced into the tank for tasting as per the procedure followed by Borquez and Cerqueira (1998).

At the start of the experiment, the fishes which were acclimated in the plastic pool were stocked in experimental tank for three to four days and later subjected to experiment. During this period the fishes were fed once daily with tubifex worms, between 1000 hrs and 1130 hrs. The feeding trials were performed between 1000 and 1200 hrs. Only one diet containing stimulant (treatment) was tested daily, with eight trials per tank. The five tanks available resulted in 40 observations (responses) per treatment. The feeding responses observed were scored from one to five adapting the methodology of Stradmeyer (1989) and expressed as following: (1) No reaction: fish remains on station without moving. (2) Orientation: rapid movement of the head pointing at pellet. (3) Approach: fish swims quickly towards pellet. (4) Capture-rejection (tasting): fish takes pellet in mouth but spits it out. (5) Capture-ingestion: fish eats pellet. Initially all trials were conducted with one fish per tank. Later the experiments were repeated using two fish per tank. Experimental diets were analysed for proximate composition by using standard methods (AOAC 1984).

Growth study

The experiment was conducted in triplicate. Twelve rectangular all glass tanks (0.6 X 0.3 X 0.3 m) with the water capacity of about 54 liters were used for experiment. A total of 20 sea bass fry, 22 (± 0.04) mm in total length and 0.23 (± 0.02) g in weight were introduced in each tank. The water temperature was maintained between 29^0 to 30^0 C. Feeding was done with the same diets used in Experiment 1 and 2 following the procedure of Borquez and Cerqueira (1998) @ 10% of body weight. Fishes were fed twice daily at 1000 hrs and 1600 hrs. The tanks were provided with continuous aeration and 25 % of water was replenished every day. Unused feed and excreta were regularly removed. The unused feed was collected, semi-dried (14 % moisture) and weighed for determination of feed consumption. The water quality parameters i.e. pH, temperature and dissolved oxygen were recorded each day. After the feeding started, the lengths and weights of fishes were measured at weekly intervals.

Statistical analysis

The data analysis was done using non-parametric Chi-square test at a significance level of 5% (Sokal and Rohalf 1981). The positives [sum of responses (3), (4) and (5)] and negatives [sum of (1) and (2) responses] were grouped for statistical analysis. For growth study, the data analysis was done using standard statistical methods as described by Snedecor and Cochran (1967). Newman Keul's multiple comparison test was used as described by Zar (1984).

Results

Experiment I. The effect of stimulants on the feeding behaviour of sea bass fry

The experiments were conducted separately with one fry per tank and two fry per tank by using five different purified diets containing one stimulant in each diet and sixth diet without any stimulant as control. The results of the five different feeding stimulants on feeding response of sea bass fry with one fry per tank and two fry per tank are presented in Table 2.

Among the various feeding stimulants tested, diet 'A' containing Bombay duck meal showed higher ingestion response (15%) with two fish per tank. The ingestion is defined as "Fish eating pellets". The ingestion response was observed to be nil in control diet. The testing response, which is defined as "Fish takes pellets in mouth and spit it out" was also highest (40.0%) in the diet 'A' in comparison to other diets with two fish per tank. The results of the group i.e. positive and negative responses are presented in Table 3. The highest sum of positive i.e. 31 was obtained in diet 'A' containing Bombay duck meal and lowest in control i.e. 04 with two fish per tank. The Chi- square statistical analysis indicated significant difference ($\pm \chi_o^2 = 31.22 > \chi_e^2 = 9.49$) among the different feeding stimulant diets used. In the experiment with one fry per tank the ingestion response was 7.5%, which increased by double i.e. 15% in two fry per tank experiment. The significant difference ($\pm \chi_o^2 = 24.44 > \chi_e^2 = 9.49$) was also observed among the feeding response and substances tested with two fry per tank experiment (Table 3). The statistical analysis of data revealed that in both experiments the variables, substances and feeding responses were dependent. When the total positive and negative responses of all the substances were compared to the number of fry in a trial, the number of positive responses for the two fry per trial were significantly higher ($\pm \chi_o^2 = 6.58 > \chi_e^2 = 3.84$) indicating that number of fishes in the trial affected the feeding response in sea bass fry (Table 3).

Experiment II. The effect of stimulants on the feeding behaviour of sea bass (Lates calcarifer) juveniles.

The similar pattern was observed in case of feeding behaviour of juveniles of sea bass where the diet 'A' containing Bombay duck meal had a significant impact on ingestion (25 %) as compared to other fish meals (Table 6) in experiment with two juvenile per tank, whereas, in case of one juveniles per tank, the ingestion was 12.5 % when fed with diet 'A'. The ingestion is almost double in the case of two juvenile per tank experiment compared to ingestion response in one juvenile per tank (Table 4). The results of the group i.e. positive and negative responses are presented in Table 5, which showed that the juveniles fed with diet A indicated the highest sum of positive responses i.e. 34 which is also higher than that of sum of positive responses observed with one juvenile per tank i.e. 30. As the ingestion response is concerned, the juveniles of sea bass had higher response i.e. 25 % with two fish per tank, where as fry had only 15 % response with two fish per tank.

There was very less feeding responses in the control diet (5) which doesn't have any feeding stimulant. However, the highest responses were in the diet 'A' containing Bombay duck meal (34), followed by diet 'D' containing Ribbon fish meal (31), diet 'E' containing shrimp head meal (26), diet 'C' containing Lesser sardine meal (21) and diet 'B' containing Anchovy meal (11) in case of juveniles of sea bass with two fish per tank experiment.

The results of growth study are given in Table 6. It was observed that the diet 'A' prepared by using Bombay duck fish meal showed the highest gain in length (20 ± 0.01 mm), weight (0.25 ± 0.01g) and FCR (1.80); over diets 'B' of Anchovy fish (gain in length 7 ± 0.02 mm, weight 0.09 ± 0.02g, FCR 3.08); 'C' of Sardine fish (length 15 ± 0.02 mm, weight 0.17 ± 0.02 g, FCR 2.4); 'D' of Ribbon fish (gain in length 17± 0.01mm, weight 0.18 ± 0.01 g, FCR 1.94); and diet 'E' of Shrimp (length 13 ± 0.03mm, weight 0.15 ± 0.01g, FCR 2.83). However, all these diets were proved to be good as the diet without any fish meal showed the least growth 'F' (length 5 ± 0.03 mm, weight 0.07 ± 0.02 also its FCR 3.12). Fry survival at the end of experiment was highest (100 %) in the case of diet A and D, while diet C and E showed 90% survival. The lowest survival was in the case of diet B and F (80%). The maximum feed

consumption was observed in diet 'A' (82%) followed by diet 'D' (74%), diet 'C' (62 %), diet 'E' (58 0%) and control 'F' (52%).

The ANOVA showed significant difference ($P< 0.05$) between the length and weight of fry of sea bass fed with different diets. Newman Keul's multiple comparison test indicated that the average gain in length and weight of fry of sea bass fed with diet 'A' was significantly higher ($P< 0.05$) than the average gain in length and weight of fry fed with other diets.

Table 2. Feeding responses of sea bass (*Lates calcarifer*) fry to different natural feeding stimulants

Sr.no.	Stimulants	Feeding responses (%)									
		1		2		3		4		5	
		I	II	I	II	I	II	I	II	I	II
1	Bombay duck meal	7.5	2.5	27.5	20	30	22.5	27.5	40	7.5	15
2	Anchovy meal	27.5	27.5	62.5	55	7.5	12.5	2.5	5	0	0
3	Lesser sardine meal	22.5	20.0	52.5	45	15	17.5	10	15	0	2.5
4	Ribbon fish meal	17.5	7.5	47.5	27.5	20	30.0	12.5	22.5	2.5	7.5
5	Shrimp head meal	25	25	57.5	50.0	10	17.5	7.5	7.5	0	0
6	Control	62.5	60.0	27.5	30.0	10	10.0	0	0	0	0

I Experiment with one fish per tank
II Experiment with two fish per tank
1) No reaction
2) Orientation
3) Approach
4) Tasting
5) Ingestion

Table 3. Results of the comparison by means of the independence test with χ^2 ($\alpha = 0.05$) of the positive and negative feeding responses of sea bass (*Lates calcarifer*) fry to different natural feeding stimulants

Sr. no.	Stimulants	One fish per tank		Two fish per tank	
		Positive*	Negative*	Positive**	Negative**
1	Bombay duck meal	26	14	31	09
2	Anchovy meal	04	36	07	33
3	Lesser sardine meal	10	30	14	26
4	Shrimp head meal	07	33	26	14
5	Ribbon fish meal	14	26	10	30
6	Control	04	36	04	36

*Experiment with one fish per tank
** Experiment with two fish per tank

Table 4. Feeding responses of sea bass (*Lates calcarifer*) juveniles to different natural feeding stimulants

Sr.no.	Stimulants	Feeding responses (%)									
		1		2		3		4		5	
		I	II	I	II	I	II	I	II	I	II
1	Bombay duck meal	5.0	0	20.0	15.0	22.5	25.0	40.0	35.0	12.5	25.0
2	Anchovy meal	25.0	22.5	60.0	50.0	7.5	15.0	5.0	10.0	2.5	2.5
3	Lesser sardine meal	15.00	12.5	47.5	35.0	25.0	17.5	7.5	27.5	5.0	7.5
4	Ribbon fish meal	12.5	2.5	37.5	20.0	22.5	32.5	20.0	30.0	7.5	15.0
5	Shrimp head meal	20	20.0	55.0	40.0	15.0	35.0	7.5	25.0	2.5	5.0
6	Control	50	55.0	25	32.5	15.0	7.5	7.5	5	2.5	0.0

I Experiment with one fish per tank
II Experiment with two fish per tank
1) No reaction
2) Orientation
3) Approach
4) Tasting
5) Ingestion

Table 5. Results of the comparison by means of the independence test with the χ^2 ($\alpha = 0.05$) of the positive and negative feeding responses of sea bass (*Lates calcarifer*) juveniles for different natural feeding stimulants.

Sr. no.	Stimulants	One fish per tank		Two fish per tank	
		Positive*	Negative*	Positive**	Negative**
1	Bombay duck meal	30	10	34	6
2	Anchovy meal	6	34	11	29
3	Lesser sardine meal	15	15	21	19
4	Shrimp head meal	10	30	31	09
5	Ribbon fish meal	20	20	26	24
6	Control	10	30	05	35

*Experiment with one fish per tank
** Experiment with two fish per tank

Table 6. Growth and feed conversion ratio of fry of sea bass (*Lates calcarifer*) fed with the diets having different natural feeding attractants for a period of four weeks. (Mean ± S.E., N = 3)

Test diets	A	B	C	D	E	F
Levels (%)	10	10	10	10	10	0
Initial average length (mm)	22 ± 0.04	22 ± 0.04	22 ± 0.04	22±0.04	22 ± 0.04	22 ± 0.04
Final average length (mm)	42 ± 0.02	29 ± 0.01	37 ± 0.02	39± 0.02	35 ± 0.01	27 ± 0.01
Gain in length (mm)	20 ± 0.01	7 ± 0.02	15 ± 0.02	17± 0.01	13 ± 0.03	5 ± 0.03
Gain in length (%)	90.9	31.8	68.18	77.27	59.09	22.72
Initial average weight (g)	0.23 ± 0.02	0.23 ± 0.02	0.23 ± 0.02	0.23± 0.02	0.23 ± 0.02	0.23±0.02
Final average weight (g)	0.48 ± 0.03	0.32 ± 0.02	0.40 ± 0.02	0.41± 0.02	0.38 ± 0.03	0.30 ± 0.01
Gain in weight (g)	0.25 ± 0.01	0.09 ± 0.02	0.17 ± 0.02	0.18 ± 0.01	0.15 ± 0.01	0.07 ± 0.02
Gain in weight (%)	108.69	39.13	73.91	78.26	65.21	30.43
Feed conversion ratio	1.80	3.08	2.4	1.94	2.83	3.12
Survival (%)	100	80	90	100	90	80

A - Bombayduck meal
B - Anchovy meal
C - Sardine meal
D - Ribbon fish meal
E - Shrimp head meal
F - Control

Discussion

The Bombay duck meal gave significantly high stimulating effect over the other natural feeding stimulants used in the diets in respect of both fry and juveniles. It was also observed that the response was significantly higher in the treatment with two fish per tank than one fish per tank (Tables 3 and 5). The 'ingestion' responses in case of fry of sea bass were 15% in the presence of two fishes, which is comparatively high than one fish per tank treatment (Tables 2 and 3). On the contrary, Borquez and Cerqueira (1998) observed no 'ingestion' response and maximum 'orientation' responses in the juvenile snook. However, Borquez and Cerqueira (1998) explained this behaviour stating that fishes try to capture any particle moving inside the water, and once in the mouth, is ingested or rejected depending on its taste and texture. Predatory nature of constant aggression on feeding followed by attempts at biting the other fish was also noted in the sea bass juveniles similar to juvenile snook (Borquez and Cerqueira 1998). The occurrence of 'ingestion' responses in sea bass fry may be due to the moist nature of feed as the acceptance for moist diet is comparatively high than dry diets (Personal observations). In the present study, the effect of number of fishes on the feeding response was significant ($\pm \chi_o^2 = 31.22 > \chi_e^2 = 9.49$) for one fish per tank experiment and ($\pm \chi_o^2 = 6.58 > \chi_e^2 = 3.84$) for two fish per tank experiment respectively. We used only two fish per tank while Carr (1976), Carr and Chaney (1976), Carr et al. (1976) and Carr et al. (1977) used groups of six to twenty fishes in different experiments and has not recorded the effect of number of fishes on feeding response. The other fish meals used in the diets for sea bass fry and juveniles had also stimulating effect but the effect of Bombay duck meal was much higher over others as the Bombay duck meal scored 26, 31 positive stimulating responses in the case of fry with one fish and two fish per tank (Table 3). The same was also high in respect of juveniles of sea bass with 30 and 34 positive responses with one and two fish per tank respectively (Table 5). It was also observed that there were more positive feeding responses in the juveniles than in the fry indicating that the acceptance level of feed is higher in the case of juveniles with Bombay duck as stimulant in the feed. While evaluating the growth performance of five fishmeal attractant diets, it was observed that the maximum percentage weight gain was in the diet 'A' containing the Bombay duck meal followed by diet 'D' and 'E' containing Ribbon fish meal and Sardine meal, respectively. The least weight gain was with diet 'F' (control) which was without any attractant.

The investigation on the chemical composition of fish meats, revealed that Bombay duck has lowest lipid content (0.7 %) than lesser sardine (11.7 %), ribbon fish (1.42%), anchovy (1.3 %) and shrimp (0.75 %) (Anon 1997). It is reported that the fish meals, which had less lipid content, and high protein content have high concentrations of various amino acids like valine, histidine, arginine and lysine (Arzel et al. 1999). Bombay duck meal has comparatively least lipid content over other fish meals, hence might have higher concentrations of valine, histidine, arginine and lysine. All these amino acids have feed attractant properties and used in aquaculture.

Conclusion

Among the five fish meals namely Bombay duck meal, Anchovy meal, Lesser sardine meal, Ribbon fish meal and shrimp head meal tested as stimulants, the Bombay duck meal

was observed to be the best feed stimulant for the fry and juveniles of sea bass. The acceptance level of feed is higher in the case of juveniles as compared to the sea bass fry.

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