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EFFECT OF STIMULANTS ON FEEDING RESPONSE, FEEDING BEHAVIOR, AND GROWTH OF FRY OF SEA BASS, *LATES CALCARIFER* (BLOCH, 1790)

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Abstract

The influence of four stimulants (glycine, proline, L-lysine, and Bombay duck fishmeal) on the feeding behavior of sea bass fry was studied. The substances were added at a level of 1% of the weight of moist pellets made from purified diets. Feeding responses were observed in glass tanks containing one or two fish.The fishmeal had significantly greater stimulating effect on the feeding behavior than the chemical substances. Feeding responses were significantly different when there were two fish in the tank. Inclusion of fishmeal at a 10% level (compared to 1%, 5%, 7.5%) produced the greatest ingestion response (50%). Compared to 7.5%, inclusion at 10% produced a significantly higher weight gain (0.54±0.03 g), FCR (1.79), and PER (1.20). The present investigation revealed that Bombay duck meal is a better natural fish feed stimulant than the tested chemicals.

Introduction

Among the various factors that influence the intake of feed, smell plays a vital role. Dietary feeding stimulants in compounded aquafeeds have received considerable attention in recent years. L-proline, L-lysine, L-histidine, glycine, and betaine have been tried as feeding stimulants to promote faster food intake. Faster intake of food means that feed remains in the water for a shorter period of time and leaching of water-soluble nutrients is reduced. Such compounds can stimulate appetite at several stages by: initiating orientation of the fish toward the food at a greater distance (“attractant” effect), helping the fish capture prey at shorter distances (“arrestant” effect), encouraging tasting of the feed (“incitant” effect), or

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promoting ingestion of the feed ("stimulant" effect; Mackie, 1982). Free amino acids and betaine are highly soluble, effectively diffuse in water, and stimulate the olfactory bulb (Hara, 1973), thereby producing the attractant and arrestant effects. As organic farming becomes more important, replacement of chemical feeding stimulants by cost-effective natural feed stimulants becomes more necessary.

In the present study, strong-smelling Bombay duck *(Harpodon nehereus* Hamilton) fishmeal was tested as a natural stimulant for the fry of Asian sea bass *(Lates calcarifer)*. The Bombay duck is a marine fish of the Scopelidae family. The species averages about 10% of India's total marine fish landings and constitutes a major fishery along the Gujarat and Maharashtra coasts of India. Nearly 98% of the Bombay duck supply comes from these two states where it is caught in the non-selective bag net locally known as a "dol". When fresh, the fish is edible but, being soft, it spoils quickly. Surplus catch is sun dried on specially built scaffoldings. When unfit for human consumption, Bombay duck is converted into manure (Bal and Rao, 1984). The dried fish are also powdered and sold as 'Bombay duck fishmeal' which is used for human and cattle feeds.

The dietary requirement for tryptophan of juvenile Asian sea bass was studied by Coloso et al. (2004). Euseblo and Coloso (2002) investigated the effect of high dietary protein intake on proteolytic activity of juvenile Asian sea bass. Identification of a suitable, effective, readily available, and economic feeding stimulant for sea bass fry is important for a number of reasons. The species is very carnivorous and prefers trash fish; it normally does not accept formulated feed when raised in ponds. However, it is not always possible and economical to provide trash fish. The poor acceptance of formulated feed and need for large quantities of trash fish deter farmers from culturing sea bass, despite the fact that good quality seed is available from hatcheries. Hence, in view of the aquaculture potential of this species, it is important to formulate a diet that is acceptable to the fry and yields a high FCR. The objectives of this study were to evaluate the efficacy of Bombay duck fishmeal as a feed stimulant for *Lates calcarifer* fry and to determine the suitable inclusion levels in their diet.

Materials and Methods

Experimental diets. Four test diets were prepared by incorporating 1% proline, glycine, L-lysine, or Bombay duck meal into a purified diet; the control contained no stimulant (Table 1). The diets contained a protein level within the range (45-55%) stipulated by Cuzon and Fuchs (1988). Bombay duck fish were procured from the local market and ground in a mixer to a fine powder. Moist pellets of uniform size (2-3 mm) were used for the experiments. Experimental diets were analyzed for proximate composition using standard AOAC (1984) methods.

Fish and tanks. Sea bass fry (15±0.02 mm standard length, 200±0.01 mg weight) were obtained from Pancham Aquaculture, Safale, District Thane, Maharashtra, India. They were held for acclimation in circular plastic pools (3 m diameter, 2 m height) at the laboratory of Taraporevala Marine Biological Research Station, Mumbai, India. The stock was gradually acclimated to fresh water within 10 days from the date of procurement. During this period, they were fed tubifex worms. The plastic pools were provided continuous aeration and 20% of the water was exchanged daily. The water temperature was maintained at 28±0.5°C and the photoperiod was natural. Water quality parameters were recorded on alternate days; pH was 7.0-7.1 and DO_2 5-6.2 mg/l, within the optimum range.

Experimental design and treatments. The acclimated fish from the plastic pools were restocked into five glass tanks (0.6 x 0.3 x 0.3 m). During a 3-4 day acclimation period, the fish were fed tubifex worms once daily, between 10:00 and 11:30. The water temperature was maintained at 28±0.5°C by a 300W thermostatic heater (Rena, Cal Basic, France).

The feeding trials were performed between 10:00 and 12:00. Feed, at 10% of the body weight of the fry, was introduced into the

Table 1. Composition of the basal diet and proximate analyses of the experimental diets.

Ingredient	*%*
Casein	31.0
Soybean meal	31.0
Rice bran	11.5
Gelatin	5.86
Carboxymethyl cellulose	5.0
Vitamin mix	2.5
Cellulose	6.74
Soybean oil	5.4

Ingredient	*Experimental diet (%)*				
	Proline	*Glycine*	*L-lysine*	*Bombay duck meal*	*Control*
Proline	1.0	-	-	-	-
Glycine	-	1.0	-	-	-
L-lysine	-	-	1.0	-	-
Bombay duck meal	-	-	-	1.0	-
Proximate composition					
Protein	46.0	46.1	46.05	46.0	46.13
Fat	8.58	8.51	8.53	8.52	8.5
Moisture	14.2	13.5	14.0	14.1	13.8
Ash	4.1	4.2	4.0	4.1	4.0

tank as per the procedure followed by Borquez and Cerqueira (1998). One substance (treatment) was tested per day, with eight trials per tank, resulting in 40 observations (responses) per treatment. The feeding responses were scored from one to five adapting the methodology of Stradmeyer (1989): (1) no reaction, fish remains on station without moving, (2) orientation, rapid movement of the head toward the pellet, (3) approach, fish swims quickly towards pellet, (4) capture-rejection (tasting), fish takes pellet in mouth but spits it out, and (5) capture-ingestion, fish eats pellet.

The first trial was conducted with one fish per tank. This trial was repeated using two fish per tank. After determining the best stimulant, various levels of the stimulant were tested using the same procedure. Finally, a 30-day growth study was conducted using the two best levels in triplicate with 15 fry per tank.

Statistical analysis. The positive (3-5) and negative (1, 2) responses in the first two experiments were added together for statistical analysis using a non-parametric Chi-square test at a significance level of 5% (Sokal and Rohalf, 1981).

Data from the 30-day trial were statistically analyzed by one-way analysis of variance (ANOVA) at $p < 0.05$ (Snedecor and Cochran, 1967).

Results

Results of the four stimulants on the feeding response of the fry with one fish per tank are presented in Table 2. Only Bombay duck meal caused a capture-ingestion response (10%) and it had the highest number of positive responses. The chi-square statistical analysis indicated significant differences ($\chi_o^2 = 31.22 > \chi_e^2 = 9.49$) among the substances used. When the experiment was repeated with two fish per tank the same pattern was observed (Table 3) and differences among substances were significant ($\chi_o^2 = 24.44 > \chi_e^2 = 9.49$). Statistical analysis of the data revealed that in both experiments the variables, substances, and feeding responses were dependent. The total number of positive responses was significantly higher ($\chi_o^2 = 6.58 > \chi_e^2 = 3.84$) in the trial with two fish per tank, indicating that the number of fish in a tank affects the feeding response. Survival was 100% in all treatments and no cannibalistic behavior was recorded.

The results of the trial using various levels of Bombay duck meal are presented in Table 4. The ingestion response was highest at the 10% inclusion level. There was only one 'no reaction', and it was at the lowest inclusion level. Statistical analysis revealed that the feeding response was significantly dependent ($\chi_o^2 = 14.51 > \chi_e^2 = 9.49$) on the level of feeding stimulant.

The two best-performing levels of Bombay duck meal were compared to a control in a 30-day growth trial (Table 5). The gains in length and weight, FCR, and protein efficiency ratio were significantly ($p<0.05$) higher in the treatment with 10% inclusion (Table 6).

Discussion

Bombay duck meal had a significantly higher stimulating effect than the other three substances. The response was significantly higher in treatments with two fish per tank than one. In the presence of two fish, 50% of the Bombay-fed fish had a 'tasting' response, whereas in tanks with only one fish, only 32.5% had a 'tasting' response. This relationship was also reported by Borquez and

Table 2. Feeding responses of sea bass *(Lates calcarifer)* fry to different stimulants when tested with one fish per tank.

Stimulant	*Feeding responses*										*Number of:*	
	No reaction		*Orientation*		*Approach*		*Tasting*		*Ingestion*		*Positive responses*[1]	*Negative responses*[2]
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*n*
Bombay duck meal	1	2.5	14	35	8	20	13	32.5	4	10	25	15
Glycine	11	27.5	14	35	10	25	5	12.5	-	-	15	25
Proline	10	25	18	45	8	20	4	10	-	-	12	28
Lysine	8	20	21	52.5	9	22.5	2	5	-	-	11	29
Control	23	57.5	15	37.5	2	5	-	-	-	-	2	38

[1] Approach + tasting + ingestion
[2] No reaction + orientation

Table 3. Feeding responses of sea bass *(Lates calcarifer)* fry to different stimulants when tested with two fish per tank.

Stimulant	*Feeding responses*										*Number of:*	
	No reaction		*Orientation*		*Approach*		*Tasting*		*Ingestion*		*Positive responses*[1]	*Negative responses*[2]
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*n*
Bombay duck meal	2	5	10	25	4	10	20	50	4	10	28	12
Glycine	5	12.5	13	32.5	12	30	10	25	-	-	22	18
Proline	2	5	19	47.5	13	32.5	6	15	-	-	19	21
Lysine	9	22.5	18	45	9	22.5	4	10	-	-	13	27
Control	20	50	12	30	8	20	-	-	-	-	8	32

[1] Approach + tasting + ingestion
[2] No reaction + orientation

Table 4. Feeding responses of sea bass *(Lates calcarifer)* fry to different inclusion levels of Bombay duck meal in the feed.

Stimulant	*Feeding responses*										*Number of:*	
	No reaction		*Orientation*		*Approach*		*Tasting*		*Ingestion*		*Positive responses*[1]	*Negative responses*[2]
	n	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*%*	*n*	*n*
1	1	2.5	10	25	12	30	12	30	5	12.5	29	11
5	-	-	5	12.5	5	12.5	18	45	12	30	35	5
7.5	-	-	2	5	5	12.5	15	37.5	18	45	38	2
10	-	-	1	2.5	6	15	13	32.5	20	50	39	1

[1] Approach + tasting + ingestion
[2] No reaction + orientation

Cerqueira (1998) who used two juvenile snook per tank. On the other hand, Carr (1976), Carr and Chaney (1976), and Carr et al. (1976, 1977) used groups of six to 20 fish in different experiments and recorded no effect of the number of fish on the feeding response.

In experiments conducted by Borquez and Cerqueira (1998) on juvenile snook, 'tasting' was nil and the highest number of fish had an 'orientation' response. Borquez and Cerqueira (1998) attributed this observation to the sight-feeding behavior of juvenile snook by which fish react to any particle that enters the water but approach it only if chemically stimulated. However, they further explained that fish try to capture any moving particle in the water and, once it is in the mouth, it is ingested or rejected depending on the taste and texture. The

Table 5. Composition of the experimental diets with different inclusion levels of Bombay duck meal (%).

Ingredient	*Level of Bombay duck meal (%)*		
	0	*7.5*	*10*
Casein	31.0	31.0	31.0
Soybean meal	31.0	31.0	31.0
Rice bran	11.5	11.5	11.5
Gelatin	6.27	3.14	2.10
Carboxymethyl cellulose	5.0	5.0	5.0
Vitamin mix	2.5	2.5	2.5
Cellulose	7.33	2.96	1.5
Cod liver oil	5.4	5.4	5.4
Bombay duck meal	-	7.5	10.0
Proximate composition			
Protein	46.5	46.5	46.5
Fat	8.90	8.68	8.56
Moisture	14	13.5	14
Ash	4.1	4.2	4.0

Table 6. Results of a 30-day growth trial of diets containing two levels of Bombay duck meal.

Ingredient	*Level of Bombay duck meal (%)*		
	0	*7.5*	*10*
Casein	31.0	31.0	31.0
Initial avg length (cm)	1.50±0.02	1.50±0.02	1.50±0.02
Final avg length (cm)	3.24±0.01	4.02±0.02	4.52±0.02
Gain in length (cm)	1.74±0.01	2.52±0.02	3.02±0.02*
Initial avg wt (g)	0.20±0.01	0.20±0.01	0.20±0.01
Final avg wt (g)	0.26±0.02	0.47±0.03	0.74±0.03
Wt gain (g)	0.06±0.02	0.27±0.03	0.54±0.03*
Feed conversion ratio	10.66	2.88	1.79*
Protein efficiency ratio	0.20	0.75	1.20*
Survival (%)	100	100	100

* Significantly different at 5%.

same sight-feeding behavior was noted in our sea bass fry. Constant aggression during feeding followed by attempts to bite other fish was also noted, similar to that observed by Borquez and Cerqueira (1998) in juvenile snook.

The ingestion responses may have been due to the moist nature of the feed. The acceptance of moist diets is comparatively higher than dry diets (personal observation). All the tested substances had a stimulating effect but the effect of Bombay duck meal was superior to the chemical stimulants. This is important as the aquaculture industry is moving towards organic fish farming and reduced use of chemicals.

The market prices of 10 g proline, glycine, lysine, and Bombay duck meal are US$2.12, 0.28, 6.97, and 0.02, respectively. The lowest price is that of Bombay duck meal which also has the best effect. Including Bombay duck meal in diets at a 10% inclusion level will cost only US$0.20 per kg feed. The Asian sea bass fetches a high market price, making the addition of Bombay duck meal to sea bass diets commercially viable.

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