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Evaluation of Tomato pulp Concentrate as a Feed Attractant for Post-larvae of *Macrobrachium rosenbergii* (De Man, 1879)

Koushik Das[1]*, Saswata Maitra[1], M. Harikrishnan[1] and Rahul Mondal[2]

[1]*School of Industrial Fisheries, Cochin University of Science and Technology, 32-Fine Arts Avenue, Cochin - 682 016, India*

[2]*Asutosh College, University of Calcutta, West Bengal, 92, Shyamaprasad Mukherjee Road, Kolkata - 700 026, India*

Abstract

Tomatoes are known to be the richest source of glutamic acid and aspartic acid which give the "Umami flavor". In this study, tomato pulp incorporated prawn feeds and control were subjected to feeding stimuli experiments in post larvae of giant freshwater prawn *(Macrobrachium rosenbergii)* (De Man, 1879) in a "Y- maze" tank. The Mean reaching time was measured as 701.4 ± 52.11 sec, in experiments with control while the same in test feeds T1 and T2 were only 278.7 ± 25.2 and 39.95 ± 3.68 sec, respectively. ANOVA results revealed a significant difference between the three treatments ($f= 100.02$, $p<0.001$). The results revealed that tomato pulp could be considered as a feed attractant for prawn feeds owing to the high content of glutamic acid in tomato. Its use can sustainably reduce feed costs besides increasing the feed consumption.

Keywords: Tomato pulp, dietary attractant, *Macrobrachium rosenbergii*, glutamic acid

Introduction

Feed attractants are small group of chemicals including free amino acids, nucleotides, nucleosides and quaternary ammonium base (Takeda & Takii, 1992; Penaflorida & Virtanen, 1996; Gomes et al., 1997; Papatryphon & Sorares, 2000). During the past decade, attempts have been made to enhance the chemical stimuli of fish larvae and juvenile towards diets by adding feed attractants.

The feed attractan helps the growing animals in culture systems to approach the feed source faster. The ingestion of food and continuation of feeding can be stimulated by the feeding stimulants (Mackie & Mitchell, 1985; Carr, 1988). As crustaceans are primarily chemosensory feeders (Akiyama & Chwang, 1989), they are attracted to the artificial feed pellets by following an attractant plume originating from the food source (Derby & Atema, 1988). Specialized literature on dietary attractants and stimulants for marine shrimp is scarce. Smith et al. (2005) examined squid, crustacean and krill meals, fish and krill hydrolysates and a betaine product as stimulants in black tiger shrimp *Penaeus monodon* (Fabricius, 1798) diets. *P. monodon* showed a significantly greater preference for feeds containing crustacean or krill meal.

Giant freshwater prawn *(M. rosenbergii)* is a bottom feeder and an omnivore (New & Singholka, 1985). The rate of diffusion of attractants from prawn feed pellet was assessed by Harpaz (1986). Experiments with chemo-attractant Betaine, HCl which is relatively inexpensive, revealed to be a powerful stimulant in *M. rosenbergii*, inducing vigorous food searching behavior (Harpaz et al., 1987; Harpaz & Steiner, 1990).

Most chemical signals identified so far among aquatic animals are relatively small soluble molecules like amino acids. Evidence that mixtures can be more stimulatory than single compounds, by either additive or synergistic interactions, have been accumulated in both behavioral and electrophysiological studies (Lee & Meyers, 1997). For many

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*E-mail: koushik_90@yahoo.com

years, the acidic amino acids, glutamate and aspartate, have been candidate excitatory neurotransmitters in both vertebrates and invertebrates (Usherwood, 1978; Di Chiara et al., 1981; McLennan, 1983). Tomatoes are naturally rich sources of both glutamic and aspartic acids commonly known as umami, present in the ideal ratio of 4:1 (Ajinomoto, 2003). Therefore, in the present study, tomato pulp incorporated experimental feed was used for the first time in prawn feed to evaluate its efficiency as a feed attractant.

Materials and Methods

Attracting behavior assays were carried out with 30 randomly selected post-larvae of *M. rosenbergii* ranging in size from 15 to 25 mm. Individuals were collected from a prawn hatchery and transported alive to the laboratory. Feed ingredients used were fishmeal, groundnut cake, rice bran, wheat flour, starch and tomato pulp in Table 1.

Table 1. Feed formulation using ingredients in g kg^{-1}

Ingredients	Control	T1	T2
Fish Meal	400	400	400
Rice bran	200	200	200
Wheat flour	150	150	150
Starch	50	50	50
Groundnut oil cake	200	200	200
Tomato pulp	0	20	30

Feeds were provided with 40% of protein level. Individual ingredients were ground properly in a mechanical grinder. A sieve of 420 micron was used to remove large particles or foreign material like stones, dirt, pieces of metal and sand. They were weighed properly according to the pre-calculated quantity on an electronic weighing balance. All the ingredients were mixed thoroughly and carefully using a mechanical mixer for 10-15 min. To enhance the feed water stability, starch was applied in gelatinized form. The starch and dry ingredients were mixed properly and cooked in a pressure cooker for about 15 min. Control feed was prepared without tomato extract while test feeds (T1 and T2) contained tomato pulp. T1 contained 2% (20 g kg^{-1} feed) by weight and T2 contained 3% (30 g kg^{-1} feed) by weight respectively.

Prawns were stocked in 1000 l tank with aeration. Before experiment prawns were transferred to 40 l glass aquarium for acclimation. Then prawns post larvae were starved for 24 h prior experiment. The present experiment was carried out in a "Y-maze" aquarium measured 1.25 m length x 0.3 m width x 0.4 m height following Nunes et al. (2006). The "Y-maze" tank was equipped with an acclimation chamber, isolated by a removable glass guillotine thickness 0.005 m to facilitate prawn locomotory activity. During the experiment, for each observation two feeds (control and experimental) were compared and the height of the water column was maintained at 0.1 m with a total volume of 0.04 m^3 water. Prawns were allowed to acclimate in "Y-maze" tank for 10 min. After each observation water was completely discarded.

The feeding stimulus was measured visually by one observer positioned on the right side of the "Y-maze" tank following the methods of Nunes et al. (2006). Observations were made during the day, in presence of low light intensity and lasted less than 30 min per experiment. The whole observation was covered by a black paper liner to minimize external disturbance to prawn post larvae. Feeding behavior was categorized according to the responses proposed by Lee & Meyers (1997) with modifications and was added together *i.e.* detection + orientation and locomotion = time of reach. Here, detection time started after the guillotine was withdrawn, which is the time elapsed between the introduction of test feed in water and the start of prawn's perception of chemical signals, such as moving of the antennules, mouth parts and pereiopods. While orientation + locomotion is the time prawn spent to find the chosen ingredient after detection; prawn starts the movement forward or backward for the feed, walking and moving quickly from one place to another, searching calmly or intensively for the feed.

Results and Discussion

30 experimental observations were recorded with starved post larvae of 15 to 25 mm size range. The mean time taken by individual post-larvae for different experimental set up are given in Table 2.

In experiments with control, reach time ranged from 454 to 900 sec (7.5 to 15 min) with mean value 701.4 ± 52.11 sec (11.68 ± 2.75 min). In experiments with tomato pulp, T1 time ranged from 172 to 445 sec (2.86 to 7.41 min) with the mean value 278.7 ± 25.2 sec (4.63 ± 0.41 min). In contrast, the T2 tomato pulp incorporated feed, the time ranged from 24.54 to 53.84 sec with the mean value of 39.95 ± 3.68 sec.

Table 2. Mean ± SE time taken (in sec) *M. rosenbergii* post larvae in reach in different treatments

Treatments	n	Mean	SE	p-Value	Minimum	Maximum
Control	30	701.4	52.11	<0.001	454	900
T1	30	278.7	25.2	<0.001	172	445
T2	30	39.95	3.68	<0.001	24.54	53.84

ANOVA revealed a significant difference between the three treatments ($f = 100.020$, $p<0.001$).

Artificial diets for crustaceans must be chemically attractive to induce their location and feeding, and an addition of small amounts of chemo-stimulants might increase ingestion rates and improve food conversion. Prawn relies on combinations of sophisticated chemosensory systems to identify and locate food. The best studied chemosensory behavior in crustaceans is the selection and acquisition of food (Carr, 1988).

In the present study, the test feed containing tomato pulp showed more significance than the control feed with regular feed ingredients. In fact, when the experiments were performed to compare the acceptance between the two feed in 'Y' tank simultaneously, the control feed was completely overlooked by the prawn individuals, in 8 instances. The T1 test feed (containing 2% tomato pulp) showed less time of detection in comparison to control feed. The T2 test feed (containing 3% tomato pulp) had an even lesser time of detection and orientation when the two feed were simultaneously supplied.

Glutamic acid is the predominant free amino acid in the tomato and progressively increases in quantity with ripening. The content of glutamic acid in full ripened red tomato is more than eight times compared to the green tomato (Inaba et al., 1980). The major nucleotide in tomato is 5'-AMP. The 5'-AMP concentration also increased with ripening, especially in locular of tomato. Immature green tomato contains only about 20 mg 100 g^{-1} of glutamic acid.

Betaine has already been established as an important feed attractant in fishes as well as in prawns (Meyers, 1987; Shankar et al., 2008; Murthy et al., 2016). Betaine (Glycine Betaine, Trimethyl Glycine and Oxyneurine) is known to stimulate the olfactory bulb of prawn (Felix & Sudharshan, 2004). Tapioca and Coconut are already used as bait in the cast nets to catch the prawn from the Vembanad Lake (Harikrishnan, 1997). Also, experiments with coconut as a popular feed attractant have also been performed which provided positive results.

The amino acid glutamic acid has also been reported to be stimulatory to bottom-feeding crustaceans (Robertson et al., 1981; Rittschof & Buswell, 1989). An earlier study of amino acid specificity in *Panulirus interruptus* antennules indicated the presence of two classes of receptors responding maximally to mono- (e.g., aminobutyric acid) or dicarboxylic (e.g., glutamic acid) amino acids (Weissburg, 1990).

The results have revealed that tomato pulp can be considered as a feed attractant. Dried form of tomato produced from centrifuged tomato pulp can also be used. This is because, dried form is more stable and is less prone to bacterial infestation than tomato pulp, which contains very high percentage of fluids. However, this may act as a very easily available and relatively cheap source of attractant, considering the relatively cheap availability of glutamic acid in tomato, it has the potential as an ingredient in prawn feed and thereby reduce feed cost and maximize profit for farmers.

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