

## Behavioral response to selected feed attractants and stimulants in Pacific white shrimp, *Litopenaeus vannamei*

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### Abstract

Nine commercial feeding attractants and stimulants for *Litopenaeus vannamei* were evaluated by observation of behavioral responses in animals allotted in one Y-maze aquarium apparatus. In the validation phase, fishmeal–Brazilian origin (FMBO); fishmeal–Peruvian origin (FMPO); blood meal (BM), meat and bone meal (MBM), squid meal (SM), fish oil (FO) and fish solubles (FS) were evaluated. There was also a control without stimulatory raw material. The tested materials were included at 3% in neutral gelatin pellets (wet basis). In each behavioral observation, two different ingredients were offered at the same time in equal quantities, being allotted in the end of each chamber's arm. In Phase II after system validation, the following commercial attractants were tested: 80% crude protein (CP) vegetable dried biomass (VDB<sub>80</sub>), 68% CP vegetable dried biomass+glutamate+betaine (VDB<sub>68</sub>), complex of amino acids (alanine, valine, glycine, proline, serine, histidine, glutamic acid, tyrosine and betaine) with enzymatically digested bivalve mollusk (CAA), condensed fish soluble protein (CFSP), squid liver meal (SLM), betaine (Bet), dried fish solubles–low biogenic amines (DFS<sub>LB</sub>), dried fish solubles–high biogenic amines (DFS<sub>HB</sub>) and whole squid protein hydrolysate (WSPH). Attractants were used at a 3% level wet basis with neutral gelatin, without any additional ingredient source available. The best four commercial attractants from this phase (CAA, CFSP, SLM and WSPH) were compared under 0.5% and 1.0% levels. In Phase I of the study, a higher percentage of choices were observed for FMPO and FMBO. BM and FO were the least chosen ingredients. In Phase II, the worst results were observed for Bet, DFS<sub>HB</sub> and, mainly, for VDB<sub>80</sub> and VDB<sub>68</sub>. When two-by-two comparisons were performed, results suggested that CFSP and CAA were the best commercial attractants tested. In the last phase, both CFSP and CAA at 1.0% level were significantly more chosen by shrimp than CFSP (0.5%), SLM (0.5 or 1.0%) or WSPH (0.5 or 1.0%). At both 0.5% and 1.0% levels, shrimp spent a similar amount of time feeding on CFSP and CAA. However, only CAA was statistically better than SLM and WSPH together. Further work is required to better elucidate the chemical drivers of chemostimulation for *L. vannamei* for each one of the attractants tested.

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### 1. Introduction

As inclusion of fishmeal is confronted in commercial shrimp feeds, the role of chemoattractants becomes

crucial to preserve feed attractiveness. High inclusion of low-cost vegetable protein sources, such as soybean meal, in shrimp diets is often linked with poor performance. This can be the result of decreased feed consumption led by low food attractability and palatability. Identifying the chemosensory stimuli which shrimp normally find palatable may assist in improving ingestion of formulated diets (Holland and Borski, 1993). Arousal of shrimp feed intake can also minimize leaching of feed nutrients caused by the animal's slow feeding behavior (Peñaflorida and Virtanen, 1996).

Behavioral, physiological, and electrophysiological responses have been employed in testing chemosensitivities of decapods (Heinen, 1980). Most aquatic chemical signals identified so far among aquatic animals are soluble molecules relatively small, where amino acids predominate. Evidence that mixtures can be more stimulatory than single compounds, by either additive or synergistic interactions, has accumulated in both behavioral and electrophysiological studies (reviewed by Lee and Meyers, 1997).

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* diets. These authors concluded that *P. monodon* showed a significantly greater preference for feeds containing crustacean or krill meal. Huang et al. (2003) conducted an experiment to investigate the dietary selectivity in Chinese shrimp, *Fenneropenaeus chinensis*. The experimental diets contained one of the following ingredients: fish flesh, shrimp flesh, clam foot or polychaete worm. A commercial formulated diet and a mixed diet were also utilized. The results showed that specific growth rates were highest in the mixed diet fed group, and food conversion efficiencies were highest at polychaete worm fed group. The authors stated that Chinese shrimp possess the ability to discriminate different diets.

Artificial diets for crustaceans must be chemically attractive to induce their location and feeding, and addition of small amounts of chemostimulants might increase ingestion rates and improve growth, survival, and food conversion (Carr, 1988). The present work was carried out to evaluate nine commercial attractants and/or stimulants in *Litopenaeus vannamei* diets. Behavioral responses of individuals allotted in a Y-maze aquarium apparatus were monitored. The objectives were (1) to know whether the Y-maze apparatus is a valid methodology to evaluate attractiveness in *L. vannamei*; and (2) to measure and compare feeding stimuli of commercial attractants fed to this species.

## 2. Materials and methods

### 2.1. Shrimp and conditioning period

Attractability behavior assays were carried out with juvenile shrimp *L. vannamei* ranging in size between 6 and 12 g in body weight. Animals were collected from a commercial shrimp farm and transported alive to the laboratory. Animals were then stocked in a 1.000-l tank equipped with a biological filter and continuous aeration. Prior to any behavioral evaluations of attractability, shrimp were first subjected to an acclimation period of 1 week to adapt to laboratory rearing conditions. During the conditioning period, animals were fed *ad libitum* with a 35% crude protein diet (Camaronina 35 hp, Agribands Purina do Brasil Ltda., São Lourenço da Mata, Pernambuco, Brazil).

### 2.2. Shrimp behavioral evaluations

To start attractability sessions, shrimp were transferred to a 50-l glass aquarium equipped with a rubber carpet laid over a biological sand filter fixed on the tank bottom. To stimulate more rapid responses in feeding arousal, animals were starved for 24-h prior to any behavior observations. Attractability bioassays began by stocking one starved animal at a time in a double choice aquarium containing filtered and disinfected seawater at 33‰ salinity.

The aquarium resembled a Y-maze and measured 1.3 m × 0.3 m × 0.4 m (length × width × height). The Y-maze apparatus was equipped with an acclimation chamber, isolated by a removable glass guillotine and a rubber bottom to facilitate shrimp locomotory activity (Fig. 1). During each session, no water exchange was used to avoid the reotaxis influence over the animal's orientation towards the food source. Aeration was only used right before the delivery of the ingredient in water. For each observation, two ingredients were compared. They were offered separately in similar amounts (2 g), placed individually in the perimeter of one of the Y-maze apparatus arms. Prior to behavioral evaluations, shrimp was stocked in the acclimation chamber and allowed to acclimate to the Y-maze system for 10 min.

For each ingredient tested, one different specimen of *L. vannamei* was used. In case food was not detected within a 7-min time limit, observation was interrupted and the animal replaced by another acclimated specimen. After each session, water was discarded and completely replaced by new filtered seawater at 78 l of total aquarium volume.

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