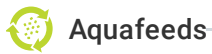




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White shrimp study compares commercial feed attractants

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Stimulatory power varies based on chemical profiles



In each behavioral observation, two different ingredients were offered to shrimp at the same time, each in equal quantity at the end of each arm of the Y-maze test apparatus.

Several studies have supported that in diets for Pacific white shrimp (*Litopenaeus vannamei*), fishmeal can be partially replaced by a number of land and vegetable protein sources without a significant cost to growth performance. However, at high substitution levels, some key nutrients can become deficient or unavailable, suppressing feed intake and deteriorating growth.

To help in the detection of food and stimulation of feed intake in low-fishmeal diets, a number of ingredients known to act as attractants and feeding incitants (squid meal, krill meal, fish solubles and bivalve meals) have been used at 5-30 kg/metric ton (MT) of feed.

In a recent study by the authors, a Y-maze aquarium apparatus was used to test the efficacy of nine commercial feeding effectors for *L. vannamei*. The Y-maze methodology distinguished the performance of the products based on shrimp behavioral responses, including the time shrimp required to detect, identify and feed on the tested feed source. Additionally, chemical analyses were carried out on the different products in order to elucidate the chemical drives in shrimp-feeding attractiveness and stimulation.

Study setup

Juvenile *L. vannamei* weighing 6 to 12 grams were allotted individually in one Y-maze aquarium. In phase I of the study, the system was validated using fishmeal made from scrap fish, anchovy fishmeal, blood meal, meat and bone meal, squid meal, fish oil and fish solubles. There was also a negative control without any stimulatory raw material. The tested ingredients were included at 3 percent in neutral gelatin pellets. In each behavioral observation, two different ingredients were offered at the same time, each in equal quantity at the end of each chamber's arm.

After system validation, the following commercial attractants were tested in phase II: 80 percent-crude protein (C.P), vegetable dried biomass (VDB₈₀), 68 percent-crude protein vegetable dried biomass plus glutamate and betaine (VDB₆₈), amino acids complex (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, dried fish solubles – low biogenic amines (DFS_{LB}), dried fish solubles – high biogenic amines (DFS_{HB}) and whole squid protein hydrolysate (WSPH).

Attractants were used at a 3 percent wet-basis level with neutral gelatin and no additional ingredient source. In this phase, soybean meal at 3 percent inclusion wet-basis was used as a negative control. In order to elucidate the chemical drivers of feeding stimulation for *L. vannamei*, each feeding effector used in phase II was evaluated in terms of soluble protein content and biogenic amines.

Results

Phase I

In the validation phase, anchovy fishmeal, fishmeal from scrap fish and squid meal were the top choices among all ingredients tested (Fig. 1) and were not rejected by *L. vannamei* on any occasion. In agreement with previous studies, this indicated their beneficial effects in stimulating shrimp feed intake.

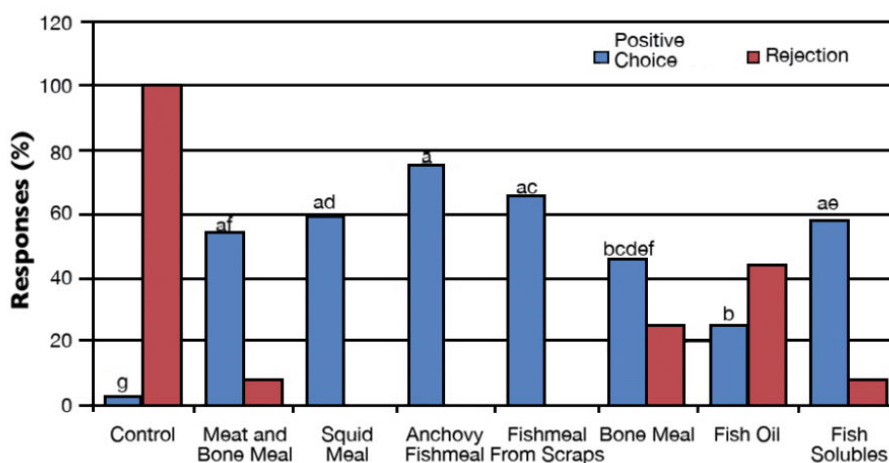


Fig. 1: Responses of individual stimulatory raw materials offered to *L. vannamei* juveniles. Blue columns with different superscript letter are statistically different ($P < 0.05$).

Ingredients of terrestrial origin were less preferred by *L. vannamei* than those of marine origin, either because of higher rejection (meat and bone meal) or lower percentage of positive choices (blood meal). Shrimp spent longer to detect fish oil than all other ingredients tested except meat and bone meal.

Fat- and oil-based materials are generally immiscible in water, and therefore are not optimal chemicals to attract, stimulate or trigger shrimp feeding responses. The minimal content of water-soluble compounds in fish oil makes it rather ineffective as a feeding activator in fish or crustacean feeds.

Phase II

In phase II, CFSP, CAA and WSPH stimulated higher feeding responses in *L. vannamei* than the other commercial attractants tested (Table 1). On the other hand, VDB₈₀ and VDB₆₈ produced the worst results for almost all experimental indicators. The remaining attractants could be categorized as medium-performance feeding stimulants.

Nunes, Attractability of commercial feed ingredients to *L. vannamei*, Table 2

Attractant	Selection (%)	Rejection (%)	Detection * (seconds)	Feeding * (seconds)	Crude Protein (%)	Soluble Protein/Crude Protein (%)
Control	20.0 ^f	22.2	–	–	46.7	66.2
VDB ₈₀	35.6 ^{ef}	37.5	381 ^b	80 ^b	79.8	13.2
VDB ₆₈	40.0 ^{def}	27.8	408 ^b	345 ^{ab}	68.1	10.1
CAA	66.7 ^{ab}	0	313 ^{ab}	495 ^a	79.6	77.9
CFSP	73.3 ^a	3.0	308 ^{ab}	374 ^{ad}	30.9	13.7

SLM	62.2 ^{abcd}	0	256 ^{ab}	364 ^{ab}	41.5	23.8
Betaine	42.2 ^{cde}	15.8	321 ^{ab}	134 ^{bcd}	70.3	0.5
DFS _{LH}	53.3 ^{abcde}	8.3	321 ^{ab}	288 ^{ab}	89.2	14.0
DFS _{HH}	46.7 ^{bcde}	19.0	363 ^b	254 ^{ab}	88.9	14.2
WSPH	60.0 ^{abcd}	0	202 ^a	406 ^{ac}	72.1	19.2

Table 1. Attractability of commercial feed ingredients to *L. vannamei*. Each comparison represents the response of one animal simultaneously exposed to two attractants

VDB80 = Vegetable dried biomass

VDB68 = Vegetable dried biomass plus glutamate and betaine

CAA = Amino acid complex with enzymatically digested bivalve mollusk

CFSP = Condensed fish-soluble protein

SLM = Squid liver meal

DFS_{LH} = Dried fish solubles – low biogenic amines

DFS_{HH} = Dried fish solubles – high biogenic amines

WSPH = Whole squid protein hydrolysate

Values with different superscripts in the same column are statistically significant ($P < 0.05$).

* Comparisons against the control diet (neutral gelatin plus soybean meal).

The superior attractiveness results for the commercial enzymatically digested bivalve mollusk product confirmed the chemosensory ability of amino acids to elicit positive feeding responses in *L. vannamei*. Further, when CAA's results were contrasted with those obtained for betaine, it evidenced that amino acids pools are better attractants for *L. vannamei* than isolated ones.

As with fish solubles, CFSP appeared to be rich in water-soluble substances that boosted feeding stimuli response. So the higher attractiveness for CFSP observed in the study is supported by the accepted premise of feeding attractants for aquatic animals.

The tested attractants ranged from vegetable biomass to squid liver meal and condensed fish protein.

Performance correlations

Regression analysis revealed no significant correlation between the soluble protein (S.P.) to crude protein (C.P.) ratio of attractants and the feeding responses they provided (Table 1). One of the highest S.P.:C.P. ratios was achieved for the gelatin pellets containing the soybean meal control, which provided the poorest feeding response. Similarly, CAA also obtained a high S.P.:C.P. ratio despite high shrimp-feeding responses.

On the other hand, there seemed to be a relationship between the levels and presence of biogenic amines and attractiveness. Tested attractants that contained only putrescine in their composition, such as the soy control and VDB₈₀, resulted in poor attractiveness. Conversely, the high feeding responses

obtained with CFSP were associated with the presence of cadaverine alone. As opposed to betaine, which also contained only cadaverine, CFSP had high concentrations of this biogenic amine.

When the sum of the total biogenic amine was either very low or very high, it also resulted in low feeding stimulation, such as observed for VDB_{68} and DFS_{HH} . The combination of putrescine with cadaverine (SLM) or cadaverine with histamine (CAA and WSPH) was also beneficial to attractiveness.

Perspectives

While the quality and freshness of feed ingredients such as fishmeal are typically measured by the total content of biogenic amines, the study revealed that some of the most powerful attractants evaluated contained some level of these compounds. Further work is required to better classify the stimulatory power of shrimp attractants based on their chemical profiles.

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