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Recent Advances on Nutrition Research of Penaeus vannamei *in Ecuador*

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ABSTRACT: This article provides a brief summary on recent advances in *Penaeus vannamei* nutrition research in Ecuador. In 1988, a study was performed to identify the lipid profile of wild shrimp larvae and broodstock, collected along the Ecuadorian coast. The average fatty acid percentages relative to total lipid content in wild larvae were: 3.24% of 18:2n-6 and 1.22% of 18:3n-3, while the percentages found for the highly unsaturated fatty acids (HUFA) eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) were 13.26 and 13.91, respectively. *P. vannamei* juveniles were found to have higher survival and growth rates (138 mg/d) when fed a diet containing 11% dietary lipid level. *P. vannamei* juveniles performed best with a diet containing 40% protein. Average weight gain was 137 mg/d. The optimum vitamin C level in artificial diets, for *P. vannamei* juveniles, was found to be 4.6 mg ascorbic acid equivalents/100 g diet. A series of experiments designed to determine digestibility of diets containing local feedstuffs are reported here.

KEY WORDS: Shrimp, *Penaeus vannamei*, protein, lipids, vitamin, digestion.

1. INTRODUCTION

Penaeid shrimp are the most important and extensively cultured crustaceans throughout the world. This is due to their great consumer demand and their high market value. They are also ideal for intensive cultivation because of their adaptability to different culture systems, rapid growth, availability of postlarvae from artificial propagation, and positive response to supplemental feeding.

In Ecuador the development of shrimp culture has been noteworthy. Shrimp culture now constitutes one of the most important sources of hard currency through exportation. Nevertheless, there is still much to learn about their nutritional requirements.

During the last decade, shrimp activity has undergone an important development in Ecuador. In 1992, 113,340 ha were being used for shrimp culture, providing a yield of approximately 113,137 metric tons, an export value of almost 526 million dollars. If we consider that food for shrimp represents around 60% of the production

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cost, food availability and quality are critical factors for the successful development of shrimp culture in Ecuador (Cámara de Productores de camarón C.P.C., 1993).

Despite the fact that the shrimp food industry for *P. vannamei* has expanded quickly, there are still some problems with respect to quality (Camba, 1992) and the cost of improving it. Frequently, due to the lack of references on nutritional requirements on this species, diets are formulated based on requirements of other penaeid shrimp. Moreover, many commercial diets contain excessive amounts of certain ingredients and often poor nutrient efficiency is observed, resulting in unfavorable nutritional balances.

This article reviews recent research on the nutritional requirements and optimization of commercial diets in Ecuador.

II. PROTEIN REQUIREMENTS

Two experiments were carried out to evaluate protein requirements of *P. vannamei* juveniles and the effectiveness of different commercial diets. The first experiment evaluated different levels of proteins in the experimental diets prepared at CENAIM, while the second compared the best protein level obtained in the first experiment with local commercial diets containing different protein levels.

For the first experiment, Camba et al. (1993) collected *P. vannamei* juveniles from the area of Palmar (Península de Santa Elena, Ecuador) and acclimated them to laboratory conditions. The animals were fed five artificial diets with protein levels ranging from 25 to 45%. Diet formulation was based on locally available animal and plant ingredients instead of using semipurified test diets. An animal:plant protein ratio of 2:1 was utilized. Biochemical analyses were performed on the five diets and shrimp juveniles (directly after collection and during the experiment).

Results showed that animals fed the three lowest protein levels (25, 30, and 35%) attained significantly ($P \leq 0.05$) lower weights (4.11, 3.50, and 3.57 g, respectively) than those fed diets containing 40 and 45% protein (6.57 and 6.52 g).

Shrimp fed diet 40% protein had the highest weight gain, significantly different ($P \leq 0.05$) from those fed the other dietary protein levels, except those fed 45% protein. No significant differences ($P \leq 0.05$) were observed among weight gains of shrimp fed the three lowest dietary protein levels (25, 30, and 35%).

In all treatments the feed conversion ratios (FCR) were relatively high, ranging from 2.30 to 3.32. The 40% protein diet provided a FCR value of 2.77. There was no difference in FCR values for the 25, 40, and 45% protein diets ($P \geq 0.05$). The protein efficiency ratio (PER = live weight gain [g]/protein eaten [g]) was high for the five treatments (0.88 to 1.34). The 25% protein diet gave a significantly higher PER value than those of the other diets. The PER values of shrimp fed at 30 to 45% protein did not differ. Colvin and Brand (1977) reported that the protein requirements of *P. californiensis, P. stylirostris,* and *P. vannamei* postlarvae were similar and varied from 30 to 35%.

P. japonicus was found to grow best when fed more than 60% protein by Deshimura and Shigeno (1976) and at least 50% purified protein according to Deshimaru and Kuroki (1975). Despite that both are *Penaeus,* protein requirements are different; otherwise, both present levels of proteins requirement over 40%.

Growth of *P. vannamei* juveniles in the present study (137 mg/d) was equal to that reported for similar-sized penaeids fed artificial diets in tanks. Fenucci et al. (1980) reported an increase of 130 mg/d obtained for *P. stylirostris* fed with a diet based on shrimp meal and Sick et al. (1972) reported a 140 mg/d increase for *P. aztecus*, fed a fish meal-based diet.

The quality of diet required to achieve maximum growth also appears to be size dependent (Chen et al., 1985); smaller shrimp being more dependent than larger ones on animal protein. The animal protein sources were shrimp head meal, fish, and squid meal. Vegetable protein sources were wheat and soybean meals and wheat bran. Under the present conditions, the best growth was obtained by feeding the 40% protein diet during 6 weeks, starting with an average weight of 0.93 g.

In the second experiment *P. vannamei* juveniles with an average weight of 1.51 ± 0.16 g were fed five artificial diets (D-40%, 35%, 40%, 38%, and 27% protein). One experimental diet (D-40%) was prepared at CENAIM, while the others were bought at local feed mills. After 8 weeks, shrimp fed diet D-40% showed the best results in terms of weight gain (6.31 g), specific growth (1.27%), and survival rate (86%) Camba et al. (1992).

The final average weight of diet D-40% (7.84 g) was significantly different ($P \leq 0.05$) from that of diet 40% (5.48 g), although both diets had equivalent protein levels. Three out of the four commercial diets tested gave significantly lower results than those of diet D-40%, in terms of weight gain, specific growth rate, and survival. The average wet weights of animals fed diets 40% and 35% were 5.48 g and 5.44 g and were not significantly different than those of diets D-40 and 38%. The animals on treatment 35% showed the lowest average wet weight (4.93 ± 0.71).

The body weight gain (BWG) in animals of diet 38% (6.86 g) was similar to that reached by diet D-40% (6.31 g). However, diet 38% obtained a weight variation coefficient (18.24%) significantly greater ($P \leq 0.05$) than that of diet D-40% (10.5 8%). The BWG of both diets were significantly different than those recorded for the other treatments (3.42, 3.99 vs. 3.94 g). The FCR's were relatively high for all diets ranging between 3.34 and 7.58. Nevertheless, the FCR of diets D-40 and 38% were significantly lower than those of the remaining treatments ($P \leq 0.05$).

According to studies by Colvin and Brand (1977), Smith et al. (1985), Akiyama (1992), and Camba et al. (1993), the optimum protein level for *P. vannamei* lays between 30 and 40%.

Therefore, all the tested diets, except diet 27%, satisfied the protein requirements; however, diet D-40 and 38% gave the best growth results. Diets D-40 and 40% showed results thoroughly different in relation of BWG, FCR, and survival, and this seems to be due to the quality of protein used in the diet's elaboration.

III. LIPIDS

A. SURVEY OF FATTY ACID PROFILES

Arellano et al. (1991) investigated fatty acid profiles of wild and hatchery *P. vannamei* larvae and broodstock. For 1 year, shrimp were collected in several zones of the Ecuadorian coast and the total lipid content and fatty acid profiles were determined in order to identify differences throughout the year and between geographical areas.

Fatty acid profiles did not fluctuate in relation to climatic changes or geographical area. The results revealed a high HUFA content compared with other fatty acids, possibly an indication of larval quality, considering the well-documented importance of these essential dietary compounds. In the case of hatchery larvae, the values of 18:2n-6 (6.67%) and 18:3n-3 (17.73%) were much higher than those observed in wild larvae, while EPA (8.08%) and DHA (1.82%) were lower than in wild larvae. Fatty acid profiles of female broodstock were also independent of season, zone, and maturation stage. Female broodstock showed the following lipidological pattern characteristics: 18:2n-9 = 15.44%, 18:2n-6 = 2.58%, 20:5n-3 = 11.10%, and 22:6n-3 = 8.38%.

We can assume that wild *P. vannamei* shrimp in Ecuadorean waters make up part of the same ecosystem, given the homogeneity of the results, at least during years with normal climatic conditions, as was the case in 1990 and 1991.

B. DIETARY LIPID UTILIZATION

In a recent study performed by León-Hing et al. (1995), lipid requirements of *P. vannamei* were investigated in a 70-d experiment in which diets of different lipid levels were fed to juvenile shrimp. Lipid analysis of the hepatopancreas was evaluated at the end of the experiment, and a determination of diet digestibility was performed with a chromic oxide analysis in feces collected during the experiment. According to different studies on lipid requirements for other species of penaeid shrimp, recommended lipid levels for commercial diets are between 6 and 8%. The level should not be higher than 10% according to Kanazawa et al. (1979).

Five experimental diets with different lipid levels of 6.31, 8.30, 11.00, 13.13, and 16.67% on dry weight base were prepared, and animals were fed twice daily. Feces were collected 1 h after feeding from day 56 onward, and stored at −20°C until analysis.

The results showed that a total lipid level between 8 and 11% can be considered suitable for *P. vannamei* juveniles. Lipid levels above 11 and below 8% produced retarded growth ($P \leq 0.05$). Sheen and Chen (1992) found that the introduction of lipid levels of 8, 10, and 12% in diets for *P. monodon* produced a significant growth gain; however, it was observed that *P. monodon* did not demonstrate a specific lipid requirement.

After 70 d, the mean wet weight of the shrimp fed a diet containing 6.31% lipid was significantly lower than mean wet weights of those fed diets with 8.30 and 11.00% lipids. There was no significant difference among diets with 6.31, 13.13, and 16.67% lipids. However, diet 11.00% had weight gains significantly higher than the others diets.

At the end of this study, survival in treatment groups ranged from 93.3 to 97.8%. Survival in the treatment with the highest lipid level (16.67%) was lower than other treatments ($P \leq 0.05$).

The best results in terms of BWG, FCR, molting frequency, and specific growth rate (SGR) were obtained using the diet with 11.00% lipid. The total lipid content of the hepatopancreas at the end of the experiment had increased from 4.07 to 14.78%, and it seemed to increase according to the dietary lipid levels. There was also a slight variation in essential fatty acid (EFA) composition, specifically of n-3

HUFA in relation to diet. The lowest lipid level produced the lowest total lipid content in the hepatopancreas ($P \leq 0.05$).

The apparent lipid digestibility values ranged from 77.53 to 90.28% and they increased with the dietary lipid level. In an experiment with diets formulated for *P. monodon* containing crude lipid levels of 9.45 to 11.92%, Catacutan (1991) observed apparent lipid digestibility values of 90 to 93%, which are higher values than those found in *P. japonicus*. Regarding dry matter digestibility, the values obtained ranged between 77.16 and 81.66% and did not increase according to the lipid level in the diet. However, the diet containing 11% lipid produced the best results (81.66% digestibility), probably due to an adequate energy:dry matter ratio.

IV. VITAMIN C REQUIREMENTS

The requirement for dietary ascorbic acid (AA) has been demonstrated in penaeid shrimp (Deshimaru and Kuroki, 1976; Guary et al., 1976; Sedgwick, 1980; Shiau and Jan, 1992; Haiqui and Lawrence, 1993). Examples of the physiological effects with insufficient levels of AA have included poor growth, poor feed conversion, reduced molting frequency or incomplete molting, decreased resistance to stress, impaired collagen synthesis and wound healing, melanized lesions underneath the exoskeleton, and high mortality (Lightner et al., 1977; Magarelli and Colvin, 1978; Hunter et al., 1979; Lightner et al., 1979; Magarelli et al, 1979; Catacutan and De la Cruz, 1989; and Merchie et al., 1995).

A stable form of vitamin C, L-ascorbyl-2-phosphate-Mg (APM), was tested for efficacy as a vitamin C source for *P. vannamei* juveniles (Montoya and Molina, 1995). Five test diets containing graded levels of APM (0, 10, 18, 33, 66 mg/100 g) were prepared for feeding trials. Molting frequency of shrimp fed 10 mg APM/100 g diet was significantly higher than that of the other groups. No differences in growth between the treatments were detected. Feed conversion ratios were not significantly different ($P \leq 0.05$) among treatments. Thus, supplementation of 10 mg APM/100 g diet was sufficient for better survival and prevention of clinical signs of vitamin C deficiency signs in *P. vannamei*.

The majority of the dead shrimp in the vitamin C-deprived treatment developed blackened lesions under the exoskeleton on the abdomen, on the carapace, in the gills, and in the foregut. Histological examination of the blackened areas in these shrimp showed the lesions typically to be present in loose connective tissues. This symptom is similar to the proposed ascorbic acid deficiency syndrome named "Black Death" (Lightner et al, 1977; Magarelli et al., 1979). This pathology was not noted in the other treatments.

V. DIGESTION

Digestibilities of various local feedstuffs for *P. vannamei* were determined by Pedrazzoli et al. (1995). Six experimental diets were evaluated. The control diet was a normal balanced artificial diet. The other treatments consisted of 50% control diet and 50% experimental ingredients that differed in each treatment. The tested ingredients were rice bran, soybean meal, fish meal, shrimp head meal, and squid meal.

Digestibility values of experimental diets, except the basal diet, ranged from 56.92 to 95.19% for dry matter; 69.80 to 93.3 1% for total lipids; and 73.09 to 90.69% for protein. Akiyama et al. (1989), working with *P. vannamei*, found apparent dry matter digestibility values for fish meal, shrimp head meal, soybean meal, squid meal, and rice bran to be 64.3, 56.8, 55.9, 68.9, and 40%, respectively.

Protein digestibility seems to coincide well with other studies by Smith et al. (1985), in which the effect of protein level and source was investigated in *P. vannamei*. Results revealed that the protein digestibility ranged between 80.7 and 84.5%. However, the lipid and dry matter digestibility they obtained were lower than those obtained in our study.

The highest negative value reported for the protein digestibility of the control diet was –113.83%. It suggests endogenous losses in the animal, involving processes such as enzymatic secretion, sloughing of gut epithelial cells, formation of the chitinous peritrophic membrane, and the secretion of other lubricative substances. It was mentioned by Akiyama et al. (1989).

The protein digestibility of the rice bran diet was high and significantly different ($P \leq 0.05$) from those of fish and shrimp head meal. For essential fatty acids, the digestibility ranged from 65.36 to 97.07%, 78.03 to 100%, 68.48 to 96.35%, and 62.57 to 95.65% for 18:2n-6, 18:3n-3, 20:5n-3, and 22:6n 3, respectively. Squid meal had the highest lipid digestibility. The squid meal, soybean meal, and fish meal in the diets proved to be most beneficial, because of their high nutritional value and their high digestibility.

VI. GENERAL CONCLUSIONS

The values reported on quantitative dietary protein requirements for *P. vannamei* ranged from 25 to 40%. Until more knowledge is available on essential amino acid

TABLE 1
Composition of Experimental Diets Containing Various Feedstuffs (Dry Weight Basis)

Componentes	D1	D2	D3	D4	D5	D6
Casein	400.00	200.00	200.30	200.00	200.00	200.00
Gelatina	100.00	50.00	50.00	50.00	50.00	50.00
Pollack liver oil	84.00	42.00	42.00	42.00	42.00	42.00
Soy oil	21.00	10.50	10.50	10.50	10.50	10.50
Lecithin	10.00	5.00	5.00	5.00	5.00	5.00
Cholesterol	5.00	2.50	2.50	2.50	2.50	2.50
Vitamin premix	35.00	17.50	17.50	17.50	17.50	17.50
Mineral premix	20.00	10.00	10.00	10.00	10.00	10.00
Dextrin	300.00	150.00	150.00	150.00	150.00	150.00
Aquatec (binder)	20.00	10.00	10.00	10.00	10.00	10.00
Chromic oxide	5.00	2.50	2.50	2.50	2.50	2.50
Experimental ingredient	—	500.00	500.00	500.00	500.00	500.00
Total	1000	1000	1000	1000	1000	1000

D1: control diet; D2: rice bran meal; D3: soybean meal, D4: shrimp head meal; D5: fish meal; D6: squid meal.

requirements, dietary protein/energy ratio and availability of isocaloric diets, determining more exact requirements will be difficult.

Recommended lipid levels for commercial shrimp feeds range from 6 to 7.5%. An optimum level of 11% is suggested. Polyunsaturated fatty acids, phospholipids, and sterols have received most attention in crustacean lipid nutrition.

Very little is known about vitamin nutrition in shrimp. Most vitamin requirements are affected by size, physiological state, environmental factors, and nutrient interrelationships. Due to the instability of ascorbic acid in feed, consideration must be given to manufacturing and storage losses sustained before feeding.

Proper nutrition of marine organisms is essential to profitable aquaculture, and effective feed depends on our knowledge of how organisms use various diet components. The types and properties of shrimp-digestive enzymes define digestive capabilities and hence the ingredients to be included in shrimp diets. Future studies will include specific mechanisms involved in digestion and assimilation.

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