Dietary protein requirement for *Litopenaeus vannamei*.[1]

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ABSTRACT: Postlarval (PL) *Litopenaeus vannamei* of 0.9 and 1.0 mg mean initial weight were reared for 20 and 21 days in fiberglass tanks in experiments 1 and 2, respectively, to determine the optimal dietary crude protein level for this species and growth stage reared in recirculating systems. Six semi-purified diets formulated to contain crude protein levels of 10, 18, and 25 %, and crude lipid levels of 3 and 11 % were evaluated in experiment 1. In experiment 2 semi-purified diets formulated to contain crude protein levels of 5, 10, 15, 20, and 25 %, and 8 % crude lipid were evaluated. Mean PL survival in experiment 1 was not significantly affected by protein level, but was significantly lower for feeds containing 11 % lipid. Mean PL survival in experiment 2 was above 90% for all treatments and no significant differences were detected. Diets with 18 and 20 % protein level were adequate to provide similar PL growth as higher protein levels in these culture conditions in experiments 1 and 2, respectively. Lipid level did not affect growth significantly in experiment 1. Quadratic regression analysis of weight gain data for experiments 1 and 2 indicated the optimal dietary crude protein level (X_{max}) to be 21.4 % and 24.5 %, respectively, while broken-line analysis indicated to be 20.2 % and 21.5 %, respectively.

KEYWORDS: protein requirement, shrimp, *L. vannamei*.

INTRODUCTION

Pacific white shrimp, *Litopenaeus vannamei* Boone 1931 (Perez-Farfante and Kensley, 1997) adapts well to a wide range of culture conditions (Lawrence *et al.* 1985) and is the species of choice for culture in the western hemisphere (Weidner and Rosenberry, 1992; Rosenberry, 1997). As shrimp farming has increased over the years, culture methods are becoming more intensive. Intensification of the culture system means higher dependence on the use of manufactured dry feed that may represent an important part, or be the single greatest expense, of the variable cost of the operation (Wyban *et al.* 1988). Protein is typically the most expensive macronutrient in shrimp feeds, thus determination of the optimal protein level is important for formulation of cost-effective feeds.

Dietary protein levels ranging from 30 to 60 % are recommended for various species and sizes of marine shrimp (Akiyama *et al*. 1992). A range of feeding habits, from carnivorous to herbivorous, has been suggested as one possible reason for the wide range in protein requirements among penaeid shrimp species (Deshimaru and Yone, 1978). Deshimaru and Shigeno (1972) showed that growth of *Penaeus japonicus* was positively correlated with the amount of protein in the diet and that squid meal was the best protein source. However, these authors recommended a mixture of protein sources to provide the required pattern of amino acids for shrimp. This recommendation was supported by Colvin (1976) who determined that a protein mixture of both fish meal and shrimp meal resulted in better growth of *P. indicus* juveniles than either fish or shrimp meal alone.

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The optimum level of dietary protein was determined to be within the range of 52-57 % for *P. japonicus* (Deshimaru and Yone, 1978) and later revised not to exceed 42 % (Koshio *et al.* 1993), 40-44 % for *P. monodon* (Alava and Lim, 1983; Shiau *et al.* 1991), and 36 % or higher for *P. vannamei* (Smith *et al.* 1985) and later revised to 30 % (Cousin *et al.* 1993) and 15 % (Aranyakananda 1993). It s generally believed that postlarval shrimp require a higher dietary protein level than older shrimp (Colvin and Brand, 1977; Chen *et al*. 1985; Goddard, 1996), and *L. vannamei* postlarvae are often fed feeds with protein levels of 35% when reared in nursery ponds with natural feed available (Villalón, 1991) whereas in intensive nursery systems they are fed diets with protein levels of 40-55% (Sturmer *et al*. 1992; Samocha *et al*. 1993; Treece and Fox, 1993). Velasco (1998) did not find significant differences between growth at 25 and 33% dietary protein levels with postlarvae.

Estimation of nutrient requirements from growth data had been determined mainly by the broken-line method and recently by nonlinear models. Robbins *et al*. (1979) compared these two methods and found that the broken-line method with its discontinuous first derivative is just a rough approximation. The consistently good fits obtained with the nonlinear models are generally preferable, despite the subjectivity that seems to enter the choice of the definition for requirement (Robbins *et al*. 1979). In a conservative approach we used both methods in the present study.

There is little information about the protein requirement during the early postlarval stage of this shrimp, thus the objective of this study was to determine the optimal dietary protein level of *Litopenaeus vannamei* postlarvae held in a recirculating culture system.

MATERIALS AND METHODS

Experiment 1

Hatchery reared *L. vannamei* postlarvae (PL_7-PL_{10}) of 0.95 ± 0.04 mg (mean ± SD) initial weight were stocked at a density of 1.5 PL L^{-1} (stocking density related to surface area was 444 PL m^{-2}) in 20 L of water in fiberglass tanks. These tanks were part of a closed-recirculating culture system equipped with a water pump, a fine sand filter, a submerged and trickling biofilters, a heat exchanger, a 50 μm cartridge filter, and UV lights. Water exchange in each tank was approximately 125% h^{-1}. Culture seawater salinity and temperature ranged from 25-27 g L^{-1} and 27-29 °C, respectively. Aeration was supplied via a single airstone within each tank and dissolved oxygen levels were maintained above 4.0 mg L^{-1}. A photoperiod of 12 h light and 12 h dark was maintained with cool fluorescent tubes by an automatic timer. Water samples were taken weekly from the sump tank and immediately analyzed for total ammonia-nitrogen (Solorzano, 1969; Spotte, 1979), and nitrite-nitrogen (Spotte, 1979; Parsons *et al.*,1989). An spectrophotometer (Model spectronic 401, Milton-Roy Co., Rochester, New York) was used to read water samples. A pH meter (Model 701A\digital ionalyzer, Orion Research Inc., Cambridge, Massachusetts) was used to measure pH.

PL were fed every 96 min with automatic feeders during the 20-day experimental period. Feed allowance was calculated according to the following feed curve, where y = mg of feed PL^{-1} day^{-1} and x = day on growth trial:

day 1 to 16 $y = 1.597 - 0.119x + 0.088x^2 - 0.003x^3$
day 17 to 20 $y = - 6.593 + 0.826x$

This feed curve was derived from previous experiments (Velasco *et al*. 1998). Daily feed amount was accurately weighed on an analytical balance (Model AE240, Mettler-Toledo Inc., Highstown, New Jersey, USA). Six semi-purified diets were formulated to contain 10, 18 and 25 % dietary protein with either 3 or 11 % lipid level (Table 1). Graded levels of protein were achieved by replacing wheat starch with wheat gluten and soybean protein isolate. Lipid level in the feed was mainly modified by replacing acid-washed diatomaceous earth with fish oil. Experimental diets were prepared by mixing dry ingredients for 25 min in a twin shell dry V-blender (maximum capacity 4 kg, Patterson-Kelley Co., East Stroudsburg, Pennsylvania, USA), and the ingredients were then transferred to a food blender (Model C-100, Hobart Manufacturing Co., Troy, Ohio, USA) where menhaden oil added into the mixture and mixed for 10 min. Then, 400 ml boiling deionized water was added per kg of diet, a further 5 min mixing was undertaken and the moist mix was extruded through a 3-mm die with a food mixer (Hobart A-200, Hobart Manufacturing Co., Troy, Ohio, USA). The resulting pellets were oven-dried at 60 °C to a moisture content of 8-10 %, crumbled to 1.0-0.5 mm particle size, and stored at -10 °C until used. Proximate analyses of diets were performed by a commercial laboratory (Woodson-Tenent Laboratories Inc., Memphis, Tennessee, USA).

Table 1 Ingredient composition and proximate analysis of test diets[1] (% as-fed basis) used in Experiment 1.

	Nominal Protein Level/Nominal Lipid Level					
	10/3	10/8	18/3	18/8	25/3	25/8
Ingredient						
Wheat starch[2]	63.4	63.4	54.4	54.4	45.5	45.5
Soybean protein isolate[2]	0	0	5.3	5.3	10.6	10.6
Wheat gluten[2]	3.5	3.5	7.4	7.4	11.3	11.3
Menhaden fish meal[3]	8.0	8.0	8.0	8.0	8.0	8.0
Krill meal[4]	4.0	4.0	4.0	4.0	4.0	4.0
Menhaden fish oil[3]	0	8.2	0	8.2	0	8.2
Lecithin[5]	1.5	1.5	1.5	1.5	1.5	1.5
Cholesterol[2]	0.5	0.5	0.5	0.5	0.5	0.5
Carboxymethylcellulose[6]	4.0	4.0	4.0	4.0	4.0	4.0
Diatomaceous earth[2,7]	8.2	0	8.2	0	8.2	0
Na_2HPO_4 reagent[8]	1.9	1.9	1.7	1.7	1.4	1.4
Mineral mixture AIN 76[2,9]	42	4.2	4.2	4.2	42	4.2
Vitamin mixture[10]	0.5	0.5	0.5	0.5	0.5	0.5
Stay-C® (25% active) [11]	0.3	0.3	0.3	0.3	0.3	0.3
Proximate analysis						
Crude protein	10.9	11.1	18.9	20.0	26.1	26.6
Crude lipid	2.7	8.2	2.6	12.3	2.9	11.1
Ash	15.6	7.3	15.5	79	154	7.3
Moisture	7.8	8.8	7.5	85	79	7.3

[1]Each diet was formulated to contain 4.0 % fiber and 1.3 % total phosphorus (Ca:P of 1.0:1.3).
[2]I.C.N. Biochemicals Inc., Cleveland, Ohio, USA.
[3]Zapata Haynei Corp., Reedville, Virginia, USA.
[4]Inual, Santiago, Chile.
[5]Central Soya, Fort Wayne, Indiana, USA.
[6]United States Biochemicals, Cleveland, Ohio, USA.
[7]Acid washed.
[8]Fisher Scientific, Houston, Texas, USA.

[9]Composition (g kg^{-1}): calcium phosphate dibasic (500.0), chromium potassium sulfate (0.55), cupric carbonate (0.3), ferric citrate (6.0), magnesium oxide (24.0), manganous sulfate (3.5), potassium citrate monohydrate (220.0), potassium iodate (0.01), potassium sulfate (52.0), sodium chloride (74.0), sodium selenite (0.01), sucrose (118.0), zinc carbonate (1.6).
[10]Dawes Laboratories, Arlington Heights, Illinois, USA. Composition (g kg^{-1}): retinol (22.2), cholecalciferol (1.1), tocopherol (10.3), menadione (2.3), thiamine (5.0), riboflavin (5.7), pyridoxine (10.1), niacin (10.9), pantothenic acid (10.9), biotin (0.2), choline (0.8), folic acid (3.5), cyanocobalamine (0.02), dextrin (917.0).
[11]L-Ascorbyl-2-Polyphosphate. Hoffman-LaRoche Inc., Nutley, New Jersey, USA.
At the beginning and end of the growth trial PL were air blotted, weighed and counted. Survival, weight gain, relative growth rate [(mean wet weight increase/mean initial wet weight) x 100] (Hopkins 1992), and feed conversion ratio (weight of dry feed fed/mean wet weight increase) were calculated.

The experiment was run as a completely randomized design with 6 replicates per treatment. Data were analyzed using two-way analysis of variance. Tuckey-Kramer Honest Significant Difference test for mean separation was used to evaluate significant differences ($P < 0.05$) among treatment means (Lentner and Bishop 1993). All statistical analyses were performed using Statistical Analysis System procedures (SAS Institute, Inc., Cary, North Carolina, 1989-91.JMP version 2.0.5). PL weight gain results were fitted to quadratic and broken-line regression models (Robbins 1986) to estimate the optimal dietary protein level.

Experiment 2

In an attempt to validate and improve the results of the first experiment, we conducted a second experiment using a new group of postlarvae (PL_7-PL_{10}) stocked at an initial weight of 1.0 ± 0.05 mg (mean ± SD). PL were stocked and managed as described for experiment 1, except culture seawater salinity and temperature ranged from 22-24 g L^{-1} and 29-31 °C, respectively.

Diets were provided according to the following feed curve, where y = mg of feed PL^{-1} day^{-1} and x = day on growth trial:

$$\text{day 1 to 21} \quad y = 0.949 + 0.287x + 0.041x^2 - 0.002x^3$$

Five semi-purified diets were formulated to contain 5, 10, 15, 20 and 25 % dietary protein, and 8 % lipid level (Table 2). Experimental conditions and procedures were the same as those described for experiment 1. Data obtained from this completely randomized design with 7 replicates per treatment were analyzed as described for experiment 1.

Table 2 Ingredient composition and proximate analysis of test diets[1] (% as-fed basis) used in Experiment 2.

	5	10	15	20	25
Ingredient[2]					
Wheat starch	71.6	66.4	61.2	56.0	50.8
Soybean protein isolate	0	5.3	10.7	16.0	21.4
Menhaden fish meal	4.0	4.0	4.0	4.0	4.0
Krill meal	4.0	4.0	4.0	4.0	4.0
Menhaden fish oil	6.4	5.9	5.3	4.8	4.2
Lecithin	1.5	1.5	1.5	1.5	1.5
Cholesterol	0.5	0.5	0.5	0.5	0.5
Carboxymethylcellulose	4.0	4.0	4.0	4.0	4.0
Diatomaceous earth	3.0	3.3	3.6	3.9	4.2
Mineral mixture AIN 76	4.2	4.2	4.2	4.2	4.2
Vitamin mixture	0.5	0.5	0.5	0.5	0.5
Stay-C® (25% active)	0.3	0.3	0.3	0.3	0.3
Methionine[3]	0	0.08	0.16	0.24	0.32
Arginine[3]	0	0.02	0.05	0.08	0.10
Proximate analysis					
Crude protein	6.0	10.7	15.4	18.2	25.1
Crude lipid	9.3	8.4	7.7	7.5	7.0
Ash	7.9	8.3	8.9	9.4	9.9
Moisture	6.0	6.0	7.3	7.4	6.0

[1]Each diet was formulated to contain 4.0 % fiber and 0.8 % total phosphorus (Ca:P of 1.1:1.0).
[2]See details in Table 1.
[3]Crystalline form.

RESULTS

Experiment 1

Average concentrations of total ammonia-nitrogen and nitrite-nitrogen throughout the trial were below 0.1 (pH = 8.0) and 0.8 mg L^{-1}, respectively. Mean PL survival (Table 3) ranged from 66 to 92% and was not significantly affected by protein level ($P = 0.0842$), but was significantly lower for diets containing 11 % ($P = 0.0158$). The interaction between these two factors was not significant ($P = 0.6656$). Mean PL weight gain (Table 3) tended, but was not significantly affected by protein ($P = 0.0539$) or lipid level ($P = 0.4584$). The interaction between these two factors was not significant ($P = 0.6988$). Because PL growth was not significantly affected by lipid level weight gain data was pooled by protein level and fitted to curvilinear and broken-line regression models. Quadratic regression analysis of weight gain data indicated the optimal protein level to be 21.4 %, while broken-line analysis indicated 20.2 % (Fig. 1).

Table 3. Experiment 1: Biological performance of *Litopenaeus vannamei* postlarvae fed experimental diets for 20 days. Entries are sample mean ± SD. Number of replicates per treatment = 6.

Lipid level (%)	3			11		
Nominal Protein level (%)	10	18	25	10	18	25
Survival (%)	75.8 ±13.7	82.2 ±8.0	92.5 ±11.0	66.7 ±6.6	74.2 ±7.4	75.8 ±13.7
Initial weight (mg)	0.95 ±0.04	0.95 ±0.04	0.95 ±0.04	0.95 ±0.04	0.95 ±0.04	0.95 ±0.04
Weight gain (mg)	64.4 ±11.8	72.4 ±10.8	76.7 ±10.9	58.3 ±5.3	74.1 ±7.0	71.4 ±12.9
Relative growth rate (%)	6776 ±1246	7618 ±1136	8078 ±1145	6141 ±557	7797 ±735	7516 ±1364
Feed conversion ratio	2.4	1.9	1.6	3.0	2.1	2.1

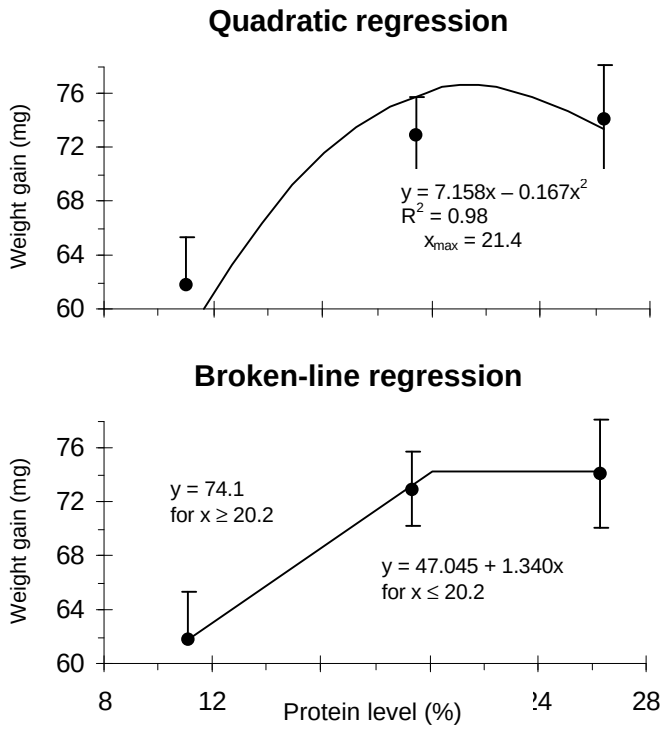

Figure 1 Quadratic and broken-line plots of weight gain (mean ± SE) of *Litopenaeus vannamei* postlarvae fed graded levels of protein as determined by proximate analysis (Table 1) in Experient 1.

Experiment 2

Average concentrations of total ammonia-nitrogen and nitrite-nitrogen throughout the trial were below 0.2 (pH = 8.2) and 0.8 mg L^{-1}, respectively. Mean PL survival (Table 4) ranged 93.3 to 98.9% and no significant differences were detected (P = 0.4666). Mean PL weight gain (Table 4) was significantly different (P = 0.0001). PL growth was significantly lower for diets containing 15 % or lower protein compared to diets with 20 and 25 % protein. Growth of PL fed diets with 20 and 25 % protein was not significantly different. Quadratic regression analysis of weight gain data indicated the optimal protein level to be 24.5 %, while broken-line analysis indicated 21.5 % (Fig. 2).

Table 4 Experiment 2: Biological performance of *Litopenaeus vannamei* postlarvae fed experimental diets for 21 days. Entries are sample mean ± SD. Number of replicates per treatment = 7. Values in the same row with different letters indicate significant differences (P < 0.05).

Nominal Protein level (%)	5	10	15	20	25
Survival (%)	93.3	98.0	98.9	95.7	98.1
	±10.3	±3.0	±2.7	±6.0	±3.2
Initial weight (mg)	1.0	1.0	1.0	1.0	1.0
	±0.05	±0.05	±0.05	±0.05	±0.05
Weight gain (mg)	47.3	56.3	67.4	81.3	87.5

	$\pm 6.3_{a}$	$\pm 2.9_{a,b}$	$\pm 3.7_{b}$	$\pm 9.7_{c}$	$\pm 9.2_{c}$
Relative growth rate (%)	4730	5629	6740	8132	8747
	$\pm 634_{a}$	$\pm 296_{a,b}$	$\pm 367_{b}$	$\pm 974_{c}$	$\pm 920_{c}$
Feed conversion ratio	2.7	2.2	1.8	1.5	1.4

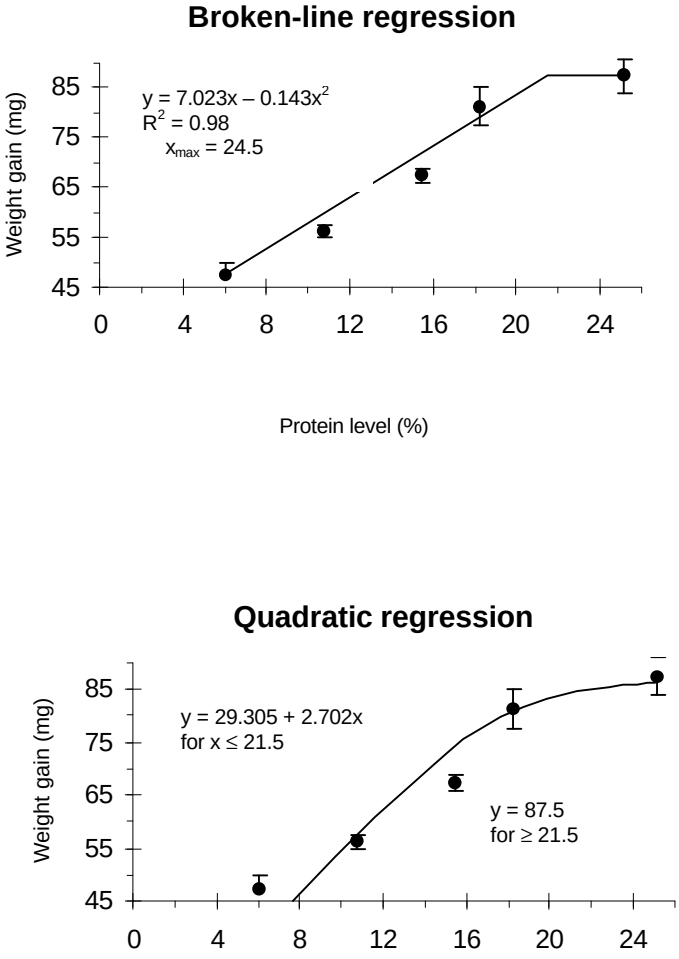

Figure 2 Quadratic and broken-line plots of weight gain (mean ± SE) of *Litopenaeus vannamei* postlarvae fed graded levels of protein as determined by proximate analysis (Table 2) in Experiment 2.

DISCUSSION AND CONCLUSIONS

During both growth trials total ammonia-nitrogen and nitrite-nitrogen concentrations in the water were below those reported as toxic for penaeid species (Wickins 1976). High postlarvae (PL) survival was obtained with these experimental conditions for both experiments. No significant differences were detected on PL weight gain for diets formulated to contain 18 % or higher protein levels in both experiments. PL growth was significantly decreased when fed diets containing 15 % protein indicating that essential amino acids were already limiting at this protein level. Only for comparison, Table 5

presents protein, intake energy, and amino acid values determined by a commercial laboratory (Woodson-Tenent Laboratories Inc., Memphis, Tennessee, USA) for diets formulated to contain 15 and 20 % protein in experiment 2. Research to optimize dietary amino acid levels based on these values (Table 5) is warranted.

Table 5 Actual protein, intake energy, and amino acids except tryptophan, of two experimental diets fed in experiment 2. Weight gain of *Litopenaeus vannamei* postlarvae was significantly lower (Table 4) when fed the diet containing 15 % protein compared to the diet containing 20 %.

Calculated protein level (%)	15 [limiting]	20 [adequate]
Protein level (%)	15.4	18.2
Intake energy level (kcal g^{-1})	3.8	4.0
Essential amino acids (g kg^{-1})		
Arginine	9.7	13.0
Histidine	3.0	4.0
Isoleucine	6.2	8.2
Leucine	10.5	13.8
Lysine	9.7	12.3
Methionine	4.3	5.4
Phenylalanine	6.5	8.6
Threonine	5.6	7.3
Valine	6.6	8.6
Non-essential amino acids (g kg^{-1})		
Alanine	6.6	8.2
Aspartate	16.1	21.5
Cystine	1.6	2.0
Glutamate	19.8	26.7
Glycine	6.6	8.3
Proline	7.0	9.4
Serine	6.7	9.0
Tyrosine	3.0	4.0

Fitting of PL weight gain data to curvilinear and broken-line models (Figs. 1 and 2) resulted in optimal dietary protein estimations of 21.4 (X_{max}) and 20.2 % for experiment 1, and of 24.5 (X_{max}) and 21.5 % for experiment 2, respectively. Calculation of the 90% of the upper asymptote of the quadratic fits as has been used by Baker *et al*. 1996 resulted in protein estimates of 19.3 and 22.0 % for experiments 1 and 2, respectively. These two protein estimates are in close agreement with those obtained with broken-line analysis especially for experiment 2, where more data points were available to fit the models. An optimal protein estimate of 22.0 % was obtained at 90% of X_{max} with quadratic regression while an estimate of 21.5 % was obtained with broken-line analysis in experiment 2. The optimum protein level estimates found in this study are lower than those previously reported for larger size shrimp of the same species (Smith *et al.* 1985; Cousin *et al.* 1993) and lower than those fed commercially (Villalón, 1991; Sturmer *et al.* 1992; Samocha *et al.* 1993; Treece and Fox, 1993), but higher than those reported by Aranyakananda (1993). However, it is important to remember that determination of the optimum dietary protein level is also affected by the level of other components in the feed (Andrews *et al*. 1972; Sedgwick 1979), quality of the protein (Smith *et al.* 1985), water quality (Shiau *et al*. 1991; Robertson *et al*. 1993a; Wyban *et al*. 1995), and experimental factors such as stocking density, daily ration size, and feeding frequency (Teshima and Kanazawa 1984; Robertson *et al*. 1993b), which makes comparison of results difficult.

Optimal protein level for *Litopenaeus vannamei* postlarvae was determined on the basis of growth response. Figures 1 and 2 show best-fit asymptotic and broken-line curves for weight gain as a function of protein level. Use of these curves and the equations describing them can be used as a basis for selection of protein level that will maximize economic returns. Further research to optimize the protein-amino acid to energy ratio for this species should be conducted.

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REFERENCES

Akiyama, D.M., Dominy, W.G., Lawrence A.L., 1992. Penaeid shrimp nutrition. In: Marine Shrimp Culture: Principles and Practices (Fast, A.L. & Lester, L.J. eds.), pp. 535-568. Elsevier Science Publishing Inc., New York, New York, USA.

Alava, V.R., Lim, C., 1983. The quantitative protein requirement of *Penaeus monodon* juveniles in a controlled environment. *Aquaculture*, 30, 53-61.

Andrews, J.W., Lowell, V.S., Garry J.B., 1972. The influence of dietary protein and energy levels on growth and survival of penaeid shrimp. Aquaculture, 1, 341-347.

Aranyakananda, P., 1993. Dietary protein and energy requirements of *Penaeus vannamei* and the optimal protein to energy ratio. Ph.D. Dissertation, Texas A&M University, College Station, Texas, USA.

Baker, D.H., Fernandez, S.R., Webel, D.M., Parsons C.M., 1996. Sulfur amino acid requirement and cystine replacement value of broiler chicks during the period three to six weeks posthatching. Poultry Sci., 75, 737-742.

Chen, H.-Y., Zein-Eldin, P., Aldrich, D.V. (1985) Combined effects of shrimp size and dietary protein source on the growth of *Penaeus setiferus* and *Penaeus vannamei.* J. World Maricult. Soc., 6, 288-296.

Colvin, P.M., 1976. Nutritional studies on penaeid prawns: protein requirements in compounded diets for juvenile *Penaeus indicus* (Milne Edwards). Aquaculture, 7, 315-326.

Colvin, L.B., Brand, C.W., 1977. The protein requirement of penaeid shrimp at various life cycle stages in controlled environment systems. J. World Maricult. Soc., 8, 821-840.

Cousin, M., Cuzon, G., Blanchet, E., Ruelle, F., AQUACOP., 1993. Protein requirements following an optimum dietary energy to protein ratio for *Penaeus vannamei* juveniles. In: Proceedings of the Aquaculture Feed Processing and Nutrition Workshop: Fish Nutrition in Practice (Kaushik, S.J. & Luquet, P. eds.), pp. 599-606. INRA, Paris, France.

Deshimaru, O., Shigueno, K., 1972. Introduction to the artificial diet for prawn *Penaeus japonicus*. Aquaculture, 1, 115-133.

Deshimaru, O., Yone, Y., 1978. Optimum level of dietary protein for prawn. Bull. of the Jpn. Soc. of Sci. Fish., 44, 1395-1397.

Goddard, S., 1996. Feed Management in Intensive Aquaculture. Chapman and Hall, New York, New York, USA.

Hopkins, K.D**.,** 1992. Reporting fish growth: a review of the basics. J. World Aquacult. Soc., 23, 173-179.

Koshio, S., Teshima, S., Kanazawa, A., Watase, T., 1993. The effect of dietary protein content on growth, digestion efficiency and nitrogen excretion of juvenile kuruma prawns, *Penaeus japonicus*. Aquaculture, 113, 101-114.

Lawrence, A.L., McVey, J.P., Huner, J.V., 1985. Penaeid shrimp culture. In: Crustacean and Mollusk Aquaculture in the United States (Huner, J.P. & Brown, E.E. eds.). AVI Publishing Co., Inc., Westport, Connecticut, USA.

Lentner, M., Bishop, T., 1993. Experimental Design and Analysis. Valley Book Company, Blacksburg, Virginia, USA.

Parsons, T.R., Maita, Y., Lalli, C.M., 1989. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press Inc., Elmsford, New York, USA.

Perez-Farfante, I., Kensley, B., 1997. Penaeoid and Sergestoid Shrimps and Prawns of the World: Keys and Diagnoses for the Families and Genera. Editions of the National Museum of Natural History, Paris, France.

Preston-Mercer, L., Dodds, S.J., Gustafson, J.M., 1986. The determination of nutritional requirements: a modeling approach. Nutr. Rep. Int., 34, 337-350.

Robbins, K. R., 1986. A method, SAS program, and example for fitting the broken-line to growth data. Univ. Tenn. Agric. Exp. Sta. Res. Rpt., 86-09.

Robbins, K. R., Norton, H.W., Baker, D.H., 1979. Estimation of nutrient requirements from growth data. *J. Nutr.*, 109, 1710-1714.

Robertson, L., Lawrence, A.L., Castille, F.L., 1993a. Interaction of salinity and feed protein level on growth of *Penaeus vannamei.* J. Appl. Acuacult., 2, 43-54.

Robertson, L., Lawrence, A.L., Castille, F.L., 1993b. Effect of feeding frequency and feeding time on growth of *Penaeus vannamei* (Boone). Acuacult. Fish. Manage., 24, 1-6.

Rosenberry, B., 1997. World Shrimp Farming (Rosenberry, B. ed.), Shrimp News International, San Diego, California, USA.

Samocha, T.M., Lawrence, A.L., Bray, W.A., 1993. Design and operation of an intensive nursery raceway system for penaeid shrimp. In: Handbook of mariculture: crustacean aquaculture (MacVey, J.P. ed.), pp. 173-210. CRC Press Inc., Boca Raton, Florida, USA.

Sedgwick, R.W., 1979. Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merguiensis* De Man. Aquaculture, 16, 7-30.

Shiau, S.-Y., Kwok, C.-C., Chou, B.-S., 1991. Optimal dietary protein level of *Penaeus monodon* reared in seawater and brackishwater. Nippon Suisan Gakkaishi, 57, 711-716.

Smith, L.L., Lee, P.L., Lawrence, A.L., Strawn, K.**,** 1985. Growth and digestibility by three sizes of *Penaeus vannamei* Boone: effects of dietary protein level and protein source. Aquaculture, 46, 85-96.

Solorzano, L., 1969. Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol. Oceanogr., 14, 799-801.

Spotte, S., 1979. Fish and Invertebrate Culture: Water Management in Closed Systems. John Wiley & Sons Inc., New York, New York, USA.

Sturmer, L.N., Samocha, T.M., Lawrence, A.L., 1992. Intensification of penaeid nursery systems. In: Marine Shrimp Culture: Principles and Practices (Fast, A.W. & Lester, L.J. eds.), pp 321-344. Elsevier Science Publishing Inc., New York, New York, USA.

Teshima, S., Kanazawa, A., 1984. Effects of protein, lipid, and carbohydrate levels in purified diets on growth and survival rates of the prawn larvae. Bull. Jap. Soc. Sci. Fish., 50, 1709-1715.

Treece, G.D., Fox, J.M., 1993. Design, Operation and Training Manual for an Intensive Culture Shrimp Hatchery. Texas A&M University Sea Grant College Program. TAMU-SG-93-505. College Station, Texas, USA.

Velasco M., 1996. Effects of dietary protein:energy ratio and phosphorus level on growth and survival of *Penaeus vannamei* postlarvae and on aquacultural water quality. Ph.D. Dissertation, Texas A&M University, College Station, Texas, USA. 102 pp.

Velasco, M., Lawrence, A.L., Neill, W.H., 1998. Development of a static-water ecoassay with microcosm tanks for postlarval *Penaeus vannamei*. Aquaculture 161: 79-87.

Villalón, J.R., 1991. Practical Manual for Semi-intensive Commercial Production of Marine Shrimp. Texas A&M University Sea Grant College Program. TAMU-SG-91-501. College Station, Texas, USA.

Weidner, D., Rosenberry, B., 1992. World shrimp farming. In: Proceedings of the Special Session on Shrimp Farming (Wyban, J. ed.). The World Aquaculture Society, Baton Rouge, Lousiana, USA.

Wickins, J.F., 1976. The tolerance of warm-water prawns to recirculated water. Aquaculture, 9, 19-37.

Wyban, J.A., Sweeney, J.N., Kanna, R.A., 1988. Shrimp yields and economic potential of intensive round pond systems. J. World Aquacult. Soc., 19, 210-217.

Wyban, J.A., Walsh, A.W., Godin, D.M., 1995. Temperature effects on growth, feeding rate and feed conversion of the Pacific white shrimp (*Penaeus vannamei*). Aquaculture, 138, 267-279.