Metabolic requirement for protein by pacific white shrimp, *Litopenaeus vannamei.*[1]

Nasir Kureshy and D. Allen Davis*

The University of Texas at Austin, Marine Science Institute, Fisheries and Mariculture Laboratory, 750 Channel View Drive, Port Aransas, Texas, 78373-5015.
*The Department of Fisheries and Allied Aquacultures, 203 Swingle Hall, Auburn University, Al. 36849-5419. Phone 334-844-9312 Fax 334-844-9208. E-mail: ddavis@acesag.auburn.edu

ABSTRACT: The dietary protein requirement of penaeid shrimp is an important nutritional consideration because it is a major limiting nutrient for growth. In most cases, research has focused on dietary protein levels rather than on the actual requirements for protein. In this study, four 28 day feeding trials were conducted to determine the maintenance requirement for protein (protein required to maintain body functions with all other nutrients provided in adequate amounts) by juvenile and sub-adult shrimp. Shrimp were offered practical diets containing 16 or 32% crude protein. In order to estimate the maintenance requirement, weight gain was regressed against daily protein ration. Juvenile shrimp were found to have maintenance protein requirements in the range of 1.8-3.8 g dietary protein/kg body weight/day (g DP/ (kg BW * d)), and sub-adult shrimp were found to have maintenance protein requirements in the range of 1.5-2.1 g DP/ (kg BW * d). Four additional 28-day trials were conducted to determine the protein requirement for maximum growth by juvenile and sub-adult shrimp. On an isoproteic basis, the 16% protein diet produced significantly lower weight gain, feed efficiency, and protein conversion efficiency values than the 32% protein diet for both the juvenile and sub-adult shrimp. The 48% protein diet produced significantly lower weight gain in the juvenile shrimp, but there was no significant effect in the sub-adult shrimp. Feed efficiency values were higher for shrimp fed the 48% protein diet as compared to those offered the 32% protein diet. Broken line analysis was conducted on the growth responses for each diet and each size of shrimp in order to determine the protein requirement for maximum growth. Protein requirement for maximum growth of juvenile shrimp was found to be 46.4 g DP/ (kg BW * d) when fed a 32% protein diet and 43.4 g DP/ (kg BW * d) when fed a 48% protein diet. Sub-adult shrimp exhibited a maximum protein requirement of 23.5 g DP/ (kg BW * d) when fed a 32% protein diet and 20.5 g DP/ (kg BW * d) when fed a 48% protein diet. In summary, FE increased with the protein content of the diet and decreased with increasing feeding rates. Weight gain corresponded to daily protein intake. Based on these results a wide range of dietary protein levels could be used to produce maximum weight gain. However, due to restriction on feed intake and consequently protein intake, low protein diets may not support maximum growth.

KEY WORDS: Protein requirements, shrimp, *L. vannamei*, weight gain.

INTRODUCTION

The dietary protein requirement of penaeid shrimp is an important nutritional consideration because protein is a major limiting nutrient for growth and is one of the primary cost components of prepared feeds. Additionally, protein content of the feed and dietary availability can affect water quality via nitrogen excretion. Protein that is assimilated for energy and not deposited for growth can contribute to

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release of nitrogen metabolites into the culture medium (Cho *et al.* 1994). A build-up of residual nitrogen metabolites can result in the eventual eutrophication of the culture medium and of effluent receiving streams. For these reasons there is an interest in developing “environmentally friendly” feeds containing the least amount of protein necessary for optimal growth.

Protein requirement has been defined, by Guillaume (1997), as the minimum or the maximum amount of protein needed per animal per day. Protein requirements change with respect to changes in biotic factors (e.g., species, physiological state, size) and dietary characteristics (e.g., protein quality, energy:protein ratio). Abiotic factors such as temperature and salinity may also affect the protein requirement (Guillaume 1997). The protein requirement of a given species is often based on the response (e.g., growth, feed efficiency, protein conversion efficiency) of the animal to varying levels of dietary protein under a given set of circumstances. Hence, the requirement is generally described as the optimal protein content of the diet. However, a low level of dietary protein could be compensated for by higher ingestion. As a result, the optimal dietary protein level could show substantial variation. For this reason, protein requirement levels would be better reported as the amount of protein needed per animal or per biomass per day and should be adjusted for digestibility of the diet utilized.

The maintenance requirement for protein can be defined as the level of protein required for maintaining body functions associated with protein metabolism, with all other nutrients having been provided in adequate amounts (Guillaume, 1997). By determining the maintenance requirement, a better understanding of the basic metabolic needs of the organism could be achieved. With this knowledge, rationing of feed to maintain basal metabolism could allow for extended holding of shrimp at minimum cost once marketable size is reached or under adverse culture conditions. The requirement for protein resulting in maximum weight gain would have to be determined if maximum growth rates are to be attained.

To date, little research has been undertaken to determine the quantitative protein requirements for maintenance and/or maximum growth of penaeid shrimp, although they have been determined for several fish species. McGoogan and Gatlin (1997) determined the metabolic requirements of juvenile red drum, *Sciaenops ocellatus*. By feeding incremental levels of a 36.5% protein diet, the maintenance requirement was estimated as 1.5-2.5 g dietary protein/kg body weight per day (g DP/(kg BW*d)). A maximum growth rate requirement of 20-25 g DP/(kg BW*d) was also determined. Similarly, Gatlin *et al.* (1986) evaluated the protein requirements of channel catfish (*Ictalurus punctatus*). In this study, incremental feeding rates, ranging from 0-5% of body weight, were used with diets containing 25% crude protein and 35% crude protein. The maintenance protein requirement was 1.32 g DP/(kg BW*d) and the maximum growth rate requirement was 8.75 g DP/(kg BW*d).

Although a quantitative requirement has not been determined for penaeid shrimp, several studies have evaluated the effect of variation in dietary protein level on growth and feed conversion. Colvin and Brand (1977) fed postlarval and juvenile *Litopenaeus vannamei* semi-purified diets of 25, 30, 35, and 40% crude protein over a four week period. Feed conversion was found to be significantly lower only for shrimp fed the 25% crude protein diet. The dietary protein requirement by postlarval shrimp was reported as 30-35%. Also, it was determined that juveniles had a dietary protein requirement of less than 30%.

Optimal dietary protein level for juvenile *L. vannamei* was also assessed by Aranyakananda *et al*. (1993). In this study, no differences in growth were shown by shrimp fed diets containing 25, 35, and 45% crude protein. When shrimp were fed diets containing 10, 15, 20, and 25% crude protein, growth of shrimp fed the 10% protein diet was significantly less than that of shrimp fed higher protein levels. Growth of shrimp fed 15% protein diets, with lipid levels at 4 and 8%, was not different from that of higher protein diets. The authors concluded that the maximum dietary protein level was 15%, with an optimal energy to protein ratio of 28.57 kcal/g protein. Since *ad libitum* feed levels were used in this study, feed consumption was not quantified. As a result, protein utilization and feed intake could not be determined.

Cousin *et al*. (1993) evaluated growth of *L. vannamei* fed feeds in which crude protein ranged from 18% to 34%. The protein source for these diets was a 1:1 mixture of casein and crab protein concentrate. The energy to protein ratio was maintained at approximately 10 kcal/g protein. Results showed a significant effect of protein level on growth. The optimal dietary protein level was approximated as 30% but an actual protein requirement for maximum growth in terms of daily intake was not determined.

It has also been shown with shrimp that size (weight) affects growth response relative to the protein content of the diet. Smith *et al*. (1984) studied the growth response of three sizes of *L. vannamei* (4.0, 9.8, and 20.8g) fed diets containing 22, 29, and 36% crude protein for a period of 30 days. Dietary protein content only affected growth for 4.0 g shrimp, with a significant increase in weight gain corresponding to the increase in dietary protein content. However, the range of protein levels of diets used in this study was too narrow to estimate an optimum protein level. Results indicated that a dietary protein level in excess of 36% would be required to yield a maximum growth rate for the 4.0 g shrimp. No relationship between dietary protein level and growth was shown by either of the two larger sizes of shrimp.

The effect of dietary protein level on growth of pond-reared *L. vannamei* has also been studied. Growth of juvenile shrimp (0.3 g and 1.9 g) was evaluated using diets containing 20 and 40% crude protein (Teichert-Coddington *et al*. 1995). Shrimp were fed according to the following relationship between feeding rate and mean individual shrimp weight:

$$Y = 11.74 - 6.79\text{Log}_{10}X$$

where Y = percentage of wet biomass fed as dry feed, and X = mean individual shrimp weight. A weekly mortality of 0.5% was assumed, and mean weight of shrimp was determined weekly. Results showed that dietary protein level had no significant effect on the final mean weight of the shrimp. This agrees with previous research in which *L. vannamei* were grown in outdoor ponds and fed feeds containing 25 to 35% crude protein (Teichert-Coddington *et al*. 1988). However, neither of these studies reported the influence of natural productivity, which would have served as an additional source of protein to the shrimp and the actual intake of feed was not determined hence feeding rates could have been in excess of the requirement.

Research on the protein requirements of penaeid shrimp, *L. vannamei* in particular, has apparently been largely concentrated within evaluations of optimal dietary protein level and not with quantitative protein requirement. Hence, the various studies reviewed have reported optimal protein level ranging from 15% to more than 36%. Since *ad libitum* feeding was used in most of these studies, the amount of protein fed and consequently the protein requirements for maintenance and maximum growth were not determined. Quantification of the requirement levels and the growth parameters associated with such feeding levels could provide information assisting in the maximization of production of *L. vannamei*.

The objectives of this study were to ascertain the maintenance requirement for protein and the protein requirement for maximum growth of juvenile and sub-adult *L. vannamei*.

MATERIALS AND METHODS

Test Diets and Feed Preparation

Diets utilized in this study were formulated to contain 16, 32, and 48% crude protein with calculated energy:protein ratios of 25.1 kcal/g protein, 12.57 kcal/g protein, and 8.90 kcal/g protein, respectively (Table 1). Feed ingredients were ground with a laboratory hammer-type mill using a #24 screen (0.609 mm). Dry ingredients and fish oil were mixed in a food mixer (Hobart Corp., Troy, Ohio), with hot water blended in to attain a consistency appropriate for pelleting. Each feed was extruded through a 2mm die in a meat grinder and dried to a moisture content of less than 10%. Feeds were refrigerated and subsequently crumbled to the desired pellet size prior to feeding.

Table 1. Composition (g/100g dry weight) of diets formulated to contain 16% (TRT 16), 32% (TRT 32), and 48% (TRT 48) crude protein.

	TRT 16	TRT 32	TRT 48
Menhaden fish meal[a]	8.00	16.00	24.00
Soybean meal[b]	16.85	33.70	50.55
Fish solubles	0.50	1.00	1.50
Menhaden fish oil[c]	3.80	3.80	8.60
Wheat gluten[d]	2.50	5.00	7.50
Wheat starch[d]	64.03	36.38	3.90
Aqualipid 95[e]	1.30	1.30	1.30
Shrimp trace mineral premix[f]	0.50	0.50	0.50
Shrimp vitamin premix[g]	2.00	2.00	2.00
Stay C (150 mgC/kg)[h]	0.12	0.12	0.05
CaP-monobasic[i]	0.40	0.20	0.10

[a]Special Select™, Zapata Protein USA Inc., Randeville, Louisiana, USA.
[b]Solvent extracted, Producers Coop, Bryan, Texas, USA.
[c]Omega Protein Inc., Reedville, Virginia, USA.
[d]United States Biochemical Corporation, Cleveland, Ohio, USA.
[e]Aqualipid 95, Central Soya Chemurgy Division, Fort Wayne, Indiana, USA
[f]g/100g premix: cobalt chloride 0.004, cupric sulfate pentahydrate 0.250, ferrous sulfate 4.0, magnesium sulfate heptahydrate 28.398, manganous sulfate monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulfate heptahydrate 13.193, filler 53.428.
[g]g/kg premix: thiamin HCL 0.5, riboflavin 3.0, pyrodoxine HCL 1.0, Dl Ca-Pantothenate 5.0, nicotinic acid 5.0, biotin 0.05, folic acid 0.18, vitamin B_{12} 0.002, choline chloride 100.0, inositol 5.0, menadione 2.0, vitamin A acetate (20,000 IU/g) 5.0, vitamin D3 (400,000 IU/g) 0.002, dL-alpha-tocopherol acetate (250 IU/g) 8.0, Alpha-cellulose 865.266.
[h]Stay C® , (L-Ascorbyl-2-Polyphosphate 25% Active C), Roche Vitamins Inc., Parsippany, New Jersey, USA.
[i]Cefkaphos® (primarily monobasic calcium phosphate) BASF Corporation, Mount Olive, New Jersey, USA

Maintenance Requirement for Protein

Four 28-day feeding trials were conducted to determine maintenance requirements. Two trials (1 and 2), with feeds containing 16 and 32% crude protein diet, were undertaken with juvenile (1.3 – 1.4 g mean initial weight) *L. vannamei*. In both trials, each of four replicate tanks was stocked with 8 shrimp of similar size. Juvenile shrimp fed the 16% crude protein diet (Table 1) were offered feed at the following rates: 0.4, 0.6, 0.8, 1.0, 1.4, 1.8, 2.2 and 2.6 g of feed per shrimp per week. Juvenile shrimp fed the 32% crude protein diet (Table 1) had feeding rates of 0.4, 0.55, 0.7, 0.85, 1.0, 1.3, 1.6 and 1.9 g of feed per shrimp per week.

Two trials (3 and 4), with feeds containing 16 and 32% crude protein diet, were undertaken with sub-adult (6.9 - 8.5 g mean initial weight) *L. vannamei*. Six shrimp were stocked in each tank and the total biomass was determined. Each of these trials was composed of four replicate tanks assigned to one of eight treatments. Shrimp fed the 16% crude protein diet had feeding rates of 0.4, 0.6, 0.8, 1.0, 1.4, 1.8, 2.6 and 3.2 g of feed per shrimp per week. Shrimp fed the 32% crude protein diet had feeding rates of 0.4, 0.55, 0.7, 0.85, 1.0, 1.3, 1.6 and 1.9 g of feed per shrimp per week.

Protein Requirement for Maximum Growth

Four growth trials were conducted with either juvenile or sub-adult shrimp to estimate protein requirements for maximum growth and to compare responses to various levels of dietary protein. Two 28-day trials were conducted with the 16 and 32% crude protein diets (Table 1) being used simultaneously in each trial. The first trial (No 5) was with juvenile shrimp (1.7 g mean initial weight) and the next (No 6) with sub-adult shrimp (5.6 g mean initial weight). In both trials, each diet was represented by five feeding rates, with each feeding rate having three replicates. The 16% protein diet was fed to the juvenile shrimp at 7, 14, 20, 26, and 32% body weight, and the 32% protein diet was fed at 7, 10, 13, 19, and 25% body weight. For the sub-adult shrimp, the 16% diet was fed at 2.6, 5.1, 7.7, 10.2, and 15.3% body weight, and the 32% diet was fed at 2.6, 3.8, 5.1, 7.7, and 10.2% body weight. In each of these trials, shrimp were weighed after two weeks, and the feed was adjusted according to the biomass of each tank.

The final two trials were conducted with juvenile shrimp (1.3 g mean initial weight) and sub-adult shrimp (8.4 g mean initial weight) over a 28 day period (No. 7 and 8, respectively). Shrimp were offered diets containing either 32 or 48% protein. Juvenile shrimp were offered the 32% protein diet at 12, 18, 24, and 32% body weight, and the 48% protein diet was fed at 2, 4, 8, 12, 16, and 24% body weight. Sub-adult shrimp were offered the 32% protein diet at 2.6, 5.1, 7.7 and 10.2% body weight, an the 48% protein diet at 1.7, 2.6, 3.4, 5.1, 6.8, and 10.2% there body weight. Shrimp were weighed after two weeks and feeding rate adjusted according to the biomass of each tank.

Experimental System

Each feeding trial was conducted using a semi-closed recirculating seawater system containing thirty-two 110L tanks, common biological filter, pressurized sand filter, and a circulation pump. The system make up water was exchanged at a rate of 100% per day. Dissolved oxygen and temperature were measured daily, and total ammonia-nitrogen, nitrite-nitrogen, and pH were measured biweekly according to Spotte (1979).

Determination of Growth Responses

At the conclusion of the growth trials, final biomass was determined, and percent weight gain (weight gain x 100/initial weight), feed efficiency (weight gain x 100/dry weight feed offered), and protein conversion efficiency (dry weight protein offered x 100/dry weight protein gained) values were calculated. Also, a sample of three shrimp was kept from each treatment tank and frozen for subsequent analysis. Dry matter content of each sample was determined by drying to a constant weight at 90^{o} C. Each sample was then ground and frozen for subsequent analysis. The micro-kjeldhal method of Ma and Zuazago (1942) was used to determine the protein content of each sample and the diets. Dry matter and protein analyses were conducted in duplicate and triplicate, respectively.

Digestibility Trials

At the conclusion of the four initial trials, a sub-sample of the shrimp were weighed and re-stocked into each of 8 tanks (6 shrimp per tank) and used for the digestibility determinations. Each of the three digestibility diets was extruded with 0.75% chromic oxide marker replacing wheat starch. The 16 and 32% protein diets were fed to both juvenile and sub-adult shrimp, while the 48% protein diet was fed only to juvenile shrimp. Shrimp were allowed to acclimate to feed containing 0.75% chromic oxide for two days after stocking. Fecal collection commenced on the third day after stocking and was conducted over three days. Feces were collected by siphoning onto a 48 mm mesh screen. Feces were rinsed with distilled water and dried at 90^{o} C. Fecal samples and feed samples were analyzed for chromic oxide and protein content. Apparent dry matter and protein digestibility was determined as described by National Research Council (1993).

Statistical Analysis

Data were analyzed using SAS methods (V6.12, SAS Institute Inc. Cary, North Carolina, USA). Two-way analysis of variance (ANOVA), was used to determine significant differences of the main effects across dietary protein levels. One-way analysis of variance (ANOVA) and the Student Newman Keuls multiple-range test were performed to determine significant differences among means of growth variables (Steel and Torrie, 1980). Growth data were analyzed using regression analysis in order to determine maintenance requirement levels. Non-linear regression analysis was also utilized, depending upon growth results. From these analyses, regression equations were developed predicting growth corresponding to various dietary protein levels.

RESULTS

All growth trials were conducted without interruption, water quality problems, or disease problems. Survival for all growth trials was above 90%. The observed water quality in each trial was suitable for uninterrupted growth of *L. vannamei* (Table 2). The only variation in water quality was low salinity, which was noted during the maintenance protein requirement trial for sub-adult shrimp offered a 16% protein diet.

Table 2. Water quality data presented as mean values ± standard deviation for each feeding trial.

Trial	Salinity (ppt)	Temperature (^{0}C)	Dissolved oxygen (mg/L)	NH_3-N (mg/L)	NO_2 (mg/L)	pH
1	34.9 ± 0.6	28.1 ± 0.5	6.1 ± 0.3	0.006 ± 0.006	0.008 ± 0.006	7.9 ± 0.1
2	34.6 ± 2.8	28.0 ± 0.5	5.8 ± 0.4	0.028 ± 0.067	0.028 ± 0.056	7.9 ± 0.1
3	28.8 ± 1.4	27.6 ± 0.7	6.1 ± 0.5	0.001 ± 0.001	0.006 ± 0.004	8.0 ± 0.1
4	21.0 ± 2.8	27.5 ± 1.1	6.3 ± 0.6	0.007 ± 0.008	0.006 ± 0.006	7.9 ± 0.1
5	30.1 ± 1.7	28.2 ± 0.7	6.2 ± 0.5	0.015 ± 0.014	0.061 ± 0.088	7.8 ± 0.1
6	30.1 ± 1.7	27.5 ± 0.8	6.3 ± 0.9	0.050 ± 0.065	0.061 ± 0.088	7.9 ± 0.1
7	31.0 ± 1.4	28.4 ± 0.5	6.2 ± 0.2	0.0 ± 0.016	0.014 ± 0.006	7.8 ± 0.1
8	33.5 ± 1.6	28.4 ± 0.8	6.0 ± 0.4	0.036 ± 0.074	0.060 ± 0.022	7.8 ± 0.1

Maintenance Requirements for Protein

Growth response of juvenile shrimp to increasing feeding rates resulted in a sequential increase in weight gain and a decrease in feed efficiency (FE) and protein conversion efficiency (PCE) with both

16 and 32% protein diets. The responses of juvenile *L. vannamei* offered the 16 and 32% protein diets at various feeding rates are presented in Tables 3 and 4, respectively. The maintenance protein requirements for juvenile shrimp is shown in Table 5. The maintenance requirement for shrimp fed the 16% protein diet was 1.8 g DP/(kg BW*d) from the following regression equation:

$$\text{weight gain} = 1.58(\log_{10}(\text{g DP/(kg BW*d)})) - 0.40, \text{ adjusted } r^2 = 0.93$$

Juvenile shrimp fed the 32% protein diet had a maintenance protein requirement of 3.8 g DP/(kg BW*d):

$$\text{weight gain} = 2.25(\log_{10}(\text{g DP/(kg BW*d)})) - 1.31, \text{ adjusted } r^2 = 0.95$$

Table 3. Four-week growth response of juvenile *L. vannamei* (1.4 g mean initial weight) fed 16% crude protein diet.[a]

G DP/(kg BW*d)[b]	%BW[c]	Weight gain (g)[d]	FE (%)[e]	PCE (%)[f]
6	4.0	0.83[u]	55.8[z]	45.6[zy]
9	5.9	1.15[v]	51.3[y]	50.3[z]
13	7.8	1.32[wv]	44.3[x]	44.0[yz]
16	10.0	1.50[w]	40.5[w]	41.3[y]
22	14.0	1.72[x]	33.0[v]	33.3[x]
28	17.7	1.93[y]	28.8[u]	31.8[x]
35	21.9	1.96[y]	24.0[t]	25.8[w]
41	25.8	2.20[z]	23.0[t]	23.0[w]
PSE[g]		0.05	0.9	1.4

[a]Values represent means of four replicates. Numbers in the same column with different superscripts are significantly different (*P*<0.05).
[b]g dietary protein/kg body weight/day
[c]g feed / 100 g body weight / day
[d]Weight gain = final weight - initial weight
[e]Feed efficiency = weight gain x 100/dry weight feed offered
[f]Protein conversion efficiency = dry weight protein gain x 100/dry weight protein offered
[g]Pooled standard error

Table 4. Four- week growth response of juvenile *L. vannamei* (1.3 g mean initial weight) fed a 32% crude protein diet.[a]

g DP/(kg BW*d)[b]	%BW[c]	Weight gain (g)[d]	FE (%)[e]	PCE (%)[f]
15	4.6	1.23[u]	83.6[z]	43.1[z]
19	6.0	1.66[v]	81.7[z]	43.1[z]
26	8.0	1.90[w]	73.7[y]	41.0[z]
30	9.5	2.03[w]	65.0[x]	35.8[y]
36	11.2	2.20[x]	59.8[w]	33.6[y]
47	14.6	2.52[y]	52.5[v]	27.2[x]
57	17.8	2.61[yz]	44.3[u]	25.1[x]
66	20.7	2.74[z]	39.2[t]	22.0[w]
PSE[g]		0.04	1.01	0.70

[a]Values represent means of four replicates. Numbers in the same column with different superscripts are significantly different (P<0.05).
[b]g dietary protein/kg body weight/day
[c]g feed/100 g body weight/day
[d]Weight gain = final weight - initial weight
[e]Feed efficiency = weight gain x 100/dry weight feed offered
[f]Protein conversion efficiency = dry weight protein gain x 100/dry weight protein offered
[g]Pooled standard error

Table 5. Maintenance requirements for protein (grams dietary protein/kilogram body weight/day = g DP/(kg BW*d)) relative to size of shrimp and diet utilized (TRT16 =16% protein, TRT32 = 32% protein).

	Juvenile		Sub-adult	
	TRT16	TRT32	TRT16	TRT32
g DP/(kg BW*d)	1.8	3.8	1.5	2.1
MSE [a]	0.02	0.01	0.12	0.12
PSE [b]	0.05	0.04	0.12	0.12

[a]Mean square error
[b]Pooled standard error

Weight gain of sub-adult *L. vannamei* offered the 16% protein diet increased significantly as feeding rate increased, with the lowest feed level producing negative weight gain (Table 6). Also, PCE and FE increased sequentially and leveled out above 2.0 grams protein per kilogram body weight (Table 6). Sub-adult *L. vannamei* offered the 32% protein diet at various feeding rates also had significant increases in weight gain as feed rate increased, while PCE and FE plateaued above 2.1 grams protein per kilogram body weight (Table 7). Maintenance protein requirements of sub-adult shrimp are presented in Table 5. When fed the 16% protein diet, sub-adults exhibited a maintenance protein requirement of 1.5 g DP/(kg BW*d):

$$\text{weight gain} = 3.18(\log_{10}(\text{g DP/(kg BW*d)})) - 0.55, \quad \text{adjusted } r^2 = 0.97$$

Utilizing the 32% protein diet, the maintenance protein requirement of sub-adult shrimp was found to be 2.1 g DP/(kg BW*d):

$$\text{weight gain} = 5.43(\log_{10}(\text{g DP/(kg BW*d)})) - 1.74, \quad \text{adjusted } r^2 = 0.92$$

Table 6. Four-week growth response of sub-adult *L. vannamei* (6.9 g mean initial weight) fed a 16% crude protein diet.[a]

g DP/(kg BW*d)[b]	%BW[c]	Weight gain (g)[d]	FE (%)[e]	PCE (%)[f]
1.3	0.8	-0.22[u]	-14.8[x]	-122.3[v]
2.0	1.2	0.48[v]	21.5[y]	-28.3[w]
2.7	1.7	0.78[w]	22.5[y]	6.5[x]
3.3	2.1	0.98[w]	24.8[y]	10.5[xy]
4.7	2.9	1.73[x]	26.0[y]	35.0[zy]
6.0	3.8	1.93[x]	26.5[y]	24.8[zy]
8.7	5.4	2.38[y]	28.8[zy]	25.0[zy]
10.6	6.6	2.70[z]	33.3[z]	22.3[z]
PSE[g]		0.06	1.2	2.7

[a]Values represent means of four replicates. Numbers in the same column with different superscripts are significantly different (P<0.05).
[b]g dietary protein/kg body weight/day
[c]g feed/100 g body weight/day
[d]Weight gain = final weight - initial weight
[e]Feed efficiency = weight gain x 100/dry weight feed offered
[f]Protein conversion efficiency = dry weight protein gain x 100/dry weight protein offered
[g]Pooled standard error

Table 7. Four-week growth response of sub-adult *L. vannamei* (8.5 g mean initial weight) fed a 32% crude protein diet.[a]

g DP/(kg BW*d)[b]	%BW[c]	Weight gain (g)[d]	FE (%)[e]	PCE (%)[f]
2.1	0.7	0.05[v]	3.1[y]	-16.5[y]
3.0	0.9	0.93[w]	45.6[z]	18.3[z]
3.7	1.2	1.29[w]	49.7[z]	26.5[z]
4.5	1.4	1.82[x]	57.8[z]	29.7[z]
5.4	1.7	2.23[xy]	59.9[z]	32.8[z]
7.0	2.2	2.47[y]	51.1[z]	33.5[z]
8.6	2.7	3.49[z]	58.8[z]	33.8[z]
10.2	3.2	3.87[z]	54.9[z]	38.8[z]
PSE[g]		0.12	4.0	3.2

[a]Values represent means of four replicates. Numbers in the same column with different superscripts are significantly different (P<0.05).
[b]g dietary protein/kg body weight/day
[c]g feed/100 g body weight/day
[d]Weight gain = final weight - initial weight
[e]Feed efficiency = weight gain x 100/dry weight feed offered
[f]Protein conversion efficiency = dry weight protein gain x 100/dry weight protein offered
[g] Pooled standard error

Protein Requirements for Maximum Growth

Juvenile shrimp fed the 32% protein diet had significantly higher weight gain, FE, and PCE on an isoproteic basis compared to juveniles fed the 16% protein diet. Also, as feed rate increased, weight gain increased, while FE and PCE decreased (Table 8). Weight gain for shrimp fed the 16% protein diet plateaued to some extent but followed a curvilinear pattern (Fig. 1). These shrimp did not reach final weights equivalent to those offered the 32% protein diet which appeared to reach a maximum after 42 g DP/(kg BW * d) (Fig. 2).

Table 8. Four week growth responses of juvenile *L. vannamei* (1.7 g mean initial weight) fed either a 16% crude protein diet (TRT16) or a 32% crude protein diet (TRT32).[a]

g DP/(kg BW*d)[b]	TRT16					TRT32				
	%BW[c]	Weight gain (g)[d]	Weight gain (%)[e]	FE (%)[f]	PCE (%)[g]	%BW[c]	Weight gain (g)[d]	Weight gain (%)[e]	FE (%)[f]	PCE (%)[g]
11	7	1.69^{x}	99^{x}	47.3^{z}	74.3^{z}					
22	14	2.40^{y}	146^{y}	32.3^{y}	46.0^{y}	7	2.61^{x}	153^{w}	69.0^{z}	48.3^{z}
32	20	2.72^{y}	163^{y}	25.0^{x}	35.3^{x}	10	3.35^{y}	199^{x}	57.3^{y}	37.3^{y}
42	26	2.84^{y}	171^{zy}	19.7^{w}	28.7^{w}	13	3.67^{y}	219^{yx}	46.7^{x}	31.7^{x}
51	32	3.27^{z}	191^{z}	17.7^{w}	24.0^{v}					
61						19	4.19^{z}	248^{zy}	35.0^{w}	23.3^{w}
80						25	4.48^{z}	261^{z}	26.7^{v}	18.0^{v}
PSE[h]		0.09	5.1	0.5	1.1		0.13	7.3	1.3	0.9

[a]Values represent means of three replicates. Numbers in the same column with different superscripts are significantly different ($P<0.05$)
[b]g dietary protein/kg body weight/day
[c]g feed/100 g body weight/day
[d]Weight gain = final weight - initial weight
[e]Percent weight gain = weight gain x 100/initial weight
[f]Feed efficiency = weight gain x 100/dry weight feed offered
[g]Protein conversion efficiency = dry weight protein gain x 100/dry weight protein offered
[h]Pooled standard error

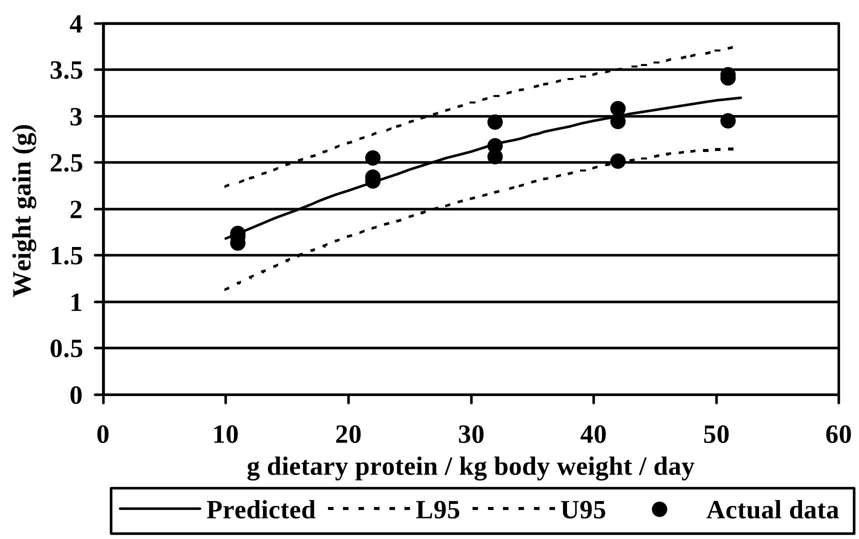

Figure 1. Linear regression of weight gain of juvenile *Litopenaeus vannamei* offered a 16% protein diet

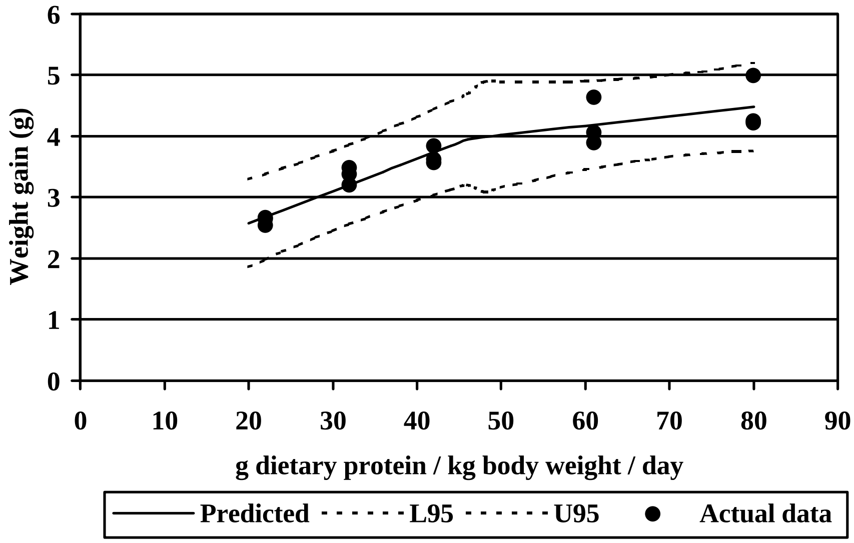

Figure 2. Non-linear regression of weight gain of juvenile *Litopenaeus vannamei* offered a 32% protein diet

In the second juvenile trial, shrimp fed the 32% protein diet exhibited significantly higher weight gain on an isoproteic basis than the 48% protein diet; however, the latter produced significantly higher FE on an isoproteic basis (Table 9). There were no significant differences in weight gain or percent weight gain for shrimp fed the 32% protein diet. FE did, however, significantly decrease as protein fed was increased. For shrimp fed the 48% protein diet, weight gain and percent weight gain significantly increased as protein fed increased, plateauing after 41 grams protein/kilogram body weight/day (Fig. 3). FE increased initially leveled and then decreased as the quantity of protein fed was increased.

Table 9. Four week growth responses of juvenile *L. vannamei* (1.3 g mean initial weight) fed either a 32% crude protein diet (TRT32) or a 48% crude protein diet (TRT48).[a]

g DP/(kg BW*d)[b]	TRT32					TRT48				
	%BW[c]	Weight gain (g)[d]	Weight gain (%)[e]	FE (%)[f]	PCE (%)[g]	%BW[c]	Weight gain (g)[d]	Weight gain (%)[e]	FE (%)[f]	PCE (%)[g]
10						2	0.34[w]	25[v]	43.3[yx]	5.3[x]
20						4	1.17[x]	90[w]	68.7[z]	23.7[z]
41	12	3.40[z]	267[z]	51.7[z]	28.0[z]	8	2.87[y]	217[x]	68.7[z]	25.0[z]
61	18	3.63[z]	279[z]	35.7[y]	18.7[y]	12	3.16[y]	235[yx]	46.7[y]	16.0[y]
82	24	3.82[z]	295[z]	27.3[x]	16.0[yx]	16	3.23[y]	249[zyx]	36.0[yx]	12.0[yx]
102	30	4.14[z]	320[z]	21.7[x]	11.7[x]	20	3.79[z]	280[z]	32.7[x]	11.0[yx]
144*						30	3.67[z]	267[zy]	20.5[w]	-
PSE[h]		0.23	18.2	1.8	1.4		0.08	5.9	1.9	1.4

[a]Values represent means of three replicates, except for * (144 g DP/(kg BW*d)) which had two replicates. Numbers in the same column with different superscripts are significantly different ($P<0.05$).
[b]g dietary protein /kg body weight/day
[c]g feed/100 g body weight/day
[d]Weight gain = final weight - initial weight
[e]Percent weight gain = weight gain x 100/initial weight
[f]Feed efficiency = weight gain x 100/dry weight feed offered
[g]Protein conversion efficiency = dry weight protein gain x 100/dry weight protein offered
[h]Pooled standard error

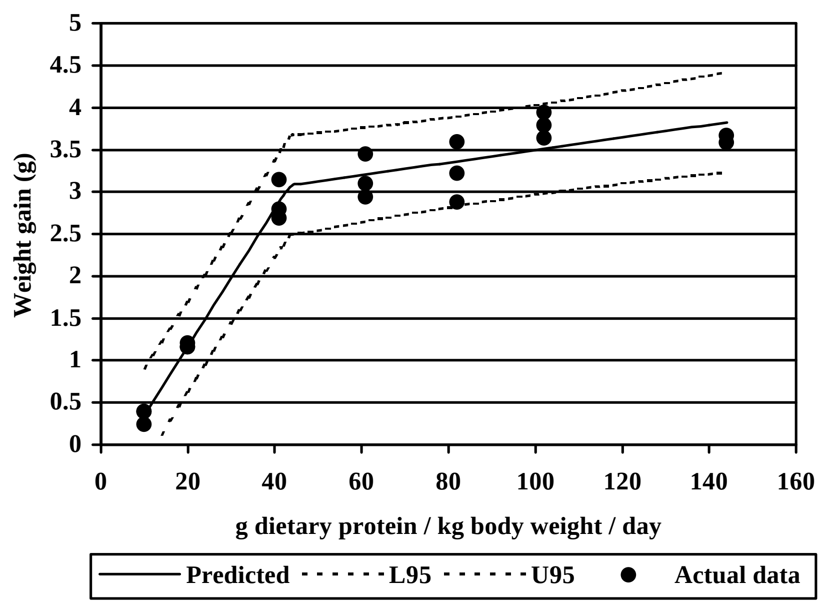

Figure 3. Non-linear regression of weight gain of juvenile *Litopenaeus vannamei* offered a 48% protein diet

Results from non-linear regression for the higher protein diets for each of these growth trials are presented in Table 10. Non-linear regression of the weight gain data for shrimp offered the 32% and 48% protein diets at various daily protein intakes indicated that the protein requirement for maximum growth was at 46.4 g DP/(kg BW * d) and 43.4 g DP/(kg BW * d).

Table 10. Protein requirements for maximum growth (grams dietary protein/kilogram body weight/day = g DP/(kg BW*d)) of *L. vannamei* relative to size of shrimp and diet utilized (TRT32= 32% protein, TRT48=48%protein) with weight gain used as the dependent variable.

	Juvenile		Sub-adult	
	TRT32	TRT48	TRT32	TRT48
g DP/(kg BW*d)	46.4	43.4	23.5	20.5
Lower 95% confidence interval	28.4	38.5	18.5	11.5
Upper 95% confidence interval	64.4	48.4	28.5	29.5

Sub-adult shrimp fed the 32% protein diet had significantly higher weight gain, FE, and PCE on an isoproteic basis than those fed the 16% protein diet (Table 11). Weight gain significantly increased in a sequential manner for shrimp fed both diets as feed rate increased, while FE and PCE significantly decreased in a sequential manner for shrimp fed both diets (Table 11). As previously seen with the juvenile shrimp, weight gain of the sub-adult shrimp offered the 16% protein diet increased in a curvilinear pattern (Fig. 4). Whereas, weight gain of shrimp offered the 32% protein diet did appear to increase to a maximum at approximately 25 g DP/(kg BW * d) (Fig. 5).

Table 11. Four-week growth responses of sub-adult *L. vannamei* (5.6 g mean initial weight) fed either a 16% crude protein diet (TRT16) or a 32% crude protein diet (TRT32).[a]

g DP/(kg BW*d)[b]	TRT16					TRT32				
	%BW[c]	Weight gain (g)[d]	Weight gain (%)[e]	FE (%)[f]	PCE (%)[g]	%BW[c]	Weight gain (g)[d]	Weight gain (%)[e]	FE (%)[f]	PCE (%)[g]
4	2.6	1.98[x]	35[x]	50.7[z]	42.0[z]					
8	5.1	2.84[y]	50[y]	34.7[y]	31.7[y]	2.6	3.12[x]	58[x]	77.7[z]	37.0[z]
12	7.7	2.81[y]	51[y]	23.3[x]	23.0[x]	3.8	3.88[y]	69[yx]	60.7[y]	37.0[z]
16	10.2	3.28[zy]	59[zy]	20.0[x]	21.0[x]	5.1	3.98[y]	69[yx]	45.7[x]	25.3[y]
25	15.3	3.73[z]	66[z]	15.0[w]	14.0[w]	7.7	4.79[z]	86[z]	36.0[w]	20.0[x]
33						10.2	4.23[y]	76[zy]	24.7[v]	14.0[w]
PSE [h]		0.12	2.6	1.1	1.5		0.12	2.5	1.2	1.4

[a]Values represent means of three replicates. Numbers in the same column with different superscripts are significantly different (*P*<0.05)
[b]g dietary protein/kg body weight/day
[c]g feed/100 g body weight/day
[d]Weight gain = final weight - initial weight
[e]Percent weight gain = weight gain x 100/initial weight
[f]Feed efficiency = weight gain x 100/dry weight feed offered
[g]Protein conversion efficiency = dry weight protein gain x 100/dry weight protein offered
[h]Pooled standard error

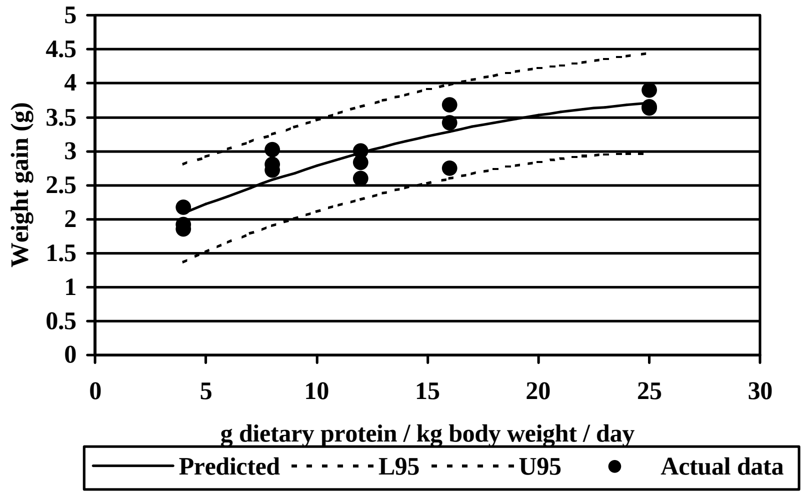

Figure 4. Linear regression of weight gain of sub-adult *Litopenaeus vannamei* offered a 16% protein diet

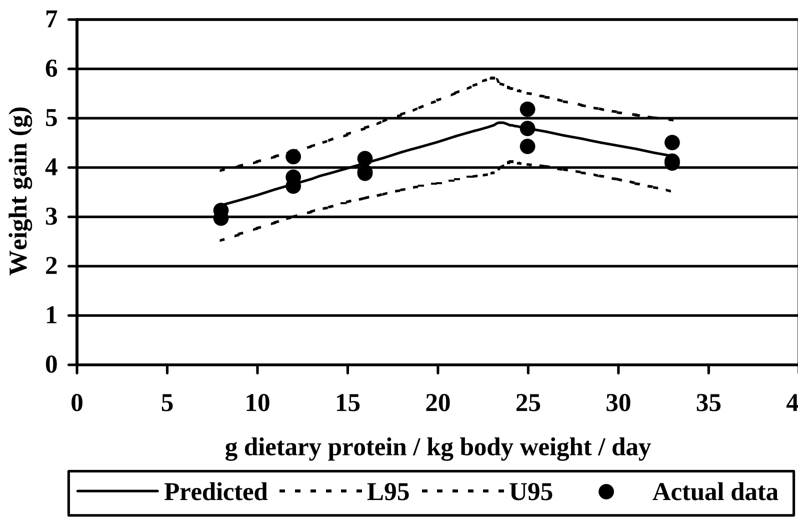

Figure 5. Non-linear regression of weight gain of sub-adult *Litopenaeus vannamei* offered a 32% protein diet

In the next sub-adult growth trial, shrimp were offered either a 32 or 48% protein diet. On an isoproteic basis, protein content of the diet did not significantly influence weight gain but did influence FE and PCE values (Table 12). For shrimp fed the 48% protein diet, weight gain increased and then plateaued when the shrimp were offered more than 25 g DP/(kg BW * d) (Fig. 6). As is the previous trials FE and PCE values significantly decreased as feeding rates increased.

Table 12. Four week growth responses of sub-adult *L. vannamei* (8.3 g mean initial weight) fed either a 32% crude protein diet (TRT32) or a 48% crude protein diet (TRT48).[a]

g DP/(kg BW*d)[b]	TRT32					TRT48				
	%BW[c]	Weight gain (g)[d]	Weight gain (%)[e]	FE (%)[f]	PCE (%)[g]	%BW[c]	Weight gain (g)[d]	Weight gain (%)[e]	FE (%)[f]	PCE (%)[g]
8	2.6	1.6^{x}	19.5^{y}	25.2^{z}	24.1^{z}	1.7	1.4^{w}	17.4^{w}	33.2^{z}	14.9^{yz}
12						2.6	2.1^{wx}	24.7^{wx}	31.2^{z}	17.9^{z}
16	5.1	2.9^{y}	34.3^{z}	20.7^{y}	16.4^{y}	3.4	2.4^{xy}	28.8^{xy}	27.6^{z}	16.1^{yz}
25	7.7	3.3^{z}	39.3^{z}	16.4^{x}	13.1^{y}	5.1	2.7^{xyz}	32.4^{xyz}	20.2^{y}	12.0^{y}
33	10.2	2.8^{y}	34.4^{z}	10.7^{w}	9.2^{x}	6.8	3.4^{z}	40.7^{z}	18.6^{y}	11.7^{y}
58						10.2	3.1^{yz}	36.6^{yz}	9.3^{x}	5.6^{x}
PSE[h]		0.12	1.68	1.27	1.04		0.23	2.73	2.06	1.22

[a]Values represent means of three replicates. Numbers in the same column with different superscripts are significantly different ($P<0.05$).
[b]g dietary protein /kg body weight/day
[c]g feed/100 g body weight/day
[d]Weight gain = final weight - initial weight
[e]Percent weight gain = weight gain x 100/initial weight
[f]Feed efficiency = weight gain x 100/dry weight feed offered
[g]Protein conversion efficiency = dry weight protein gain x 100/dry weight protein offered
[h]Pooled standard error

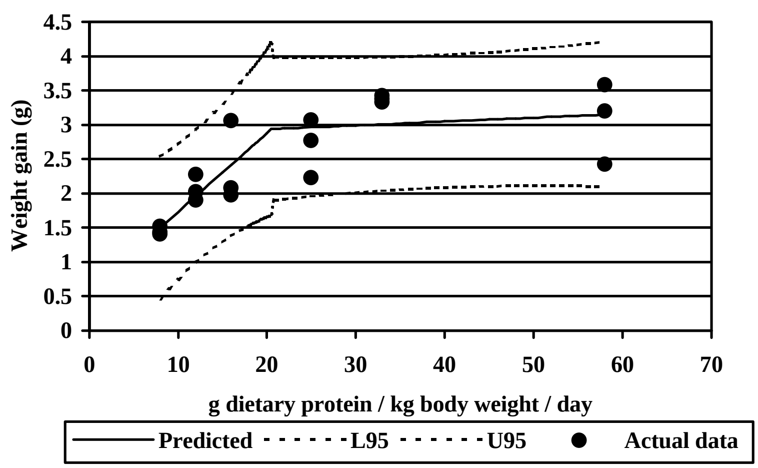

Figure 6. Non-linear regression of weight gain of sub-adult *Litopenaeus vannamei* offered a 48% protein diet

Results from non-linear regression for the higher protein diets for each of these growth trials are presented in Table 10. Non-linear regression of the weight gain data for shrimp offered the 32% and 48% protein diets at various daily protein intakes indicated that the protein requirement for maximum growth was at 23.5 g DP/(kg BW * d) and 20.5 g DP/(kg BW * d), respectively (Table 10).

Digestibility

Apparent dry matter digestibility (ADD) of juvenile shrimp fed 16, 32, and 48% protein diets were 61.6, 59.5, and 66.9% for the 16, 32, and 48% protein diets, respectively. Apparent protein digestibility (APD) of the same diets fed to the same shrimp significantly increased with dietary protein level: 65.9, 74.7, and 83.2% for the 16, 32, and 48% protein diets, respectively. ADD values for diets fed to sub-adult shrimp were statistically different at 68.3 and 56.7% for the 16 and 32% protein diets, respectively, while APD values were not statistically different (76.4 and 75.4%, respectively). These data are presented in Table 13.

Table 13. Apparent dry matter digestibility (ADD)[a] and apparent protein digestibility (APD)[b] values for juvenile and sub-adult *L. vannamei* offered diets containing 16% (TRT16), 32% (TRT32), or 48% (TRT48) crude protein and Cr_2O_7 as a marker.[c]

	Juvenile		Sub-adult	
	ADD	APD	ADD	APD
TRT16	61.6[y]	65.9[x]	68.3[y]	76.1[z]
TRT32	59.5[y]	74.7[y]	56.7[z]	75.1[z]
TRT48	66.9[z]	83.2[z]	NA	NA
PSE[d]	1.23	0.79	2.13	1.41

[a]Apparent dry matter digestibility = 100 - (100 x (Cr_2O_7 in feed/Cr_2O_7 in feces))
[b]Apparent protein digestibility = 100 - (100 x (Cr_2O_7 in feed/Cr_2O_7 in feces) x (protein in feces/ protein in feed))
[c]Values represent mean of four replicates. Numbers in the same column with different superscripts are significantly different (P<0.05).
[d]Pooled standard error

DISCUSSION

Maintenance Requirements for Protein

The first four trials of this study were conducted to determine maintenance protein requirements of juvenile and sub-adult shrimp. Log transformation of the independent variable was found to provide the best correlation coefficient values when regression analysis was performed. Both juvenile and sub-adult shrimp fed the 32% protein diet had higher maintenance protein requirement values (Table 5). This is probably due to higher growth rates associated with feeding the 32% protein diet. In general, it can be stated that juvenile shrimp have a maintenance protein requirement in the range of 1.8 – 3.8 g DP/(kg BW*d), and sub-adult shrimp have a maintenance protein requirement in the range of 1.5 – 2.1 g DP/(kg BW*d). The maintenance protein requirement of sub-adult shrimp fed a 16% protein diet (1.5 g DP/(kg BW*d)) may have been affected by low salinity (21.0 ± 2.8) that occurred during the growth trial.

Although this type of feeding trial has not been performed with shrimp, it has been used in studies with fish. Results with shrimp were similar to those obtained by McGoogan and Gatlin III (1998) for red drum. When fed a 36.5% protein diet, juvenile red drum (~3.4 g initial weight) were found to have a maintenance requirement of 1.5 g DP/(kg BW*d), and larger red drum (~5.5 g initial weight) exhibited a maintenance requirement of 2.5 g DP/(kg BW*d). Similarly, fingerling channel catfish have been shown to have a maintenance requirement of 1.32 g DP/(kg BW*d), when fed diets containing either 25 or 35% crude protein (Gatlin III *et. al.* 1986).

Protein Requirements for Maximum Growth

Juvenile shrimp exhibited significantly higher weight gain, percent weight gain, FE, and PCE when fed the 32% protein diet as compared with the 16% protein diet (Table 8). Juveniles fed the 16% protein diet did not reach a point at which growth did not increase as protein ration increased, and a polynomial regression best described the growth curve (Fig. 1). The poor growth response seen with the 16% protein diet, is probably primarily due to the large quantity of feed which must be consumed to meet daily nutrient requirements. This could also be due to reduced digestibility of protein in the test diet. Growth of juveniles offered the 32% protein diet did level off at 46.4 g DP/(kg BW*d), which corresponds to a feeding rate of ~ 13% body weight. (Fig. 2).

The 32% protein diet also produced significantly higher weight gain and percent weight gain of juvenile shrimp than the 48% diet on a protein fed basis. However, the 48% protein diet had higher FE on an isoproteic basis. The lower weight gain for the 48% diet is possibly due to low energy:protein ratio of the diet, which would cause shrimp to utilize protein as a source of energy. Use of protein as an energy source is relatively inefficient as compared with lipids (Hochachka, 1991) and would reduce the amount of protein available for deposition in tissues. Shrimp exhibit a higher FE when fed the 48% diet, because, compared to the 32% diet, a smaller quantity of the 48% diet has to be fed to provide a given amount of protein and energy. Protein requirement for maximum growth of shrimp offered the 48% diet was found to be 43.4 g DP/(kg BW*d) (Table 10 and Figure 3). This value is very similar to the maximum requirement exhibited by juveniles offered the 32% diet (46.4 g DP/(kg BW*d)). Thus, these two diets can be said to provide adequate protein. However, FE at feeding levels associated with maximum growth is roughly 20% higher with the 48% diet. Although the 32% diet produced higher growth, a higher protein diet may be more efficient in achieving similar growth.

Growth of sub-adult shrimp fed the 16% protein diet did not appear to reach a maximum as the two highest feeding rates produce increases in weight gain; however, growth did appear to be leveling off. As with the juvenile shrimp, the best fitting line was found to be with a polynomial regression (Figure 4). In both cases, the poor performance of shrimp offered the 16% diet is probably due to the inability of the shrimp to effectively assimilate sufficient protein, resulting in a limitation of nutrient intake. Also, shrimp would have to expend more energy to ingest an equal amount of protein when fed the 16% protein diet as compared to the 32% protein diet (i.e. twice the volume would be required for the 16% protein diet). Non-linear regression analysis on the growth curve of shrimp fed the 32% protein diet showed that the protein requirement for maximum growth was 23.5 g DP/(kg BW*d) (Table 10 and Figure 5). This corresponds to a feeding rate of approximately 7% body weight. Interestingly, weight gain decreased after a maximum was reached. This response was seen in both experiments with the 32% protein diet. One potential explanation for this is that the shrimp unnecessarily expended energy to ingest excess feed and digestion efficiency may have decreased due to increased passage rate from excess feed intake. A similar response was seen with the 48% protein diet (Table 12). In this experiment, non-linear regression predicted maximum growth at 20.51 g DP/(kg BW * d) a value similar to that predicted with the 32% protein diet. On an isoproteic basis, dietary protein did not significantly influence growth but it did influence FE and PCE values. As expected, FE values were higher for the 48% protein diet. It should be noted that two-way ANOVA indicated significant difference in PCE values due to both protein intake and the level of protein in the diet without a significant interaction. At levels near the requirement, PCE values were very similar. Thus, indicating that the energy to protein ratio's and lipid content of the diets were reasonable for sub-adult shrimp.

In general, juvenile and sub-adult shrimp had higher weight gain, percent weight gain, FE, and PCE on a protein fed basis when fed the 32% protein diet compared to the 16 % protein diet. Increases in growth and PCE could be due to shifts in APD values of the diets. Although, the same protein sources were used for all the diets, APD values did increase with increasing protein content of the diet for juvenile shrimp (Table 13). This could partly explain the poor response of the shrimp to the low protein diet. However, it does not explain the poor response of the shrimp to the 48% diet. Although APD values were not determined for the 48% protein diet in sub-adult shrimp, there were no significant differences in APD values for the 16 and 32% protein diets (76.1 and 75.1%, respectively). Consequently, there are probably several reasons for the differences in growth rates seen for shrimp offered the test diets. For the low protein diet, ingestion rate and digestibility were probably the primary factors influencing the response. Alternately, the poor response to the 48% protein diet was probably more due to the protein to energy ratio's of the diet.

Physiological factors affecting and resulting from ingestion and assimilation can also provide insight into nutritional requirements. Taboada *et al.* (1998) estimated optimal dietary protein level for *Litopenaeus setiferus* by measuring changes in oxygen consumption and nitrogen excretion relative to fasting and feeding. The hypothesis used was that the diet, which allowed for the shortest time to reach peak oxygen consumption and nitrogen excretion by the shrimp was the diet being assimilated most efficiently. The shortest time to peak oxygen consumption was observed for shrimp fed 30 and 50% protein diets, and the shortest time to peak nitrogen excretion was noted for shrimp fed the 30% protein diet. Shrimp offered feeds containing 10 and 20% dietary protein were found to have the highest oxygen consumption, which would indicate surplus energy expenditures for assimilation of feed. Also, shrimp fed 40 and 50% protein diets had higher nitrogen excretion peaks than those fed diets containing 20 and 30% dietary protein. It is likely that excess nitrogen excretion in the high protein diets was due to protein being used for energy. These observations help to explain the reduced growth of *L. vannamei* fed diets containing 16 and 48% protein in our feeding trials.

CONCLUSION

Maintenance protein requirement levels determined by this study should help in establishing feed rations when adverse culture conditions are encountered or if shrimp need to be held at harvest size. In trials designed to determine maximum protein requirements, a 32% protein diet was found to induce superior growth in juvenile and sub-adult *L. vannamei* as compared to 16 and 48% protein diets. However, the 48% protein diet did produce higher feed efficiency than the 32% protein diet when fed to juvenile shrimp, indicating that the optimum protein level is probably higher than 32%. The maximum protein requirements were found to be similar for juvenile shrimp fed a 32% protein diet (46.4 g DP/(kg BW*d)) and juvenile shrimp fed a 48% protein diet (43.4 g DP/(kg BW*d)). The maximum protein requirements were also found to be similar for sub-adult shrimp fed a 32 % protein diet (23.5 g DP/(kg BW*d)) or a 48% protein diet (20.5 g DP/(kg BW*d)). Based on the results of this study, future research is to warranted to evaluate optimal dietary protein level in terms of growth, feed efficiency, and protein conversion efficiency when feeding rates are based on the reported daily protein requirement.

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