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Aquaculture 204 (2002) 125–143

Aquaculture

www.elsevier.com/locate/aqua-online

Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei*

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Received 2 January 2001; received in revised form 9 April 2001; accepted 9 April 2001

Abstract

The dietary crude protein requirement of penaeid shrimp is an important nutritional consideration because protein is often the major limiting nutrient for growth. In most cases, research has focused on total dietary protein levels rather than a daily requirement for protein. This research utilized three practical diets containing 16%, 32%, and 48% protein and various daily rations to estimate daily requirements for protein. In the first series of studies, 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 subadult 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 per day (g DP/(kg BWd)), and subadult shrimp were found to have maintenance protein requirements in the range of 1.5–2.1 g DP/(kg BWd). Four additional 28-day feeding trials were conducted to determine the protein requirement for maximum growth by juvenile and subadult shrimp. These studies, utilized three practical diets containing 16%, 32%, and 48% dietary protein. On an isonitrogenous basis, the 16% protein diet produced significantly lower weight gain, feed efficiency (FE), and protein conversion efficiency values, than the 32% protein diet for both the juvenile and subadult shrimp. The 48% protein diet produced significantly lower weight gain in the juvenile shrimp, but there was no significant effect in the subadult 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

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PII: S0044-8486(01)00649-4

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 BWd) when fed the 32% protein diet and 43.4 g DP/(kg BWd) when fed the 48% protein diet. Subadult shrimp exhibited a maximum protein requirement of 23.5 g DP/(kg BWd) when fed a 32% protein diet and 20.5 g DP/(kg BWd) when fed a 48% protein diet. In summary, FE increased as the protein concentration of the diet increased, and decreased as feeding rates (offered feed/day) increased. Weight gain reflected daily protein intake. Based on these results, a wide range of dietary protein levels could be used to produce maximum weight gain of juvenile and subadult shrimp. Due to a restriction of feed intake and consequently protein intake, low protein diets did not support maximum weight gain.

Keywords: Shrimp; Protein; Intake; Requirement studies

1. 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 utilized for energy and not deposited for growth contributes to the 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 “environment-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., weight gain, 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.

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). Determining the maintenance requirement provides a better understanding of the basic metabolic needs of the organism. With this knowledge, rationing of feed to supply minimum metabolic needs could allow for extended holding of shrimp at minimum cost once they reach marketable size or under adverse culture conditions. The requirement for protein resulting in maximum growth would have to be determined if maximum growth rates are to be attained.

To date, few studies have been undertaken to determine the quantitative protein requirements for maintenance and/or maximum weight gain of penaeid shrimp, although they have been determined for several fish species. McGoogan and Gatlin (1998) determined the metabolic requirements of juvenile red drum. 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/day (g DP/(kg BWd)). A maximum growth rate requirement of 20–25 g DP/(kg BWd) was also determined. Similarly, Gatlin et al. (1986) evaluated the protein requirements of channel catfish. In that study, incremental feeding rates, ranging from 0% to 5% of body weight, were used with diets containing 25% and 35% crude protein. The maintenance protein requirement was 1.32 g DP/(kg BWd) and the maximum weight gain requirement was 8.75 g DP/(kg BWd).

Although a quantitative requirement for protein 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 containing 25%, 30%, 35%, and 40% crude protein over a 4-week period. Feed conversion was found to be significantly better 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 and Lawrence (1993). In that study, shrimp fed diets containing 25%, 35%, and 45% crude protein showed no differences in weight gain. When shrimp were fed diets containing 10%, 15%, 20%, and 25% crude protein, weight gain of shrimp fed the 10% protein diet was significantly less than that of shrimp fed diets containing higher protein levels. Weight gain 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 shrimp were fed ad libitum in that study and feed consumption was not quantified, protein intake could not be determined.

Cousin et al. (1991) evaluated weight gain of *L. vannamei* fed diets 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 dietary energy to protein ratio was maintained at approximately 10 kcal/g protein. Results showed a significant effect of protein level on weight gain. The optimal dietary protein level was approximated as 30%, but an actual protein requirement for maximum weight gain in terms of daily intake was not determined.

Shrimp size (weight) affects growth response relative to the protein content of the diet. Smith et al. (1984) studied the response of three sizes of *L. vannamei* (4.0, 9.8, and 20.8 g) fed diets containing 22%, 29%, and 36% crude protein for a period of 30 days. Dietary protein content only affected weight gain for 4.0-g shrimp, with a significant increase in weight gain corresponding to the increase in dietary protein content. However, the range of dietary protein levels 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 maximal weight gain of 4.0-g shrimp. No relationship between dietary protein level and weight gain was found for the two larger sizes of shrimp.

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

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

where Y = percentage of wet biomass fed as dry feed, and X = mean individual shrimp weight. Weekly mortality (0.5%, assumed) and mean weight of shrimp was determined. Dietary protein level had no significant effect on the final mean weight of the shrimp. Results agree with previous research in which *L. vannamei* were grown in outdoor ponds and fed feeds containing 25–35% crude protein (Teichert-Coddington and Arrue, 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 the quantitative protein requirement. Hence, various studies 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 weight gain, could not be determined. Quantification of the requirement levels and the growth parameters associated with such feeding levels could provide information assisting in the maximization of *L. vannamei* production. The objectives of this study were to ascertain the maintenance requirement for protein, and the protein requirement for maximum growth of juvenile and subadult *L. vannamei*.

2. Materials and methods

2.1. Test diets and diet preparation

Diets utilized were formulated to contain 16%, 32%, and 48% crude protein with calculated energy/protein ratios of 25.1, 12.57, 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, Troy, OH), with hot water blended in to attain a consistency appropriate for pelleting. Each diet was extruded through a 2-mm die in a meat grinder and dried to a moisture content of less than 10%. Diets were refrigerated and subsequently crumbled to the desired pellet size prior to feeding.

Table 1
Composition (g/100 g dry weight) of diets formulated to contain 16% (TRT16), 32% (TRT32), and 48% (TRT48) crude protein.

	TRT16	TRT32	TRT48
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 mg C/kg)[h]	0.12	0.12	0.05
CaP-monobasic[i]	0.40	0.20	0.10

[a] Special Select™, Zapata Protein USA, Randeville, LA, USA.
[b] Solvent extracted, Producers Coop, Bryan, TX, USA.
[c] Omega Protein, Reedville, VA, USA.
[d] United States Biochemical, Cleveland, OH, USA.
[e] Aqualipid 95, Central Soya Chemurgy Division, Fort Wayne, IN, USA.
[f] g/100 g 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, pyridoxine 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 D_3 (400,000 IU/g) 0.002, DL-alpha-tocopheryl acetate (250 IU/g) 8.0, alpha-cellulose 865.266.
[h] Stay C®, (L-Ascorbyl-2-polyphosphate 25% Active C), Roche Vitamins, Nutley, NJ, USA.
[i] Cefkaphos® (primarily monobasic calcium phosphate) BASF, Mount Olive, NJ, USA.

2.2. Maintenance requirement for protein

Four 28-day feeding trials were conducted to determine maintenance requirements. Two trials (1 and 2), with diets containing 16% and 32% crude protein, 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 eight shrimp of similar size. Juvenile shrimp fed the 16% crude protein diet (Table 1) were fed the following rations: 0.4, 0.6, 0.8, 1.0, 1.4, 1.8, 2.2 and 2.6 g of diet 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 diet per shrimp per week. Due to the small ration size at the lowest feeding rates the ration was split into two feedings per day, and for the remaining treatments the daily ration was split into four feedings per day.

Two trials (3 and 4), with diets containing 16% and 32% crude protein, were undertaken with subadult (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 each 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 diet 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 diet per shrimp per week.

2.3. Protein requirement for maximum growth

Four growth trials were conducted with either juvenile or subadult shrimp to estimate protein requirements for maximum growth and to compare responses to various levels of dietary protein. Daily rations were split into four equivalent feedings per day. 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) utilized juvenile shrimp (1.7-g mean initial weight) and the second (no. 6) utilized subadult 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 subadult 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 2 weeks, and the ration 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 subadult shrimp (8.4-g mean initial weight) over a 28-day period (nos. 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. Subadult shrimp were offered the 32% protein diet at 2.6%, 5.1%, 7.7%, and 10.2%

Table 2
Four-week growth response of juvenile *L. vannamei* (1.4-g mean initial weight) fed 16% crude protein diet Values represent means of four replicates. Numbers in the same column with different superscripts are significantly different ($P < 0.05$).

g DP/(kg BWd)[a]	Ration (%)[b]	Weight gain (g)[c]	FE (%)[d]	PCE (%)[e]
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[f]		0.05	0.9	1.4

[a] g dietary protein/kg body weight/day.
[b] g feed/100 g body weight/day.
[c] Weight gain = final weight − initial weight.
[d] Feed efficiency = weight gain × 100/dry weight feed offered.
[e] Protein conversion efficiency = dry weight protein gain × 100/dry weight protein offered.
[f] Pooled standard error.

Table 3
Four-week growth response of juvenile *L. vannamei* (1.3-g mean initial weight) fed a 32% crude protein diet Values represent means of four replicates. Numbers in the same column with different superscripts are significantly different ($P < 0.05$).

g DP/(kg BWd)[a]	Ration (%)[b]	Weight gain (g)[c]	FE (%)[d]	PCE (%)[e]
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[f]		0.04	1.01	0.70

[a] g dietary protein/kg body weight/day.
[b] g feed/100 g body weight/day.
[c] Weight gain = final weight − initial weight.
[d] Feed efficiency = weight gain × 100/dry weight feed offered.
[e] Protein conversion efficiency = dry weight protein gain × 100/dry weight protein offered.
[f] Pooled standard error.

Table 4
Four-week growth response of subadult *L. vannamei* (6.9-g mean initial weight) fed a 16% crude protein diet Values represent means of four replicates. Numbers in the same column with different superscripts are significantly different ($P < 0.05$).

g DP/(kg BWd)[a]	Ration (%)[b]	Weight gain (g)[c]	FE (%)[d]	PCE (%)[e]
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[f]		0.06	1.2	2.7

[a] g dietary protein/kg body weight/day.
[b] g feed/100 g body weight/day.
[c] Weight gain = final weight − initial weight.
[d] Feed efficiency = weight gain × 100/dry weight feed offered.
[e] Protein conversion efficiency = dry weight protein gain × 100/dry weight protein offered.
[f] Pooled standard error.

body weight, and the 48% protein diet at 1.7%, 2.6%, 3.4%, 5.1%, 6.8%, and 10.2% body weight. Shrimp were weighed after 2 weeks and feeding rate adjusted according to the biomass of each tank.

Table 5
Four-week growth response of subadult *L. vannamei* (8.5-g mean initial weight) fed a 32% crude protein diet
Values represent means of four replicates. Numbers in the same column with different superscripts are significantly different ($P < 0.05$).

g DP/(kg BWd)[a]	Ration (%)[b]	Weight gain (g)[c]	FE (%)[d]	PCE (%)[e]
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[f]		0.12	4.0	3.2

[a] g dietary protein/kg body weight/day.
[b] g feed/100 g body weight/day.
[c] Weight gain = final weight − initial weight.
[d] Feed efficiency = weight gain × 100/dry weight feed offered.
[e] Protein conversion efficiency = dry weight protein gain × 100/dry weight protein offered.
[f] Pooled standard error.

2.4. Experimental system

Each feeding trial was conducted using a semi-closed recirculating seawater system containing 32 tanks (110 l each), common biological filter, pressurized sand filter, and a

Table 6
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).
Values represent means of three replicates. Numbers in the same column with different superscripts are significantly different ($P < 0.05$).

g DP/(kg BWd)[a]	TRT16					TRT32				
	Ration (%)[b]	Weight gain (g)[c]	Weight gain (%)[d]	FE (%)[e]	PCE (%)[f]	Ration (%)[b]	Weight gain (g)[c]	Weight gain (%)[d]	FE (%)[e]	PCE (%)[f]
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[g]		0.09	5.1	0.5	1.1		0.13	7.3	1.3	0.9

[a] g dietary protein/kg body weight/day.
[b] g feed/100 g body weight/day.
[c] Weight gain = final weight − initial weight.
[d] Percent weight gain = weight gain × 100/initial weight.
[e] Feed efficiency = weight gain × 100/dry weight feed offered.
[f] Protein conversion efficiency = dry weight protein gain × 100/dry weight protein offered.
[g] Pooled standard error.

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).

2.5. Determination of growth responses

At the conclusion of the growth trials, final biomass was determined, and percent weight gain (weight gain × 100/initial weight), feed efficiency (FE = weight gain × 100/dry weight diet offered), and protein conversion efficiency (PCE = dry weight protein offered × 100/dry weight protein gained) values were calculated. For each experiment, an initial subsample of shrimp was frozen for proximate analyses. At the conclusion of each experiment, 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°C. Each sample was then ground and frozen for subsequent analysis. The micro-Kjeldahl 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.

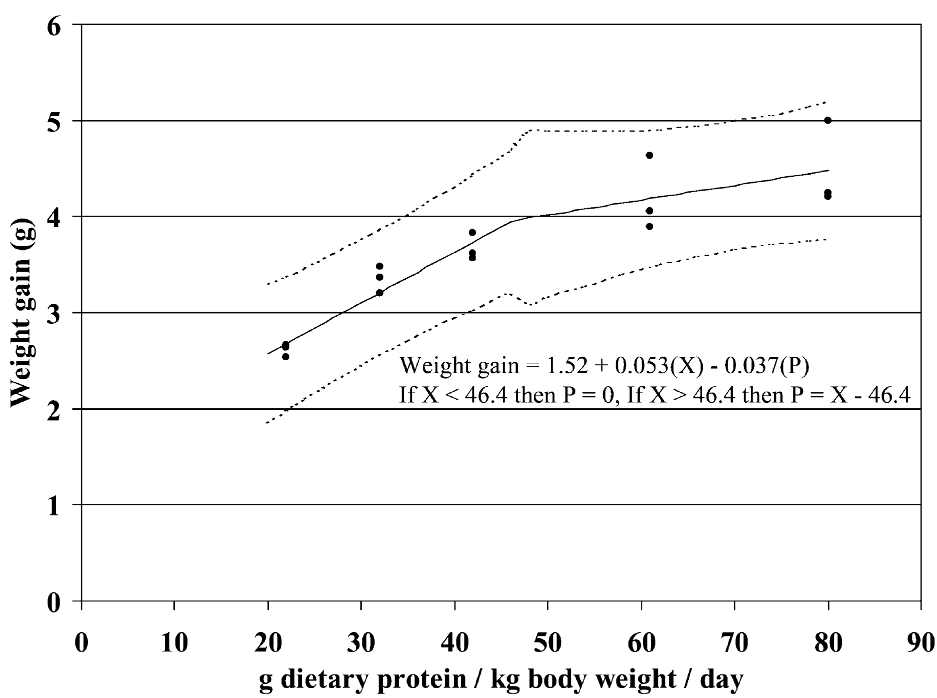

Fig. 1. Nonlinear regression of weight gain of juvenile *L. vannamei* offered a 32% protein diet. Dotted line represents upper and lower 95% confidence interval about the predicted line.

2.6. Digestibility trials

At the conclusion of the four initial trials, a subsample of the shrimp was weighed and restocked into each of eight tanks (six shrimp per tank) and used for the digestibility determinations. Each of the three diets used in determining digestibility was extruded with 0.75% chromic oxide marker replacing wheat starch. The 16% and 32% protein diets were fed to both juvenile and subadult shrimp, while the 48% protein diet was fed only to juvenile shrimp. Shrimp were allowed to acclimate to diets containing 0.75% chromic oxide for 2 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°C. Fecal samples and feed samples were analyzed for chromic oxide (McGinns and Kasting, 1964) and protein content. Apparent dry matter and protein digestibility was determined as described by National Research Council (1993).

2.7. Statistical analysis

Data were analyzed using SAS methods (V6.12, SAS Institute, Cary, NC, USA). Two-way analysis of variance (ANOVA), was used to determine significant differences of the main effects across dietary protein levels. One-way 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, as measured by weight gain, was analyzed using regression analysis in order to determine maintenance

Table 7
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).
Values represent means of three replicates, except for * (144 g DP/(kg BWd)), which had two replicates. Numbers in the same column with different superscripts are significantly different ($P < 0.05$).

g DP/(kg BWd)[a]	TRT32					TRT48				
	Ration (%)[b]	Weight gain (g)[c]	Weight gain (%)[d]	FE (%)[e]	PCE (%)[f]	Ration (%)[b]	Weight gain (g)[c]	Weight gain (%)[d]	FE (%)[e]	PCE (%)[f]
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[g]		0.23	18.2	1.8	1.4		0.08	5.9	1.9	1.4

[a] g dietary protein/kg body weight/day.
[b] g feed/100 g body weight/day.
[c] Weight gain = final weight − initial weight.
[d] Percent weight gain = weight gain × 100/initial weight.
[e] Feed efficiency = weight gain × 100/dry weight feed offered.
[f] Protein conversion efficiency = dry weight protein gain × 100/dry weight protein offered.
[g] Pooled standard error.

requirement levels. Nonlinear regression analysis was also utilized, depending upon weight gain. From these analyses, regression equations were developed predicting weight gain corresponding to various dietary protein levels.

3. Results

All growth trials were conducted without interruption, water quality, or disease problems. Survival in each growth trial was above 90%. A summary of water quality data for each experiment could be found in the thesis of Kureshy (1999). Average water quality parameters across all experiments were: salinity, 30.5 ppt; temperature, 28.0 °C; dissolved oxygen, 6.12 mg/l; total ammonia-nitrogen, 0.02 mg/l; nitrite-nitrogen, 0.03 mg/l; and pH, 7.8. The observed water quality in each trial was suitable for uninterrupted growth of *L. vannamei*. The only variation in water quality was low salinity (21.0 ppt), which was noted during the maintenance protein requirement trial for subadult shrimp offered a 16% protein diet.

3.1. 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

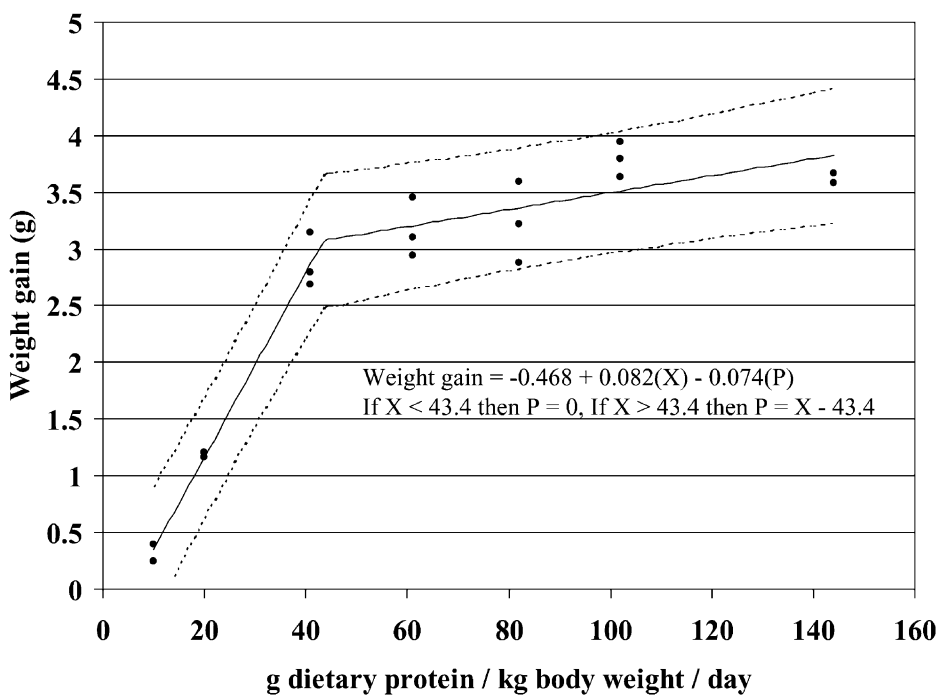

Fig. 2. Nonlinear regression of weight gain of juvenile *L. vannamei* offered a 48% protein diet. Dotted line represents upper and lower 95% confidence interval about the predicted line.

conversion efficiency (PCE) with the 16% and 32% protein diets (Tables 2 and 3, respectively). The maintenance protein requirement for shrimp fed the 16% protein diet was estimated to be 1.8 g DP/(kg BWd) and was derived from the following regression equation: weight gain = 1.58($\log_{10}$(g DP/(kg BWd))) − 0.40; adjusted $r^2 = 0.93$, mean square error (MSE) = 0.02. Juvenile shrimp fed the 32% protein diet had a maintenance protein requirement of 3.8 g DP/(kg BWd): weight gain = 2.25($\log_{10}$(g DP/(kg BWd))) − 1.31; adjusted $r^2 = 0.95$, MSE = 0.01.

Weight gain of subadult *L. vannamei* offered the 16% protein diet increased significantly as feeding rate increased, with the lowest feeding level producing negative weight gain (Table 4). Also, PCE and FE increased sequentially and leveled out above 2.0 g protein/kg body weight (Table 4). Subadult *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 g protein/kg body weight (Table 5). When fed the 16% protein diet, subadults exhibited a maintenance protein requirement of 1.5 g DP/(kg BWd). This was derived from the following equation: weight gain = 3.18($\log_{10}$(g DP/(kg BWd))) − 0.55; adjusted $r^2 = 0.97$, MSE = 0.12. Utilizing the 32% protein diet, the maintenance protein requirement of subadult shrimp was found to be 2.1 g DP/(kg BWd). This was derived from the following equation: weight gain = 5.43($\log_{10}$(g DP/(kg BWd))) − 1.74; adjusted $r^2 = 0.92$, MSE = 0.12.

3.2. Protein requirements for maximum growth

Juvenile shrimp fed the 32% protein diet had significantly higher weight gain, FE, and PCE on an isonitrogenous basis, compared to juveniles fed the 16% protein diet.

Table 8
Four-week growth responses of subadult *L. vannamei* (5.6-g mean initial weight) fed either a 16% crude protein diet (TRT16) or a 32% crude protein diet (TRT32).
Values represent means of three replicates. Numbers in the same column with different superscripts are significantly different ($P < 0.05$).

g DP/ (kg BWd)[a]	TRT16					TRT32				
	Ration (%)[b]	Weight gain (g)[c]	Weight gain (%)[d]	FE (%)[e]	PCE (%)[f]	Ration (%)[b]	Weight gain (g)[c]	Weight gain (%)[d]	FE (%)[e]	PCE (%)[f]
4	2.6	1.98^{x}	35^{x}	48.7^{z}	40.7^{z}					
8	5.1	2.84^{y}	50^{y}	33.6^{y}	30.7^{y}	2.6	3.12^{x}	58^{x}	75.0^{z}	35.7^{z}
12	7.7	2.81^{y}	51^{y}	22.6^{x}	22.3^{x}	3.8	3.88^{y}	69^{yx}	58.6^{y}	35.3^{z}
16	10.2	3.28^{zy}	59^{zy}	19.3^{x}	20.0^{x}	5.1	3.98^{y}	69^{yx}	44.3^{x}	24.3^{y}
25	15.3	3.73^{z}	66^{z}	14.3^{w}	13.7^{w}	7.7	4.79^{z}	86^{z}	35.0^{w}	19.3^{x}
33						10.2	4.23^{y}	76^{zy}	23.8^{v}	13.7^{w}
PSE[g]		0.12	2.6	1.34	1.89		0.12	2.5	1.76	1.56

[a] g dietary protein/kg body weight/day.
[b] g feed/100 g body weight/day.
[c] Weight gain = final weight − initial weight.
[d] Percent weight gain = weight gain × 100/initial weight.
[e] Feed efficiency = weight gain × 100/dry weight feed offered.
[f] Protein conversion efficiency = dry weight protein gain × 100/dry weight protein offered.
[g] Pooled standard error.

Also, as feeding rate increased, weight gain increased, while FE and PCE decreased (Table 6). Weight gain for shrimp fed the 16% protein diet plateaued to some extent but followed a curvilinear pattern, which could be described by the following equation: weight gain $= 1.06 + 0.067x - 0.0005x^2$ where $x =$ g DP/(kg BWd), adjusted $r^2 = 0.86$. 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 BWd) (Fig. 1).

In the second trial, juvenile shrimp fed the 32% protein diet exhibited significantly higher weight gain on an isonitrogenous basis than the 48% protein diet; however, the latter produced significantly higher FE on an isonitrogenous basis (Table 7). 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 the amount of protein fed was increased. For shrimp fed the 48% protein diet, weight gain and percent weight gain significantly increased as the amount of protein fed was increased, plateauing after 41 g DP/(kg BWd) (Fig. 2). FE increased initially, leveled and then decreased as the quantity of protein fed was increased.

Nonlinear regression of the weight gain data for juvenile shrimp offered the 32% protein diets at various daily protein intakes, indicated that the protein requirement for maximum growth was at 46.4 g DP/(kg BWd). The upper and lower 95% confidence intervals were 28.4 and 64.4 g DP/(kg BWd). Analysis of data for shrimp offered the 48% protein diet indicated a protein requirement for maximum growth of 43.4 g DP/(kg

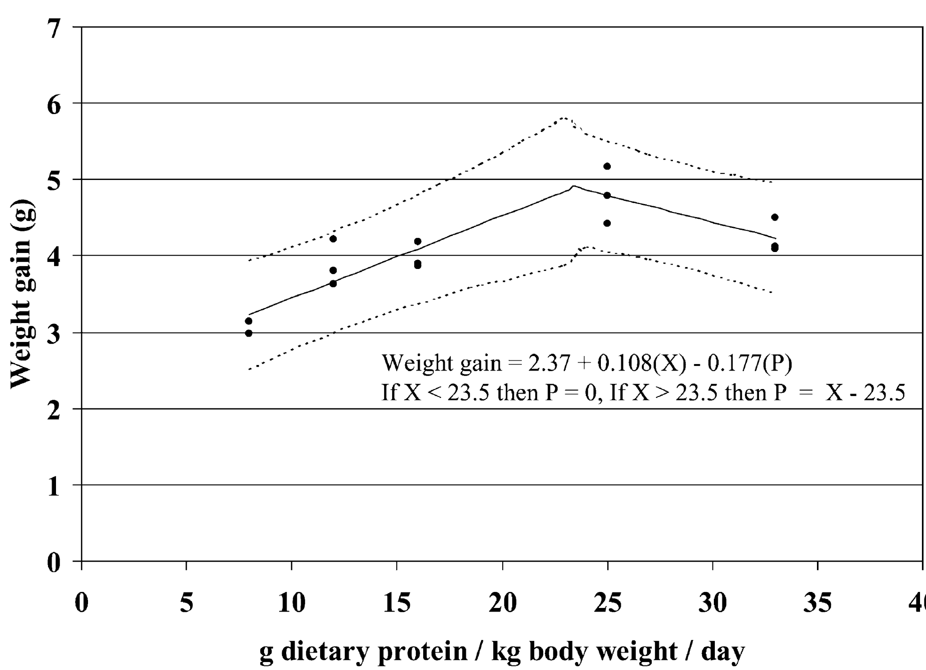

Fig. 3. Nonlinear regression of weight gain of subadult *L. vannamei* offered a 32% protein diet. Dotted line represents upper and lower 95% confidence interval about the predicted line.

BWd). The upper and lower 95% confidence intervals were 38.5 and 48.4 g DP/(kg BWd).

Subadult shrimp fed the 32% protein diet had significantly higher weight gain, FE, and PCE on an isonitrogenous basis than those fed the 16% protein diet (Table 8). Weight gain significantly increased in a sequential manner for shrimp fed both diets as feeding rate increased, while FE and PCE significantly decreased in a sequential manner for shrimp fed both diets (Table 8). As previously seen with the juvenile shrimp, weight gain of the subadult shrimp offered the 16% protein diet increased in a curvilinear pattern. This response can be described by the following equations: weight gain $= 1.53 + 0.152x - 0.00258x^2$ where $x =$ g DP/(kg BWd), adjusted $r^2 = 0.79$. Whereas, weight gain of shrimp offered the 32% protein diet did appear to increase to a maximum at approximately 25 g DP/(kg BWd) (Fig. 3).

In the next subadult growth trial, shrimp were offered either a 32% or 48% protein diet at various feeding rates. On an isonitrogenous basis, protein content of the diet did not significantly influence weight gain but did influence FE and PCE values (Table 9). For shrimp fed the 48% protein diet, weight gain increased and then plateaued when the shrimp were offered more than 25 g DP/(kg BWd) (Fig. 4). As in the previous trials, FE and PCE values significantly decreased as feeding rates increased.

Nonlinear regression of the weight gain data for subadult shrimp offered the 32% protein diets at various daily protein intakes, indicated that the protein requirement for maximum growth was at 23.5 g DP/(kg BWd). The upper and lower 95% confidence intervals were 28.5 and 18.5 g DP/(kg BWd). Similarly, analyses of the data for shrimp offered the 48% protein diets indicated that the protein requirement for maximum

Table 9
Four-week growth responses of subadult *L. vannamei* (8.3-g mean initial weight) fed either a 32% crude protein diet (TRT32) or a 48% crude protein diet (TRT48).
Values represent means of three replicates. Numbers in the same column with different superscripts are significantly different ($P < 0.05$).

g DP/ (kg BWd)[a]	TRT32					TRT48				
	Ration (%)[b]	Weight gain (g)[c]	Weight gain (%)[d]	FE (%)[e]	PCE (%)[f]	Ration (%)[b]	Weight gain (g)[c]	Weight gain (%)[d]	FE (%)[e]	PCE (%)[f]
8	2.6	1.6[x]	19.5[y]	27.8[z]	26.5[z]	1.7	1.4[w]	17.4[w]	37.6[z]	16.8[yz]
12						2.6	2.1[wx]	24.7[wx]	34.5[z]	19.6[z]
16	5.1	2.9[y]	34.3[z]	22.9[y]	18.3[y]	3.4	2.4[xy]	28.8[xy]	30.3[z]	17.6[yz]
25	7.7	3.3[z]	39.3[z]	18.3[x]	14.5[x]	5.1	2.7[xyz]	32.4[xyz]	22.9[y]	13.7[y]
33	10.2	2.8[y]	34.4[z]	12.2[w]	10.4[w]	6.8	3.4[z]	40.7[z]	21.2[y]	13.3[y]
58						10.2	3.1[yz]	36.6[yz]	10.6[x]	6.4[x]
PSE[g]		0.12	1.68	0.95	1.04		0.23	2.73	1.96	1.17

[a] g dietary protein/kg body weight/day.
[b] g feed/100 g body weight/day.
[c] Weight gain = final weight − initial weight.
[d] Percent weight gain = weight gain × 100/initial weight.
[e] Feed efficiency = weight gain × 100/dry weight feed offered.
[f] Protein conversion efficiency = dry weight protein gain × 100/dry weight protein offered.
[g] Pooled standard error.

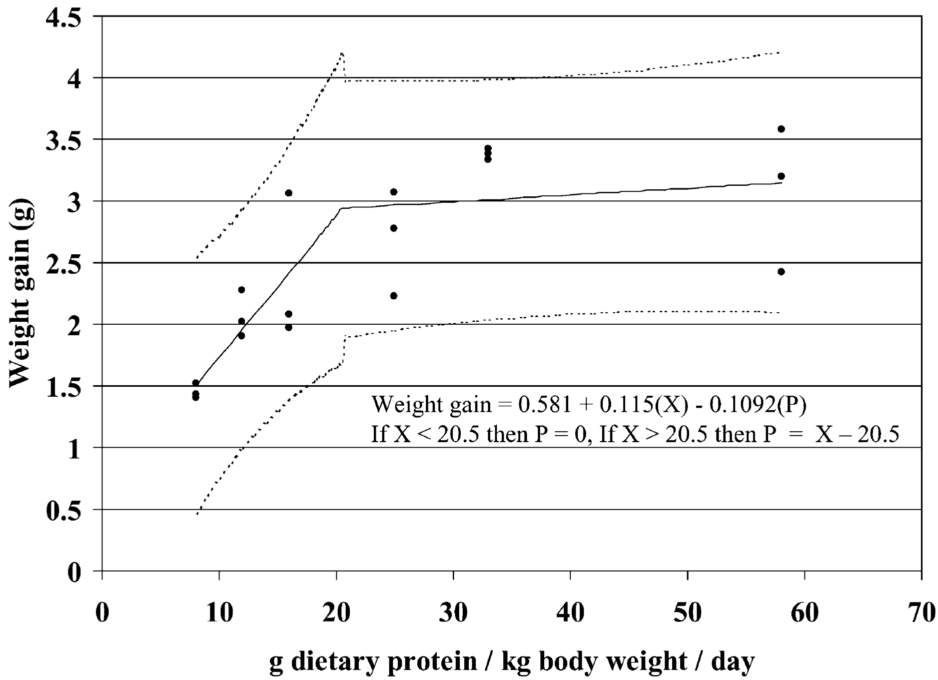

Fig. 4. Nonlinear regression of weight gain of subadult *L. vannamei* offered a 48% protein diet. Dotted line represents upper and lower 95% confidence interval about the predicted line.

growth was at 20.5 g DP/(kg BWd) with an upper and lower confidence interval of 29.5 and 11.5 g DP/(kg BWd).

3.3. 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 subadult 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 (76.1% and 75.1%, respectively).

4. Discussion

4.1. Maintenance requirements for protein

The first four trials of this study were conducted to determine maintenance protein requirements of juvenile and subadult shrimp. Log transformation of the independent variable was found to provide the best correlation coefficient values when regression analysis was performed. Both juvenile and subadult shrimp fed the 32% protein diet had higher maintenance protein requirement values. This is probably due to higher growth rates associated with feeding the 32% protein diet but could also be due to simple variation in the data. In general, juvenile shrimp have a maintenance protein requirement in the range of 1.8–3.8 g DP/(kg BWd), and subadult shrimp have a maintenance protein requirement in the range of 1.5–2.1 g DP/(kg BWd). The maintenance protein requirement of subadult shrimp fed a 16% protein diet (1.5 g DP/(kg BWd)) may have been affected by low salinity (21.0 ± 2.8) that occurred during the growth trial. However, since the determined maintenance values were relatively similar and the water was still hyper-osmotic, it is unlikely that the differences in salinity influenced the results.

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 (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 BWd), and larger red drum (~5.5-g initial weight) exhibited a maintenance requirement of 2.5 g DP/(kg BWd). Similarly, fingerling channel catfish have been shown to have a maintenance requirement of 1.32 g DP/(kg BWd), when fed diets containing either 25% or 35% crude protein (Gatlin et al., 1986).

4.2. 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. Juveniles fed the 16% protein diet did not reach a point at which growth clearly leveled as protein ration increased. A polynomial regression equation best described the growth curve. The poor growth response, which resulted from feeding the 16% protein diet, is probably due to the large quantity of feed, which must be consumed to meet daily nutrient requirements. The poor growth response could also be due to reduced digestibility of protein in the test diet. Growth of juveniles offered the 32% protein diet plateaued at 46.4 g DP/(kg BWd), which corresponds to a feeding rate of ~13% body weight.

Feeding the 32% protein diet resulted in a significantly greater weight gain and percent weight gain of juvenile shrimp than the diet containing 48% protein on a protein fed basis. However, the 48% protein diet had higher FE on an isonitrogenous basis. The lower weight gain, which resulted from feeding the diet containing 48% protein, is possibly due to the low energy to protein ratio of the diet, which would cause shrimp to utilize protein as a source of energy. Using protein as an energy source is relatively inefficient as compared with lipids (Hochachka, 1991) and would reduce the amount of protein available for tissue deposition. Shrimp exhibit a better FE when fed the 48% protein diet, because compared to the 32% protein diet, a smaller quantity of the 48% protein diet has to be fed to provide a given amount of protein and energy. Protein requirement for maximum growth of shrimp offered the 48% protein diet was found to be 43.4 g DP/(kg BWd). This value is very similar to the maximum requirement exhibited by juveniles offered the 32% protein diet (46.4 g DP/(kg BWd)). Based on these results, protein was provided at adequate levels by these two diets. However, FE at feeding levels associated with maximum growth is approximately 20% higher with the 48% protein diet. Although the 32% protein diet produced greater growth, a higher protein diet may be more efficient in achieving similar growth.

Growth of subadult shrimp fed the 16% protein diet did not appear to reach a maximum as the two highest feeding rates resulted in increases in weight gain; however, growth did appear to plateau. As with the juvenile shrimp, the best fitting line was found to be with a polynomial regression. In both cases, the poor performance of shrimp offered the 16% protein diet may have resulted from the inability of the shrimp to effectively consume sufficient feed, resulting in a limitation of protein 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 quantity would be required to equalize protein intake for the 16% protein diet). Nonlinear 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 BWd). This corresponds to a feeding rate of approximately 7% body weight. Interestingly, weight gain decreased after reaching a maximum. This response was seen in both experiments with the 32% protein diet. One possible explanation is that the shrimp had to unnecessarily expend energy to ingest excess feed, and digestion efficiency may have decreased due to faster passage rates associated with high rates of feed intake. A similar response was seen with the 48% protein diet. In this experiment, nonlinear regression predicted maximum growth at 20.51 g DP/(kg BWd), a value similar to that predicted with the 32% protein diet. Based on two-way ANOVA of the data, dietary protein did not significantly influence growth, but it did influence FE and PCE values. FE values clearly improved with increasing protein content or nutrient density of the diet. Although, there was not a significant interaction, PCE values were somewhat more variable. However, at protein levels near the apparent requirement (25 and 33 g DP/(kg BWd), PCE values were very similar possibly indicating that the energy to protein ratio of the diets were within the tolerance of the shrimp.

In general, juvenile and subadult shrimp had greater 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 increased with increasing protein content of the diet for juvenile shrimp. The poor protein digestion observed for the low protein diet could partly explain the poor response of shrimp to this 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 subadult shrimp, no significant differences were found in APD values for the 16% and 32% protein diets (76.1% and 75.1%, respectively). Consequently, based on the data collected in this study, several reasons for the differences in growth rates seen for shrimp offered the test diets are suggested. For the low protein diet, ingestion rate and digestibility were probably the primary factors influencing performance. Alternately, the poor response to the 48% protein diet was probably due more to the protein to energy ratio 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 *L. setiferus* by measuring changes in oxygen consumption and nitrogen excretion relative to fasting and feeding. The hypothesis proposed was that, the diet that 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 required to reach 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. Excess nitrogen excretion in the high protein diets was likely due to protein having to be used for energy as well as increased nitrogen intake. These observations help to explain the reduced growth of *L. vannamei* fed diets containing 16% and 48% protein in our feeding trials.

5. Conclusion

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

Acknowledgements

The authors would like to extend their thanks to those who have taken the time to critically review this manuscripts, as well as to the students and staff who help support the research at The University of Texas at Austin, Marine Science Institute Fisheries and Mariculture Laboratory. This research was supported in part by the Institutional Grant NA96RG0058 (project R/M-62), by the Texas Sea Grant College Program from the National Oceanic and Atmospheric Administration, U.S. Department of Commerce.

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