

## Effect of the Dietary Ratio of Digestible Energy to Crude Protein on Growth and Feed Conversion in Juvenile Pacific White Shrimp *Litopenaeus vannamei* under Similar Levels of Daily Protein Consumption

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**Abstract.**—We evaluated the effect of the dietary ratio of digestible energy (DE):crude protein (CP) on growth performance and nutrient utilization in juvenile Pacific white shrimp *Litopenaeus vannamei* fed various diets with a constant daily protein input. Juveniles (weight =  $0.94 \pm 0.04$  g [mean  $\pm$  SE];  $n = 30$ ) were stocked in an indoor recirculating-water system (173-L polyethylene tanks; 20 shrimp/tank) and were assigned to four different treatments. Two treatments consisted of 30% and 40% CP diets (30 isoenergetic [ISO]-100% and 40 ISO-75%, respectively) with a constant level of DE (3.28 kcal/g). Two treatments consisted of 30% and 40% CP diets with variable levels of DE (2.70 and 3.6 kcal/g, respectively) that maintained a constant DE:CP ratio of 9 kcal/g of protein (30 ratio [RAT]-100% and 40 RAT-75%, respectively). At the end of the 49-d trial, final weight and weight gain did not differ among the treatments. However, feed conversion ratio (FCR) was significantly lower ( $P < 0.01$ ) for the 40 RAT-75% and 40 ISO-75% groups than for the 30 ISO-100% and 30 RAT-100% groups. Final body composition (dry matter, CP, and gross energy) and protein conversion efficiency were not significantly different among the four treatments. However, energy conversion efficiency was significantly higher for the 40 ISO-75% group than for the 30 ISO-100% group. This study demonstrates that when the level of energy is appropriate, increasing dietary protein content allows for reduced feed inputs without affecting shrimp growth performance, while actually improving FCR and water quality due to presumably lower wastage of feed.

Protein is one of the major nutrients for shrimp growth and represents one of the primary costs in a compound feed formulation (Tacon and Akiyama 1997). Protein is also one of the nutrients that most contribute to water quality problems and pollution potential in shrimp farms. Nitrogen, the product of protein metabolism when this nutrient is not deposited as growth, is generally the most limiting nutrient for algae growth in marine and brackish water environments (Persson 1991). Hence, when shrimp farm effluents are released to coastal waters, the solid wastes and nutrients, especially N, can stimulate algae growth and cause serious eutrophication problems (Persson 1991; Cho and Bureau 2001).

The quantity and quality of dietary protein are factors that influence N excretion and waste. Therefore, feeding the optimal amount of protein in a nutritionally balanced and highly digestible diet is required to reduce the potential of N wastes. To optimize protein input, the amount of protein should be determined based on the protein requirement of the species and should be adjusted for energy in the diet. Traditionally,

to determine an animal species' optimal nutrient requirement, researchers have focused on the optimal level of the major nutrients in the diet (e.g., protein). However, it has been demonstrated that animals have a daily quantitative physiological requirement that can be provided by a variety of diets with different levels of the nutrients (Lawrence and Lee 1997; Lupatsch et al. 1998, 2001; McGoogan and Gatlin 1998; Kureshy and Davis 2002). Consequently, feed input will vary and should depend on the nutrient density of the diet.

Based on this approach, it has been suggested that the nutrient density of the diet be increased to reduce feed input and associated problems (Cho and Bureau 2001). The nutrient density of the diet can be increased by limiting the use of low-protein and low-lipid ingredients, such as grain by-products that are rich in starch and fiber, and by increasing the amount of ingredients with a high level of digestible protein and energy. These diets should be balanced for energy and nutrients.

The level of dietary energy in these nutrient-dense diets is critical because if the daily feed allowance is determined based on the species' nutrient requirements, the level of energy should be appropriate to avoid feeding suboptimal levels of energy that would depress growth or increase the use of protein as an energy source (Johnsen et al. 1991; NRC 1993; Steffens

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1996). It has been demonstrated that increasing the dietary ratio of digestible energy (DE):crude protein (CP) by increasing dietary nonprotein energy content results in an increase in N retention efficiency due to decreased catabolism of amino acids used for production of energy (protein sparing; Kaushik and Cowey 1991). Hence, an optimal energy:protein ratio has been suggested for shrimp diets to maximize growth and protein utilization.

Shrimp nutrition research on the use of high-nutrient-density diets at reduced feed allowance is limited in the literature. Similarly, studies analyzing the effect of the DE:CP ratio under limited feed allowances are also limited. Our objective was to evaluate growth performance and feed and nutrient utilization in Pacific white shrimp *Litopenaeus vannamei* fed four isonitrogenous diets (30% or 40% CP): two containing the same level of energy and two containing the same DE:CP ratio.

### Methods

This study was conducted at the Claude Petet Mariculture Center, Gulf Shores, Alabama, during summer 2004. Juvenile (mean weight =  $0.94 \pm 0.04$  g;  $n = 30$ ) Pacific white shrimp from a commercial hatchery (GMSB, Inc., Hatchery, Key West, Florida), initially maintained in nursery tanks for 22 d and then in ponds for 3 weeks, were pooled and distributed in an indoor recirculating-water system at a density of 20 shrimp/tank. Shrimp were fed four experimental diets (Tables 1, 2) that contained two different CP percentages (30% and 40%). Diets were assigned to four different experimental treatments that supplied similar amounts of N (CP) at differing feed rates. Two treatments consisted of 30% and 40% CP diets with a constant level of DE (3.28 kcal/g) fed at a full (100%) or reduced (75%) ration (30 isoenergetic [ISO]-100% and 40 ISO-75%, respectively). The other two treatments consisted of 30% and 40% CP diets with variable levels of DE (2.70 and 3.6 kcal/g, respectively) but a constant DE:CP ratio (RAT) of 9 kcal/g of protein (30 RAT-100% and 40 RAT-75%, respectively). Each treatment was assigned four replicate tanks. During the first week, the full ration was  $1.2 \text{ g} \cdot \text{shrimp}^{-1} \cdot \text{week}^{-1}$  (assumed feed conversion ratio [FCR] = dry weight of feed offered/wet weight gained = 2.0; growth = 0.6 g/week). Thereafter, the shrimp were offered  $1.62 \text{ g} \cdot \text{shrimp}^{-1} \cdot \text{week}^{-1}$  (assumed FCR = 1.8; growth = 0.9 g/week; Kureshy and Davis 2002).

The recirculating-water system consisted of 16 rectangular ( $61 \times 61 \times 61$ -cm) polyethylene tanks (Polytank, Inc., Litchfield, Minnesota). Each tank had approximately 10 cm of freeboard and was covered with a 0.5-cm nylon screen to prevent shrimp from

TABLE 1.—Composition of experimental diets (g/100g dry weight) fed to juvenile Pacific white shrimp in 2004: two isoenergetic (ISO) diets containing 30% or 40% crude protein (CP) and two diets with the same ratio (RAT) of digestible energy (DE) to CP and that also contained 30% or 40% CP. Expected values of DE, CP, and DE:CP are also shown.

Ingredient	30 ISO	40 ISO	30 RAT	40 RAT
Menhaden fish meal <sup>a</sup>	17	21.3	17	21.3
Soybean meal <sup>b</sup>	27	38.8	27	38.8
Menhaden fish oil <sup>c</sup>	3.2	2.6	0.7	7.5
Wheat gluten <sup>d</sup>	5.5	6.9	5.5	6.9
Wheat starch <sup>d</sup>	32.8	13.1	20.7	9.3
Whole wheat <sup>d</sup>	10	12	10	12
Trace mineral premix <sup>e</sup>	0.5	0.5	0.5	0.5
Vitamin premix <sup>f</sup>	1.8	1.8	1.8	1.8
Choline chloride <sup>d</sup>	0.2	0.2	0.2	0.2
Stay C (250 mg C/kg) <sup>g</sup>	0.07	0.07	0.07	0.07
CaP-monobasic <sup>d</sup>	1.3	1	1.3	1
Lecithin <sup>d</sup>	0.5	0.5	0.5	0.5
Cellulfil <sup>d</sup>	0	1.1	14.6	0
Cholesterol <sup>d</sup>	0.2	0.2	0.2	0.2
Formulated to contain				
CP (%)	30	40	30	40
Fat (%)	6.04	6.04	3.5	11
DE (kcal/g)	3.28	3.28	2.7	3.6
DE:CP ratio (kcal/g)	10.9	8.2	9	9

<sup>a</sup> Special Select, Omega Protein, Inc., Randeville, Louisiana.

<sup>b</sup> De-hulled solvent-extracted soybean meal, Southern States Cooperative, Inc., Richmond, Virginia.

<sup>c</sup> Omega Protein, Reedville, Virginia.

<sup>d</sup> United States Biochemical Company, Cleveland, Ohio.

<sup>e</sup> Trace minerals (in g/100 g of premix): cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250; ferrous sulphate, 4.0; magnesium sulphate heptahydrate, 28.398; monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulphate heptahydrate, 13.193; filler, 53.428.

<sup>f</sup> Vitamins (in g/100 g of 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 A acetate (20,000 international units [IU]/g), 5.0; vitamin D3 (400,000 IU/g), 0.002; DL tocopherol acetate (250 IU/g), 8.0; cellulose, 865.266.

<sup>g</sup> Stay C (L-ascorbyl-2-polyphosphate 35% active C); Roche, Inc., Parsippany, New Jersey.

jumping out. Each tank was equipped with a screened central standpipe (3.2-cm diameter; 65-cm length) that was set to maintain a water depth of 60 cm or a 16-L volume. The system was connected to a common circular tank that contained a biological filter and a 0.33-hp circulation pump. The recirculation rate for each tank was set at 4.1 L/min. Aeration was provided by two air stones connected to a common air supply from a 1.0-hp regenerative blower (Sweetwater Aquaculture, Inc., Lapwai, Idaho). The system was filled with filtered (AREA, Homestead, Florida; Model TR100), full-strength seawater. About half of the total water volume was changed every week with filtered seawater.

Pacific white shrimp were fed twice daily (i.e., 50% of daily feed allowance per feeding). In addition to

TABLE 2.—Gross energy; crude protein (CP); apparent digestibility of the energy (ADE) or protein (ADP) component; digestible energy (DE); digestible protein (DP); DE:CP ratio; and daily feed, DE, and DP inputs from experimental diets fed to juvenile Pacific white shrimp for 49 d during 2004. Two isoenergetic (ISO) diets contained 30% or 40% CP, and two diets with the same DE:CP ratio (RAT) also contained 30% or 40% CP.

Variable	30 ISO	40 ISO	30 RAT	40 RAT
Gross energy (kcal/g)	4.49	4.76	4.2	5.44
CP (%)	30.6	39.3	29.8	40.1
ADE (%)	77.8	78.3	70.1	77.6
ADP (%)	85.6	87.6	87.4	83
DE (kcal/g)	3.49	3.73	2.94	4.22
DP (%)	26.2	34.4	26.1	33.3
DE:CP ratio (kcal/g protein)	11.4	9.5	9.9	10.5
Daily feed input (g/shrimp) <sup>a</sup>	0.2081	0.1561	0.2081	0.1561
Daily DE input (kcal/shrimp) <sup>a</sup>	0.73	0.58	0.62	0.66
Daily DP input (g/shrimp) <sup>a</sup>	0.062	0.062	0.062	0.062

<sup>a</sup> Values averaged over 7 weeks.

daily observations for mortality, we counted shrimp in each tank every 2 weeks, and the daily ration was readjusted as needed. Dissolved oxygen (DO), pH, and temperature were measured twice daily (at 0600 and 1600 hours) with a YSI 556 DO meter (Yellow Springs Instrument Co., Yellow Springs, Ohio). Total ammonia N and nitrite-N were determined once per week. Water samples were taken from the middle of the water column in the filter and in two tanks selected at random. Total ammonia N was measured with a spectrophotometer (Spectronic Instrument, Inc., Rochester, New York; Spectronic 20 Genesys) and the Nesslerization method (APHA 1989). Nitrite-N was determined using a Model PLN code test kit from LaMotte (Chestertown, Maryland).

Diets were prepared by mixing all the dry ingredients into a food mixer (Hobart, Troy, Ohio) for 20 min and then adding menhaden oil and mixing for an additional 10 min. Boiling water was added to the mixer to attain a mash of consistency appropriate for pelleting. The mash was passed through a 3-mm die in a meat grinder (Hobart), and the pellets were dried in a forced-air drier set for a maximum temperature of 45°C until between 8% and 10% moisture was obtained. Diets were ground and sieved to an appropriate size and stored in a freezer at -15°C until use.

To evaluate Pacific white shrimp growth performance, we measured weight gain (initial weight - final weight); percent survival; FCR; total body dry matter, energy, and CP; protein conversion efficiency (PCE = 100 × [dry weight protein gain/dry weight protein offered]); energy conversion efficiency (ECE = 100 × [dry weight energy gain/dry weight energy offered]). Shrimp samples were collected at the beginning (pooled sample) and end of the experiment (6

shrimp/tank) and were kept in sealed plastic bags in a freezer at -15°C until analyzed.

To analyze the samples, frozen Pacific white shrimp were cut into pieces of about 2 cm and dried to constant weight in an oven at 90°C using standard methods (AOAC International 1990). The dry samples were then ground with a coffee grinder and stored in a freezer. The shrimp CP content was analyzed using the micro-Kjeldahl method (Ma and Zuazago 1942). Total energy content was determined using a microcalorimetric adiabatic bomb with benzoic acid as standard (Parr, Moline, Illinois; Model 1425).

*Digestibility determination.*—Chromic oxide was used as an inert marker to determine digestibility coefficients of the test diets. Digestibility determinations took place at the North Auburn Fisheries Research Station in Auburn, Alabama. At the end of the feeding trial, 12 groups of 10 Pacific white shrimp (~6-g mean weight) were stocked into twelve 60-L glass aquaria with closed recirculating systems. Every experimental diet was assigned three tanks. The four experimental diets were prepared using the same formulations (Table 1) and procedures except that 0.5% of the wheat starch was replaced by an equal amount of chromic oxide.

The shrimp were allowed to acclimate for 3 d before collection of feces was initiated. Prior to each feeding, the tanks were cleaned. The shrimp were then offered an excess of feed. One hour after feeding, feces were collected by siphoning onto a 500-µm-mesh screen. Shrimp were offered 5 feedings/d; however, feces obtained after the first feeding were discarded. Only well-formed, stable fecal filaments were collected to avoid contamination of the sample with food particles. Feces were rinsed with distilled water, stored in sealed plastic containers, and kept in a freezer. Samples collected from each tank over a 4-d period were pooled and kept frozen until analyzed. Dry matter, CP, and total energy were determined for the fecal and diet samples according to procedures previously described. Chromic oxide was analyzed by the methods of McGinns and Kasting (1964).

Apparent digestibility coefficients (ADCs) of the dry matter, protein, and energy were calculated according to Maynard et al. (1979):

$$\text{ADC}(\%) = 100 \times \left[ 1 - \left( \frac{\text{dietary Cr}}{\text{fecal Cr}} \right) \times \left( \frac{\text{fecal nutrient level}}{\text{dietary nutrient level}} \right) \right]$$

*Statistical analysis.*—Data were analyzed by a one-way analysis of variance. Significant differences

TABLE 3.—Water quality in experimental tanks containing juvenile Pacific white shrimp. Values are averages ( $\pm$  SD) of daily and weekly determinations (min = minimum; max = maximum).

Variable	Mean $\pm$ SD	Min, max
DO (mg/L)	6.5 $\pm$ 0.9	4.8, 8.4
pH	7.9 $\pm$ 0.1	7.5, 8.1
Temperature ( $^{\circ}$ C)	26.7 $\pm$ 1.7	21.2, 29.8
Salinity (g/L)	31.3 $\pm$ 1.6	28.1, 33.5
Total ammonia N (mg/L)	0.23 $\pm$ 0.27	0, 0.81
Nitrite-N (mg/L)	0.36 $\pm$ 0.46	0, 1.5

among treatment means ( $\alpha = 0.05$ ) were determined by the Student–Newman–Keuls multiple comparison test (Steel and Torrie 1980). Weight gain was regressed against feed input, dietary protein, and protein input. Analyses were performed in the Statistical Analysis System version 8.2 (SAS Institute, Inc., Cary, North Carolina).

### Results

Average DO, temperature, pH, and salinity in the aquaria were 6.5 mg/L, 26 $^{\circ}$ C, 7.5, and 31.5 g/L, respectively (Table 3). No water quality problems or diseases were observed during the trial. Total ammonia N ranged between 0.00 and 0.81 mg/L, and nitrite-N ranged between 0.0 and 1.5 mg/L (Table 3). Survival was 92.5–98.5% and did not differ among treatment groups (Table 4).

Formulated values for DE and CP are presented in Table 1, and the experimentally determined values for DE are presented in Table 2. Shrimp weight increased by between 542% and 574% relative to initial values, and no significant differences in weight among the treatments were observed (Table 4). For example, we found no significant differences in final weight or weight gain produced by the 30 ISO-100% and 40 ISO-75% diets (Table 4). However, FCR was significantly lower in the 40 ISO-75% group than in the 30 ISO-100% group. Similar to results for the isoenergetic diets, final weight and weight gain did not differ between the 30 RAT-100% and 40 RAT-75% groups (Table 4). Under a constant DE level, the FCR significantly improved from 2.18 to 1.66 when the feed allowance was reduced from 100% (30 ISO-100%) to 75% (40 ISO-75%; Table 4). At a constant DE:CP ratio, the FCR also improved when the feed rate was reduced from 100% (30 RAT-100%) to 75% (40 RAT-75%).

There were no significant differences in dry matter, body energy, body protein, and PCE among Pacific white shrimp offered the four experimental diets (Table 5). However, when the feed allowance in the isoenergetic diets was reduced from 100% (30 ISO-

TABLE 4.—Final weight (FW), overall (49-d) weight gain (WG; FW – initial weight [IW]), WG per week ((FW – IW)/7), FCR, and survival of juvenile Pacific white shrimp (IW = 0.94  $\pm$  0.04 g [mean  $\pm$  SR]) fed four experimental diets containing 30% or 40% crude protein (CP) and administered at 100% or 75% ratio: two diets were isoenergetic (ISO) and two diets had the same ratio (RAT) of digestible energy (DE) to CP. Within a column, means with differing letters are significantly different ( $P \leq 0.05$ ).

Diet or statistic	FW (g)	WG (g)	WG/week	FCR	Survival (%)
30 ISO-100%	6.1 z	5.1 z	0.73 z	2.18 z	97.8 z
40 ISO-75%	6.4 z	5.4 z	0.78 z	1.66 y	92.5 z
30 RAT-100%	6.2 z	5.2 z	0.74 z	2.09 z	94.3 z
40 RAT-75%	6.3 z	5.3 z	0.76 z	1.62 y	98.5 z
<i>P</i>	0.6734	0.5754	0.5706	0.0082	0.1534
Pooled SE	0.1829	0.1705	0.0243	0.1139	1.9659

100%) to 75% (40 ISO-75%), the ECE improved significantly, from 14.6% to 19.6% (Table 5). Energy conversion efficiency was not significantly different between the 30 RAT-100% and 40 RAT-75% diets.

Stepwise regression analyses between weight gain and feed rate, dietary protein, protein input, or energy input were not significant. However, regressions between energy fed and ECE ( $P = 0.0005$ ;  $R^2 = 0.5925$ ) and between feed rate and FCR ( $P = 0.0004$ ;  $R^2 = 0.5991$ ) were significant.

### Discussion

In a clear-water system, the only available source of nutrients for shrimp growth is the diet, since other sources of nourishment (e.g., invertebrates, organic detritus) that are normally present in ponds and other natural environments are not available (Leber and Pruder 1988; Moss et al. 1992; Moss 1995). Therefore, under clear-water tank conditions, it becomes para-

TABLE 5.—Final body dry matter (DM), crude protein (CP), gross energy (GE; calories [cal]/g of body weight [BW]), protein conversion efficiency (PCE), and energy conversion efficiency (ECE) of juvenile Pacific white shrimp fed four experimental diets containing 30% or 40% CP and administered at 100% or 75% ratio: two diets were isoenergetic (ISO) and two diets had the same ratio (RAT) of digestible energy (DE) to CP. Each value is a mean of four replicates. Within a column, means with differing letters are significantly different ( $P \leq 0.05$ ).

Diet or statistic	DM (%)	CP (%)	GE (cal/g BW)	PCE (%)	ECE (%)
30 ISO-100%	26.7 z	18.8 z	1.27 z	26.3 z	14.6 y
40 ISO-75%	25.6 z	18.4 z	1.20 z	25.6 z	19.6 z
30 RAT-100%	26.4 z	18.4 z	1.24 z	26.5 z	13.8 y
40 RAT-75%	26.6 z	18.5 z	1.25 z	25.8 z	15.8 y
<i>P</i>	0.077	0.6677	0.3501	0.9736	0.0087
Pooled SE	0.2788	0.2763	0.0245	1.5923	1.0273

mount to feed the optimal amount of a complete diet balanced for nutrients and energy. We demonstrated that when the diet is balanced for DE and protein and when feed allowance is determined based on nutrient and DE requirements, Pacific white shrimp can be raised using diets with different levels of CP without affecting growth performance. The results show that feed allowance can be reduced by 25% when dietary CP is increased from 30% to 40% without affecting weight gain.

Similar results in fishes have been reported by other investigators (Cho et al. 2001; Cho and Lovell 2002). However, for juvenile Pacific white shrimp, Kureshy and Davis (2002) reported opposite results. They obtained a significantly higher weight gain in shrimp fed a 32% CP diet than in shrimp fed a 16% or 48% CP diet when the three diets were offered on an isonitrogenous basis. The lower growth for the 16% CP diet was probably caused by low nutrient levels (e.g., protein and energy), which would require consumption of about twice the amount of the diet to match the protein intake of the 32% CP diet. Conversely, the low consumption of the 48% CP diet (66.7%) necessary to match the protein input of the 32% CP diet probably limited the amount of energy and other essential nutrients available for growth.

The dietary DE level is important because if the energy provided at a particular feed allowance is lower than the daily energy requirement, shrimp will utilize protein as an energy source (Johnsen et al. 1991; NRC 1993; Steffens 1996). In this study, the energy level for the two sets of diets (ISO and RAT) seemed to be appropriate for the two feed allowances provided (75% and 100%). If the energy level in the two diets with similar DE (30 ISO and 40 ISO) had been insufficient, growth would have been lower when the higher-protein diet (40 ISO) was offered at 75% of standard ration. However, final weight and body composition (dry matter, gross energy, and CP) did not differ between shrimp assigned these feeding regimes. Therefore, the 40 ISO-75% diet provided enough energy even though the energy allowance was reduced by 20% relative to the 30 ISO-100% diet. The ECE for the 40 ISO-75% group was improved, as would be expected, because less energy was offered but the same growth was attained. Shrimp did not have higher body energy when fed the 30 ISO-100% diet. In channel catfish *Ictalurus punctatus*, an increase in dietary DE:CP ratio led to an increase in body fat but growth was not improved (Cho and Lovell 2002). Although we did not measure shrimp body fat, we believe, based on body energy values, that fat was not higher in the 30 ISO-100% group than in the 40 ISO-75% group.

The two diets with similar DE:CP ratio at 100% (30

RAT) and at 75% (40 RAT) produced no differences in growth performance. This would be expected if the energy:protein ratio was adequate, as at both feed allowances the levels of protein and energy should be similar. Effectively, no significant differences in final weight and weight gain were observed. Even though the determined DE:CP ratios of these two diets were slightly different from the expected ratio of 9 kcal/g (9.9 kcal/g for 30 RAT; 10.5 kcal/g for 40 RAT), the daily energy supplied by these treatments was very similar ( $0.62$  and  $0.66 \text{ kcal} \cdot \text{g}^{-1} \cdot \text{shrimp}^{-1}$ , respectively). These DE:CP ratios are close to the optimal values for Pacific white shrimp and other shrimp species. Cousin et al. (1993) reported an optimal DE:CP ratio of 11.9 kcal/g for Pacific white shrimp, and they observed depressed growth at a ratio of 3.37 kcal/g. Aranyakananda and Lawrence (1993) reported that Pacific white shrimp had a protein dietary requirement of 15% and an optimum DE:CP ratio of 28.57 kcal/g when fed at libitum 15 times/d. For the grass prawn *Penaeus monodon*, Bautista (1986) and Shiau and Peng (1992) concluded that the optimum energy:protein ratio was 7.97 kcal/g. Literature values of optimal dietary protein and energy:protein ratios for shrimp and fish are conflicting, even within the same species. This is due to variations in the procedures and species used. Among these, the use of fish or shrimp of different sizes, selection of ingredients with different levels of digestibility and palatability, and variation in feeding rate and dietary energy account for much of the difference (Lupatsch et al. 2001). Some authors suggest that no specific energy:protein ratio or shrimp growth rate is optimal because energy or protein level controls the effect of the energy:protein ratio on growth (Cuzon and Guillaume 1997). Dietary protein must be adjusted according to DE level and feed allowance (Lupatsch et al. 2001). For example, the DE:CP ratio will be greater for larger shrimp due to the energy:protein ratio of the gain and the greater energy required for maintenance relative to smaller shrimp.

The energy contents of the diets we used did not appear to limit the amount of feed consumed by Pacific white shrimp. Some authors suggest that dietary energy level regulates food consumption in shrimp, as in other animal species (Sedgwick 1979; Cuzon and Guillaume 1997; Lupatsch et al. 2001). If the energy level of the 40 ISO-75% diet was appropriate, then the shrimp were fed an excess of energy when offered the 30% CP-100% diet. The excess energy could have limited food consumption and prevented shrimp from consuming enough food to meet protein requirements, which could have affected growth. However, shrimp growth did not differ between the two treatments. Another possible explanation is that the amount of protein fed was in

excess of the daily requirement. In this case, shrimp fed the 30 ISO diet would have met their protein requirement even if the higher energy level caused a reduction in food consumption. Kureshy and Davis (2002) reported that the minimum daily protein requirement was 46 g CP•kg body weight (BW)<sup>-1</sup>•d<sup>-1</sup> for juvenile Pacific white shrimp and 24 g CP•kg BW<sup>-1</sup>•d<sup>-1</sup> for subadults. In our research, the daily CP offered ranged between 15.8 g CP•kg BW<sup>-1</sup>•d<sup>-1</sup> for the last week of the trial and 61.1 g CP•kg BW<sup>-1</sup>•d<sup>-1</sup> for the first week. An average (geometric mean) of 30.8 g CP•kg BW<sup>-1</sup>•d<sup>-1</sup> was offered during the 49-d trial. Based on these values, it is likely that protein was not fed in excess and that shrimp consumed an appropriate amount of protein when fed the 40 ISO-75% diet. Furthermore, there were no significant differences in PCE among the different treatments. Since the same amount of CP was offered in all treatments, differences in PCE could have been expected if nonprotein energy was limiting. For example, the 30 ISO-100% group (higher energy input) did not attain higher PCE (26.3%) than the 40 ISO-75% group (lowest energy input; PCE = 25.6%). All of the protein offered was probably used for growth, and the extra energy provided by 30 ISO-100% did not help to spare protein; thus, energy was probably not limiting in any of the diet–ration combinations.

We have demonstrated that as long as feed allowance is adjusted for dietary energy and protein density, the nutrient requirements of Pacific white shrimp can be met by different diets with variable levels of protein and energy. As has been demonstrated in other species (Lawrence and Lee 1997; Lupatsch et al. 1998, 2001; McGoogan and Gatlin 1998; Kureshy and Davis 2002), shrimp have a daily quantitative physiological requirement that can be provided by a variety of diets with different nutrient densities. Our results clearly indicate that Pacific white shrimp can tolerate a variety of dietary energy levels and, as long as energy is not limited, their nutrient requirements can be met with a variety of protein and feed input levels.

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### References

- AOAC International. 1990. Official methods of analysis, 15th edition, volume 1. AOAC International, Arlington, Virginia.
- Aranyakananda, P., and A. L. Lawrence. 1993. Dietary protein and energy requirements of the white-legged shrimp, *Penaeus vannamei*, and the optimal protein to energy ratio. From discovery to commercialization. European Aquaculture Society, Oostende, Belgium.
- Bautista, M. N. 1986. The response of *Penaeus monodon* juveniles to varying protein/energy ratios in test diets. *Aquaculture* 53:229–242.
- Cho, C. Y., and D. P. Bureau. 2001. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *Aquaculture Research* 32:349–360.
- Cho, S. H., J.-Y. Jo, and D. S. Kim. 2001. Effects of variable feed allowance with constant energy and ratio of energy to protein in a diet for constant protein input on the growth of common carp (*Cyprinus carpio* L.). *Aquaculture Research* 32:349–356.
- Cho, S. H., and R. T. Lovell. 2002. Variable feed allowance with constant protein input for channel catfish (*Ictalurus punctatus*) cultured in ponds. *Aquaculture* 204:101–112.
- Cousin, M., G. Cuzon, E. Blanchet, and F. Ruelle. and AQUACOP. 1993. Protein requirements following an optimum dietary energy to protein ratio for *P. vannamei* juveniles. Pages 599–606 in S. J. Kaushik and P. Luquet, editors. *Fish nutrition in practice*. Institut National de la Recherche Agronomique, Paris, France.
- Cuzon, G., and J. Guillaume. 1997. Energy and protein : energy ratio. Pages 51–70 in L. R. D'Abramo, D. E. Conklin, and D. M. Akiyama, editors. *Crustacean nutrition, advances in world aquaculture, volume 6*. World Aquaculture Society, Baton Rouge, Louisiana.
- Johnsen, F., M. Hillestad, and E. Austreng. 1991. High energy diets for Atlantic salmon, effects on pollution. Pages 391–401 in S. J. Kaushik and P. Luquet, editors. *Fish nutrition in practice*. Institut National de la Recherche Agronomique, Paris, France.
- Kaushik, S. J., and C. B. Cowey. 1991. Dietary factors affecting nitrogen excretion by fish. Pages 3–19 in C. B. Cowey and C. Y. Cho, editors. *Proceedings of the first international symposium on nutritional strategies in management of aquaculture waste*. Fish Nutrition Research Laboratory, University of Guelph, Ontario.
- Kureshy, N., and D. A. Davis. 2002. Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture* 204:125–143.
- Lawrence, A. L., and P. G. Lee. 1997. Research in the Americas. Pages 566–587 in L. R. D'Abramo, D. E. Conklin, and D. M. Akiyama, editors. *Crustacean nutrition, advances in world aquaculture, volume 6*. World Aquaculture Society, Baton Rouge, Louisiana.
- Leber, K. M., and G. Pruder. 1988. Using experimental

- microcosms in shrimp research: the growth enhancing effect of shrimp pond water. *Journal of the World Aquaculture Society* 19:197–203.
- Lupatsch, I., G. W. Kissil, D. Sklan, and E. Pfeffer. 1998. Energy and protein requirements for maintenance and growth in gilthead seabream (*Sparus aurata* L.). *Aquaculture Nutrition* 4:165–173.
- Lupatsch, I., G. W. Kissil, D. Sklan, and E. Pfeffer. 2001. Effects of varying dietary protein and energy supply on growth, body composition and protein utilization in gilthead seabream (*Sparus aurata* L.). *Aquaculture Nutrition* 7:71–80.
- Ma, T. S., and G. Zuazago. 1942. Micro-Kjeldahl method for organic nitrogen. Academic Press, New York.
- Maynard, L. A., J. K. Loosli, H. F. Hintz, and R. G. Warner. 1979. *Animal nutrition*, 7th edition. McGraw-Hill, New York.
- McGinnis, A. J., and R. Kasting. 1964. Colorimetric analysis of chromic oxide used to study food utilization by phytophagous insects. *Food Chemistry* 12:259–262.
- McGoogan, B. B., and D. M. Gatlin. 1998. Metabolic requirements of red drum, *Sciaenops ocellatus*, for protein and energy, based on weight gain and body composition. *Journal of Nutrition* 128:123–129.
- Moss, S. M. 1995. Production of growth-enhancing particles in a plastic-lined shrimp pond. *Aquaculture* 132:253–260.
- Moss, S. M., G. Pruder, K. M. Leber, and J. A. Wyban. 1992. The relative enhancement of *Penaeus vannamei* growth by selected fractions of shrimp pond water. *Aquaculture* 101:229–239.
- NRC (National Research Council). 1993. *Nutrient requirements of fish*. National Academy Press, Washington, D.C.
- Persson, G. 1991. Eutrophication resulting from salmonid fish culture in fresh and salt waters: Scandinavian experience. Pages 163–185 in C. B. Cowey and C. Y. Cho, editors. *Proceedings of the first international symposium on nutritional strategies in management of aquaculture waste*. Fish Nutrition Research Laboratory, University of Guelph, Ontario.
- Sedgwick, R. W. 1979. Influence of dietary protein and energy on growth, food consumption and food conversion efficiency in *Penaeus merguensis*. *Aquaculture* 16:7–30.
- Shiau, S. Y., and C. Y. Peng. 1992. Utilization of different carbohydrates at different dietary protein levels in grass prawn *Penaeus monodon* reared in sea water. *Aquaculture* 101:240–250.
- Steel, R. G. D., and J. H. Torrie. 1980. *Principles and procedures of statistics: a biometrical approach*. McGraw-Hill, New York.
- Steffens, W. 1996. Protein sparing effect and nutritive significance of lipid supplementation in carp diets. *Archives of Animal Nutrition* 49:93–98.
- Tacon, A. G. J., and D. M. Akiyama. 1997. Feed ingredients. Pages 411–472 in L. R. D'Abramo, D. E. Conklin, and D. M. Akiyama, editors. *Crustacean nutrition, advances in world aquaculture, volume 6*. World Aquaculture Society, Baton Rouge, Louisiana.