PERFORMANCE OF THREE DIETS WITH DIFFERENT PROTEIN: ENERGY RATIOS ON THE CULTURE OF THE PACIFIC WHITE SHRIMP, *LITOPENAEUS VANNAMEI*, UNDER PRACTICAL DESCENDING TEMPERATURE CONDITIONS

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RESUMO

Desempenho de três dietas com diferente relações proteína: energia na cultura do camarão branco de pacífico, *Litopenaeus vannamei*, sob condições de diminuição da temperatura

Três dietas com diferente relações de proteína:energia: 66,7, 77,8, e 87,5 mg de PC/kcal foram avaliados para cultivo de *Litopenaeus vannamei* em um experimento de 10 semanas durante a estação de diminuição de temperatura no Nordeste do México. As unidades experimentais consistiram em nove tanques de plástico de 4000-L. Não foram detectadas diferenças significativas entre os tratamentos em termos de ganho de peso (2,60 a 2,83 g), taxa de crescimento semanal (0,26 a 0,28 g/semana), percentual de ganho de peso (176 a 188 %), taxa se conversão alimentar (2,9 a 3,2), e sobrevivência (64,3 a 72,5%). Os dados foram analisados separadamente, nas primeiras cinco semanas e nas últimas semanas, detectando-se diferenças significativas. Nas primeiras 5 semanas (temperatura> 18℃), não foram observadas diferenças entre dietas no que diz respeito a ganho de peso (2.20 to 2.42 g), taxa de crescimento semanal (0.44 to 0.48 g/week), percentual de ganho de peso (147 to 161 %) e taxa de conversão alimentar (2.1 to 2.2). Nas últimas 5 semanas (Temperatura <17℃), todos os parâmetros de produção foram muito baixos e diferenças significativas foram encontradas entre. Os melhores resultados foram observados no tratamento 66,7 mgCP/Kcal. Os resultados preliminares obtidos no presente estudo sugerem uma forte relação entre a razão proteína:energia e o desempenho produtivo do camarão-branco do Pacífico em baixas temperaturas.

PALAVRAS CHAVE: Aqüicultura de inverno; *Litopenaeus vannamei*; nutrição de camarão; relações de proteína:energía; baixa temperatura.

ABSTRACT

Three diets with different protein:energy ratios: 66.7, 77.8, and 87.5 mg CP/kcal were evaluated for *Litopenaeus vannamei* farming in a 10-week trial during the fall season in Northwestern Mexico when temperature was descending. Experimental units consisted of nine 4000-L plastic tanks. For the complete 10-week set of data, no significant differences in total weight gain (2.60 to 2.83 g), weekly growth (0.26 to 0.28 g/week), percent of weight gain (176 to 188 %), fed conversion ratio (2.9 to 3.2), and survival (64.3 to 72.5%) were found among diets. Data analyzed separately, considering the first and the last 5 weeks of study, showed to be different. For the first 5 weeks (temperature > 18℃), no differences in weight gain (2.20 to 2.42 g), weekly weight gain (0.44 to 0.48 g/week) percent weight gain (147 to 161 %), and FCR (2.1 to 2.2) were observed among diets. For the last 5 weeks (Temperature < 17℃), all the production parameters were very poor and significant differences were found between diets, with the best results observed in the treatment 66.7 mgCP/kcal and without differences between the other two diets. The preliminary results found in the present study strongly suggest an effect of protein:energy ratio on the productive performance of Pacific white shrimp at low temperature.

KEY WORDS: *Litopenaeus vannamei*; low temperature; protein:energy ratio; shrimp nutrition; winter aquaculture.

INTRODUCTION

Shrimp aquaculture in Northwestern Mexico has emerged in the last 2 decades as one of the most important economic activities, in spite of adverse environmental conditions during at least a third part of the year. Temperature in this region is adequate for farming *Litopenaeus vannamei* during only 8 months of the year (from March to October). This species is now being farmed in many other countries of the world such as China and Brazil where temperature in some regions are sometimes out of the range considered suitable for its culture. Some farmers run two short growing cycles a year and harvest shrimps around 15 g. Others run only one long growing cycle, getting shrimps over 25 g. Some times it is necessary to extend the culture period to months with suboptimal low temperature in order to reach a little greater size which can represent a difference in price, or simply to maintain the organisms alive, waiting for a better opportunity in the market. In this respect, commercially available shrimp feeds may not perform at lower temperatures as adequately as they do under optimal conditions.

Protein is the most important nutrient in the diet of any aquatic organism, and also the most expensive component of formulated feeds. They are an important part of muscles and connective tissue of crustaceans; can be used as energy source, and also to build antibodies for the immune system of the organisms (Lehninger 1995). The protein content of feed has an important effect on the digestive enzymatic activity of shrimp (Le Moullac et al. 1996). The excess, as well as the deficiency of protein may have negative effects on the productive response of farmed organisms. Guzman et al. (2001) reported massive mortalities of *L. setiferus* fed on a diet with high protein content, as a result of the accumulation of ammonia in hemolymph. Schmitt and Santos (1999) found that metabolism of *Farfantepenaeus paulensis* was affected by nitrogen metabolites and excess of protein in feed was the main source of them.

Although not considered a nutrient, energy is a necessary element for all the metabolic functions of any organism. Energy is the product of absorption and metabolism of macronutrients contained in feed (Rodríguez 1999). It is very important to formulate feeds containing the necessary amount of energy for the farmed organisms, not more, not less. Hajra et al. (1988) found that in feeds for *Penaeus monodon*, levels of energy lower or higher than optimal, negatively affected its growth.

Shrimp can obtain energy from protein, lipid and carbohydrate (Rosas-Vásquez 1996). In this context, an adequate formulation is necessary in order to maximize the use of proteins for tissue building and growth, rather than a source of energy. This strategy is adequate not only from an economic viewpoint because of the high cost of protein sources, but also from an ecological perspective, because metabolism of protein produces nitrogen metabolites such as ammonia, which can be environmentally dangerous at relatively low levels (Bautista 1986; Akiyama et al. 1993; Lin and Chen 2003).

Over 30 years ago, Andrews et al. (1972) pointed out the importance of the protein:energy (P:E) ratio for aquaculture organisms. Unfortunately, few studies in this field have been focused on penaeid shrimp. Rosas et al. (2001) and Cousin et al. (1993) evaluated the P:E requirements for *L. vannamei*, Guzman et al. (2001) for *L. setiferus*, and Alava and Pascual (1987) and Hajra et al. (1988) for *P. monodon*. However, all of these studies have been conducted under controlled environmental conditions, i.e., within optimal environmental ranges for the studied species. It is plausible to think that when temperature and/or salinity are out of optimal ranges, the requirements for protein, energy and their ratio must be different. It has been shown that some fishes require more energy to maintain their metabolism when farmed at low temperature or high salinity (Watanabe et al. 1993; El-Sayed 2006). Fox and Lawrence (2004) found that *L. vannamei* use more protein as energy source when farmed at low salinity.

In semi-intensive shrimp farming, temperature and other environment parameters, are not controlled and varied over the time in a range depending on the region. In northwest Mexico, the variation of temperature during the farming period (from April to November) may be over 18 °C. The most critical period is from June to November when it may descend from 32-33 to 15-17 °C. It is very important to know what the performance of diets is under these descending temperature conditions, and not only in controlled laboratory experiments.

Pacific white shrimp grows optimally when temperature ranges from 25 to 30 °C, but its growth rate decreases as temperature descends, and stops at temperatures under 18 °C (Martinez-Cordova 1999).

The present study is a preliminary approach on the performance of three diets with different P:E ratios on the productive response of Pacific white shrimp, *L. vannamei*, farmed under practical descending temperature conditions in Northwest Mexico.

MATERIALS AND METHODS

The study was carried out during 10 weeks in the facilities of the Kino Bay Experiment Station (KBES), Department of Scientific and Technological Research, University of Sonora, at Kino Bay, Sonora, Northwest Mexico. Nine outdoor 4,000-L plastic tanks were used as experimental units. A 3-5 cm layer of soil was added to each plastic tank in order to simulate shrimp ponds. Postlarvae of *L. vannamei* were obtained from a commercial hatchery located in Kino Bay and maintained in a nursery phase in plastic tanks at the KBES. The experiment started when juveniles reached approximately 1.5 g of wet body weight and they were stocked at 100 juveniles per tank (25 shrimp/m^3).

Treatments consisted of three experimental diets formulated to have P:E ratios of 66.7, 77.8 and 88.5 mgCP/kcal (treatments 1, 2 and 3, respectively). Diets of treatment 2 and 3 had the same protein level (around 35%), and a lower level for the treatment 1 (around 30%).

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Feeds were prepared by cold extrusion using a Hobart A-2000 Extruder™ (Hobart, Troy, Ohio, USA) and then analyzed to determine their chemical proximate composition according to standardized procedures (AOAC, 1999). Table 1 shows the formulation and proximate composition of the diets.

Table 1. Ingredient composition and determined proximate composition of experimental diets (% of dry weight) with different P/E ratios for *L. vannamei*.

	P/E ratio (mgCP/kcal)		
	66.7	77.8	88.5
Wheat starch[1]	12.00	14.00	9.50
Fish meal[2]	35.00	40.00	22.00
Soy paste[3]	10.00	17.00	15.00
Sorghum[4]	28.20	14.91	25.00
Blood meal[5]	0	0	14.20
Fish oil[6]	2.00	2.00	2.50
Soy oil[7]	3.00	2.29	2.50
Soy lecithin[8]	3.00	3.00	2.50
Vitamin premix[9]	0.50	0.50	0.50
Vitamin C stable[10]	0.30	0.30	0.30
Mineral premix[11]	2.00	2.00	2.00
Alginate[12]	4.00	4.00	4.00
TOTAL	100.00	100.00	100.00
Proximate composition			
Moisture	4.4	5.2	5.4
Crude protein	29.4	34.5	34.3
Crude fat	12.1	12.5	11.3
Ash	9.6	10.8	9.6
Fiber	4.7	4.6	5.0
Gross energy (kcal/Kg)[13]	4458.7	4520.3	4100.7
P/E	65.9	76.4	83.5
Digestible energy(kcal kg^{-1})[14]	3638.1	3694.2	3181.3

[1]Wheat starch (ICN102955); [2]Sardine meal (HPS9901-65); [3]Soy paste (SP9901-01); [4]Sorghum moiled (SM9901-0019); [5]Blood meal (Rendimientos Proteicos, S.A. de C.V., Culiacán, Sinaloa, Mexico); [6]Fish oil (APES9901); [7]Soy oil (ASA. USA); [8]Soy lecitin (LSY9901); [9]Vitamin premix (units/kg feed) Vitamin A, 5000 UI; Vitamin D, 4000 UI; Vitamin E, 100 mg; Vitamin K, 5 mg; Thiamin, 60 mg; Rivoflavin, 25 mg; Pyridoxine, 50 mg; Pantothenic acid, 75 mg; Niacin, 50 mg, Biotin, 1 mg; Inositol, 400 mg, Cyanocobalamine, 0.2 mg; Folic acid, 10 mg; [10]Star-C (Roche); [11]Mineral premix (mg/kf feed), KCl, 0.5; $MgSO_4$, 0.5; $ZnSO_4$, 0.09, $MnCl_2$, 0.0234; $CuSO_4$, 0.005; KI, 0.005; $CoCl_2$, 0.0025; Na_2HPO_4, 2.37. [12]Alginate (Faga Lab S.A. de C.V., Mazatlan, Sinaloa, Mexico); [13]Determined values with a bomb calorimeter.; [14]Estimated values with the feed formulation program AgriData LTD, Tipperary, Ireland.

Starting with a feed rate of 5% of wet body weight, feed was supplied twice a day in feeding trays, adjusting the daily ration according to apparent consumption as suggested by Salame (1993).

Temperature, salinity, pH and dissolved oxygen were recorded twice a day by means of a bulb thermometer, a refractometer (type salinometer), a pen pH meter, and a membrane oxygen meter, respectively. Approximately 10% daily water exchange was applied to plastic tanks. Total ammonium nitrogen (TAN), nitrates, nitrites, and ortophosphates were measured weekly by colorimetric methods, using a programmable spectrophotometer Hach DR 4000™ (Hach Co. Loveland, CO., USA).

Weight gain of shrimp was monitored each week by weighing a sample of 10 individuals of each experimental unit in a digital Sartorious™ balance. Final weight, survival, weight gain (final-initial weight), % weight gain, growth rate, and feed conversion ratio (FCR) was known at the end of the study when all the organisms were harvested.

Data were analyzed by one-way ANOVA with a significance level of $\underline{P} < 0.05$. Duncan tests were applied to data, as they showed to have a normal distribution and a homogeneous variance. A first analysis was made for the complete 10-week set of data and a second analysis was applied separately to data from the first 5 weeks (when mean temperature remained over 18℃) and from the last 5 weeks (when temperature was under 17℃).

RESULTS

Table 2 shows means and standard deviation of environmental and water quality parameters in the treatments during the study. Mean temperature remained over 25 ℃ only the first 2 weeks, subsequently it was descending towards the end of the culture, when a temperature of 14.2 ℃ was recorded. Mean salinity remains always over 47 ppt. Mean pH ranged between 8.4 and 8.5. No significant differences in temperature, salinity and pH were recorded among treatments. Mean dissolved oxygen was higher in treatment 3 with a value of 7.5 mg/L, and no differences were observed between the other two treatments, both with a mean of 6.8 mg/L. Mean concentration of nitrates, nitrites and orthophosphates were not significantly different among diet treatments. Nitrates varied from 1.3 to 1.5 mg/L, the mean concentration of nitrites was 0.001 mg/L in all treatments, and ortophosphates ranged from 0.66 to 0.96 mg/L. Total ammonium nitrogen was higher in treatment 3, with a mean concentration of 0.049 mg/L, and no differences were observed between the other two treatments, with mean concentrations of 0.030 and 0.031 mg/L.

Table 2. Means (± SD) of environmental and water quality parameters during the study.

	P/E ratio (mgP/kcal)		
	66.7	77.8	87.5
Temperature (℃)	18.26 ± 3.1^a	18.29 ± 3.2^a	18.25 ± 3.0^a
Salinity (‰)	47.7 ± 0.4^a	47.7 ± 0.4^a	47.4 ± 0.3^a
DO (mg/L)	6.8 ± 0.2^a	6.8 ± 0.1^a	7.5 ± 0.3^b
pH	8.5 ± 0.1^a	8.5 ± 0.1^a	8.4 ± 0.1^a
$N\text{-}NO_3$ (mg/L)	1.30 ± 0.50^a	1.30 ± 0.60^a	1.50 ± 0.50^a
$N\text{-}NO_2$ (mg/L)	0.01 ± 0.001^a	0.01 ± 0.001^a	0.01 ± 0.001^a
TAN (mg/L)	0.031 ± 0.004^a	0.030 ± 0.003^a	0.049 ± 0.003^b
$P\text{-}PO_4$ (mg/L)	0.96 ± 0.37^a	0.87 ± 0.46^a	0.66 ± 0.25^a

Means with different superscripts in the same row are significantly different ($\underline{P} < 0.05$).

For the complete 10-weeks set of data, total weight gain, weekly growth rate, and percent of growth of shrimp were similar in all treatments. Slightly higher values, but not significant, were recorded in the treatment 1. Feed conversion ratio (FCR) was somewhat lower in treatment 1, as compared to the two other, but without significant differences. Survival was slightly higher in treatment 3 (72.3%) but without significant differences with the values obtained in treatment 1 (62.3%) and treatment 2 (64.5%) as shown in Table 3.

Table 3. Means (± SD) of production parameters of *L. vannamei* fed diets with different P/E ratios during the 10 weeks.

	P/E ratio (mgP/kcal)		
	66.7	77.8	87.5
Initial weight (g)	1.50 ± 0.01[a]	1.50 ± 0.01[a]	1.50 ± 0.01[a]
Final Weight (g)	4.33 ± 0.17[a]	4.09 ± 0.02[a]	4.13 ± 0.30[a]
Weight gain (g)	2.83 ± 0.18[a]	2.60 ± 0.03[a]	2.63 ± 0.29[a]
% weight gain	188.60 ± 12.80[a]	173.40 ± 2.60[a]	176.80 ± 18.40[a]
weekly weight gain (g/week)	0.28 ± 0.10[a]	0.26 ± 0.01[a]	0.26 ± 0.10[a]
Survival	64.3 ± 3.75[a]	64.5 ± 3.66[a]	72.5 ± 4.87[a]
FCR	2.91 ± 0.30[a]	3.20 ± 0.09[a]	3.10 ± 0.40[a]

Means with different superscripts in the same row are significantly different ($\underline{P} < 0.05$).

When data were analyzed separately for the first 5 weeks (using initial weight and weight at week 5), when mean temperature was over 18 ℃, and the last 5 weeks (using weight at weeks 5 and 10), when it was under 17℃, results were different as shown in Table 4. For the first 5 weeks, no differences in total weight gain (2.20 to 2.42 g), weekly growth rate (0.42 to 0.48 g/week), percent weight gain (147 to 161%) and FCR (2.1 to 2.2) were found among diet treatments. For the last 5 weeks, all the growth parameters (total weight gain, weekly growth rate, and percent of weight gain) were significantly higher, and FCR lower for the treatment 1, and no differences between the two other treatments were found. In general, the production parameters observed in the last 5 weeks of study were very poor. Total weight gain ranged from 0.30 to 0.70 g; weekly growth rate from 0.06 to 0.09 g/week; percent weight gain from 2.1 to 19.4 %, and FCR from 3.3 to 3.7.

Table 4. Production parameters (means ±) of *L. vannamei* fed diets with different P/E ratios during the first 5 and the last 5 weeks of study.

	P/E ratio (mgCP/kcal)		
	66.7	77.8	87.5
Weight gain first 5 weeks	2.20 ± 0.15[a]	2.31 ± 0.16[a]	2.42 ± 0.16[a]
Weight gain last 5 weeks	0.70 ± 0.08[b]	0.32 ± 0.05[a]	0.30 ± 0.04[a]
Weekly weight gain (g), first 5 weeks	0.44 ± 0.03[a]	0.46 ± 0.03[a]	0.48 ± 0.04[a]
Weekly weight gain (g), last 5 weeks	0.09 ± 0.01[b]	0.06 ± 0.01[a]	0.06 ± 0.01[a]
% weight gain first 5 weeks	147 ± 11.12[a]	154 ± 12.09[a]	161 ± 12.97[a]
% weight gain last 5 weeks	19.4 ± 1.21[b]	2.3 ± 0.21[a]	2.1 ± 0.18[a]
FCR first 5 weeks	2.2 ± 0.16[a]	2.1 ± 0.16[a]	2.1 ± 0.15[a]
FCR last 5 weeks	3.3 ± 0.12[a]	3.7 ± 0.11[b]	3.7 ± 0.11[b]

Means with different superscripts in the same row are significantly different ($\underline{P} < 0.05$).

DISCUSSION

Significant differences were detected for dissolved oxygen (DO) and TAN. However, neither oxygen was limiting nor TAN was found at toxic levels for shrimp from any of the treatments. Thus, the environmental and water quality parameters, except for temperature and salinity, remained within ranges considered suitable for the culture of *L. vannamei* (Boyd and Tucker 1998; Martinez-Cordova 1999, 2002). Salinity was above the recommended levels for the species throughout the experimental period (Bray et al. 1994). Temperature values within optimal levels were recorded only during the first 2 weeks, while values under the recommended range were observed the remainder of the study. This condition negatively affected the productive performance of shrimp. The growth rate during the first 2 weeks was around 1 g/week, comparable to what is generally obtained in most commercial semi-intensive farms (Clifford 2003). However, for the complete trial, the mean growth rate was from 0.26 to 0.28 g/week, very poor for commercial purposes (Leung and Engle 2006). Survival obtained in the three treatments for the 11 weeks trial ranged on the values acceptable for commercial shrimp farms (Martinez Cordova 2008). For the first 5 weeks of study, when mean temperature ranged from 18 to 25 °C, the growth rate ranged from 0.44 to 0.48 g/week, which can be considered acceptable, especially if only small additional weight gain is required for shrimp to reach a size with improved market value. Under these conditions, no differences in productive parameters of shrimp were found among the three experimental diets. This means that a diet with 30% crude protein and energy content of 4,500 kcal/Kg was as good as other with 35% crude protein and the same energy content. This can represent an important savings in feed cost. When temperature was lower than 17 °C, the production parameters were extremely poor and the culture under those circumstances is not economically viable, unless farmers need to maintain the organisms alive, awaiting for a better price in the market or for some other reason. In that case, the lowest P:E ratio of 66.7 showed to be better than the two other treatments, probably because of the lower protein content (30% vs. 35% in the other treatments). At very low temperature metabolism is depressed and the requirements for protein diminish (Chen and Lai 1993; Jiang et al. 2000). Furthermore, an excess of protein may cause a depression in shrimp growth, as described by Millamena et al. (1998). With respect to the treatments 77.8 and 87.5, the results ruled out an effect of the energy content at this protein level (35%). Cruz-Suárez et al. (2000) reported a P:E ratio of 67 mgP/kcal of digestible energy (DE) as optimal for *L. vannamei*. Dokken (1987) and Cousin et al. (1993) coincided at an optimal value of 83.2 mgP/kcal (DE) for the same species. Rosas-Vásquez (1996) found optimal values between 26-36 mgP/kJ of gross energy (GE) (108-150 mgP/kcal) for *L. vannamei*. The P:E ratios used in the present study, as those reported by Rosas-Vásquez (1996), are expressed on a GE-basis. Thus, they seem to be considerably lower. On the other hand, when they are expressed on an estimated DE (DE values from Table 1)-basis (82.8, 95.2, and 110.9 mg CP/kcal for treatments 1, 2, and 3, respectively), the lowest P:E ratio is almost identical to the optimal value reported by Dokken (1987) and Cousin et al. (1993), which may help to explain its better performance. However, these comparisons are only theoretical, since digestible energy values were estimated but not determined, and perhaps more importantly, the experiment was conducted in tanks simulating ponds with the participation of natural food, while the previous studies were conducted under clear-water laboratory conditions. It has been widely documented that natural feed, including zooplankton, benthos and inclusively microorganisms, can supply up to 70% or more of the nutritional requirements of shrimp semi-intensively farmed in ponds, pens or outdoor tanks (Anderson et al. 1981; Martinez-Cordova et al. 1999, 2002; Molina et al. 2006, Ballester et al. 2007; Fernandez da Silva et al. 2008).

The preliminary results found in the present study strongly suggest an effect of protein:energy ratio on the productive performance of Pacific white shrimp at low temperature. However a more complete study is necessary to clearly establish optimum levels, using a wider range of the P/E ratio, dietary protein content, and temperature.

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