OPTIMIZATION OF DIETARY NUTRIENT INPUTS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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OPTIMIZATION OF DIETARY NUTRIENT INPUTS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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OPTIMIZATION OF DIETARY NUTRIENT INPUTS FOR PACIFIC WHITE

SHRIMP *Litopenaeus vannamei*

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DISSERTATION ABSTRACT

OPTIMIZATION OF DIETARY NUTRIENT INPUTS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

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A series of studies were conducted with Pacific white shrimp, *Litopenaeus vannamei*, with the objective of optimizing feed input and improving feeding management strategies to reduce wastes and the potential of negative environmental impacts of shrimp aquaculture. In the first study, two trials were conducted with juvenile (2.2 g ± 0.2, n = 30) and sub-adult (11.1 ± 0.4, n = 30) shrimp. Digestible protein (DP) and digestible energy (DE) requirements for maintenance of shrimp were determined using a factorial procedure. The DE requirement for juvenile shrimp was 41.1 Kcal/kg BWd (body weight per day) and the DP requirement was 2.82 g/kg BWd. For juvenile shrimp, the efficiencies of DE and DP utilization were 0.29 and 0.54, respectively. The DE requirement for sub-

adult shrimp was 50.3 Kcal/kg BWd and the DP requirement was 3.6 g DP/kg BWd. The efficiencies of DE and DP utilization and were 0.42 and 0.72, respectively. Data on DE and DP requirements as well as the partial efficiencies of utilization and deposition of dietary DE and DP were used to formulate a bioenergetics model to calculate daily feed allowance for shrimp culture. Values of feed allowance determined using the bioenergetics model showed that shrimp could be fed with diets with different levels of DE and DP, but the levels of feed input had to be adjusted to the energy and nutrient density of the diets as well as the growth rate of shrimp under the specific biotic and abiotic conditions. In order to verify this statement in practical conditions, the growth response of juvenile shrimp to a 30 and a 40% CP diet offered at the same input of nitrogen was evaluated in tank and pond trials. In a tank trial, both the 30 and the 40% CP diets were offered at three feeding rates (100, 75, and 50% standard feeding). At the conclusion of the trial (56 days) there were no significant differences in final weight between shrimp offered the 30% CP diet fed at 100% of the ration and the 40% CP diet offered at 75% of the ration (~10.3 g, mean weight), which provided the same protein input. These results demonstrated that under controlled experimental conditions, shrimp can be fed with diets containing different levels of nutrients or energy, but the levels of feeding have to be adjusted to the energy and nutrient density of the diets. In the pond trial the same two diets (30% and 40% CP) were offered to juvenile shrimp (0.04 ± 0.04 g, n=56) at two feeding rates (100 and 75%). At the conclusion of the trial (114-121 days), there were no significant differences in final weight among shrimp from the different feeding treatments (19.7-21.7 g). However, production was significantly higher for the treatment 30-100% than for the treatment 30-

75% (6,482 versus 5,054 kg/ha). Yield for the 40-75 treatment tended to be lower than that of the 30% CP diet offered at standard rate (30-100%). However, this statement was not evaluated statistically due to loss of two ponds of the 40-75% treatment. In order to evaluate the effect of the DE/CP ratio under these isonitrogenous feeding regime, a third study was performed with juvenile shrimp in a clear water recirculating system. Two treatments consisted of 30% and 40% CP diets (30 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 Kcal/g and 3.6 Kcal/g, respectively) to maintain a constant DE/CP ratio of 9 Kcal/g of protein (30 RAT-100% and 40 RAT-75%, respectively). At the conclusion of the trial (49 days) there were no significant differences among the four feeding treatments, which indicated appropriate levels of energy inputs for all the treatments. However, the feed conversion rate (FCR) was lower for the low feeding rate treatments (75% feed rate) (1.48 and 1.50 versus 2.09 and 2.06). Results obtained in this study with the development and experimental application of a bioenergetics model support the principle that shrimp feed input should be based not on constant nutrient requirements referred as a dietary level of the nutrient, as traditionally has been used, but as a daily quantitative requirement that can be supplied with diets with variable levels of nutrient and energy.

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CHAPTER I

INTRODUCTION

World aquaculture production has been experiencing a steady and continued expansion. Excluding aquatic plants, production from 1990 to 2003 increased from 13.1 mmt (million metric tonnes) to 42.30 mmt (FAO, 2000, 2005). Production in 2003 was estimated to have a US $ 60.98 billion value and represented 29.3 % of the total fisheries landing (FAO, 2005). In terms of total production by weight, the primary groups included, finfish (63.4 %), mollusks (29.1 %), and crustaceans (6.6 %).

As part of the global growth of aquaculture, the world production of crustaceans has also experienced a continuous expansion that is expected to continue as world population increases and demand for quality sea food continues to rise (FAO, 2000; Tacon *et al.*, 2000). Crustaceans contributed represented 19.8 % of the total aquaculture production by value (FAO, 2005). Marine shrimp were the third most important world aquaculture species in 2003, with a value of US $ 9.32 billion (FAO, 2005). Additionally, marine shrimp production represented 64.7% of the total crustacean production of which most was represented by a few species, mainly *Penaeus monodon* and *Litopenaes vannamei*.

Paralleling the growth of the industry has been an expansion in feed production (Tacon *et al.*, 2000), production intensity and an increase in the levels of feeding. The increased input of nutrients, has the potential of producing negative impacts in the environment. During the most recent years the environmental impact of aquaculture operations, including those of crustaceans, that may cause eutrophication of natural waters, has become a matter of concern (Persson 1991; Cho and Bureau, 2001). In order to insure long-term sustainability of the aquaculture industry, environmental impacts have to be minimized.

Feed represents one of the primary costs associated with the production of shrimp under semi-intensive and intensive conditions (Chanratchakool *et al.*, 1994, Lovell, 1998), and the initial source of pollutants. Hence, overfeeding or poor quality feeds can severely impact water quality, production and economics (Boyd and Tucker, 1998). The main sources of pollutants from the feed are solid wastes, phosphorus and nitrogen. Solid wastes from the feed can have a negative impact on the benthic ecosystem when they deposit on the sediments (Gowen *et al.*, 1991).

Nitrogen wastes from feed are generally a greater concern in marine environments since this nutrient is often the most limiting for algae growth (Persson, 1991). Nitrogen is a waste product of protein metabolism, especially when protein is not deposited as growth and is utilized as energy. In intensive shrimp culture systems, Briggs and Funge-Smith (1994) estimated that 95% of the nitrogen input was in the form of feed and fertilizers, and only 24% of that nitrogen was retained in the shrimp production. Similarly, in semi-intensive shrimp ponds Green *et al.* (1997) found that about 47% of the nitrogen was derived from the feed and fertilizer and about 37% was returned in shrimp production.

The quantity and quality of dietary protein are factors that influence feed costs as well as nitrogen excretion and waste production. Therefore, feeding the appropriate amount of protein in a nutritionally balanced high digestible diet is required to reduce the potential of nitrogen wastes. Adequate dietary intake of high quality protein is required to support rapid growth of shrimp (Guillaume, 1997) which in turn affects revenues. The protein requirement for shrimp is often described as the optimal protein content of the diet (ie., 20,30, 40% crude protein) and its determination is usually based on the response of experimental animals (ie., growth performance) to varying levels of dietary protein under some specific conditions (dose-response method) (Colvin and Brand, 1977; Kanazawa, 1990; Cousin *et al.*, 1991; Shiau *et al.*, 1991; Aranyakananda and Lawrence, 1993; Guillaume, 1997). However, by this method different investigators have reported different levels of protein as the best level, resulting in controversy with regards to optimal dietary levels. For example, for Pacific white shrimp, *Litopenaeus vannamei*, some authors (Aranyakananda and Lawrence, 1993) suggested 15% protein as the optimal level while others (Colvin and Brand, 1977; Cousin *et al.*, 1991) suggested levels of 30-35%. These variations are due the variation of different biotic (ie., shrimp size, genetic strain) and abiotic (ie., temperature, salinity) factors during the determinations, especially in terms of food intake provided by the diet or from other food sources. Normally, to obtain an adequate nutrient intake using a poor quality diet, total food consumption is increased (Brett and Groves, 1979). Consequently, dietary protein requirements are met as a function of nutrient density (eg., protein level) of the diet and feed intake. Therefore, expressing protein requirement in terms of percentage of the diet can be misleading (Ogino, 1980; Lupatsch *et al.*, 2001).

The traditional dose-response to various levels of dietary protein, most used in experiments, may have limited utility due to the specific conditions under which the requirements were determined that make it difficult to extrapolate to a variety of different conditions (Baker, 1986). As an alternative to the variability and limitations of this method, some authors (Gatlin *et al.*, 1986; McGoogan and Gatlin, 1998; Lupatsch *et al.*, 1998; Lupatsch *et al.*, 2001; Kureshy and Davis, 2002) suggested that the required protein should be expressed as the amount of digestible protein that the animal should consume on a per animal or per biomass daily basis.

The factorial method has been proposed to determine daily requirements of protein and energy and allows optimization of feed input. This mechanistic method, contrary to the dose-response method, takes in consideration the physiological processes that affect the requirements and can be applied to a broad variety of conditions (Baldwin, 1985). By the factorial approach, requirements for energy and protein in growing animals can be quantified from the sum of the amounts of consumed energy and protein retained as growth plus the amount of the same nutrients simultaneously lost from the body as results of partial efficiency of utilization and deposition of these nutrients (Pfeffer and Pieper, 1979; Brett and Groves, 1979; Shearer, 1995). The daily feed input required to reach determined levels of growth must match these requirements and should be adjusted for digestible or availability of the energy and protein of the feed. Under this approach the nutrient requirements are not expressed as percentages in the diet but as daily feed intake per unit of body weight or as weight gain.

The factorial method has been used frequently in nutrition of land animals (Shearer, 1995), but less commonly in fish (Braaten, 1974; Pfeffer and Pieper, 1979) and shrimp (Kureshy and Davis, 2002). Gatlin *et al.* (1986) determined the daily requirements of channel catfish (*Ictalurus punctatus*) by feeding 2 diets (25% and 35% CP) of known digestible protein (DP) and digestible energy (DE) at specific feeding rates (from 0 to 5% of body weight/day) and measuring the growth rate and whole body energy and protein. By non-linear regression analysis they determined the minimal amount of DE and DP required for maintenance and for maximum growth based on changes on weight and whole body energy or protein. McGoogan and Gatlin (1998) working with red drum *Sciaenops ocellatus* and Kureshy and Davis (2002) with juvenile *L. vannamei* applied similar methodology to determine the daily protein requirement for maintenance and for maximum growth. Lupatsch *et al.* (1998) used a similar procedure to determine DE and DP requirements for gilthead seabream (*Sparus aurata)*, but they expressed the requirements as metabolic weight instead of gross weight. They pointed out that the metabolic weight is a more appropriate way to express the requirement of fish because the metabolic expenditure for maintenance at a constant temperature is mainly a function of the metabolic body weight of the fish.

A common application of the factorial method in studies with animals is the use of bioenergetic models. These models follow the partitioning of ingested energy through the use of balanced energy equations (NRC, 1981). Using these balanced equations, an energy budget can be constructed for any time period from the entire life cycle or for a specific period of time (Hurwitz and Bornstein, 1973) and the amount of food and nutrients required (energy and protein) can be estimated.

Improvement of diet formulation is another strategy suggested to reduce solid and nutrient wastes in shrimp farms. It can be accomplished by replacing low digestible by high digestible ingredients and by improving the nutritional balance of the feeds. Limiting the use of low protein and lipid ingredients, such as grain by products rich in starch and fiber, and increasing the amount of ingredients with high levels of digestible protein and energy, allows formulation of high nutrient and energy density diets (Cho and Bureau, 2001). Under this approach less feed will be required to meet the nutrient requirement (eg., protein), and the feed efficiency would be improved.

Some studies have shown the benefits of feeding high nutrient density diets at lower feeding rates in warm water fish and shrimp. Cho and Lovell (2002) did not find significant differences between juvenile channel catfish (*Ictalurus punctatus*) raised and pond fed a diet with 28% crude protein (CP) fed at satiation (100%) and a diet with 32% CP fed at 87.5%. A significantly higher feed efficiency was observed with the 32% CP diet. Further increase of the protein level to 36% and reduction in the feeding rate to 77.8% depressed growth in the same experiment, probably due to deficience in energy supply. Similar results were obtained by Cho and Kim (2001) in common carp (*Cyprinus carpio*) when they reduced feeding to 77.8% of satiation by increasing the level of protein of the feed from 35% to 40% and feeding on an isonitrogenous basis. A significant lower growth rate than the 35% protein diet fed at satiation was observed when they fed a diet containing 40% CP at 77.8% of the satiation rate. Kureshy and Davis (2002) reported a significant higher weight gain and feed efficiency for juvenile *L. vannamei* raised in tanks when fed a diet with 32% of CP compared to a diet with 16% CP. They also reported a significant higher weight gain for shrimp fed a 32% CP diet on an isonitrogenous basis as compared to a 48% CP diet, but the feed efficiency was significantly higher for the later regimen. This last study was designed to determine the daily requirement of DP of juvenile *L. vannamei* and the question of the effect of reducing the feeding level by increasing the nutrient density of the diet was not addressed.

When feeding feeds with a higher nutrient or energy density (eg., protein, energy) the balance between digestible protein and digestible energy of the diet (DP/DE ratio) has to be taken into consideration. Especially when the level of feeding is decreased due to the use of a high protein diets, the level of energy should be appropriate to avoid feeding sub-optimal levels of energy that would depress growth or increase the use of protein as energy. It has been demonstrated that decreasing the dietary DP/DE ratio by increasing dietary non-protein energy content, results in an increase in N retention efficiency, due to a decrease in the catabolism of amino acids used for production of energy (protein sparing) (Kaushik and Cowey, 1991).

The general objective of this study was to expand our understanding of shrimp nutrition and to demonstrate the applicability of high nutrient density diets on *L. vannamei* production. It was reached through the following specific objectives:

- to determine daily DE and DP requirements of juvenile and sub-adult *L. vannamei* by factorial methods and to propose a bioenergetics model that allows determination of optimal feed allowance based on the nutrient availability of the feed and on the nutrient requirements of shrimp to reach specific levels of production under specific conditions.

- to evaluate the effect of adjusting the daily feed allowance to the protein requirements of *L. vannamei* when utilizing diets with different nutrient densities.
- to evaluate the response on growth performance, feed and nutrient utilization of *L. vannamei*, fed on a isonitrogenous basis, two diets (30 and 40% CP) containing the same level of energy and two diets (30 and 40% CP) containing the same digestible energy/crude protein ratio.

CHAPTER II

DETERMINATION OF THE DIGESTIBLE PROTEIN AND DIGESTIBLE ENERGY REQUIREMENTS OF PACIFIC WHITE SHRIMP *Litopenaeus vannamei* AND IMPROVEMENT IN THE FEEDING STRATEGIES OF THE SPECIES THROUGH THE USE OF A BIOENERGETICS MODEL

Abstract

This study was conducted to determine the digestible protein (DP) and digestible energy (DE) requirement for maintenance and growth of Pacific white shrimp (*Litopenaeus vannamei*) using a factorial method and to propose a bioenergetics model to determine daily feed allowance for optimal production of shrimp. Two experiments were conducted with juvenile shrimp *L. vannamei*. In a first trial, juvenile shrimp (2.2 g ± 0.2, n=30) were stocked in an outdoor semi-closed recirculating system at a density of 30 shrimp/tank. Five feeding regimes or treatments (0, 0.25, 0.5, 1.0, and 2.0 g/shrimp/week) were assigned to 3 replicated tanks per treatment. The second trial was conducted under the same conditions and in the same system as trial 1, but with sub-adult shrimp (11.1 g ± 0.4, n = 30). The feeding treatments for this trial were: 0, 0.5, 1.0, 2.0, and 4.0 g/shrimp/week. For juvenile shrimp, significant differences were observed between the final weight and weight gain of shrimp under the different treatments. Increased feeding input produced significantly higher final weight and weight gain for each feeding level.

Growth data did not reach a plateau at the highest feeding inputs. The feed conversion ratio (FCR), protein conversion efficiency (PCE), and energy conversion efficiency (ECE) were also significantly affected by the feeding levels. For sub-adult shrimp, increased feeding input produced significantly higher final weight and weight gain for each feed level until reaching a plateau for the last two feeding rates. Significant differences in FCR, PCE and ECE of shrimp fed the different feeding treatments were observed. These significant differences in growth, FCR, PCE, and ECE, showed that shrimp were fed sub-optimal (under maintenance requirement), at maintenance, and above maintenance levels of DE and DP in both trials. The DE requirement for juvenile shrimp determined by regression analysis of DE fed and body energy retention was 41.1 Kcal/kg BWd **(**kg body weight per day**)** and 2.82 g DP/kg BWd for DP. The efficiencies of DE and DP utilization were 0.29 and 0.54, respectively. The DE requirement for sub-adult shrimp was 50.3 Kcal/kg BWd and 3.6 g DP/kg BWd for DP. The efficiencies of DE and DP utilization and deposition were 0.42 and 0.72, respectively. A bioenergetics model to determine daily feed allowance was formulated using values on DE and DP requirements and efficiencies of DE and DP utilization. Values of feed allowance estimated by using the bioenergetics model showed that the amount of feed input to shrimp has to be adjusted to the DE and DP of the feed and to the expected growth rate of shrimp under the specific culture conditions.

Introduction

Feed is one of the primary variable production costs in shrimp aquaculture, especially under semi-intensive and intensive conditions (Chanratchakool *et al.*, 1994;

Lovell, 1998; Lovell, 2002). Feed is also a source of nutrients (eg., nitrogen, phosphorus) and solid wastes that can be released to effluent waters and cause pollution and eutrofication problems in coastal ecosystems (Gowen *et al.*, 1991). These effects can be increased when feed is provided in excess of the daily requirement or when the quality (eg., digestibility) and physical properties (eg., water stability) of the feed are poor. Furthermore, feed that is not assimilated in shrimp production can cause deterioration of the culture water which leads to reduction in shrimp growth and survival (Boyd and Tucker, 1998), with a subsequent reduction in production and economical performance. For these reasons there is an interest in optimizing feed input to improve economic return in shrimp farms and to reduce the potential negative effects on the environment.

In order to optimize feed input, daily feed allowance should be based on the nutrient requirements of the shrimp species and should be adjusted for nutrient availability. Traditionally, nutrient requirements of shrimp have been determined by the dose-response method. By this method graded levels of a nutrient are fed under specific conditions and the requirements are considered as the level of nutrient or energy where the animal reaches maximum growth or growth becomes constant (NRC, 1993). By the dose-response method, animals are often fed *at libitum*, which leads to substantial variation in feed intake since the level of energy or nutrient in the diet could regulate feed consumption (Lee and Putnam, 1972; Jobling and Wandsvik, 1983; Paspatis and Boujard, 1996; Lupatsch *et al.*, 2001). Furthermore, this method is normally time-consuming and the results are only valid for the specific biotic and abiotic conditions under which the determinations took place (Baker, 1986). Due to these variations, published information on nutrient requirements of shrimp, based on the dose-response method are variable and controversial with regards to optimal level of nutrients. For example, for *L. vannamei* some authors (Aranyakananda and Lawrence, 1993) suggested 15% protein as the optimal level while others (Colvin and Brand, 1977; Cousin *et al.*, 1991) suggested levels of 30-35%. (Colvin and Brand, 1977; Baker, 1986; Cousin *et al.*, 1991).Therefore, expressing nutrient requirement in terms of percentage of the diet can be misleading (Ogino, 1980; Lupatsch et al, 2001).

Alternatives to the dose-response method are found in energetics analyses. Energetics can be defined as the quantification of the exchange and transformation of energy and matter between living organisms and their environment (Blaxter, 1989; Lucas, 1993). In order to understand the nutrient requirements of an animal species, the dynamic of growth or tissue deposition and energy and nutrient supply must be understood. The components of this dynamic exchange system are normally expressed in energy balance equations. These equations are based in the energy partition scheme proposed by the National Research Council (NRC, 1981), outlined in the following general equation:

$$IE = FE+(UE+ZE)+HiE+HeE+HjE+RE$$

Where IE is the ingested energy contained in the food; FE energy lost as feces after digestion processes, the remaining energy is called digestible energy (DE); (UE+ZE) is the energy lost through the gills and urine after metabolism of proteins, the remaining energy is the metabolic energy (ME); HiE or heat increase of feeding is the energy lost as heat due to the post-absorptive processes related to an ingested feed that include processes of synthesis, transformation and elimination of wastes from metabolism; HeE is the basal metabolism or energy necessary to maintain life body functions; HjE is the energy spent for voluntary or resting activity; and RE is the portion of recovered energy or the ME from the diet that is not dissipated as heat and is retained in the body as new tissue.

The different components of the energy equation described above can be determined empirically. For example, there are procedures to determine digestibility of feed ingredients and diets for an animal species (NRC, 1993). The values of UN + ZE can be measured directly in the water, but their determination is difficult by this method. Cho and Kaushik (1985) suggested determination of this value through carcass analysis by obtaining the difference between digestible nitrogen (DN) fed and retained nitrogen (RN). As pointed out earlier, the part of the ME that is not deposited as growth is dissipated as heat. This heat production can be determined by direct or indirect calorimetric methods. By direct methods, the heat produced by animals placed in a calorimetric chamber is measured. For fish and other aquatic organisms this method is difficult to apply due to the high specific heat of the water (Bureau *et al.*, 2002). By indirect methods, the respiration rate is measured by determination of consumption of O_2 or production of CO_2 by animals confined in a closed chamber (Blaxter, 1989). By this method, respiration in fish is usually expressed as the heat or energy produced per liter or gram of consumed oxygen after transformation of oxygen consumption per shrimp using an oxycalorific coefficient, which value is based in the type of fuel used as energy. In fish and other aquatic organisms, an oxycalorific coefficient of 3.25 Kcal/g O_2 consumed is used by most authors (Cho and Kaushik, 1990). This value corresponds to the mean heat production released from catabolism of amino acids and lipids, which are the most abundant source of energy for these organisms.

Another indirect procedure used to measure heat production, especially in fish, is the comparative carcass analysis (Cho and Kaushik, 1985). In this procedure, heat production is determined by the difference between IE and the sum of FE, UE+ZE and RE. The energy lost due to basal metabolism (HeE) can be determined by variation in body energy when experimental animals are subject to fasting for a determined period of time. For this reason this standard practical measurement of minimal metabolism is known as fasting heat production (Hef) (Blaxter, 1989; Cho and Kaushik, 1990). The energy of maintenance (HEm) is the level of energy at which the energy balance of the animal is zero (RE=0). Normally HEm corresponds to the HeE plus the HiE associated with the utilization of nutrient from the diet to compensate for energy losses associated with basal metabolism. The most common method used for estimating HEm consists of feeding fish at different levels from under-optimal to excessive feed supply and using regression of RE as a function of DE or ME intake that results in RE=0 (Bureau *et al.*, 2002). The last component of the model, RE or growth, consists in the accumulation and increase in the body components. Growth depends on the size or life stage of the animal and on the type of tissue deposited. Energy is available for growth only after all the other components of the energy budget have been met.

Based on energy balances, factorial methods have been proposed as an alternative to the dose-response method to determine energy and nutrient requirement of animals and to determine daily allocation of food (Baldwin, 1985). The factorial method allows dividing energy requirements into different components or fractions that vary with the specific environmental conditions and biotic components (eg., species, life stage, genetic potential, health). This mechanistic method, contrary to the dose-response method, takes into consideration the physiological processes that affect the requirements and can be applied to a broad variety of conditions (Baldwin, 1985). By the factorial approach, requirements for energy and protein in growing animals can be quantified from the sum of the amounts of consumed energy and protein that is retained as growth plus the amount of the same nutrients simultaneously lost from the body due to partial efficiencies of utilization (Pfeffer and Pieper, 1979; Brett and Groves, 1979; Shearer, 1995). The daily feed input required to reach determined levels of growth and production can be estimated if the digestible energy and digestible protein contents of a feed are known. Under this approach the nutrient requirements are not expressed as percentages in the diet but as daily feed intake per unit of body per day and weight gain. A factorial model can be used to estimate the dietary requirement of a nutrient for an animal at any specific stage or whole life cycle under specific environmental and dietary conditions (Hurwitz and Bornstein, 1973).

The factorial method has been used frequently in nutrition of land animals (Shearer, 1995), but less commonly in fish (Braaten, 1979; Pfeffer and Pieper, 1979) and shrimp (Kureshy and Davis, 2002). With regards to warm water species, Gatlin *et al.* (1986) determined the daily requirements of channel catfish (*Ictalurus punctatus*) by feeding two diets (25% and 35% CP) of known digestible protein (DP) and digestible energy (DE) at 0 to 5% of body weight/day and measuring the growth rate and whole body energy and protein. By non-linear regression analysis they determined the minimal amount of DE and DP required for maintenance and for maximum growth based on changes in weight and whole body energy or protein. McGoogan and Gatlin (1998) determined DE and DP of red drum *Sciaenops ocellatus* using a similar procedure. Lupatsch *et al.* (1998) also used a factorial method to determine DE and DP requirements for gilthead seabream (*Sparus aurata)*, but they expressed the requirements as metabolic weight instead of gross weight. They pointed out that the metabolic weight was a more appropriate way to express the requirement of fish, because the metabolic expenditure for maintenance at a constant temperature was mainly a function of the body weight of the fish.

Kureshy and Davis (2002) applied a similar methodology to determine the daily protein requirement for maintenance and for maximum growth of *L. vannamei* feeding diets of 16, 32, and 48% CP. They reported that CP requirement for maintenance and maximum growth of juvenile *L. vannamei* was 1.8-3.8 and 43.4-46.4 g CP/kg body weight per day (BWd), respectively. For sub-adult shrimp these values were 1.5-2.1 and 20.5-23.5 g CP/ BWd. The authors did not report daily DE requirement for the species. Cousin (1995) reported the optimal DP requirement of *L. vannamei* to be 33 g DP/kg BWd. Information on energy requirement for shrimp determined by the factorial method is limited in the literature.

A practical application of factorial methods is found in the formulation of bioenergetics models. Bioenergetics models are systems that allow accurate and practical prediction of the energy balance of animals based on biotic (eg., body weight, age, and physiological stage and activity among others) and abiotic factors (eg., environmental conditions and the amount and nutritional composition of the diet).These models can be useful tools for diet formulation and estimation of optimal feed input to reach specific growth and production goals (Baldwin and Bywater, 1984). The advantage of using the factorial approach in formulation of bioenergetics models is that it allows division of the animal energy requirement into different components or fractions, whose magnitudes vary with levels of growth performance, food composition, and life stage.

In aquaculture, several bioenergetics models have been designed to predict the energy requirement and growth of several fish species under specific conditions (Gatlin *et al.*, 1986; Lupatsch *et al.*, 1998; McGoogan *et al.*, 1998; Elliot and Hurley, 1999; Cui and Xie, 1999; Watanabe *et al.*, 2000; Lupatsch *et al.* 2001). However, models based on factorial methods for crustaceans are limited. Consequently, the objective of this study was to determine daily DE and DP requirements of juvenile and sub-adult *L. vannamei* by factorial methods and to propose a bioenergetics model that allows determination of optimal feed allowance based on the nutrient availability of the feed and on the nutrient requirements of shrimp to reach specific levels of production under specific conditions.

Materials and Methods

This research was conducted at the Claude Peteet Mariculture Center, in Gulf Shores, Alabama from May through mid August 2003. Two trials with two different sizes of white Pacific shrimp *Litopenaeus vannamei* were conducted to determine digestible energy (DE) and digestible protein (DP) requirements for maintenance and specific growth levels.

Shrimp post-larvae (PL) obtained from a commercial hatchery (GMSB Inc. hatchery /Key West, FL) were kept in a nursery system for 21 days and in production ponds afterward. An experimental diet containing 30% CP and 3766 kcal DE/kg (Table 1 and Table 2) was fed to shrimp in both feeding trials. In the first trial, juvenile shrimp (2.19 ± 0.16 g, n=15) at a density of 20 shrimp/tank were offered the following rations: 0, 0.036, 0.071, 0.143, and 0.286 g feed/shrimp/day for 38 days. For the second trial, sub-adult shrimp (11.08 ± 0.42, n=15) at a density of 15 shrimp/tank were offered: 0, 0.071, 0.143, 0.286, and 0.571 g feed/shrimp/day for a 35-day period. For each trial, three replicates per treatment were assigned to 15 circular tanks in a completely randomized experimental design.

The trials took place in an outdoor semi-closed recirculating system that consisted of 16 circular polyethylene tanks (0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter), a common reservoir with a biological filter, and a 1/3-hp circulation pump. The recirculation rate for each tank was set at 2.92 L/min (n=3). Each tank was equipped with a center drain, 3.2 cm stand pipe which was 75 cm long, screened and set to maintain a water depth of 61 cm or 570 L volume. Aeration was provided by two air stones connected to a common air supply from a 1 hp regenerative blower (Sweetwater Aquaculture Inc. Lapwai, ID, USA). The recirculating system was filled with a mixture of well water and brackish water (~12 ppt) from an intracoastal waterway, a shipping channel

Table 1. Formulation of the experimental diet (g/100g dry weight) and analyzed composition (crude protein and gross energy).

Ingredient	Percentage
Menhaden fish meal[1]	17.1
Soybean meal[2]	30.5
Menhaden fish oil[3]	2.7
Wheat starch[4]	14.5
Whole wheat[4]	30
Trace Mineral premix[5]	0.5
Vitamin premix w/o choline	1.8
Choline chloride[6]	0.2
Stay C (250 mg C/kg)[7]	0.07
CaP-monobasic[4]	1.9
Cholesterol[4]	0.2
Lecithin[4]	0.5
Analyzed composition	
Crude protein (%)	31
Gross energy (Kcal/kg)	4639

[1]Special Select™, Omega Protein™, USA Inc., Randeville, LA, USA
[2]De-hulled solvent extracted soybean meal, Southern Sates Cooperative Inc., Richmond Virginia, USA.
[3]Omega Protein™, Reedville, VA, USA
[4]United States Biochemical Company, Cleveland, OH, USA
[5]g 100 g premix: cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferros 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
[6]g Kg_{-1} Premix: Thiamin HCl 0.5, riboflavin 3.0, pyridoxine HCL 1.0, DL Ca-pantothenate 5.0, nicotinc acid 5.0, biotin 0.05, folic acid 0.18, vitamin A acetate (20 000 IU g-1) 5.0, vitamin D3 (400 000 IU g_{-1}) 0002, DL-"-tocopheryl acetate (250 IU g_{-1}) 8.0
[7]Stay C®, (L-ascorbyl-2-polyphosphate 35% active C) Roche Vitamins Inc., Parsippany, NJ, USA.

Table 2. Apparent digestibility coefficients (ADC) of dry matter (ADDM), protein (ADPC), and energy (ADEC) of the 30%-CP diet fed to sub-adult (10.1 ± 0.3 g, n=15) *Litopenaeus vannamei*.

ADC	Mean ± std. dev.
ADDM	75.25 ± 0.15
ADPC	87.01 ± 0.43
ADEC	81.20 ± 0.84

that connects Bon Secour Bay with Wolf Bay in Gulf Shores and Orange Beach. About 50% of the total volume was changed every 3-4 days.

The diet was prepared by mixing all the dry ingredients in a food mixer (Hobart, Troy, OH, USA) for 20 minutes and then adding the menhaden oil and mixing for 10 more minutes. Boiling water was then blended with the mixture while it was still mixing to attain a mash of consistency appropriate for pelleting. The mash was passed through a 3-mm die in a meat grinder (Hobart, Troy, OH, USA), and the pellets dried in a forced-air dryer for a maximum temperature of 45 ºC until moisture content between 8-10% was obtained. The diet was ground and sieved to an appropriate size and stored at -15 ºC until used.

Shrimp were fed three times a day. The population in each tank were counted every two weeks and the daily ration readjusted. Dissolved oxygen (DO), pH and temperature were measured twice a day (~ 0600 and 1600 hours) with a YSI 556 DO meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonia-nitrogen (TAN) and nitrite-nitrogen were determined once a week. Water samples were taken from the mid water column in the reservoir and in two tanks selected at random. Total ammonia-nitrogen was measured with a spectrophotometer (Spectronic 20 Genesys, Spectronic Instrument Inc. Rochester, NY, USA) following the Nesslerization method (APHA, 1989). Nitrite-nitrogen was determined using a model PLN code test kit from LaMotte (Chestertown, MD, USA).

At the end of each trail, the weight gain (WG = initial weight -final weight), percentage survival and feed conversion rate (FCR = dry weight of feed offered/wet weight gain) were determined. Shrimp samples were collected at the beginning (pooled sample) and at the end of the experiment (6 shrimp/tank) to determine changes in body composition (dry matter, protein and total energy). The data on initial body composition, in addition to data on body composition analyses of shrimp of different sizes collected every two or thee weeks from a semi-intensive production pond, were used to determine the association between shrimp size and body composition (CP, energy, and moisture). Based on proximate analyses, protein conversion efficiency (PCE = dry weight protein gain x 100/dry weight protein offered), energy conversion efficiency (ECE = dry weight energy gain x 100/dry weight energy offered), energy retention (ER = body energy x WG/days), and protein retention (PR = body protein x WG/days) were determined.

Samples were stored in sealed plastic bags in a freezer at -15 ºC until analyzed. Shrimp samples were dried in an oven at 90 ºC to a constant weight, using the methods described by the A.O.A.C.(1990), and then ground in a coffee grinder and stored. Crude protein content of shrimp was analyzed using the micro-Kjeldahl method (Ma and Zuazago 1942). Total energy content was determined using a micro-calorimetric adiabatic bomb using benzoic acid as standard (Parr 1425, Moline, IL, USA).

Digestibility determination

Chromic oxide was used as an inert marker to determine digestibility coefficients of the test diet. Nine groups of 10 shrimps (10.1 ± 0.3 g, n=15) selected at the end of the first trial were stocked in a closed recirculating system consisting of nine 60-L glass aquaria, biological filter, reservoir, circulation pump, and supplemental aeration. Water temperature was 28.2 ± 1.5 ºC and salinity was 5 ppt. The test diet used to determine digestibility coefficients was prepared using the same formulation as the diet used in the growth trials (Table 1), but replacing 0.5% of wheat starch by equal amount of chromic oxide. The diet was prepared as previously described.

Shrimp were allowed to acclimate for three days before starting the collection of feces. 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 um mesh screen. Shrimp were offered five feedings per day, however, feces obtained after the first feeding were discarded. Collected feces were rinsed with distilled water and stored in sealed plastic containers at -15 ºC. Samples from three tanks were pooled (r = 3) and kept frozen until analyzed. Dry matter, crude protein, total energy was determined for the fecal and the diet samples according with procedures previously described. Chromic oxide was analyzed following the McGinns and Kasting (1964) procedures. Apparent digestibility coefficients (ADC) of the dry matter, protein, and energy were calculated according to Maynard *et al.* (1979).

ADC (%) = 100 x[1- (diet. Cr level/fecal Cr level) x (fecal nutrient/dietary nutrient)]

Respirometry determinations

Flow-through respirometry chambers were used to determine values of energy required for maintenance in 24-h fasted shrimp. These values were used to evaluate the results of DE requirements for maintenance obtained in the experimental trials. The determinations took place at the Fish Nutrition Processing laboratory at the North Auburn Fisheries Station located in Auburn, AL. A Strathkelvin respirometer (model 928) was used to estimate oxygen consumption in shrimp.

Four respirometry chambers were constructed using 5-cm diameter transparent plexiglass pipe cut into 9-cm sections and capped with quick-disconnect PVC caps and fitted with a small mesh false bottom (~2-L volume). The two ends of the chamber were drilled and connected to vinyl air tubing to provide air and water outlets. Each chamber contained a micro stir-bar and was set on a magnetic stir plate to maintain a smooth movement of the water inside the chamber. An oxygen probe connected to the respirometer was fitted through the top of each chamber. Clean water at 6 ppt salinity, 28 ºC and at oxygen saturation was supplied by gravity to each chamber. The water was stored in a 60-L glass tank and was disinfected with ~1.5 ml Na-hypochlorite (5.25%) 12 hours before determinations to reduce bacteria activity. Sodium thiosulfate was added to the tank to deactivate residues of chlorine until no color change was detected using ortho toluidine. Water flow from the chamber was controlled by a flow-restrictor that was attached to the tubing exiting the chamber. Before using the respirometer the instrument was calibrated using a zero oxygen solution (prepared with de-ionized water that was added a small amount of sodium sulfite) and oxygen saturated water.

Shrimp were weighed and placed individually in the chambers. Oxygen consumption of four shrimps for each of two size groups (~4 g and ~11 g) were determined. The oxygen probe was connected to the chamber and left undisturbed for about five to seven hours or until respiration rate appeared stable for at least one hour with readings taken every 15 minutes. The oxygen rate in mg/L was registered, also flow rate (L/h). Respiration rate in mg O_2/g shrimp/hour was calculated by the following equation:

$$R = Q \times ([O_2\ \text{initial}]-[O_2\ \text{final}])/W$$

Where Q is flow rate (L/h) and W is the weight of the shrimp (g). To transform oxygen consumption into energy consumption an oxycalorimeter index of 3.25 Kcal/g O_2 consumed was used (Bureau *et al.*, 2002).

Fasting trial

To determine effect of shrimp size on metabolic rate, six groups (1.10 ± 0.3, 2.11 ± 0.16, 2.78 ± 0.41,7.28 ± 0.51, 11.18 ± 0.42 , and 14.10 ± 0.88 g) of 15 similar-sized shrimp were selected. Initially, five shrimp from each group were sacrificed and stored in a freezer at -15 ºC for body composition analysis. The remaining population was maintained in a recirculating tank system for 30 days without food supply. At the end of the experiment, shrimp were sacrificed and kept in a freezer at -15 ºC for body composition analysis. The recirculating system consisted of 16 plastic (61 x 61 x 61 cm) polyethylene tanks (Polytank Inc., Litchfield, MN, USA). The system was connected to a common circular tank that had a trickling biological filter, and a 1/3-hp circulation pump. The recirculation rate into each tank was set at 4.1 L/min (n=3). Aeration was provided by two air stones connected to a common air supply. Water temperature was 28.3 ± 1.2 ºC and salinity was 5.1 ppt.

Data Analyses

Data were analyzed by a one-way analysis of variance (ANOVA). Significant differences among treatment means at a probability level of $P \leq 0.05$ were determined by the Student-Newman-Keuls multiple comparison test (Steel and Torrie, 1980). Analyses were done using SAS program version 8.2 (SAS Institute Inc., Cary, NC). Growth (weight gain) was analyzed using regression analysis or non-linear regression analysis in order to determine maintenance levels for digestible protein (DP) and digestible energy (DE). Data on shrimp size and body composition adjusted better to a regression of the log transformation of the values. Allometric curves of the form $y = ax^b$ were determined for the regressions of shrimp size and body composition (body protein, moisture, and body gross energy).

Results

Average water quality parameters of trial 1 were as follows: temperature, 27.15 ± 0.12 ºC; DO, 6.97 ± 0.09 ppm; pH, 7.86 ± 0.02; salinity, 5.56 ± 0.05 ppt; and TAN, 0.31 ± 0.04 ppm (Table 3). For trial 2, water quality parameters were: temperature, 27.29 ± 0.13 ºC; DO, 7.30 ± 0.04 ppm; pH, 7.25 ± 0.05; salinity, 4.45 ± 0.02 ppt; and TAN, 0.37 ± 0.06 ppm (Table 3). These were typical values for water quality for this system and would be expected to support adequate growth for this species. No water quality problems or diseases were observed during the trial. Survival rate was between 68 and

Table 3. Water quality parameters recorded for trial 1 and trial 2. Values are mean ± standard deviation of daily and weekly determinations. Values below in parenthesis represent minimum and maximum.

Parameter	Trial 1	Trial 2
Dissolved oxygen (mg/l)	6.97 ± 0.09 (5.16, 8.82)	7.30 ± 0.04 (6.59, 8.01)
pH	7.86 ± 0.02 (7.16, 8.05)	7.25 ± 0.05 (6.61, 8.79)
Temperature (ºC)	27.15 ± 0.12 (23.05, 30.11)	27.29 ± 0.13 (24.78, 30.89)
Salinity (g/l)	5.56 ± 0.05 (4.64, 6.39)	4.45 ± 0.02 (4.22, 4.81)
Total ammonia-N (mg/l)*	0.31 ± 0.04 (-, 0.61)	0.37 ± 0.06 (- , 0.78)
Nitrite-N (mg/l)*	0.45 ± 0.11 (-, 0.91)	0.48 ± 0.09 (-, 1.2)

82% for trial 1 due to loss of part of the population that jumped out of the tanks (Table 4). Survival rate for trial 2 ranged between 93 and 100% with no significant differences among the treatments.

At the conclusion of trial 1 (day 38) there were significant differences between the final weight and weight gain of shrimp offered varying feed inputs (Table 4). Significantly higher final weights were observed as feed inputs increased with no apparent plateau being reached at the higher levels of feed inputs. At the conclusion of trial 2, significantly higher final weights and weight gain were observed for the sub-adult shrimp at each feed levels until reaching a plateau for the last two feeding rates (Table 4). There were no significant differences in weight gain between treatments 4 (0.286 g/sh/d) and 5 (0.571 g/sh/d) (14.29 vs 14.45g), but these values were significant higher than those for shrimp maintained on the other treatments (Table 4). There were also significant differences in FCR, PCE and ECE of shrimp fed the various feed inputs (Table 4 and Table 5). The treatment with the highest feeding level (treatment 5) showed the highest FCR (6.2). The best value for FCR (2.7) was obtained for treatment 4 (0.286 g/sh/d).

With respect to proximate analyses of whole shrimp, crude protein and energy changed significantly with increased body size but changes in moisture were not significant (Figure 1a and Figure 1b). The relationship between shrimp weight (kg) and body protein content can be described by the following lineal regression equation (after log-log transformation):

$$\text{Body crude protein (g CP/100g body weight)} = 24\ (\text{kg})^{0.05336} \qquad (1)$$

$$R^2 = 0.6290$$

Table 4. Final weight (FW), geometric mean weight (GMW), weight gain (WG), feed conversion ratio (FCR), and survival of juvenile (2.2 g, trial 1) and sub-adult shrimp (11.1 g, trial 2) *L. vannamei* fed varying levels of a 30%-CP diet over a 38 or a 35-day period, respectively. Values are means of three replicates. Means in the same column with different superscripts were significantly different.

Ration (g/sh/d[1])	Ration (% GMW[2])	FW (g)	GMW[2] (g)	WG[3] (%)	FCR[4]	Survival (%)
Trial 1						
0	0	2	2.1	-8	0	68.3[a]
0.036	1.4	2.9[d]	2.5[d]	28[d]	2.3[ab]	78.3[a]
0.071	2.4	3.9[c]	2.9[c]	72[c]	1.7[b]	78.3[a]
0.143	4.4	5.0 [b]	3.3[b]	136[b]	1.9[b]	75.0[a]
0.286	7.9	6.2[a]	3.6[a]	193[a]	2.7[a]	81.7[a]
P value		<0.0001	<0.0001	<0.0001	0.007	0.1171
PSE		0.1633	0.0606	4.322	0.1465	3.25
Trial 2						
0	0	8.8[d]	9.9[d]	-21[d]	0	93.3[a]
0.071	0.7	10.4[c]	10.7[c]	-6[c]	-5.6[b]	95.5[a]
0.143	1.2	12.5[b]	11.8[b]	13[b]	3.5[a]	93.3[a]
0.286	2.3	14.3[a]	12.6[a]	32[a]	2.7[a]	97.8[a]
0.571	4.5	14.5[a]	12.7[a]	28[a]	6.2[a]	100.0[a]
P value		<0.0001	<0.0001	<0.0001	0.002	0.2246
PSE		0.278	0.1444	0.2118	1.3716	2.223

[1] g/sh/d= g feed/shrimp/day
[2] Geometric mean weight= (initial weight x final weight)$^{1/2}$
[3] Weight gain = (final weight - initial weight) ×100/initial weight
[4] FCR = dry weight of feed offered/wet weight gain

Table 5: Energy conversion efficiency (ECE), protein conversion efficiency (PCE), retained energy (RE), and retained protein (RP) of juvenile (2.2-g, trial 1) and sub-adult (11.1 g, trial 2) *L. vannamei* in response to varying levels of a 30%-CP diet over a 38 or a 35-day period, respectively. Values are means of three replicates. Means in the same column with different superscripts were significantly different.

Ration (g/sh/d[1])	Ration (% GMW[2])	ECE[3] (%)	PCE[4] (%)	RE[5] (Kcal/kg BWd	RP[6] (g/kg BWd)
Trial 1					
0	0	0	0	-14.4	-2
0.036	49	5.3[b]	20.9[b]	3.3[d]	0.9[d]
0.071	2.4	17.6[a]	36.9[a]	18.5[c]	2.8[c]
0.143	4.4	16.5[a]	33.9[a]	31.7[b]	4.7[b]
0.286	7.9	11.7[a]	23.6[b]	39.7[a]	5.8[a]
P value		0.0019	0.0026	<0.0001	<0.0001
PSE		1.5461	2.277	1.3012	0.2317
Trial 2					
0	0	0	0	-23.2	-3.1
0.071	0.7	-40.6[b]	-56.8[c]	-11.3[c]	-1.1[c]
0.143	1.2	3.0[b]	11.9[ab]	1.5[b]	0.4[b]
0.286	2.3	10.3[a]	20.7[a]	9.9[a]	1.4[a]
0.571	4.5	5.1[ab]	10.3[b]	9.5[a]	1.4[a]
P value		<0.0001	<0.0001	<0.0001	<0.0001
PSE		2.012	2.2572	0.431	0.0686

[1] g/sh/d= g feed/shrimp/day
[2]Geometric mean weight= (initial weight x final weight)$^{1/2}$
[3]Energy conversion efficiency= dry weight energy gain x 100/dry weight energy offered
[4]Protein conversion efficiency= dry weight protein gain x 100/dry weight protein offered
[5]Retained energy= initial body energy- final body energy)/days
[6]Retained protein (g/shrimp/day)=initial body protein- final body protein/days

Figures 1a and 1b: Crude protein (CP), water, and body energy of *L. vannamei* of different weights.

Figure 1a

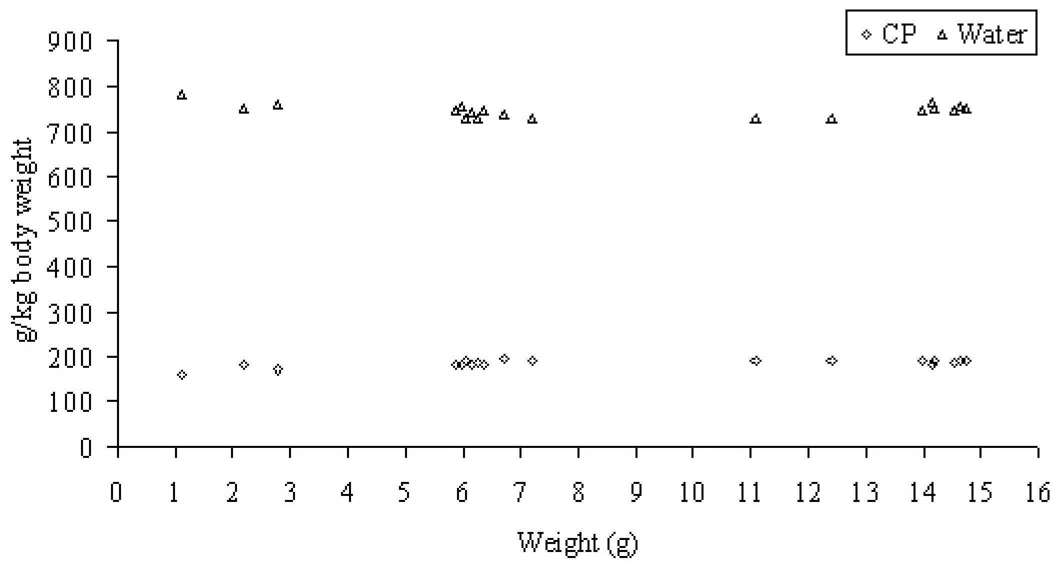
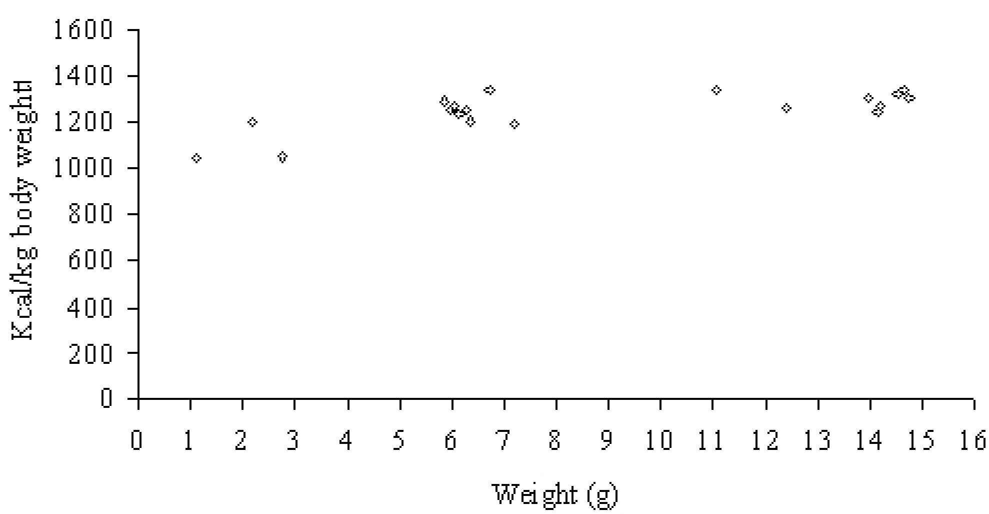


Figure 1 b



The following lineal regression equation was fitted to the data for shrimp weight (kg) and body energy. After log-log transformation:

$$\text{Body energy (Kcal/kg)} = 1831\ (\text{kg})^{0.07842} \quad (2)$$

$$R^2 = 0.6283$$

To determine the effect of shrimp size on metabolic rate, data of energy and protein loss per day after fasting shrimp of different sizes for 30 days were transformed to a log-log function to obtain the regression equation that best fit the data. The antilog of these functions provided the allometric relationship that described the function. For daily energy loss the allometric equation was:

$$\text{Energy loss (Kcal/kg BWd)} = 2.5252\text{BW(kg)}^{1.0276} \quad (3)$$

For body protein loss the equation was:

$$\text{Protein loss (g/kg BWd)} = 23977\text{BW(kg)}^{1.0682} \quad (4)$$

The coefficients of these equations are the metabolic weight of shrimp under the experimental conditions. For energy and protein the coefficients were estimated to be 1.0276 and 1.0682 , respectively.

Similar results to those of final weight and weight gain were observed for retained energy (RE) and protein retention (PR) both for juvenile and sub-adult shrimp (Table 5). The following lineal regression equations describe the relationship between DE intake (Kcal/kg BWd) and energy retention (Kcal/kg BWd) for both juvenile and sub-adult shrimp:

Juvenile (Figure 2a):

$$RE = 0.29147\ (\pm\ 0.0227)\ x\ (\ Kcal/kg\ BWd) - 11.97641\ (\pm\ 2.1539) \qquad (5)$$

$$R^2 = 0.9426,\ P \leq 0.0001$$

Sub-adult (Figure 2b):

$$RE = 0.41501\ (\pm\ 0.0371)\ x\ (Kcal/kg\ BWd) - 20.86393\ (\pm\ 1.73646 \qquad (6)$$

$$R^2 = 0.9260,\ P \leq 0.0001$$

For DP intake (g/kg BWd) and protein retention (g/kg BWd) the lineal regression equations were:

Juvenile (Figure 3a)

$$PR = 0.53826\ (\pm\ 0.0404)\ x\ (g/kg\ BWd) - 1.51701\ (\pm\ 0.29816) \qquad (7)$$

$$R^2 = 0.9468,\ P \leq 0.0001$$

Sub-adult (Figure 3b):

$$PR = 0.71531\ (\pm\ 0.073)\ x\ (g/kg\ BWd) - 2.61795\ (\pm\ 0.2677) \qquad (8)$$

$$R^2 = 0.9050,\ P \leq 0.0001$$

The slopes of these regression curves are considered the efficiency of utilization of DE and DP by *L. vannamei*. For juvenile shrimp the efficiency of DE was 0.29 and 0.49 for sub-adult shrimp. The efficiency of utilization of DP were 0.54 and 0.72 for juvenile and sub-adult shrimp, respectively.

Both sets of equations were used to determine g DP and DE requirement for maintenance. For juvenile shrimp the DE requirement for maintenance was: 41.1 Kcal DE/kg BWd and 50.3 Kcal DE/kg BWd for sub-adult shrimp. Digestible protein for

Figures 2a and 2b: Daily energy retention (RE) per unit of body weight (Bwd). Trial 1 (Figure 2a) was conducted over 38 days using juvenile (2.2 g initial weight) shrimp. Trial 2 (Figure 2b) was conducted over 35 days using sub-adult (11.2 g initial weight) shrimp.

Figure 2a

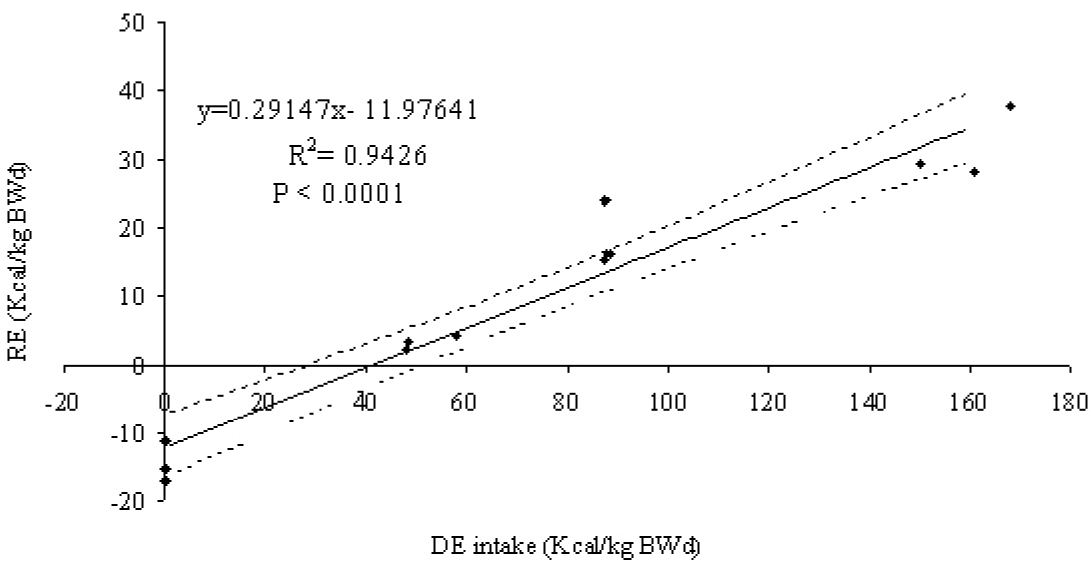
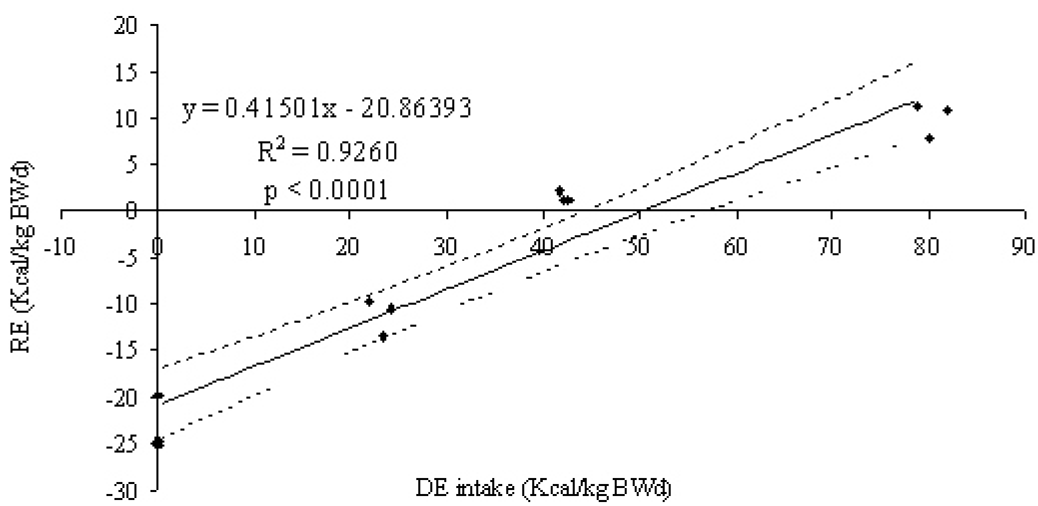


Figure 2b



Figures 3a and 3b: Daily protein retention (PR) per unit of body weight (BWd) of *L. vannamei* fed increasing levels of a 30%-CP.Trial 1 (Figure 3a) was conducted over 38 days using juvenile (2.2 g initial weight) shrimp. Trial 2 (Figure 3b) was conducted over 35 days using sub-adult (11.2 g initial weight) shrimp.

Figure 3a

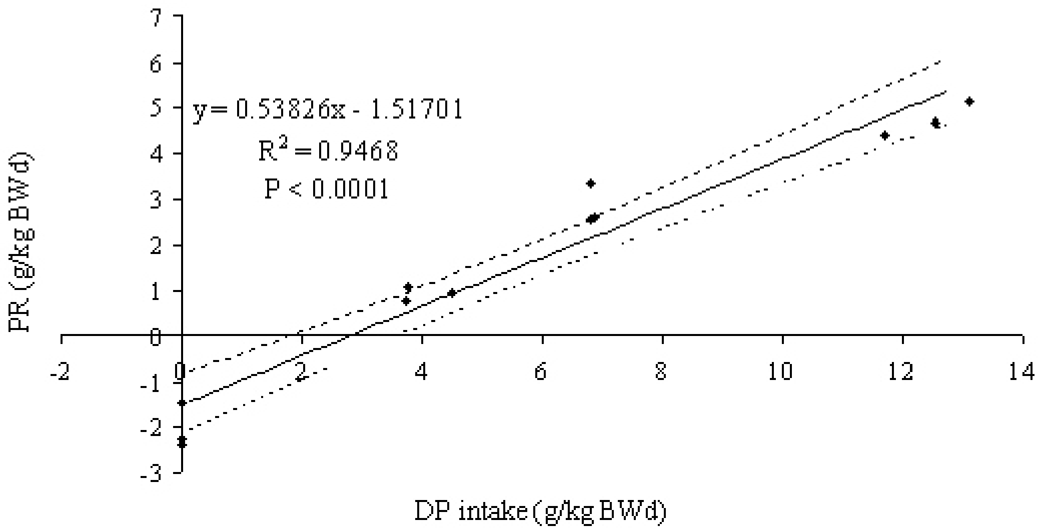
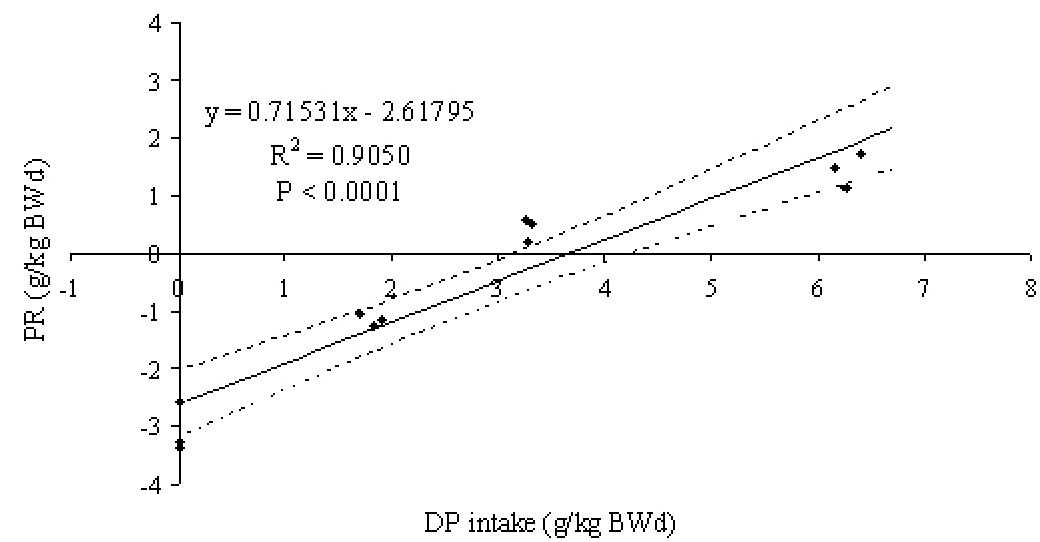


Figure 3b



maintenance was 2.82 g DP intake/kg BWd for juvenile shrimp and 3.6 g DP intake/kg BWd for sub-adult shrimp.

Equations for DE (Kcal/kg BWd) or DP (g/kg BWd) fed and weight gain of juvenile shrimp are presented in Figure 4a and Figure 5a, respectively. The equations are:

Juvenile (Figure 4a):

$$WG = 0.16030 (\pm 0.00672) \times (\text{Kcal/kg BWd}) - 1.52679 (\pm 0.63639 \qquad (9)$$

$$R^2 = 0.9827, P \leq 0.0001$$

Juvenile (Figure 5a):

$$WG = 2.05305 (\pm 0.08629) \times (\text{g DP/kg BWd}) - 1.52518 (\pm 0.6377) \qquad (11)$$

$$R^2 = 0.9826, P \leq 0.0001$$

Sub-adult (Figure 4b):

$$WG = 0.18868 (\pm 0.01411) \times (\text{Kcal/kg BWd}) - 6.07194 (\pm 0.66022) \qquad (10)$$

$$R^2 = 0.9470, P \leq 0.0001$$

Sub-adult (Figure 5b):

$$WG = 2.41609 (\pm 0.18067) \times (\text{g DP/kg BWd}) - 6.07054 (\pm 0.66016) \qquad (12)$$

$$R^2 = 0.9470, P \leq 0.0001$$

Digestible energy and DP for maintenance using these equations are: for juvenile shrimp the DE requirement for maintenance was: 9.5 Kcal DE/kg BWd and 32.2 Kcal DE/kg BWd for sub-adult shrimp. Digestible protein for maintenance was 0.74 g DP intake/kg BWd for juvenile shrimp and 2.5 g DP intake/kg BWd for sub-adult shrimp.

Figures 4a and 4b: Regression curve of weight gain of *L. vannamei* in response to varying levels of digestible energy input (DE). Trial 1 (Figure 4a) was conducted over 38 days using juvenile (2.2 g initial weight) shrimp. Trial 2 (Figure 4b) was conducted over 35 days using sub-adult (11.2 g initial weight) shrimp.

Figure 4a

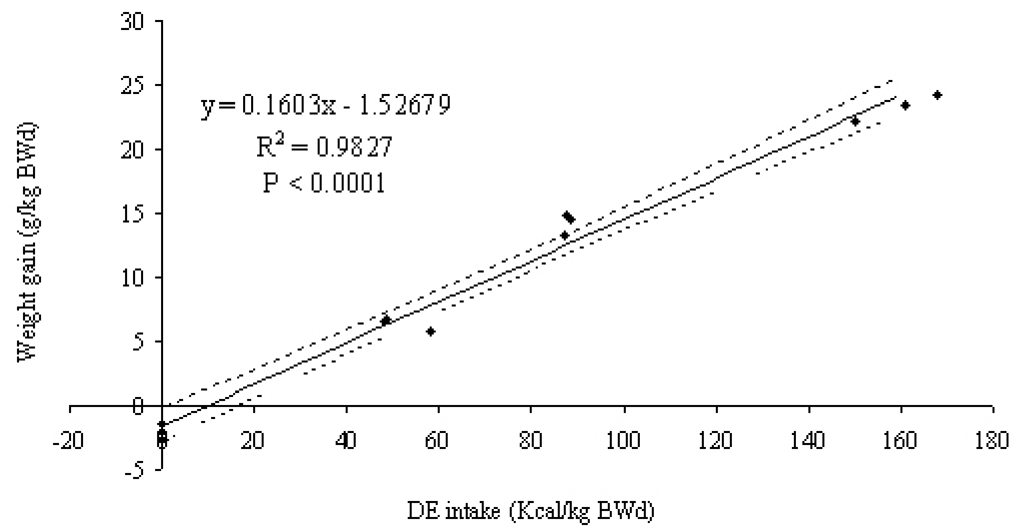
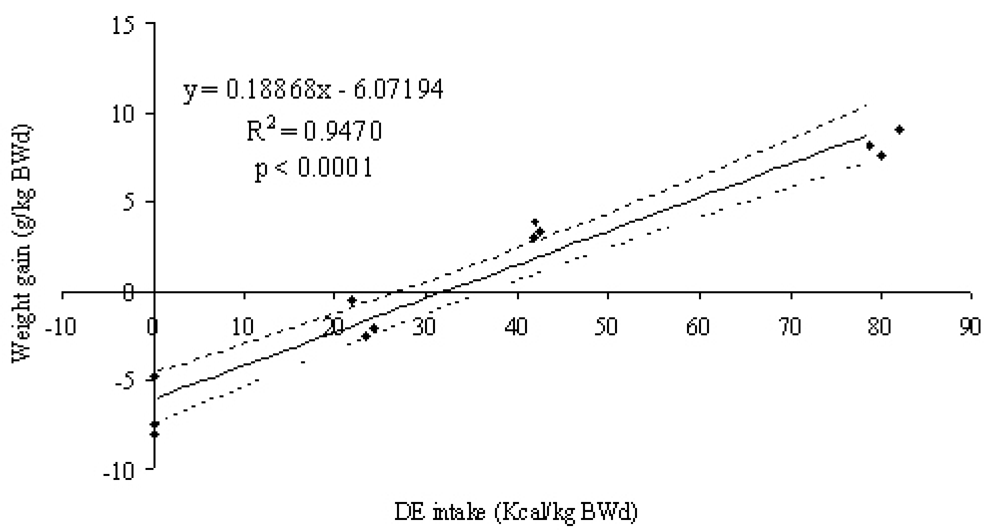


Figure 4b



Figures 5a and 5b: Regression curve of weight gain of *L. vannamei* in response to varying levels of digestible protein input (DP). Trial 1 (Figure 5a) was conducted over 38 days using juvenile (2.2 g initial weight) shrimp. Trial 2 (Figure 5b) was conducted over 35 days using sub-adult (11.2 g initial weight) shrimp.

Figure 5a

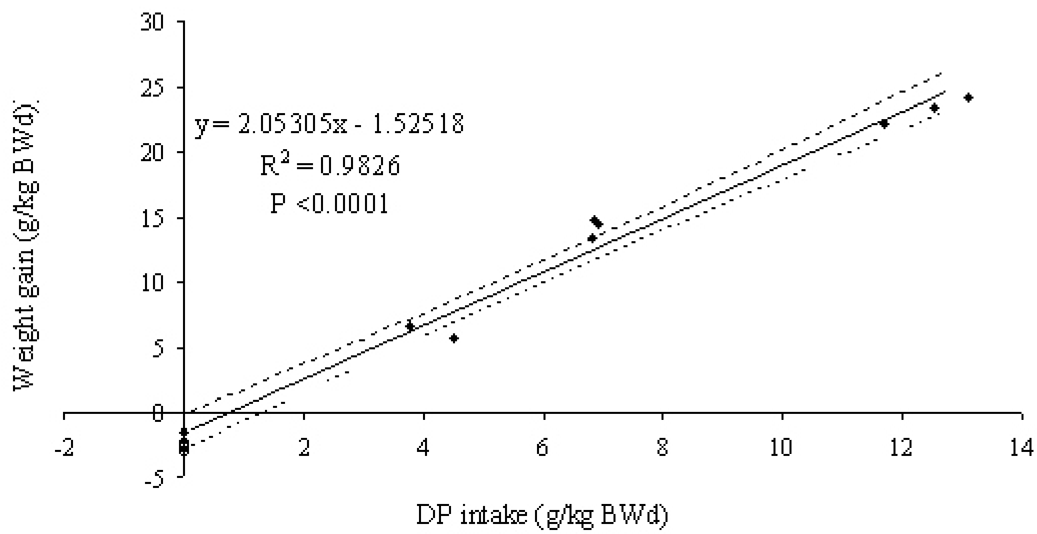
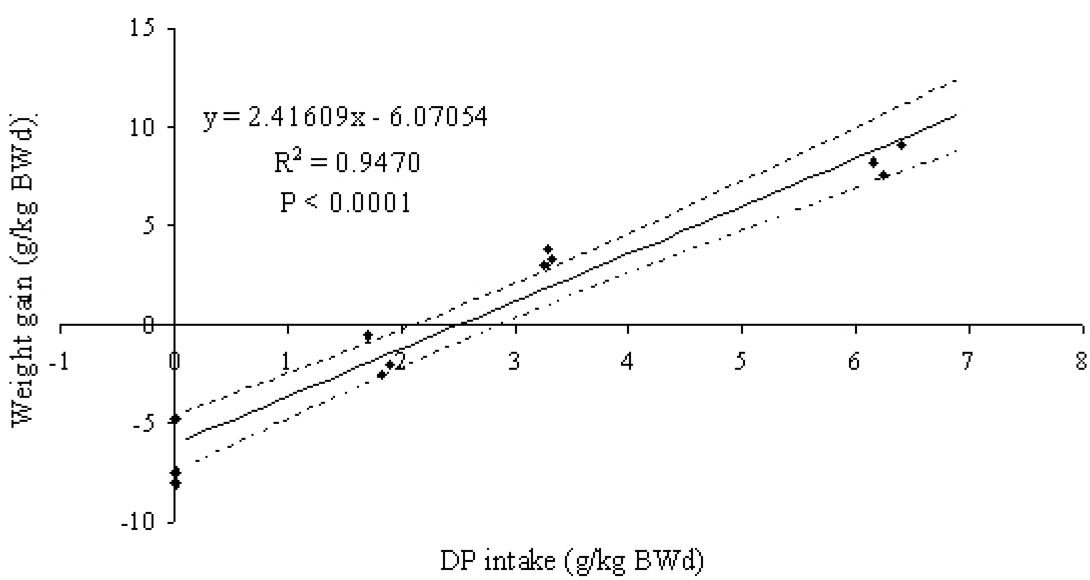


Figure 5b



The DE requirement for maintenance (energy of fasting) from respirometry was 28.1-35.5 Kcal/kg BWd for juvenile shrimp (4.3 g, mean weight) and 14.1-19.5 Kcal/kg BWd for sub-adult shrimp (12 g, mean weight).

In Table 6 is presented an example of daily feed allowance to shrimp of three body sizes with diets of three different levels of energy and DP calculated with a bioenergetics model. This model was designed with the values of DE and DP requirements for maintenance and the values of partial efficiencies of utilization of DE and DP determined in this research.

Discussion

The factorial method was used to determine daily DE and DP requirement for maintenance and growth of *L. vannamei*. Reports of daily nutrient requirements of crustaceans determined by factorial methods are very limited in the literature. By factorial methods the requirements are expressed as a daily feed intake per unit of weight or body components (eg., protein, energy) gain, rather than a percentage in the diet as is traditionally expressed using the dose-response method. In this study the requirements for protein and energy in growing shrimp were determined from the sum of the energy retained as growth plus the amounts of energy and protein lost from the body due to partial efficiencies of utilization and allocation of these body components.

The factorial procedure used involved offering graded levels of feed to supply nutrients and energy requirement below and above maintenance. In response to variations

Table 6. Determination of daily feed allowance (g/shrimp/day) using a bioenergetics model based on digestible energy (DE) or digestible protein (DP) of the diet for 2-g shrimp having a growth rate of 0.5,1.0, 1.5, or 2.0 g/week.

Diet	Expected growth (g/week)			
DE (Kcal/kg)	0.5	1	1.5	2
3000	0.119	0.211	0.303	0.395
3500	0.102	0.181	0.259	0.338
4000	0.089	0.158	0.227	0.296
4500	0.079	0.141	0.202	0.263
DP (%)				
25	0.114	0.205	0.296	0.387
30	0.095	0.171	0.247	0.323
35	0.081	0.146	0.211	0.277
45	0.07	0.128	0.185	0.242

in the level of feeding, shrimp growth decreased, remained constant, or increased for both trials. By regressing DE or DP offered and weight or body components gained (energy or crude protein), regression equations were determined, which allowed the estimation of DE and DP requirements. The daily DE requirement of maintenance for juvenile *L. vannamei* was 41.1 and 50.3 Kcal/kg BWd for sub-adult shrimp. Similarly, requirement of DP for juvenile shrimp was 2.82 and 3.6 g/Kg BWd for sub-adult shrimp. Values of DE requirements on *L. vannamei* determined by factorial methods are not reported in the literature. However, Kureshy and Davis (2002) reported a DP requirement of 2.8 g DP/kg BWd (3.8 g CP) for juvenile shrimp and 1.6 g DP/kg BWd (2.1 g CP) for sub-adult shrimp when feeding a 32% protein diet. Daily requirement of DP for *L. vannamei* determined in this study are similar to those reported for Kureshy and Davis (2002), especially if we adjust their values to the geometric mean and not to initial weight as presented by these authors. Values of DP and DE requirements for maintenance in this study tend to be higher for sub-adult shrimp than for juvenile shrimp. The reasons for this discrepancy between the two procedures are unknown. However, due to the low energy of maintenance of shrimp, this difference may be negligible in practical terms because it can be the result of experimental error associated with the procedure.

The same trend was not observed in the respirometry experiment of this study. Data showed a higher value of energy of fasting for juvenile shrimp (28.1-35.5 Kcal/kg BWd for 4.3 g average weight shrimp) than for sub-adult shrimp (14.1-19.5 for 11-g shrimp). Overall, the values of DE requirement for maintenance of juvenile shrimp obtained by the factorial approach seem to be similar to those obtained by respirometry

(41.1 Kcal/kg BWd versus 28.1-35.5 Kcal/kg BWd). For sub-adult shrimp the difference between DE of maintenance determined using both procedures is higher (50.3 Kcal/kg BWd determined by factorial procedure versus 14.1-19.5 Kcal/kg BWd determined by respirometry). The reasons for these discrepancies are unknown. However, lower values of DE requirements should be expected when using the respirometry procedure because by this method only values of basal metabolism (HeE) are determined. Animals are fasted 24 hours before the determination of DE and the effect of heat increase for feeding (HiE) should not be present. In addition the movement of shrimp inside the respirometry chamber is minimal, which reduces the energy spent due to activity. In contrast shrimp move freely in the tanks when DE is determined by factorial procedures, increasing the energy expenditure under those conditions. Overall these results demonstrate that both procedures can be used to determine energy requirement of *L. vannamei*.

Values of respirometry are in agreement with values in the literature reported for other crustaceans and shrimp. For 30-g crayfish (*Cherax tenuimanus*), Villareal (1990), reported the energy of fasting (HeE) to be 6.21 kcal/kg BWd at 22 ºC. For *L. Stylirostris*, Tchung (1995) reported a heat production of fasting of 14.8 kcal/kg $BW^{0.66}$d at 28 ºC. Gauquelin (1996) determined a HeE of 10.8 kcal/kg BWd for *L. stylirostris* of 20-30 g. These values are also close to those reported by other authors for fish (Bureau *et al.*, 2002). It seems that at optimal temperature energy of fasting and energy for maintenance of shrimp are similar to that of fish.

The DE and DP protein requirements determined were expressed in terms of total body weight. However, it is usually recommended to express rate of metabolism in terms of metabolic weight because expenditure for maintenance, at a constant temperature, is a function of the body weight of the animal (Brett and Groves, 1979; Blaxter, 1989). Normally, animals of smaller size have a higher metabolic rate per unit of body weight than animals of larger size. This is reflected in the linear increase observed when logarithm of the HeE is plotted against the logarithm of the body mass (Blaxter, 1989). Usually the slope of the regression equations resulting from these type of graphs is lower than 1, which means that animals of smaller size spend more energy per unit of body weight than larger animals. The value of the exponent for fish usually ranges between 0.5 to 1. An average of 0.83 was estimated for fish (Brett and Gloves, 1979; Hepher, 1988; Lupatsch *et al.*, 1998).

Information in the literature about metabolic weight of shrimp or other crustaceans is limited. Gauquelin (1996) reported a coefficient of 1 for *L. vannamei*. In this study, shrimp of four different body sizes were fasted for 30 days and the regression of body components used to meet energy requirements and body weight were determined. Shrimp had a coefficient of approximately 1.0 both for protein and energy loss. This indicates that, apparently, the size of the shrimp (from 1 to 12 g) did not affect the metabolic rate for the conditions of this trial. Probably the range of size of shrimp was not large enough to affect the metabolic rate of the animals tested. This was reflected in the results of DE and DP requirements for maintenance determined by using protein or energy retention values, which tended to be similar for both juvenile and sub-adult shrimp. Pacific white shrimp, *L. vannamei* under production conditions in ponds normally grow from an average of 1 g to

20-25g. Therefore, due to this narrow size range under culture conditions, the effect of body size on metabolic rate probably is not as important for this species as occurs in fish.

By using the factorial approach, the daily requirement of protein or energy in fish and shrimp have been determined using either total weight gain or specific components of growth, such as CP and recovered energy (RE). Traditionally RE has been used as a measure of nutrient deposition and growth in most feed requirement models (Bureau *et al.*, 2002). Retained energy is preferred to weight gain because body composition in fish and higher animals, especially lipids, tend to change with body size. The energy content of lipids is considerably higher than that of protein, which tends to exaggerate the differences of energy content of weight gain. In addition, retention of lipid reduces the moisture content of the body and changes the energy level per unit of body weight (Bureau *et al.*, 2000). These differences between retained energy and weight gain are important for land animals and fish. Larger or older fish tend to accumulate more body fat than smaller ones. The level of crude protein tends to be more constant ranging from 154-179 g/Kg BW; however the level of fat tends to vary considerably between young and active growing and older animals (Shearer, 1994; Lupatsch *et al.*, 1998).

In shrimp, body composition changes little with body size (Bureau *et al.,* 2000). In this study, the level of crude protein and body energy showed narrow variation for shrimp of body size between 1 and 12 g. Crude protein content ranged between 16 and 18.5% of body weight and body energy varied between 1000 and 1200 Kcal/kg BW (Figure 1a and Figure 1b). Hence, due to relative constant body composition, values of DE and DP determined by using either retained protein and energy or weight gain should be similar. However, in this study values determined from both procedures were different. For example DP requirements of juvenile shrimp were 0.74 g/kg BWd and 2.75 for sub-adult shrimp when using WG as the dependant variable. These values increased to 2.82 and 3.6 g/kg BWd when using retained protein as the dependant variable. These results demonstrate that weight gain was not a good indicator to determine specific nutrient requirements in shrimp because the body composition of shrimp changed as response to feeding rates. Shrimp that were fasted or received a food allowance below their daily requirements had values of dry matter, CP, and energy below shrimp that was fed above maintenace. The composition of the gain, especially of the nutrient whose requirements are being investigated seems to be more appropriate.

It is known that the partial requirement of food for gain depends mainly on the amount and composition of the accumulated tissue as well as the efficiency of utilization and deposition of these tissues. Due to these variations in nutrient composition and tissue deposition, some authors suggest a specific system based on the type of nutrient retained (eg., protein or lipid). By factorial methods, the efficiency of utilization of ME or DE are determined empirically by feeding graded levels of feed and regressing DE or DP intake and RE or CP retention, respectively. The slope of the regression equation is normally the efficiency of utilization of energy or protein. Usually this relation adjusts to a significant linear regression between DE and RE. The slope of this equation usually ranges between 0.5 and 0.75 in most studies (Cho and Kaushik, 1990; Azevedo *et al.*, 1998; Lupatsch *et al.*, 1998; Ohta and Watanabe, 1998). By these procedures, partial efficiency of ME utilization for protein deposition in common carp (*Cyprinus carpio*) has been reported to be of 0.56 and 0.72 for lipid deposition (Schwartz and Kirchgessner, 1995). Lupatsch *et al.* (2001) reported a constant energy utilization for growth of 0.5 for *Spaurus aurata*, but variable efficiency of protein utilization, which ranged between 0.33 and 0.6 depending on the DP/DE ratio of the diets. However, they found that the optimum protein efficiency was 0.47. For *Penaeus monodon* the efficiency of energy deposition was estimated around 0.5 for a 35% CP diet (Warukamkul *et al.*, 2000). Using similar approach in this study, we determined the efficiency of utilization at maintenance of DE for *L. vannamei* to be 0.29 for juvenile shrimp and 0.42 for sub-adult shrimp. Similarly, the efficiency of utilization and deposition of DP was 0.54 and 0.71 for sub-adult shrimp. Sub-adult shrimp had higher values of efficiency both for DE and DP than juvenile shrimp. This may be due to the most limited food allowance offered to this group. Shrimp lost weight at the second feeding rate, indicating that food supply was not deficient at this level.

Contrary to fish, shrimp showed higher efficiency of utilization of DP than DE. It may be due to the body composition of shrimp. Between 70 and 75% of shrimp dry matter was crude protein and the level of body fat, although not measured, was assumed to be less than 2% (based on the level of body energy as well as published values (Bureau *et al.*, 2001). Hence, the main body component stored by shrimp was crude protein, and it was also the main contributor of the body energy. It should be expected that dietary energy components fed in excess to the energy requirement of shrimp and that were not stored as protein, were lost. It would explain the lower efficience for DE than from DP deposition. Also, due to the relatively constant composition of growth of shrimp, the limitations indicated for the factorial methods due to variations in the composition of the gain, might be less significant for crustaceans of this size range.

The practical application of the factorial method is the development of bioenergetics models that allow determination of feed allowance to reach determined levels of growth. A bioenergetics model for shrimp was formulated (Appendix 1 and Table 6) using determined values for DE and DP for maintenance plus efficiency of energy and protein deposition to reach specific levels of growth. The model shows that feed allowance varies with the composition of the diet (DE and DP) and with the rate of shrimp growth. Therefore, this system developed empirically shows that shrimp do not have a specific requirement for a level of protein or energy in the diet, but a daily requirement that can be supplied with diets with variable levels of DE and DP. Consequently, the energy requirement can not be expressed as an absolute amount of nutrient or energy in a diet, but as a daily requirement that can vary depending on biotic and abiotic factors.

Conclusions

The use of bioenergetics models to estimate feed allowance may be an important tool to optimize feed input and to reduce feed wastage in shrimp production. Data generated by growth and feed requirement models can be used to compare with current performance in a shrimp farm (eg., weekly growth, FCR). This information could be used to adjust production conditions and to improve feeding and husbandry practices (Cho and Bureau, 1998) to reach biologically achievable goals. However, it must be clear that there is not a unique bioenergetics model than can be applied to every production condition.

Maximum production and optimal growth of an animal species are factors that depend on the genetic, diet, environmental conditions (eg., temperature), and animals health conditions, among others. Hence, nutrient requirements should be determined for specific values of performance, feed composition and life stage. In this context, on-site generated information and production records of a production unit are very important to develop bioenergetics models adjusted to the local conditions. Therefore, these models will be useful only when they have been developed and adjusted to the specific biotic and environmental conditions of the production operation.

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CHAPTER III

VARIABLE FEED ALLOWANCE WITH CONSTANT PROTEIN INPUT FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei* UNDER SEMI-INTENSIVE CONDITIONS IN TANKS AND PONDS

Abstract

In shrimp aquaculture feed input can be reduced by increasing the protein density of the diet. To evaluate this strategy, two growth trials were conducted with juvenile *Litopenaeus vannamei*. In an outdoor tank trial, juvenile (0.57 g ± 0.01, n = 30) shrimp were reared for 56 days and fed two practical diets formulated to contain 30% and 40% crude protein. Each diet was offered at three feeding rates (50, 75, and 100% ration). At the end of the trial, final weight of shrimp offered the 30% CP diet ranged from 8.1-10.3 g and 8.7-11.3 for shrimp fed the 40% CP diet. Final weight, weight gain, feed conversion rate (FCR), and protein conversion efficiency (PCE) was reduced significantly at every reduced feed ration (50 and 75%). There were no significant differences in final weight (10.3 g, mean weight) of shrimp offered the two treatments with similar protein input (30-100 and 40-75%). However, FCR was significantly lower for the 40-75% treatment (1.1 versus 1.4). To demonstrate variable feed allowance under practical pond conditions 12 ponds were stocked at 35 shrimp/m^2 (0.04 ± 0.04 g, n = 56) and assigned three treatments (30-100, 30-75, and 40-75%). A fourth treatment (40-100%) was initiated two weeks later to allow observational data collection. At day 107 final weight ranged between

19.7 and 23 g and there were not significant differences between the treatments. Due to adverse environmental conditions two ponds of the treatment 40-75% were lost. Therefore, results from this treatment were not included in the statistical analyses. At the end of the trial (114-121 days) the final weight (19.7-21.7 g), FCR (1.0) and survival (75-88%) between the treatments 30-100% and 30-75% were no significantly different. However, production was significantly higher for the treatment 30-100% than for the treatment 30-75% (6,482 versus 5,054 kg/ha). Although yield was higher for the 40-75 treatment than for the 30-75 treatment, it tended to be lower than that of the 30% CP diet offered at standard rate (30-100%). This study demonstrates that increasing the nutrient density (protein content) of the shrimp feed allows to reduce feeding input without affecting the growth performance of shrimp in tanks. Unfortunately, due to lost of some experimental units in the pond trial results of the tank trial could not be verified statistically. These results show that lab research by itself can not provide all the solutions to problems that occur under production conditions, but it still represents an important tool that can provide some useful information that helps to understand the production systems.

Introduction

Commensurate with the growth of the crustaceans aquaculture industry has been a shift toward high intensity systems and an increase in feed inputs (FAO, 2000; Tacon *et al.*, 2000). Feed represents one of the primary variable costs associated with the production of shrimp. In intensive operations it has been estimated that feed accounts for about 55 to 60% of the total variable costs and under semi-intensive conditions this value has been estimated to be about 40% (Chanratchakool *et al.*, 1994; Lovell, 1998; Lovell, 2002). The feed also has the potential of producing wastes that can cause negative impacts to the environment when they are released to effluent waters (Persson, 1991; Cho and Bureau, 2001). The amount of these wastes is increased when shrimp farmers follow inappropriate feed management strategies and overestimate feed inputs. This excess of feed input can cause deterioration of water quality that leads to poor growth and survival with a consequent reduction in production and economic return. For these reasons there is an interest in optimizing feed input and feed management to improve economic return in shrimp farms and to reduce the potential of environmental impact.

In marine shrimp culture, optimal daily supply of protein is one of the aspects of greatest interests in a feed management program. Protein is one of the major nutrients for shrimp growth and represents one of the primary costs in a compound feed formulation. It is also one of the nutrients that most contributes to water quality problems and pollution potential (Persson, 1991). Nitrogen, the product of protein metabolism when it is not deposited as growth, is generally the most limiting nutrient for algae growth in marine and brackish water environments (Persson, 1991). In intensive shrimp culture systems Briggs and Funge-Smith (1994) estimated that 95% of the nitrogen input was in the form of feed and fertilizers and only 24% of that nitrogen was retained in the shrimp production. Similarly, in semi-intensive shrimp ponds Green *et al.* (1997) found that about 47% of the nitrogen was derived from the feed and fertilizer and about 37% was retained in shrimp production. The quantity and quality of dietary protein as well as the non-protein energy content are factors that influence nitrogen excretion and waste. Therefore, feeding the optimal amount of protein in a nutritional balanced and high digestible diet is required to reduce the potential of nitrogen wastes. In order to optimize protein input, the amount of protein should be determined based in the protein requirement of the species.

Traditionally, to determine nutrient requirement of an animal species researchers have focused on manipulating the level of the major nutrients in the diet (e.g., protein). However, it has been demonstrated that animals have a daily quantitative requirement that can be met with a variety of diets with different levels of the nutrients (Lawrence and Lee, 1997; McGoogan and Gatlin, 1998; Kureshy and Davis, 2002). Therefore, feed input will vary and should depend on the nutrient density of the diet. For example, to meet the daily nutrient requirement, a reduced ration of a diet with high nutrient density could be used or a low nutrient-dense diet could be used but would require a higher consumption rate. Based on this approach it has been recommended to increase the nutrient density of the diets 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 rich in starch and fiber, and by increasing the amount of ingredients with high level of digestible protein and energy.

Some studies have shown the benefits of feeding diets with increased protein density at lower feeding rates in fish and shrimp. Cho and Lovell (2002) did not find significant differences between juvenile channel catfish (*Ictalurus punctatus*) fed in ponds a diet with 28% CP fed at satiation (100%) and a diet with 32% CP fed at 87.5 of satiation. A significantly higher feed efficiency was observed with the 32% CP diet.

Further increase of the protein level to 36% and reduction in the feeding rate to 77.8% depressed growth in the same experiment, probably due to deficiency in energy supply. Similar results were obtained by Cho *et al.* (2001) in common carp (*Cyprinus carpio*) when they reduced feeding to 77.8% of satiation by increasing the level of protein of the feed from 35% to 40% and feeding on an isonitrogenous basis. A significant lower growth rate was observed when they fed a diet containing 40% CP at 77.8% of the satiation rate. Kureshy and Davis (2002) reported a significant higher weight gain and feed efficiency for juvenile *L. vannamei* raised in tanks when fed a diet with 32% of CP compared to a diet with 16% CP. They also reported a significant higher weight gain for shrimp fed a 32% on an isonitrogenous basis than a 48% CP diet, but the feed efficiency was significantly higher for the later regimen.

An improvement in feed efficiency (FE) and feed conversion ratio (FCR) is observed when a high nutrient density diet is fed at reduced rate. Since the FCR is the amount of feed offered to produce a unit of shrimp, a lower amount of feed and consequently lower FCR is expected when feeding a diet with higher concentration of nutrients to produce the same amount of growth. For example, if it is assumed that 30% of the total protein offered in the feed is retained as shrimp tissue (Funge-Smith,1994; Green *et al.* 1997; Halver and Hardy 2001) and shrimp body contains about 18% crude protein, the estimated FCR is 2:1 for a 30%-protein diet (e.g. ,100 g diet provides 30 g CP of which 30% or 9 g are retained as shrimp tissue which represents 50 g of live shrimp). For a 40%-protein diet the estimated FCR would be 1.5:1.

Knowing the expected FCR based on the nutrient density of the diets helps to determine the amount of feed input in a shrimp production system. However, other factors must be considered to optimize feed input and reduce wastes. Traditionally, feeding tables are the most common method used in shrimp farms to determine feed allowance. However, they usually lead to over-estimation of feed input and high FCR (Jory *et al.*, 2001). As an alternative to the feeding tables some strategies have been suggested to improve feed management (Garza, 2001; Zelaya 2005). These include the use of previous production records to obtain information on production variables, such as growth rates, FCR, and survival. Based on this information, expectations of production could be determined and feed input could be estimated accordingly. The estimated daily feed input will be dependant on accurate determinations of the growth of the shrimp and on the size of the population. Since population estimates are often difficult to determine and inaccurate (Hutching *et al.*, 1980), it is common to evaluate and adjust daily feed input of a culture system based on a variety of factors, such as: actual weekly growth rates, environmental conditions (eg., morning dissolved oxygen and temperature), quantity of feed remaining on feed trays, and nutrient density of the diets (Garza, 2001; Zelaya, 2005). This feed management plan allows the use of diets with different levels of nutrients, including diets of high nutrient density.

As expected, the use of high nutrient density diets reduces the amount of feed necessary to reach the production goals. Although this practice has been recommended and research has been conducted with some aquatic species (Watanabe *et al.*, 1979; McGoogan and Gatlin, 1999; Cho and Kim, 2001; Cho and Lovell, 2002), its use and research has been more limited for shrimp, especially under pond production conditions. Often, shrimp nutrition research is conducted in tanks or aquaria under laboratory controlled conditions. Controlled conditions are important because it allows control over external variables that are not being studied. Laboratory is good for research, however, the contributions of natural foods present under typical production conditions are not present (Lawrence and Lee, 1997). Therefore, feed input and feed management research in ponds is encouraged because it reflects closer operations under commercial conditions (Lawrence and Lee, 1997). However, pond research is less attractive to investigators due to the effect and possible influence of variables difficult to control (e.g., environmental conditions), limitation to obtain adequate amount of similar experimental units necessary for good replication, and high operation costs.

In order to overcome the limitations of clear water laboratory controlled experiments and pond research a compromise between both systems can be reached. This is accomplished by using systems that have some primary productivity but are small and easily replicated. For example, primary productivity can be incorporated into a tank system by supplying green water from a production pond. Adding green water to shrimp tanks has shown improvement in shrimp growth , presumably due to the addition of detritus and organic particles that can be assimilated by shrimp (Leber and Pruder, 1988; Moss *et al.*, 1992; Moss, 1995).

There is little information in the literature with regard to the effect of feeding high nutrient density diets (e.g., high protein) at various feed allowances in shrimp culture. Consequently, the objective of this study was to evaluate the effect of adjusting the daily feed allowance to the protein requirements of *L. vannamei* when utilizing diets with different nutrient densities.

Materials and Methods

This research was conducted at the Claude Peteet Mariculture Center, in Gulf Shores, Alabama, from May through mid September 2004. Two experiments were conducted in parallel; one in plastic tanks that received water from a shrimp production pond and the other in replicated ponds.

Approximately 1 million post-larva Pacific white shrimp *L. vannamei* (4.3 ± 2.3 mg, mean ± standard deviation; n=57) were received from a commercial hatchery (GMSB Inc. hatchery, Key West, FL). The post-larvae (PL) were shipped in styrofoam boxes that contained double plastics bags with about 6 liters of 15 ppt salt water at 21 °C and 13.1 mg/L dissolved oxygen (DO) at an approximate density of 1,500 PL/L**.** The bags were placed in a 940-L acclimation tank that was filled with 15 ppt sea water and allowed to float until the temperatures were equilibrated. The PL were released after the water in the bags and the acclimation tank were within 0.5 °C of each other. The PL were pooled together in the acclimation tank and newly hatched nauplii of *Artemia salina* (INVE Americas, Inc., Salt Lake City, UT, USA) were fed. After approximately 1 h, the PL were concentrated and quantified volumetrically (Hardin *et al.*, 1985; Juarez *et al.*, 1996). The whole PL population was concentrated in a 57-L container. Oxygen was provided and delivered through airstones. The water in the container was mixed vigorously with both hands before taking sub-samples with a 60-ml beaker. The average number of PL from three sub-samples were determined. If the CV was >15% then additional sub-samples were counted. The average number of PL was then used to estimate the whole population in the 57-L container. The population was divided in six groups of approximately the same number of PL and stocked in each of six nursery tanks.

The nursery phase was carried out in six fiberglass tanks (3.0 x 1.5 x 0.9 m) that were located in a clear polyethylene plastic covered greenhouse. The nursery system was designed as a semi-closed recirculating system containing six culture tanks, common biological filter, a rapid-rate sand filter (Model TR100, AREA, Homestead, FL, USA) and a circulation 2-hp high head pump (AREA, Homestead, FL, USA). Post-larvae were fed Artemia nauplii (~100 nauplii/PL) and a commercial PL feed (Zeigler Bros, Inc., Gardners, PA, USA) four times a day during the first three days (Table 1). From day four to day five equal proportion of the commercial PL feed and a crumbled commercial shrimp feed (Rangen® Inc., Buhl, ID) were fed four times a day. Thereafter, four feedings per day of the crumbled feed (Rangen® Inc., Buhl, ID) of various sizes were fed following the schedule shown in Table 1.Temperature, DO, pH, and salinity were monitored twice daily (at 0800 and 1600 H) using a YSI 556 DO meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonia-nitrogen (TAN) was measured every three days with a spectrophotometer (Spectronic 20 Genesys, Spectronic Instrument Inc. Rochester, NY, USA) following the Nesslerization method (APHA 1989). Every week about 50% of the total water volume was exchanged with filtered sea water. Water quality parameters were as follows: temperature, 27.4 ± 1.55 ºC; DO, 5.98 ± 1.67 ppm; pH, 7.47 ± 0.33; salinity, 33.62 ± 0.33 ppt; and TAN, 0.81 ± 0.54 ppm. The survival rate, FCR (dry weight of feed

Table 1. Feeding regimen utilized during a 22-day nursery period of *Litopenaeus vannamei*. Feed rates as percentage of the biomass were based on an expected survival rate of 70% (during 22 days) and average shrimp weights.

Days	Feed type	Feed rate (% biomass)
1 to 3	Artemia nauplii [a](100/PL)and PL Ready[b]	25
4 to 6	PL Ready[b] (1) and Crumble[c] #1(1)	25
7 to 10	Crumble[c] #1	15
11 to 15	Crumble[c] #1	10
16 to 19	Crumble[c] #1 (3/4), Crumble[d] #3(1/4)	10
20 to 21	Crumble[d] #3	10

[a]INVE Americas , Inc., Salt Lake City, UT, USA
[b]PL Ready 50% Protein, Zeigler Bross, Inc., Gardners, PA, USA
[c, d]Rangen 45% protein, Rangen Inc., Buhl, Idaho, USA

offered/wet weight gained), and final weight at the end of a 22-day nursery period were (mean ± standard deviation) 67.48% ± 19, 1.83 ± 0.79, and 0.0413 ± 0.0091g, respectively. At the conclusion of nursery culture the juvenile shrimp were stocked into ponds.

Tank trial

Juveniles shrimp used in this trial were selected and hand-sorted for size from production ponds and stocked into the production system five days prior to the initiation of the growth trial. Juvenile (0.57 g ± 0.01, n=30) reared for 56 days in an outdoor semi-closed recirculating system and fed two practical diets (Table 2 and Table 3) formulated to contain 30% and 40% crude protein and produced at a commercial feed mill using extrusion technologies (Rangen® Inc., Angleton, TX) and offered at three different feeding rates (50%, 75%, and 100%). Six feeding treatments were assigned to 4 replicate tanks per treatment at a density of 30 shrimps/tank. The standard feed rate (100% ration) was based on an expected FCR of 1.8 and a growth rate of 1 g/wk or 0.26 g/shrimp/day. The 40% CP diet fed at 75% feeding level (40-75%) matched the protein input of the control (30% CP diet at 100% ration). The treatments 40-100% and 30-75% matched the feed input of the control and the 40-75% treatments, respectively.

The semi-closed recirculating system consisted of 24 circular polyethylene tanks (0.85 m height x 1.22 m upper diameter, 1.04 m lower diameter), a common reservoir with a biological filter, and a 1/3-hp circulation pump. The recirculation rate for each tank was set at 2.92 L/min (n=3). The reservoir received water from a shrimp production pond during 6 hours per day at a flow rate of 3 L/min. Each tank was equipped with a center

Table 2. Composition (g/100g as fed) of experimental diets formulated to contain 30% CP- 6% lipid and 40% CP-8% lipid. Diets were commercially manufactured (Rangen® Inc., Angleton, TX).

Ingredient	D30	D40
Menhaden fish meal Select	7.5	9.99
Poultry by-product meal 62%	18.8	24.9
Milo	54.5	29.6
Soybean meal	14.5	30.7
Fish oil	1.07	2.37
Bentonite	1.5	1.5
Trace mineral premix	0.065	0.085
Vitamin premix	0.28	0.38
Copper sulfate	0.01	0.013
Stay C (30% active)	0.006	0.008
Dicalcium phosphate (21% P)	1.583	0.583
Mold inhibitor	0.15	0.15

Table 3. Proximate composition (crude protein and lipids), apparent digestibility coefficients of dry matter (ADDM), protein (ADP), and energy (ADE), digestible energy (DE), digestible protein (DP), DE/CP ratio, DE/DP ratio and DE of a 30% CP and a 40% CP diet fed to *Litopenaeus vannamei* at 50, 75, and 100% feeding rate.

ADC	30% CP	40% CP
Crude protein	32.2	42.5
Lipids	10.3	11.9
ADDM	60.8	63.8
ADP	69.3	76.9
ADE	66.7	75.4
Digestible protein (%)	22.3	31.1
Digestible energy (Kcal/g diet)	3.12	3.66
Kcal DE/g CP	9.96	9.04
Kcal DE/g DP	13.99	11.76
DE fed at 100% rate (Kcal/shrimp/day)	0.8	0.94
DE fed at 75% rate (Kcal/shrimp/day)	0.6	0.71
DE fed at 50% rate (Kcal/shrimp/day)	0.4	0.47

drain, a stand pipe of 3.2 cm diameter which was 75 cm long, screened and set to maintain a water depth of 61 cm or 570 L volume. Aeration was provided by two air stones connected to a common air supply from a 1 hp regenerative blower (Sweetwater Aquaculture Inc. Lapwai, ID, USA).

Shrimp were fed twice a day, half of the daily feed allowance each feeding. Shrimp in each tank were counted every two weeks and the daily ration readjusted. Dissolved oxygen, pH and temperature were measured twice a day (at the 0600 and the 1600 hours) with a YSI 556 DO meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonia-nitrogen and nitrite-nitrogen were determined once a week. Water samples were taken from the mid water column in the reservoir and in two tanks selected at random. Total ammonia-nitrogen was measured with a spectrophotometer (Spectronic 20 Genesys , Spectronic Instrument Inc. Rochester, NY, USA) following the Nesslerization method (APHA, 1989). Nitrite-nitrogen was determined using a model PLN code test kit from LaMotte (Chestertown, MD, USA).

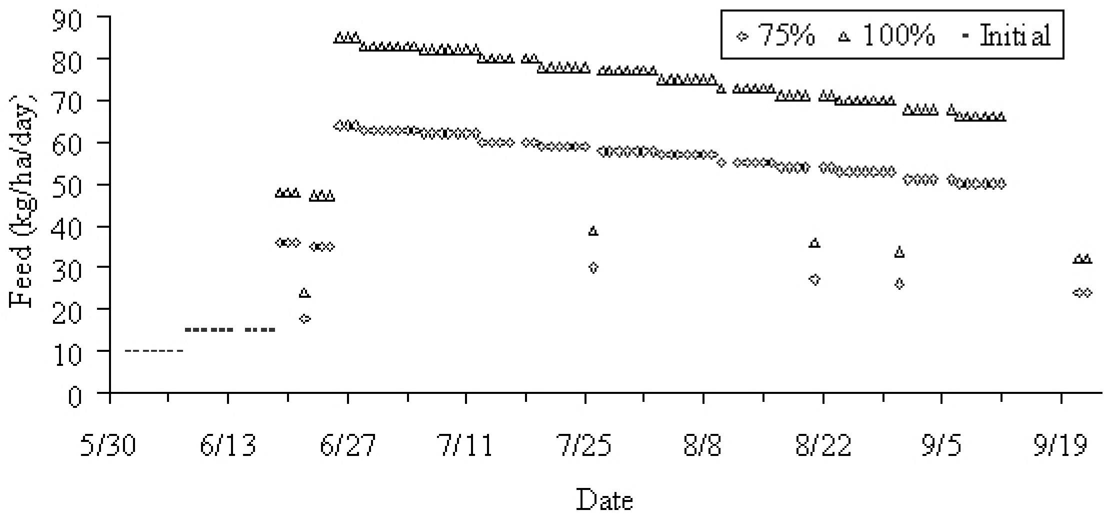
At the end of the experiment, the weight gain (WG = initial weight - final weight), percentage survival, and FCR were determined. Shrimp samples were collected at the beginning (pooled sample) and at the end of the experiment (6 shrimp/tank) to determine changes in body composition (dry matter, protein and total energy). Based on proximate analyses, protein conversion efficiency (PCE = dry weight protein gain x 100/dry weight protein offered) and energy conversion efficiency (ECE = dry weight energy gain x 100/dry weight energy offered) were determined.

Samples were kept frozen in sealed plastic bags in a freezer at -15 ºC until analyzed. Shrimp samples were dried in an oven at 90 ºC to a constant weight, using the methods described by the A.O.A.C.(1990), and then ground in a coffee grinder and stored in a freezer. Crude protein content of shrimp was analyzed using the micro-Kjeldahl method (Ma and Zuazago, 1942). Total energy content was determined using a micro-calorimetric adiabatic bomb using benzoic acid as standard (Parr 1425, Moline, IL, USA).

Pond trial

Juvenile (0.04 ± 0.04 g, n = 56) Pacific white shrimp *L. vannamei* were reared in the first 12 ponds for 114-121 days and fed two practical diets of 30 and 40% crude protein (Rangen® Inc., Angleton, TX) at two different feeding levels (75, and 100%). Once the shrimp reached approximately 1 g (day 18), three feeding treatments were assigned to the 12 ponds, allowing four replicate ponds per treatment. The first treatment (30-100%) received a 30% CP feed at a typical feed rate (100% ration) to obtain an expected FCR of 1.8 and a growth rate of 1 g/wk, which was based in previous production records in the same experimental units with the same species (Zelaya 2005) (Figure 1). The second treatment (30-75%) was fed the 30% CP diet, but it was offered at 75% of the feed allowance of a typical ration. The third treatment (40-75%) consisted of feeding a 40% CP diet at 75% of the feed allowance. This matched the total protein inputs of treatment 1 (30-100%). Two weeks after the initial stocking two additional ponds were added to the study. This allowed an observational review of the fourth treatment, which received the 40% CP diet fed at 100% ration.

Figure 1. Daily feed input (kg/ha/day) of *Litopenaeus vannamei* fed two practical diets (30 and 40% crude protein) at two feeding levels (75 and 100%) and raised in ponds at 35 shrimp/m^2.



The ponds have approximately 1-m depth, were lined with 1.52-mm thick high-density polyethylene sheeting (Grundle Lining System, Inc., Houston Texas, USA), and had about 25-cm layer of sandy-loam soil. Each pond had a concrete catching basin and was drained through a 20-cm diameter screened standpipe located inside the catching basin. Pond preparation consisted of draining the water and letting the pond bottoms dry for several weeks. They were then tilled using a garden tiller set for a depth of 10-15 cm. This exposed the soil to the air and sunlight to improve oxidation and mineralization of the organic matter. Brackish (~24 ppt) water from the intracoastal waterway, a shipping channel that connects Bon Secour Bay with Wolf Bay in Gulf Shores and Orange Beach, was used to fill the ponds 2-3 weeks before stocking. The water was filtered through a 250-um nylon filter sock (Domestic Lace Mfg., Inc.) that prevented the introduction of potential predators. Liquid inorganic fertilizers were applied at 303 ml of 10-34-0 and 1,697 ml of 32-0-0 per pond that provided 573 ml N and103 ml of P_2O_5 to stimulate plankton growth and natural productivity (Boyd and Tucker, 1998). Two weeks after the initial fertilization a second fertilization was applied if a secchi disk reading of 25-40 cm was not reached.

Shrimp were fed twice a day (about 50% of total daily feeding each time) spread uniformly throughout the pond area at approximately 0900 h and at 1600 h. All ponds were fed equally during the first 18 days of culture (Figure 1). During the first seven days, 1000 g of 35%-CP feed (crumbles # 4, Rangen® Inc., Buhl, ID) was fed daily to each pond. From day 8 to day 18 shrimp were fed 1500 g per day of the same feed. Feeding the treatment diets started at day 19 at half of the total feed input per treatment and continue

until day 22. The complete daily ration, for the assigned treatment, was fed after day 23. The amount of daily feed input in each pond was calculated based on the ration for each treatment (75 or 100%) and adjusted weekly for mortalities (assuming an expected mortality of 30% over a 17-week growth-out period). Feeding ceased 9 days before harvesting due to the interruption of electrical service caused by hurricane Ivan.
No water exchange were conducted until the last 2 weeks of the pond production trial. Dissolved oxygen, pH and salinity, were measured three times a day (at 0600, 1200, and 2000 hours). Total ammonia-nitrogen, nitrite-nitrogen, and secchi disk reading were determined once a week. Water samples were taken from the mid water column and taken to the lab in closed plastic 1-L containers. These water quality parameters were determined according to previously described procedures. Total phosphorus levels of the water were determined two weeks before the end of the trial for the first three treatments. To determine total phosphorus, samples were digested by the potassium persulfate method (Boyd and Tucker, 1992) and analyzed for soluble ortho-phosphate by the ascorbic acid method (APHA *et al.*, 1989).

Base aeration (7.5kW/ha) was provided at night to keep the level of DO at 3 mg/L or higher using either a 1-hp spiral paddle wheel aerator (Little John Aerator, Southern Machine Welding Inc. Quinton, AL) or a 1-hp (11.2 Ampers) or 2-hp aspirator (20 Ampers) (Aire-O_2, Aeration Industries International, Inc. Minneapolis, Minnesota). Aeration was also provided during the day when the levels of DO fell bellow the target levels (e.g., cloudy days or after a pond phytoplankton community had collapsed).

Additional aeration (up to 30 hp/ha) was provided when the standard aeration was not enough to maintain the expected DO.

Mean weight of the shrimp were determined on a weekly basis starting two weeks after stocking. The first sample was taken by seine, thereafter ~80 shrimp per pond were captured by cast net (monofilament net, 1.22 m radius and 0.95 cm opening).

Pond harvest was initiated at day 107, but the activity had to be interrupted due to mandatory evacuation orders and the anticipated arrival of a hurricane. The rest of the ponds were harvested at days 114-115. One night before harvesting, approximately 70% of the water was drained. The following day, the remaining water was pumped out through a hydraulic fish pump with a 25-cm suction (Aqualife-Life pump, Magic Valley Heli-arc and Mfg, Twin Falls, Idaho, USA) placed in the catch basin. The shrimp were pumped from the pond basin to a dewatering tower, then to a harvest truck that was used to transport the shrimp to a cleaning table and weighing station. At harvest, mean weight and size distribution of 100 shrimps selected at random from each pond were determined. Average final weight, percent survival, FCR, size distribution and yield were then determined.

Digestibility trial

Chromic oxide was used as an inert marker to determine digestibility coefficients of the test diets. Six groups of 10 shrimps (~ 10.2 g mean weight) were stocked in a closed recirculating system consisting of six 60-L glass aquaria, biological filter, reservoir, circulation pump, and supplemental aeration. Water temperature was 27.2 ± 1.3 ºC and salinity was 12 ppt. The two practical diets (30 and 40% CP, Table 2) were ground to a powder and then re-extruded after adding 0.5% of chromic oxide. Each ground diet was transferred to a food mixer (Hobart, Troy, OH, USA) where they were mixed for about 5 min. Boiling water was then added to the mixture while it was still mixing to attain a mash of consistency appropriate for pelleting. The mash was passed through a 3-mm die in a meat grinder (Hobart, Troy, OH, USA), and the pellets were let in a drier at 65 ^{o}C overnight to reach a moisture content between 8-10%. Diets were ground and sieved to an appropriate size and stored in a -20 ^{o}C freezer until used.

Shrimp were allowed to acclimate for three days before starting the collection of feces. 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 um mesh screen. Shrimp were offered five feedings per day, however, feces obtained after the first feeding were discarded. Feces obtained after the first feeding were discarded. Collected feces were rinsed with distilled water and stored in sealed plastic containers and stored in a freezer. Samples from three tanks were pooled (r=3) and kept frozen until analyzed. Dry matter, crude protein, total energy was determined for the fecal and the diet samples according with procedures previously described. Chromic oxide was analyzed following the McGinns and Kasting (1964) procedures. Apparent digestibility coefficients (ADC) of the dry matter, protein, and energy were calculated according to Maynard *et al.* (1979).

ADC (%)= 100x[1- (dietary Cr level/fecal Cr level)x(fecal nutrient /dietary nutrient)]

Statistical Analyses

Final weight, weight gain, final body composition, FCR, PCE and ECE were analyzed for significant differences ($P \leq 0.05$) between treatment means by a one-way analysis of variance (ANOVA). A two-way ANOVA was used to analyze the effect of feed rate and protein level (diet) and their interaction on shrimp growth. Significant differences among treatment means were determined by the Student-Newman-Keuls multiple comparison test (Steel and Torrie, 1980). Stepwise regression analysis was used to evaluate the effect and interaction of feed, protein, and energy inputs on shrimp growth. Analyses were conducted using SAS program version 8.2 (SAS Institute Inc., Cary, NC).

Results

Tank trial

Water quality parameters observed over the 56-day growth trial are presented in Table 4. Water quality problems or diseases were not observed during the experiment and there were no significant differences in survival (Table 5).

A two-way ANOVA was used to analyze the effect of feed rate (100, 75, and 50%) and dietary protein level (30 and 40% CP) and their possible interaction on final weight. Analyses indicated a significant effect for both feed rate ($P<0.0001$) and dietary protein ($P=0.0002$), but not for the interaction. At each protein level, growth decreased as the ration was reduced (Table 5). For example, the lowest final weight and weight gain

Table 4. Water quality of a tank recirculating system (tank trial) and a pond trial used to raise *Litopenaeus vannamei* juveniles fed diets with 30% or 40% protein at three different feeding levels (50, 75, and 100%). Values are mean ± standard deviation of daily and weekly determinations. Values below in parenthesis represent minimum and maximum.

Parameter	Tanks	Ponds			
		30-100%	30-75%	40-75%	40-100%
Dissolved oxygen (mg/L)	6.93 ± 1.95 (5.03-10.78	5.90 ± 2.79 (1.07-15.52)	6.12 ± 2.60 (0.86-16.63)	5.97 ± 2.78 (0.22-15.76)	6.20 ± 2.59 (1.14-15.17)
pH	7.53 ± 0.42 (5.86-8.11)	7.88 ± 0.61 (5.89-9.49)	7.92 ± 0.66 (5.74-9.58)	8.04 ± 0.64 (5.92-9.80)	8.09 ± 0.59 (6.27-9.29)
Temperature (°C)	27.03 ±1.96 (20.16-30.39)	30.21 ± 1.81 (24.51-34.05)	30.31 ± 1.86 (24.63-34.15)	30.09 ± 1.80 (24.63-34.26)	30.25 ± 1.84 (24.6-33.94)
Salinity (g/L)	18.56 ± 1.73 (15.69-20.68)	18.93 ± 2.46 (9.78-24.84)	18.39 ± 2.66 (9.72-24.73)	18.92 ± 2.48 (10.81-24.79)	14.02 ± 1.49 (9.42-18.32)
Total ammonia-N (mg/L)	0.73 ± 0.18 (0.2-2.55)	1.25 ± 0.28 (0-7.7)	1.08 ± 0.27 (0-9.02)	1.07 ± 0.29 (0-10.19)	1.01 ± 0.31 (0-4.63)
Nitrite-N (mg/L)	1.92 ± 0.43 (0-0.6)	0.07 ± 0.13 (0-0.6)	0.08 ± 0.18 (0-0.8)	0.07 ± 0.18 (0-0.8)	0.04 ± 0.09 (0-0.3)
Total P (mg/L)	-	0.43 ± 0.06 (0.34-0.48)	0.61 ± 0.16 (0.45-0.77)	0.88 ± 0.21 (0.73-1.18)	-

Table 5: Final weight (FW), weight gain (WG), weigh gain per week (WG/wk), and feed conversion ratio (FCR) of *L. vannamei* fed two diets (30 and 40% CP) at three different feeding levels (50, 75, and 100%) in a green-water recirculating system[1].

Protein (%)	Ration (%)	FW (g)	WG (g)	WG/wk (g/wk)	FCR[2]	Survival (%)
	100	10.3[b]	9.8[b]	1.22[b]	1.38[a]	98[a]
30	75	9.5[c]	8.9[c]	1.11[c]	1.15[c]	93[a]
	50	8.1[d]	7.6[d]	0.95[d]	0.89	94[a]
	100	11.3[a]	10.9[a]	1.37[a]	1.24[b]	99[a]
40	75	10.3[b]	9.5[b]	1.19[b]	1.07[d]	99[a]
	50	8.7[d]	8.7[d]	1.02[d]	0.83[f]	95[a]
P-value		<0.0001	<0.0001	<0.0001	<0.0001	0.1353
PSE[3]		0.1566	0.1569	0.0196	0.0192	1.7922

[1]Values are means of four replicates. Means in the same column with different superscripts are significantly different ($P \leq 0.05$).
[2]FCR= dry weight of feed offered/wet weight gained
[3]PSE = pooled standard error

was observed for the 50% ration, both for the 30 and the 40% CP diet. Also, shrimp growth was significantly higher for the 40% CP than for the 30% CP when offered either the 75 or the 100% ration.

To further identify if the response was due to effect of treatments the data was analyzed by one-way ANOVA and SNK means separation. Across all treatments, shrimp fed the 40% CP diet at 100 % feeding level (40-100%) showed a significant higher final weight than the other treatments. The FCR of the 40-100% treatment was significantly higher than those from the 30-75% and the 40-75% treatments. There were not significant differences between the final weight, PCE, and ECE of shrimp fed the 30% CP diet at 100% ration and the 40% CP diet at 75% (similar nitrogen input) (Table 5 and Table 6). However, the feed conversion ratio (FCR) was significantly lower for the 40% CP at reduced feeding (75%) than the 30 % CP diet at 100% feeding. Final weight was not significantly different among the two treatments (30% and 40% CP) fed at 50% of the ration, but they were significantly lower than the shrimp fed larger rations (75% and 100%). Stepwise regression procedure was used to evaluate protein input, energy input, and feeding ration as a predictor of final weight. Results indicate that protein input was the factor that best fit to the model (Figure 2). This variable produced a significant regression ($P<0.0001$) with a R^2 value of 0.87%.

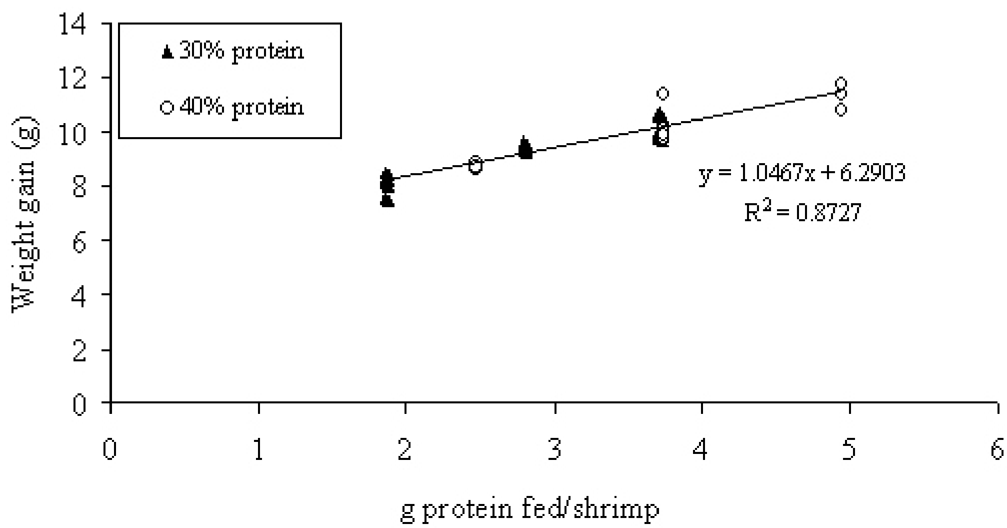
The final body protein and body energy of shrimp were not affected by the treatment levels (Table 6). Final body protein ranged between 17.1 and 19.1% (Table 6). The PCE and ECE values ranged between 42 to 78% and between 21 to 33%, respectively (Table 6). When feeding the 30% CP diet PCE decreased as the ration was

Table 6: Final body protein (% wet body weight), final body energy (Kcal/g wet weight), protein conversion efficiency (PCE), and energy conversion efficiency (ECE) of *L. vannamei* fed two diets (30 and 40% CP) at three different feeding levels (50, 75, and 100%) in a green-water recirculating system[1].

Protein (%)	Ration (%)	Body protein (% wet BW)	Body energy (Kcal./g wet BW)	PCE[2] (%)	ECE[3] (%)
30	100	18.3[a]	1.23[a]	48.8[c]	21.2[b]
	75	19.1[a]	1.25[a]	60.9[b]	25.8[b]
	50	17.1[a]	1.16[a]	78.2[a]	33.0[a]
40	100	18.9[a]	1.26[a]	41.8[c]	24.7[b]
	75	18.2[a]	1.19[a]	43.5[c]	25.7[b]
	50	18.0[a]	1.23[a]	58.6[b]	32.9[a]
P- value		0.4167	0.3428	<00001	<00001
PSE[4]		0.6291	0.044	2.334	1.2409

[1]Values are means of four replicates. Means in the same column with different superscripts are significantly different ($P \leq 0.05$).
[2]Protein conversion efficiency = dry weight protein gain x 100/dry weight protein offered
[3]Energy conversion efficiency = dry weight energy gain x 100/dry weight energy offered
[4]PSE = pooled standard error

Figure 2: Weight gain of *Litopenaeus vannamei* in response to varying levels of protein intake offered a 30 or a 40% CP diet at three different feeding rates (100, 75, and 50%) in a tank recirculating system.



reduced (Table 6). For the 40% CP diet the PCE was significantly higher at the lowest feeding rate (50%), but it was not significant different between the 75 and the 100% feeding rates. Energy conversion efficiency was significantly higher for the lowest ration (50%) for both levels of protein (Table 6).

Values of digestible protein (DP), digestible energy (DE) and DE fed to the different treatments are presented in Table 3. The digestibility coefficients of the dry matter, protein, and energy were higher for the 40% CP diet than for the 30% CP diet (Table 3). The observed digestible energy/crude protein ratio (DE/CP) was 9.94 and 9.09 for the 30 and the 40% CP diet, respectively.

Pond trial

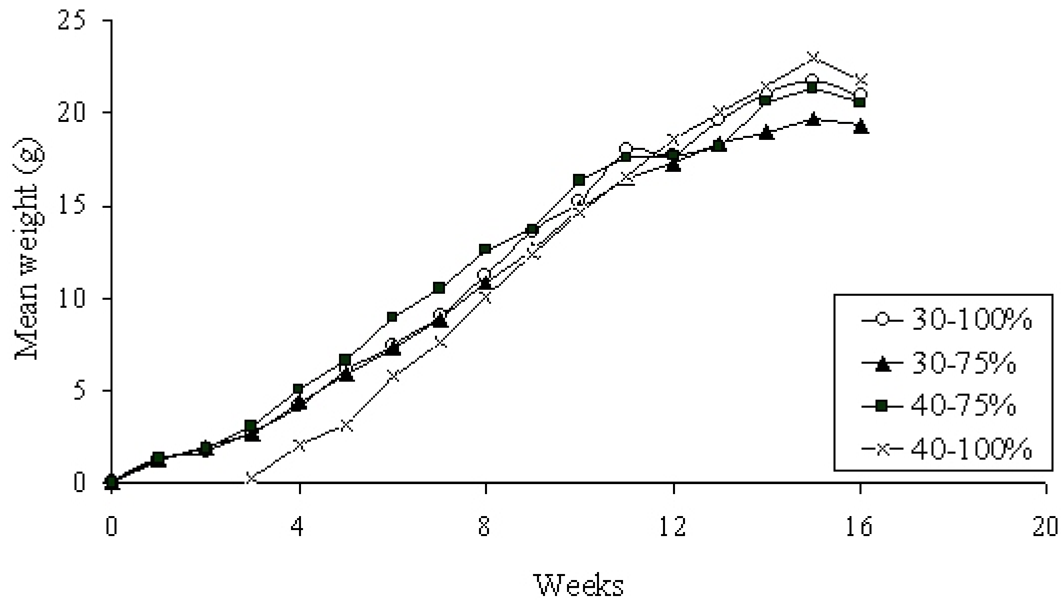
The observed water quality values for each treatment are summarized in Table 4. Average water quality parameters among all the experimental ponds were: DO, 6.02 mg/L; temperature, 30.21 °C; salinity, 18.22 ppt; pH, 7.96; total ammonia-nitrogen, 1.10 ppm; nitrite-nitrogen, 0.065 ppm; and total phosphorus, 0.64 ppm. The initial salinity for the first three treatments was 24.5 ppt. The two ponds that were stocked late (40-100%) had an initial salinity of 14 ppt. At the end of the experiment, salinity had been reduced through precipitation to about 11 ppt for all the ponds. The observed values for water quality are typical for this pond system and provided adequate conditions for *L. vannamei* growth. Collapses of the phytoplankton population resulting in depressed oxygen levels were observed after 54 days of culture and were not related to the experimental treatments.

Table 7. Final weight (FW), weight gain per week (WG/wk), yield, feed conversion ratio (FCR), and survival at 107-121 days of *L. vannamei* fed two diets (30% and 40% CP) at two different ration levels (75, and 100%) in 0.1-ha ponds (pond trial)[1].

Protein (%)	Ration (%)	FW[2] (g)	WG/wk (g)	Yield (kg/ha)	FCR[4]	Survival (%)
30	100	21.7[a]	1.38[a]	6,482[a]	1.0[a]	88[a]
30	75	19.7[a]	1.28[a]	5,054[b]	1.0[a]	75[a]
40	75	22.1[a]	1.40[a]	5,660[3]	0.9[3]	82[3]
40	100	23.0[3]	1.79[3]	5,408[3]	1.0[3]	71[3]
P-value		0.7591	0.3967	0.0374	0.7318	0.1136
PSE[5]		1.0671	0.0635	378	0.0097	25.64

[1]Values are means of four replicates, except where indicated. Means in the same column with different letter superscripts are significantly different ($P \leq 0.05$)
[2]Based on samples taken with cast net on day 106
[3]Means of two replicates (values for this treatment were not included in the statistical analyses).
[4]FCR= dry weight of feed offered/wet weight gained
[5]PSE = pooled standard error

Figure 3: Growth curve of *Litopenaeus vannamei* fed two practical diets (30 and 40% crude protein) at two feeding levels (75 and 100%) and raised in ponds at 35 shrimp/m^2 for 114-121 days.



At day 106 (week 15) cast net samples from all ponds were taken. At this point there were no significant differences between mean weights of shrimp under the different treatments (Table 7, Figure 3). By the end of the 107-114 day trial there were no significant differences between the final weight, FCR's and survival of shrimp under the 30-100% and the 30-75% treatments. However, production was significantly higher for the 30-100% than for the 30-75% treatment. Due to low oxygen and environmental constraints (Hurricane Ivan), two ponds were lost for the treatment 40-75%. Results from the two remaining ponds showed that production was higher for the 40-75% treatment than for the 30-75% treatment, but it was lower than the control (30-100%), although its statistical significance could not be evaluated.

The size and production distribution of the final shrimp samples were no significantly different between the two treatment that were analyzed statistically (30-100% and 30-75%) for all size ranges, except for the count of 31-35 shrimp/lb (Figure 4a and Figure 4b). The treatments offered 100% of the ration (30-100% and 40-100%) tended to have a higher proportion of the larger shrimp (16-20 shrimp/lb) than those offered 75% of the ration (30-75% and 40-75%). When the feeding ration was reduced to 75%, especially for the 30% CP diet, a larger proportion of smaller shrimp (26-30 shrimp/lb) was observed. However, for all the treatments, the largest proportion of shrimp was located in the count of 21-25/lb (from 48% to 62%).

Figures 4a and 4b. Size distribution (number of shrimp/lb) of *Litopenaeus vannamei* fed two diets (30% and 40% crude protein) at two feeding levels (75% and 100%) and raised in ponds at 35 shrimp/m^2. Within a size range means with different superscript letters are statistically different ($P \leq 0.05$).

Figure 4a

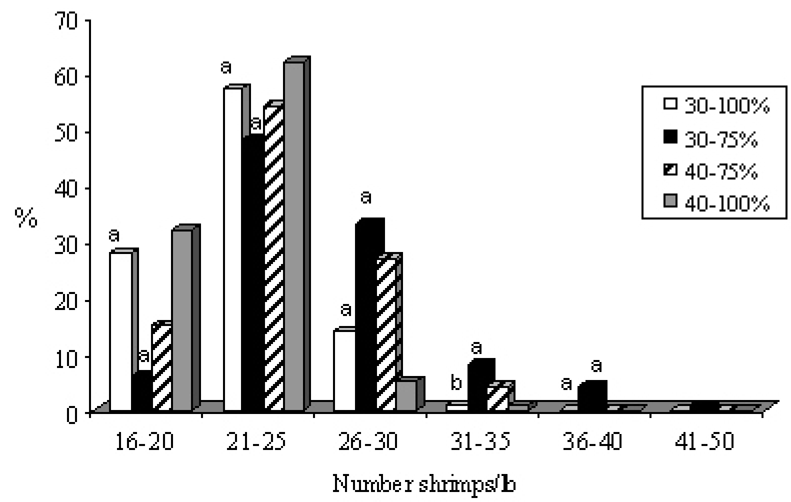
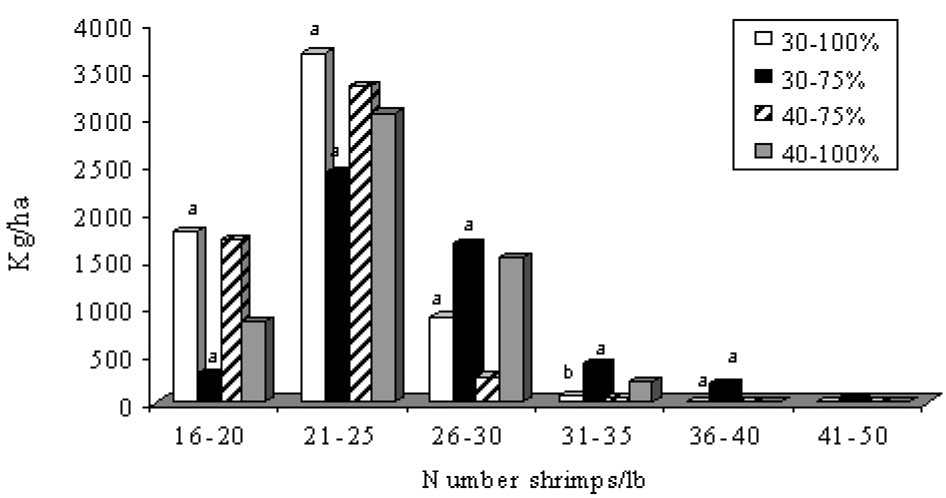


Figure 4b



Discussion

This research was conducted using both tank and pond culture systems. The tanks received water from one of the production ponds to allow the incorporation of additional sources of food from the pond's natural productivity (Leber and Pruder, 1988; Moss *et al.*, 1992; Moss, 1998). As should be expected for this type of system, the tanks provided a more replicated environment than the ponds, where the test variables were better controlled and isolated from the possible effect of uncontrollable factors. The pond trial, although more variable, provided useful information under production conditions. Research in ponds is encouraged because it provides information on the nutrient and feed management requirements under practical farming conditions (Lawrence and Lee, 1997). However, due to pond to pond variation as well as uncontrolled factors, pond work is often less reliable.

The results of the tank trial demonstrated that by increasing the nutrient density of the diet (eg, protein) feed input can be reduced without affecting growth or net yield of shrimp. Shrimp offered a 40% CP diet at 75% of the ration had similar final weight as compared to shrimp fed a 30% CP diet at 100% of the ration. These two feed inputs delivered the same amount of protein and hence produced the same amount of growth. Since growth was not reduced it may be assumed that the diets were balanced for energy and other nutrients and were no limiting. Under similar approach Cho and Kim (2001) did not find significant differences between weight gain of carp (*C. carpio*) fed a 35% protein diet to satiation and a 40% protein diet at 87.5% of the satiation rate that provided similar amounts of protein. However, they reported a significant reduction in weight gain when two 45% protein diets were fed at 77.8% of the satiation level. They observed that increasing the level of energy of the second 45% protein diet did not improve growth at 77.8% feeding, but the level of body fat in the fish was increased. Cho and Lovell (2002) found similar results in catfish (*I. punctatus*) reared in ponds and fed a 28 a 32% and a 36% protein diet at 100, 87.5, and 77.8 % of satiation, respectively. In shrimp, Kureshy and Davis (2002) observed significant differences between juvenile *L. vannamei* reared in tanks that were offered three diets (16, 32, and 48% crude protein) at the same nitrogen input. They reported that weight gain of shrimp fed the 16 and the 48% diets was significantly lower than shrimp fed the 32% diet. They concluded that the low nutrient level of the 16% protein diet restricted the amount of protein and energy that shrimp could consume since they would have to consume twice the amount of the diet in order to match the protein intake of the 32% protein diet. On the other hand, the low level of feeding of the 48% protein diet (66.7%), necessary to match the protein input of the 32% protein diet, probably limited the amount of energy and other essential nutrients available for shrimp growth. These results indicated that although various nutrient densities and feed inputs can be used they have limits.

This tank study demonstrates that, as long as the level of energy is appropriate, the daily food allowance can be reduced by 25% if we substitute 30% for a 40% CP, diet without affecting shrimp growth. The levels of reduction of feeding reported in the literature for other species have been more limited that the values obtained in this research. There are some possible explanations to the observed differences between this research and the other studies. First the nature of the species studied, second the level of feeding of the lower protein diet (satiation versus below satiation) and third the capability of the species to utilize natural sources of food in the culture environment.

Although information from research conducted with fish can be applied to crustaceans, there are some limitations due to the particular characteristics of each group. For example, there are physiological differences between fish and shrimp in relation to the metabolic processes to utilize and store energy. Fish can store large amounts of excessive energy in the form of lipids (Shearer, 1994; Lupatsch *et al.*, 1998; Sargent *et al.*, 2002). Contrary, the ability of shrimp to store lipids is more limited (Cuzon and Guillaume, 1997; Bureau *et al.*, 2000). Cho and Kim (2001) observed an increase in body lipid of carp, but not in growth, when the level of energy (lipids) of a 45% protein diet was increased and fed at a restricted feeding rate (77.5%). Similar results were obtained by Cho and Lovell (2002) in catfish. In the present research there were no differences in the level of body energy between shrimp offered any of the combinations of feeding rates and dietary protein levels.

Based on work conducted with fish, in order for a reduced feed input of high nutrient density diets to work, it is recommended to feed an amount of feed slightly below the optimal feeding rate and the diets must be balanced for energy and protein (Cho and Bureau, 2001). Allowing the fish to feed to satiation reduces the feed efficiency and growth (Minton, 1978; Andrews, 1979; Munsiri and Lovell, 1993). Also, when fish are fed to satiation they respond better to lower dietary protein, but when they are fed below satiation they grow faster on higher dietary protein (Mangalik, 1986; Li and Lovel, 1992). Cho and Kim (2001) and Cho and Lovell (2002) fed their control fish (100% ration) at satiation level and that could have intervened in their limited success at the 77.5% ration. Similar responses should be expected for shrimp. In this research, data show that the level of feeding of the 30-100% treatment was below the optimal input. The highest growth rate was observed for the 40-100% treatment. This indicates that shrimp growth can be improved when extra sources of protein are added over the amount of protein supplied by the 30-100% treatment. Therefore, the 30-100% treatment did not provide maximum growth and can be considered below satiation.

The third factor that can affect the effectiveness of a reduced feed input with increased nutrient density of the diets, is the capability of the species to utilize available natural sources of food in the culture environment. Aside the improvement in FCR due to lower feed allowance (Cho and Kim, 2001; Cho and Lovell, 2002; Kureshy and Davis, 2002), additional sources of natural food in the culture environment can further improve apparent FCR as it provides additional sources of nutrients that allow optimal growth. In the previously reported research, the trials took place in clear water tank systems (Cho and Kim, 2001; Kureshy and Davis, 2002) or with a species that can not utilize efficiently natural productivity in a pond, such as catfish (Kim and Lovell, 2002). Although not evaluated, shrimp seems to have been able to benefit from those additional sources of nutrients.

For both the tank and the pond trials the values of FCR obtained were better than those expected. The complete ration (100%) was calculated based in an expected FCR of 1.8:1. The largest FCR value obtained in this research was 1.38:1 in the tank system (for the 30-100% treatment) and 1.03:1 in the ponds (for the 30-75% and the 40-100%

treatments). These improved FCR's are most likely the result of additional sources of nutrients going into the system, probably provided by natural productivity in the pond or from the green water provided to the tanks from the ponds. The contribution of natural productivity in shrimp ponds has been well documented. Lawrence and Lee (1997) pointed out that in spite of an increased use of feed in shrimp production, natural productivity still account for more than 25% of the nutritional requirement for shrimp. Anderson *et al.* (1987) estimated that the contribution of feed to production of *L. vannamei* at levels below 5 mt/ha/crop, was only between 23 and 47%. At the same production level, Lawrence and Houston (1993) estimated that value to be 24 to 31% for *L. vannamei* and 17 to 23% for *L. setiferus*. For a production of about 4 mt/ha/crop the contribution of feed to *L. setiferus* production was estimated to be about 48% (Robertson *et al.*, 1993). By using ^{15}N-enriched, Cam *et al.* (1991) found that *Marsupenaeus japonicus* retained between 4 and 10% of the ^{15}N-enriched that had been assimilated by the natural biota. By using the same technique Burford et at. (2002) reported that 15.2 g *Penaeus monodon* retained about 21% of the dietary ^{15}N-labeled (43.75% CP diet). Funge-Smith and Stewart (1996) estimated this value in 18 to 27% in intensive shrimp ponds.

Similarly, the contribution of natural productivity on shrimp growth in tank systems that received green water has been reported. Leber and Pruder (1988) and Moss *et al.* (1992) demonstrated that shrimp growth rates can be increased by 53-89% over shrimp grown in clear well water when unfiltered water from a intensive shrimp pond is added. The enhancing materials were mainly microalgae and microbial-detrital aggregates of sizes between 0.5 and 5.0 um. Moss (1995) suggested that shrimp could be able to consume directly suspended organic particles from the water column without being processed in a detrital food web. In this research the results of the FCR, PCE, ECE, and the growth rate seem to indicate that natural productivity also improved shrimp growth and feed and nutrient utilization as has been reported from other investigators. For example the rate of growth in the tank system ranged between 1.22 to 1.35 g/week for the standard feed rate treatments (30-100% and 40-100%) (Table 5). In the pond trial these values increased to 1.38 to 1.79 g/week. Although the conditions of the experiments may have not been the same, Wasielesky at al. (2005) reported values of weekly growth lower than those obtained in this research when *L. vannamei* was raised in a filtered water system (0.94 g/week). Similarly, Moss *et al.* (1992) reported a growth rate of 0.97 g/week for *L. vannamei* raised in well water in contrast with 1.83 g/week when pond water was added to the tanks. Values of PCE and ECE observed in this research are higher than those commonly reported in the literature for aquatic species (Table 7). Halver and Hardy (2001) reported an average value for PCE between 25-30%. The values of PCE in salmon culture has been improved to about 45% from 22-25% in the 1980's by feeding high energy density diets (Halver and Hardy, 2001). In this research the PCE values ranged between 42 and 78% probably due to the contribution of natural productivity.

As previously pointed out, the contribution of natural productivity in the tank system probably increased shrimp growth and improved FCR, PCE, and ECE as evidenced by the data. However, the levels of nutrients that shrimp obtained from these sources were not high enough to compensate for the reduced feed input. Regardless of dietary protein, reductions in feed inputs resulted in reductions in growth. Thus, feed inputs were more limiting than natural productivity. Final growth of shrimp offered the 30% CP diet at 50 and 75% ration was significantly lower than that of shrimp offered the 30% CP diet at 100% ration. The observed growth increase and protein and energy utilization of shrimp raised in green water or ponds may be due to an improvement in the quality of the food source and nutritional balance (e.g., essential amino acids, micronutrients) rather than the quantity of macronutrients (protein, carbohydrates, and lipids) provided by these sources (Burford *et al.* 2004).

To evaluate the effect of the tested variables and interaction among them, stepwise regression analyses and two-way ANOVA analyses were made. These analyses indicated that although energy input and dietary protein level had a significant effect on shrimp growth, these effects were not significant when either of these variables were included in a multiple regression analysis with protein input. Therefore, protein input was the most important variable in shrimp growth and production. Shrimp growth responded linearly to increasing levels of dietary protein independently of the source of dietary protein (Figure 4). For example, shrimp offered the same amount of protein (30-100 and 40-75%), had no significant differences in final weight, even though the two diets had different levels of protein. When both diets were offered at 100% of the feed rate, animals responded better to the 40%-CP diet, probably because this diet provided more protein than the 30% CP. This effect should be expected since the 30-100% was selected to provide a sub-optimal level of feed input as discussed earlier.

Analyses on the level of energy supplied under the different treatments provided further evidence that protein input was the most important factor in shrimp growth. For example, when the ration was reduced from 100 to 75% by replacing a 30 for a 40% CP diet, protein input remained constant but the level of DE input was reduced from 0.80 to 0.71 Kcal/shrimp/day (Table 3). The lower DE level did not affect shrimp growth. This evidence that although energy allowance was partially reduced with reductions in feed inputs, this factor was not limiting between the isonitrogenous treatments (treatments 30-100% and 40-75%). Even though the DE/CP ratio of both diets seems to be different (9.96 for the 30% CP diet versus 9.04 for the 40% CP diet), the increase in dietary protein for the 40% CP was compensated with an increase in dietary energy. An amount of 0.71 Kcal/shrimp/day fed at 75% ration (40-75%) was not sub-optimal in comparison with the 0.80 Kcal/shrimp/day supplied by the 30-100% treatment.

The DE/CP ratio is important in shrimp diets because an adequate level of energy must be fed to prevent using protein as energy source that would deplete growth (Johnsen *et al.*, 1991; Steffens, 1996). The diets used in this study seemed to be balanced for energy and protein. The DE/CP ratio of the diets was 9.9 and 9.1 Kcal/g protein for the 30 and 40% CP diets, respectively. These DE/CP ratios are lower than that 11.9 Kcal/g protein reported as optimum (Cousin *et al.,* 1993; Cuzon and Guillaume, 1997) for *L. vannamei*, but, they are higher than the 3.37 kcal/g protein reported as depressing growth in this species (Cousin *et al.,* 1993).

Pond feeding management

The feed management strategy followed in this research proved to be an effective alternative for shrimp production under pond conditions. In general, this management strategy involved the use of historical records to determine expected FCR, weekly growth and survival and a continuos evaluation and adjustment of feed input to those parameters based on the dynamic of environmental variables (eg., morning DO, temperature, cloudy days). The utilization of this feed management plan has increased progressively the production levels in the research facilities since its implementation. Garza (2001) compared growth between a traditional feed table and a fixed FCR and did not observe significant differences in growth and production and obtained a FCR of 2.03. However, Zelaya (2005) in a series of pond experiments following a similar feeding and pond management as the one applied in this research, obtained FCR between 1.5-2.0 when feed was offered between 60 to 91 kg/ha/day. In one of his experiments, he evaluated three feeding schedules that consisted in an early aggressive feeding (EAF) followed by reduced feeding, a late aggressive feeding (LAF) with reduced feed input in the beginning, and an intermediary feeding input (IF), similar to the standard rate of this research. The average feed input was between 65 kg/ha/day for the EAF and 79.6 kg/ha/d for the IF treatment. He did not report significant differences among the treatments, but the feed input was reduced by about 17% when compared with programs based on feeding tables and the FCR averaged 1.5-1.8 with production between 4,328 and 4,398 kg/ha. Similarly, in this study values of production (5,054-6,482 kg/ha) and FCR (0.9-1.0) were better than previous values reported when using feeding tables. A possible explanation of the improvement in production and FCR could be found in the feed input. The levels of feed input has been reduced from a high of 140 kg/ha/day, using feeding tables, to 60 kg/ha/day using a high protein diet (40% CP). For this type of system a maximum daily feed load of 100-120 kg/ha/day has been recommended when night-time mechanical aeration is provided (Boyd, 1989; Boyd and Tucker, 1998). The maximum feed input in this research was 85 kg/ha/day for the 100% feed rate, which was below the maximum capacity of feed load suggested for ponds and values used in previous trials. Lower feed levels might improve water quality that might have lead to higher survival and production. Overall, in this research, production and FCR were improved when compared with production record data of *L. vannamei* in the same units during previous studies. The reason for these results are unknown. However, it is suspected that variables such as genetic pools of PL, climatic conditions, water quality, temperature, higher salinity, improved pond bottom conditions, and diet are all contributing factors.

By the end of the 107-114 day trial there were no significant differences between the final weight, FCR's and survival of shrimp maintained on the 30-100% and 30-75% treatments. However reduction of the feed rate to 75% (30-75%) significantly reduced production as compared with the 30-100% treatment. Although the 40-75% treatment was not included in the statistical analyses due to loss of two ponds, results from the two remaining ponds indicated that production was higher for the 40-75% treatment than for the 30-75% treatment, but it was lower than the 30-100% treatment. These results of shrimp reared on 30-100% and 40-75% treatments (iso-nitrogenous) tended to be different from those obtained for the same treatments in the tank trial. Since the level of protein allowance and energy were similar for both the tank and the pond trails, the observed differences in production could have been due to the effect of other variables, such as natural productivity or distribution of feed. In production conditions the higher feed input of the lower protein diet may have stimulated natural productivity possibly due to carbon enrichment. It is also possible that a larger amount of feed allowed a better distribution of the feed in the pond making it easier for shrimp to reach the feed, reducing competition. The combined effect of these variables may have affected production, however, further research under pond conditions is warranted to analyze these effects.

Under pond conditions, the size and production distribution of the final shrimp samples were no significantly different between the two treatment that were analyzed statistically (30-100% and 30-75%) for all size ranges, except for the count of 31-35 shrimp/lb. However, only a small portion of the population was located on the 31-35 size range (4.6 and 1.3% for the 30-100% and the 30-75%, respectively). Overall, more than half of the population for each feeding treatment was located in the 21-25 size range. However, a reduction of feed inputs to 75% for both protein levels (30-75% and 40%-75%) tended to produce a higher concentration of shrimp in the smaller size range, which is undesirable for shrimp production. However, shrimp in the size range of 26-30/lb are still a commercial product of good quality. In addition, although yield was numerically lower under a reduced feeding level (75%) when feeding a high protein diet (40-75%), the levels of production (5,660 kg/ha), FCR (0.9) and size distribution are still considered excellent values for commercial production of shrimp.

Conclusions

Under laboratory controlled conditions these studies demonstrate that shrimp have a daily requirement of protein that can be supplied with diets with different levels of protein. This is in agreement with previously reported in other studies with *L. vannamei* or for other species (Lawrence and Lee, 1997; McGoogan and Gatlin, 1998; Kureshy and Davis, 2002). Based of this, the use of diets with high nutrient density is encouraged for this type of system because a smaller quantity of feed would be required to produce the same amount of growth (Bureau and Cho, 2002). Lower amounts of feed and better feed management would lead to higher efficiency of the use nutrients with a subsequent reduction in wastes from the feed. This may improve the economic return in the farm and reduce the potential of water pollution and environmental impact on ecosystems receiving the effluents. In this study we attempted to duplicate lab result under semi-intensive conditions in ponds. Unfortunately, controlled statistical research becomes very difficult under pond conditions. The intervention of uncontrollable variables makes it difficult to isolate and evaluate specific study variables. These results show that lab research by itself can not provide all the solutions to problems that occur under production conditions, but it still represents an important tool that can provide some useful information that helps to understand production systems.

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CHAPTER IV

EFFECT OF THE DIGESTIBLE ENERGY/CRUDE PROTEIN RATIO (DE/CP) ON GROWTH AND FEED CONVERSION RATIO (FCR) OF JUVENILE PACIFIC WHITE SHRIMP *Litopenaeus vannamei*

Abstract

This study was conducted to evaluate the effect of digestible energy/crude protein (DE/CP) ratio of the diets of juvenile Pacific white shrimp (*Litopenaeus vannamei*) fed various diets based on constant daily protein input. Juvenile (0.94 ± 0.04, n = 30) shrimp were stocked in an indoor recirculating system (polyethylene tanks of 173 L at 20 shrimp/tank) and fed diets that contained 30 or 40% crude protein (CP) at two rates (100 and 75%, respectively) to allow the inputs of equal levels of protein. Two treatments consisted of 30 and 40% CP diets (30 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 variables levels of DE (2.70 Kcal/g and 3.6 Kcal/g, respectively) to maintain a constant DE/CP ratio of 9 Kcal/g of protein (30 RAT-100% and 40 RAT-75%, respectively). Each treatment was assigned four replicated tanks. At the end of the 49-day trial, there were no significant differences ($P>0.05$) in final weight (6.1-6.4 g) and weight gain among the treatments. However, feed conversion rate (FCR) was significantly lower ($P\leq0.01$) for the two treatments fed the 40% CP diets at 75% feeding level and two

different levels of energy (40 RAT-75% and 40 ISO-75%) when compared with the treatments fed the 30% CP diet at 100% feeding rate (30 ISO-100% and 30 RAT-100%) (1.48 and 1.50 versus 2.09 and 2.06, respectively). Final body composition (dry matter, CP, and gross energy) and protein conversion efficiency (PCE) values were no significantly different among the four treatments. However, for the two diets that contained similar levels of energy (30 ISO and 40 ISO) the energy conversion efficiency (ECE) was significantly higher for shrimp fed the 40% protein diet at a reduced feeding allowance (19.9 versus 24.8%). This study demonstrated that when the level of energy is appropriate, increasing the protein content of the shrimp diet allows for reduced feed inputs without affecting the growth performance of the shrimp, but improving the FCR and presumably water quality due to lower potential of wastes from the feed.

Introduction

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

The quantity and quality of dietary protein are factors that influence nitrogen 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 nitrogenous wastes. In order to optimize protein input, the amount of protein should be determined based on the protein requirement of the species and adjusted for energy in the diet.

Traditionally, to determine the optimal nutrient requirement of an animal species, 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 with a variety of diets with different levels of the nutrients (Lawrence and Lee, 1997; Lupatsch *et al.*, 1998; McGoogan and Gatlin, 1998; Lupatsch *et al.*, 2001; Kureshy and Davis, 2002). Consequently, feed input will vary and should depend on the nutrient density of the diet. For example, to meet the daily nutrient requirement of a species, lower amount of a diet with high nutrient density would be required than when feeding a diet of lower nutrient density.

Based on this approach it has been suggested that the nutrient density of the diet should 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 rich in starch and fiber, and by increasing the amount of ingredients with high level of digestible protein and energy. These diets should be balanced for energy and nutrients (eg., protein).

The level of dietary energy in nutrient-dense diets is critical because if daily feed allowance is determined based on nutrient requirements of the species (eg., protein), the level of energy should be appropriate to avoid feeding sub-optimal levels of energy that would depress growth or increase the use of protein as an energy source (Johnsen *et al.*, 1991; NRC, 1993; Steffens, 1996). It has been demonstrated that increasing the dietary digestible energy/crude protein ratio (DE/CP), by increasing dietary non-protein energy content, results in an increase in N retention efficiency, due to a decrease in the 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.

Cousin *et al.* (1993) reported an optimal digestible energy/crude protein (DE/CP) ratio of 11.9 kcal/g protein for *Litopenaeus vannamei*. They observed depressed growth when the ratio was 3.37 kcal/g protein. Aranyakananda and Lawrence (1993) reported that *L. vannamei* had a protein dietary requirement of 15% and an optimum DE/CP ratio of 28.57 kcal/g protein when shrimp were fed *at libitum* 15 times a day. For *Penaeus monodon*, Bautista (1986) and Shiau and Peng (1992) concluded that the optimum E/P ratio was 7.97 kcal/g protein. Values in the literature are controversial about optimal dietary protein and energy/protein ratios in shrimp and fish, 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, variations in feeding rates and levels of energy of the diets, account for a great part of the variation (Lupatsch *et al.*, 2001). Some authors suggest that there is not a specific E/P ratio and growth rate because the levels of energy or protein of the diets controls the effect of the E/P ratio on shrimp growth rate (Cuzon and Guillaume, 1997).

The protein level of the diets must be adjusted according to the level of digestible energy and feed allowance (Lupatsch *et al.*, 2001). For example, the DE/CP ratio will increase for larger shrimp due to the energy to protein ratio of the gain and the increase in energy required for maintenance in larger shrimp.

The effect of the protein and dietary energy balance has been observed in some studies that involved the use of diets with high nutrient density fed at lower feeding rates in fish and shrimp. Cho and Lovell (2002) did not find significant differences between juvenile channel catfish (*Ictalurus punctatus*) reared in ponds fed a diet with 28% CP fed at satiation (100%) and a diet with 32% CP fed at 87.5% of satiation. A significantly higher feed efficiency was observed with the 32% CP diet. Further increase of the protein level to 36% and reduction in the feeding rate to 77.8%, depressed growth in the same experiment probably due to deficience in energy supply. Similar results were obtained by Cho and Kim (2001) in common carp (*Cyprinus carpio*) when they reduced feeding to 77.8% of satiation by increasing the level of protein of the feed from 35% to 40% and feeding on an isonitrogenous basis. A significantly lower growth rate was observed when they fed a diet containing 40% CP at 77.8% of the satiation rate. Kureshy and Davis (2002) reported a significantly higher weight gain and feed efficiency for juvenile Pacific white shrimp *L. vannamei* raised in tanks when fed on an isonitrogenous basis a diet with 32% of CP compared to a diet with 16% CP. They also reported a significantly higher weight gain for shrimp fed a 32% CP diet than a 48% CP diet.

Shrimp nutrition research on the use of high nutrient-density diets at reduced feed allowance is limited in the literature. Similarly, studies aimed to analyze the effect of the digestible energy to protein ratio under these limited feed allowance plans are also limited. The objective of this study was to evaluate the response on growth performance, feed and nutrient utilization of *L. vannamei*, fed on a isonitrogenous basis, two diets (30 and 40% CP) containing the same level of energy and two diets (30 and 40% CP) containing the same energy/crude protein ratio.

Materials and Methods

This study was conducted at the Claude Peteet Mariculture Center, in Gulf Shores, Alabama during the summer of 2004. Juvenile (0.94 ± 0.04 g, n = 30) shrimp from a commercial hatchery (GMSB Inc. hatchery /Key West, FL), initially maintained in nursery tanks for 22 days and then in ponds for 3 weeks, were pooled and distributed in an indoor recirculating system.

Shrimp were fed four experimental diets (Table 1 and Table 2) that contained two percentages of crude protein (30 and 40%). Diets were assigned to four different experimental treatments that supplied similar amounts of nitrogen (crude protein) by adjusting feed rate. Two treatments consisted of 30 and 40% CP diets (30 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 variables levels of DE (2.70 Kcal/g and 3.6 Kcal/g, respectively) to maintain a constant DE/CP ratio of 9 Kcal/g of protein (30 RAT-100% and 40 RAT-75%, respectively). Each treatment was assigned four replicated tanks. During the first week, the full ration (100%) was 1.2 g/shrimp/week (assumed FCR 2.0

Table 1. Composition of experimental diets (g/100g dry weight) and expected values of digestible energy (DE), crude protein (CP), and DE/CP ratios.

Ingredient	30 ISO	40 ISO	30 RAT	40 RAT
Menhaden fish meal[1]	17	21.3	17	21.3
Soybean meal[2]	27	38.8	27	38.8
Menhaden fish oil[3]	3.2	2.6	0.7	7.5
Wheat gluten[4]	5.5	6.9	5.5	6.9
Wheat starch[4]	32.8	13.1	20.7	9.3
Whole wheat[4]	10	12	10	12
Trace mineral premix[5]	0.5	0.5	0.5	0.5
Vitamin premix w/o choline[6]	1.8	1.8	1.8	1.8
Choline cloride[4]	0.2	0.2	0.2	0.2
Stay C (250 mg C/kg)[7]	0.07	0.07	0.07	0.07
CaP-monobasic[4]	1.3	1	1.3	1
Lecithin[4]	0.5	0.5	0.5	0.5
Cellufil[4]	0	1.1	14.6	0
Cholesterol[4]	0.2	0.2	0.2	0.2
Formulated to contain				
CP (%)	30	40	30	40
Fat (%)	6	6	3.5	11
Digestible energy (Kcal/g)	3.28	3.28	2.7	3.6
DE/CP ratio (Kcal/g)	10.9	8.2	9	9

[1]Special Select™, Omega Protein™, USA Inc., Randeville, LA, USA
[2] De-hulled solvent extracted soybean meal, Southern Sates Cooperative Inc., Richmond Virginia, USA.
[3]Omega Protein™, Reedville, VA, USA
[4]United States Biochemical Company, Cleveland, OH, USA
[5]g $100g^{-1}$ 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
[6]g Kg^{-1} 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 IU g-1) 5.0, vitamin D3 (400 000 IU g-1) 0002, DL-"-tocopherol acetate (250 IU g-1) 8.0, cellulose 865.266.
[7]Stay C®, (L-ascorbyl-2-polyphosphate 35% active C) Roche Vitamins Inc., Parsippany, NJ, USA.

Table 2. Determined values of gross energy, crude protein (CP), apparent digestibility of the energy (ADE), apparent digestibility of the protein (ADP), digestible energy (DE), feed input, DE input, and the DE/CP ratio of four experimental diets offered to *L. vannamei* for 49 days.

	Diet			
Parameter	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
ADEC (%)	77.8	78.3	70.1	77.6
ADPC (%)	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
Feed input (g/shrimp/day)[1]	0.2081	0.1561	0.2081	0.1561
DE input (Kcal/shimp/day)[1]	0.73	0.58	0.62	0.66
DP input (Kcal/shrimp/day)[1]	0.062	0.062	0.062	0.062

[1]Average values during 7 weeks

and growth 0.6 g/week). Thereafter, the shrimp were offered 1.62 g/shrimp/week (assumed FCR 1.8 and growth 0.9 g/week).

The recirculating system consisted of sixteen rectangular (61 x 61 x 61 cm) polyethylene tanks with a water volume of 173 L (Polytank Inc., Litchfield, MN, USA). Each tank had approximately 10 cm freeboard, and was covered with a 0.5-cm nylon screen to prevent the animals from jumping out. Each tank was equipped with a screened central drain, 3.2 cm diameter stand pipe which was 65 cm long, screened and set to maintain a water depth of 60 cms or a 16- L volume). The system was connected to a common circular tank that contained a biological and a 1/3-hp circulation pump. The recirculation rate for each tank was set at 4.1 L/min (n=3). Aeration was provided by two air stones connected to a common air supply from a 1 hp regenerative blower (Sweetwater Aquaculture Inc. Lapwai, ID, USA). The system was filled with filtered (Model TR100, AREA, Homestead, FL, USA) full-strength sea water. About half of the total water volume was changed with filtered sea water every week..

Shrimp were fed twice a day. Half of the daily feed allowance was provided at each feeding. In addition to daily observations for mortality, shrimp in each tank were counted every two weeks and the daily ration readjusted as needed. Dissolved oxygen (DO), pH and temperature were measured twice a day (at the 0600 and the 1600 hours) with a YSI 556 DO meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonia-nitrogen (TAN) and nitrite-nitrogen were determined once a week. Water samples were taken from the mid water column in the filter and in two tanks selected at random. Total ammonia-nitrogen was measured with a spectrophotometer (Spectronic 20

Genesys , Spectronic Instrument Inc. Rochester, NY, USA) following the Nesslerization method (APHA, 1989). Nitrite-nitrogen was determined using a model PLN code test kit from LaMotte (Chestertown, MD, USA).

Diets were prepared by mixing all the dry ingredients in a food mixer (Hobart, Troy, OH, USA) for 20 minutes and then adding the menhaden oil and mixing for 10 more minutes. Boiling water was then blended with the mixture while it was still mixing to attain a mash of consistency appropriate for pelleting. The mash was passed through a 3-mm die in a meat grinder (Hobart, Troy, OH, USA), and the pellets dried in a forced-air drier set for a maximum temperature of 45 ºC until a moisture content between 8-10% was obtained. Diets were ground and sieved to an appropriate size and stored in a -15 ºC until used.

To evaluate shrimp growth performance the following parameters were determined: weight gain (WG = final weight - initial weight); percentage survival; feed conversion ratio (FCR = dry weight of feed offered/wet weight gained); total body dry matter, energy and crude protein; protein conversion efficiency (PCE = dry weight protein gain x 100/dry weight protein offered); energy conversion efficiency (ECE = dry weight energy gain x 100/dry weight energy offered). Shrimp samples were collected at the beginning (pooled sample) and at the end of the experiment (6 shrimp/tank) and kept in sealed plastic bags in a freezer at -15 ºC until being analyzed.

To analyze the samples, frozen shrimp were cut in pieces of about 2 cm and dried in an oven at 90 ºC until constant weight, using the methods described by the A.O.A.C.(1990). Then the dry samples were ground with a coffee grinder and stored in a freezer. Crude protein content of shrimp was analyzed using the micro-Kjeldahl method (Ma and Zuazago, 1942). Total energy content was determined using a micro-calorimetric adiabatic bomb using benzoic acid as standard (Parr 1425, Moline, IL, USA).

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 shrimps (~ 6 g average weight) each were selected and restocked in a 2500-l closed recirculating system consisting of 60-L glass aquaria with a central biological filter, biological filter, reservoir, circulation pump, and supplemental aeration. Water temperature was 27.6 ± 1.6 ºC and salinity was 32 ppt. The four experimental diets were prepared using the same formulations (Table 1), but replacing 0.5% of wheat starch by equal amount of chromic oxide and using the same procedure as previously described.

Shrimp were allowed to acclimate for three days before starting collection of feces. 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 um mesh screen. Shrimp were offered five feedings per day, however, feces obtained after the first feeding were discarded. Feces were rinsed with distilled water and stored in sealed plastic containers and stored in a freezer. Samples from three tanks were pooled (r = 3) and kept frozen until analyzed. Dry matter, crude protein, total energy was determined for the fecal and the diet samples according to procedures previously described. Chromic oxide was analyzed following the McGinns and Kasting (1964) procedures. Apparent digestibility coefficients (ADC) of the dry matter, protein, and energy were calculated according to Maynard *et al.* (1979).

ADC (%) = 100 x [1- (dietary Cr level/fecal Cr level) x (fecal nutrient /dietary nutrient)]

Statistical analyses

Data were analyzed by a one-way analysis of variance (ANOVA). Significant differences among treatment means at a probability level of $P \leq 0.05$ were determined by the Student-Newman-Keuls multiple comparison test (Steel and Torrie, 1980). Regression analyses between feed input, dietary protein, and protein input against the dependant variable weight gain were conducted. Analyses were done using SAS program version 8.2 (SAS Institute Inc., Cary).

Results

Average water quality parameters for DO, temperature, pH, and salinity were 6.5 mg/L, 26 °C, 7.5, 31.5 g/L, respectively (Table 3). There were no observed water quality problems or diseases during the trial. The total ammonia-nitrogen ranged between 0-0.81 mg/L and the nitrogen-nitrite ranged between 0-1.5 mg/L (Table 3). There were no significant differences in survival rate among shrimp assigned to the different treatments. Survivals ranged between 92.5 and 98.5% (Table 4).

Formulated values for DE and DP are presented in Table 1 and the experimentally determined values for DE are presented in Table 2. Shrimp increased their initial weight between 542 and 574% with no significant differences in weight gain among the treatments (Table 4). For example, when the diets with the same level of DE were offered

Table 3. Water quality in tanks. Values are averages ± standard deviation of daily and weekly determinations. Values below in parenthesis represent minimum and maximum.

Parameter	Mean ± Std. dev.
Dissolved oxygen (mg/L)	6.48 ± 0.86 (4.77, 8.37)
pH	7.89 ± 0.14 (7.51, 8.13)
Temperature (ºC)	26.73 ±1.66 (21.24, 29.75)
Salinity (g/L)	31.34 ± 1.58 (28.06, 33.49)
Total ammonia-N (mg/L)	0.23 ± 0.27 (0, 0.81)
Nitrite-N (mg/L)	0.36 ± 0.46 (0, 1.5)

Table 4. Final weight (FW), weight gain (WG), weight gain per week (WG/wk), feed conversion ratio (FCR), and survival of juvenile (0.94 ± 0.04 g) *L. vannamei* fed four experimental diets at two different feeding levels (75 and 100%). Means in the same column with different letter superscripts are significantly different ($P \leq 0.05$).

Diet	Ration	FW (g)	WG[1] (%)	WG/wk[2]	FCR[3]	Survival
30 ISO	100	6.1[a]	5.1[a]	0.73[a]	2.09[a]	97.8[a]
40 ISO	75	6.4[a]	5.4[a]	0.78[a]	1.48[b]	92.5[a]
30 RAT	100	6.2[a]	5.2[a]	0.74[a]	2.06[a]	94.3[a]
40 RAT	75	6.3[a]	5.3[a]	0.76[a]	1.50[b]	98.5[a]
P-value		0.6734	0.5754	0.5706	<0.0001	0.1534
PSE[4]		0.1829	0.1705	0.0243	0.1421	1.9659

[1]Weight gain = final weight - initial weight
[2]Weight gain/week = (final weight - initial weight)/7
[3]FCR= dry weight of feed offered/wet weight gained
[4]PSE= pooled standard error

at 100% (30% ISO-100%) and at 75% of the feeding rate (40-ISO-75%), there were no significant differences in the final weight and weight gain among the shrimp (6.1 versus 6.4 g, respectively) (Table 4). However, shrimp offered the lowest feeding rate (75%) had significant lower FCR than shrimp fed 100% of the ration (1.48 versus 2.09). Similarly, when the DE/CP ratio was maintained constant, there were no significant differences in final weight and weight gain of shrimp fed the 30-RAT at 100% and the 40-RAT at 75% of the feeding rate (Table 4). At a constant DE/CP ratio the FCR also improved when feeding was reduced from 100% (30-RAT-100%) to 75% (40-RAT-75%) (2.06 versus 1.50).

There were no significant differences in shrimp dry matter, body energy, body protein and PCE among shrimp offered the four experimental diets (Table 5). However, when the feed allowance of the two diets containing the same levels of energy was reduced from 100% (30-ISO-100%) to 75% (40-ISO-75%) the ECE improved significantly (from 19.9 to 24.8 %) (Table 5). ECE was not significantly different for the two diets that had similar DE/CP radio (30-RAT-100 and 40-RAT-75%).

Stepwise regression analyses between either feed rate, dietary protein, protein input, or energy input and weight gain or PCE were no significant. However, the regression was significant between energy fed and ECE ($P=0.0002$, $R^2=0.63$) and between feeding rate and FCR ($P<0.0001$, $R^2=0.93$).

Table 5: Final shrimp dry matter (DM), crude protein (CP), gross energy (GE), protein conversion efficiency (PCE), and energy conversion efficiency (ECE) of *L. vannamei* fed two commercial diets (30 and 40% CP) at two different feeding levels (75 and 100%) in a recirculating system. Values are means of four replicates. Means in the same column with different letter superscripts are significantly different (P≤0.05).

Diet	Ration (%)	DM (%)	CP (%)	GE (cal./g BW)	PCE[1] (%)	ECE[2] (%)
30 ISO	100	26.7[a]	18.8[a]	1.27[a]	30.9[a]	19.9[b]
40 ISO	75	25.6[a]	18.4[a]	1.20[a]	31.9[a]	24.8[a]
30 RAT	100	26.4[a]	18.4[a]	1.24[a]	30.6[a]	23.3[b]
40 RAT	75	26.6[a]	18.5[a]	1.25[a]	31.6[a]	22.6[b]
P-value		0.077	0.6677	0.3501	0.8814	0.005
PSE[3]		0.2788	0.2763	0.0245	0.4694	0.7619

[1]Protein conversion efficiency= dry weight protein gain x 100/dry weight protein offered
[2]Energy conversion efficiency= dry weight energy gain x 100/dry weight energy offered
[3]PSE =pooled standard error

Discussion

In a clear water system the only available source of nutrients for shrimp growth is the diet since others sources of nourishment (e.g., invertebrates, organic detritus), 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 paramount to feed the optimal amount of a complete diet, balanced for nutrients and energy. In this study it was demonstrated that when the diet is balanced for DE and protein and feed allowance is determined based on nutrient and DE requirement of the species, shrimp can be raised with diets with different levels of crude protein without affecting their growth performance. The results demonstrated that feed allowance can be reduced by 25% when the level of protein of the diet is increased from 30 to 40% without affecting weight gain. Similar results have been reported for other investigators for common carp (*C. carpio*) (Cho and Kim, 2001) and channel catfish (*I. punctatus*) (Cho and Lovell, 2002). For *L. vannamei* juvenile, Kureshy and Davis (2002) reported opposite results. They obtained a significantly higher weight gain of shrimp fed a 32% CP diet compared to a 16% CP and a 48% CP diet when the three diets were offered on an isonitrogenous basis. The lower growth for the 16% CP diet was probably due to the low level of nutrients of this diet that could have restricted the amount of protein and energy that shrimp could consume since they would have to consume twice the amount of the diet in order to match the protein intake of the 32% protein diet. On the other hand, the low level of feeding of the 48% protein diet (66.7%), necessary to match the protein input of the 32% protein diet, probably limited the amount of energy and other essential nutrients available for shrimp growth.

The DE level of the diet is important because if the level of energy provided by the diet, at a particular feed allowance, is lower than the daily energy requirement, shrimp will utilize protein as a source of energy (Johnsen *et al.*, 1991; Steffens, 1996). This normally leads to depressed growth, increased potential of water pollution, and higher production costs because protein is one of the major nutrients in the diet and the most expensive ingredient in a feed formulation. In this study, the level of energy for the two groups of experimental diets (similar energy level and similar DE/CP ratio) seemed to be appropriate for the two feed allowances provided (75 and 100%). If the level of energy would have not been high enough for the two diets with similar DE (30-ISO and 40-ISO), shrimp growth would have been lower when the higher protein diet (40-ISO) was offered at 75% of the standard ration. However, there were no significant difference in final weight and body composition (dry matter, gross energy, and crude protein) between shrimp assigned both feeding regimes. Therefore, the 40-ISO diet offered at 75% provided enough energy to the shrimp, even though the energy allowance of this treatment was reduced by 20%. The ECE for this treatment improved, as it should be expected, because less energy was offered to produce the same growth. Shrimp did not have higher body energy when fed the 30-ISO diet at 100% ration. In channel catfish it has been observed that increasing the DE/CP ratio of the diet leads to an increase in body fat, but not growth improvement (Cho and Lovell, 2002). Although shrimp body fat was not measured in this trial, based on body energy, it is assumed that shrimp body fat was not higher for the 30-ISO 100% treatment than for the 40-ISO 75%.

When feeding the two diets with similar DE/CP ratio at 100% (30-RAT) and at 75% (40-RAT) also resulted in no differences in growth performance. This would be expected if E/P ratio was adequate as at both feed allowances the levels of protein and energy should be similar. Effectively, there were no observed significant differences among final weight and weight gain of shrimp. Even though, the determined DE/CP ratios of these two diets were slightly different from the 9 Kcal/g expected ratio (9.9 and 10.5 for the 30-RAT and the 40-RAT, respectively), the energy supplied for both treatments was very similar (0.62 and 0.66 Kcal/g/shrimp/day). These DE/CP ratios are close to the optimal DE/CP ratio reported as optimal for *L. vannamei* (Cousin *et al.*, 1993; Cuzon and Guillaume, 1997). Also, no differences in body energy were found when the 30 and the 40% CP diets that had similar DE/CP ratio were fed on an isonitrogenous basis (30-RAT 100% and 40-RAT 75%). In channel catfish, Cho and Lovell (2002) reported a reduction in weight gain and increase in body fat when fish were fed a 35% CP diet at 77.5% feeding rate in comparison with fish fed a 28% protein diet at satiation. Both diets had similar DE/CP ratio, consequently the protein and energy input for both diets were similar. These observed differences between fish and shrimp may be due to physiological differences between both groups in relation to the metabolic processes to utilize and store energy. Fish can store large amounts of excessive energy in the form of lipids (Shearer, 1994; Lupatsch *et al.*, 1998; Sargent *et al.*, 2002). In contrast, the ability of shrimp to store lipids is more limited (Cuzon and Guillaume, 1997; Bureau *et al.*, 2000). Also, the source of energy can influence the ability of the animals to use dietary energy. In fish the energy density of the diets normally is increased by increasing the level of lipids, which can easily be stored as body fat. In shrimp, however, levels of lipids higher than 12% tend to affect growth (Deshimaru, 1981) and the aggregation and physical stability of the food particle. Hence, the levels of energy are increased by adding carbohydrates, especially starch, to the formulation (Cuzon and Guillaume, 1997). The ability of shrimp to use starch varies with the levels of inclusion and processing and degree of gelatinization (Davis and Arnold, 1993). The diets used in this study had levels of lipids between 3.5 and 11% and the level of wheat starch ranged between 32.8% for the 30%-RAT diet and 9.3% for the 40 RAT.

It seems that the energy contents of the diets used in this study did not limit the amount of feed consumed by shrimp. Some authors suggest that the level of energy in the diets regulate food consumption in shrimp, as occurs with other animal species (Sedgwick, 1979; Cuzon and Guillaume, 1997; Lupatsch *et al.*, 2001). If the level of energy of the 40-ISO fed at 75% of the ration was appropriate, shrimp were fed an excess of energy when offered the 30% CP diet at 100% ration. This excess of energy could have limited food consumption and shrimp may not have been able to consume enough food to meet their protein requirements, which could have affected their growth. However, shrimp growth was not significantly different among both treatments. Another possible explanation is that the amount of protein fed was in excess of the daily requirement of shrimp. In this case, shrimp could have met their protein requirement even if the higher level of energy of the 30-ISO diet would have reduced food consumption. Kureshy and Davis (2002) reported that the minimum daily protein requirement for juvenile *L. vannamei* was 46 g CP/kg body weight/day (BWd) and 24 g CP/kg BWd for sub-adult shrimp. In this research the daily CP offered ranged between 15.8 g CP/kg BWd for the last week of the trial and 61.1 g CP/kg BWd for the first week. An average of 30.8 g of CP/kg BWd was offered during the 49-day trial (use only one value with geometric mean). Based on these values it is likely that protein was not fed in excess and shrimp consumed an appropriate amount of protein at the reduced feed rate (40-ISO 75%). Furthermore, there were no significant differences in PCE values among the different treatments. Since all the treatments were offered the same amount of crude protein, differences in PCE could have been expected if non protein energy was limiting. For example, the feeding treatment that provided the higher energy input (30-ISO 100%) did not have shrimp with better PCE (30.9 versus 31.9%) than the treatment with the lowest energy input (40 ISO 75%). Probably all the protein offered was used for growth and the extra energy provided by the treatment 30 ISO-100% did not help to spare protein. Thus indicating that energy was not limited in any of diet/ration combinations.

Conclusions

This study demonstrates that as long as the feed allowance is adjusted for energy and protein density, the nutrient requirements of *L. vannamei* can be met by different diets with variable levels of protein and energy. As demonstrated with other species (Lawrence and Lee, 1997; McGoogan and Gatlin, 1998; Lupatsch *et al.* 1998; Lupatsch *et al.*, 2001; Kureshy and Davis, 2002), shrimp have a daily quantitative physiological requirement that can be provided with a variety of diets with different nutrient densities. These results clearly indicate that shrimp have a wide tolerance to levels of dietary energy and as long as energy is not limited, a variety of protein and feed input levels can be used to meet nutrient requirements.

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V. SUMMARY AND CONCLUSIONS

Feed represent one of the primary costs associated with the production of shrimp under semi-intensive and intensive conditions. The feed is also the initial source of pollutants; hence, overfeeding or poor quality feeds can severely impact water quality and production. In order to improve production of shrimp under semi-intensive conditions and reduce waste outputs it is critical to optimize feed allowance and to develop appropriate feeding management plans. One method to do this is by determining feed allowance using bioenergetics models and by reducing feed input by increasing the nutrient content of the diets.

In this study the factorial method was used to develop a bioenergetics model to determine daily feed allowance of Pacific white shrimp *Litopenaeus vannamei*. This model was developed on site with data generated empirically through feeding graded levels of diet to juvenile (2.2 g) and sub-adult shrimp (11.1 g). Application of the model showed that the daily feed allowance of shrimp varies with the expected level of growth of shrimp under the specific conditions and the energy and nutrient density of the diet (DE and DP).

These values of DE and DP required to reach specified levels of growth generated with the model provided the theoretical support of the concept that shrimp do not have a dietary protein level requirement, but a quantitative daily protein requirement that can be provided with diets with different levels of crude protein. That statement was evaluated and validated under controlled experimental conditions in tanks. Shrimp were offered diets with two levels of dietary protein (30 and 40%), whose levels of feeding were determined on an isonitrogenous base. In two different tank trials, one receiving green water from a shrimp production pond and the other utilizing clear sea water, juvenile shrimp growth was not affected when a 40% CP diet was offered at 75% of the ration of a standard 30% CP diet. Although final weight and growth rate were not significantly different, the FCR of shrimp fed the 40% CP diet at reduced rate was significantly lower, indicating that a lower amount of feed was required to produce the same amount of growth. Reduced feed input may improve water quality with a subsequent improvement in shrimp health, survival and production. The use of high nutrient diets at reduced feed input has been recommend as a practice to reduce and control aquacultural wastes that can cause environmental problem to surrounding ecosystems.

When the level of feed input in shrimp production is based on meeting the daily quantitative nutrient requirement of shrimp, such as digestible protein, the levels of other nutrients or energy must be proportionate to the specific nutrient in order to avoid feeding sub-optimal levels of the other components. In this study it was observed that when the levels of DE of the diets vary in proportion to the levels of CP (DE/CP ratio) of the diets reducing feed input by 25% feeding a higher protein diet did not affect shrimp growth.

The final objective of laboratory research should be to translate the results to the field where the findings can improve production conditions. In this study, attempts to duplicate lab result under semi-intensive conditions in ponds were done. Unfortunately, controlled statistical research becomes very difficult under pond conditions. The intervention of uncontrollable variables makes it difficult to isolate and evaluate specific study variables. For example, in this study, although there were no significant differences in final weight among the different feeding treatments, shrimp offered the 30% CP diet at 100% ration tended to have higher production than the corresponding isonitrogenous treatment (40% CP at 75% of the ration). Although this statement could not be evaluated statistically due to the lost of two experimental units of the 40% CP diet offered at 75% rate. It seems that in production conditions the higher feed input of a lower protein diet may stimulate natural productivity possibly due to carbon enrichment. It is also possible that a larger amount of feed allowed a better distribution of the feed in the pond making it easier for shrimp to reach the feed and reducing competition. Further research under pond conditions is warranted to verify these statements. These results show that lab research by itself can not provide all the solutions to problems that occur under production conditions, but it still represents an important tool that can provide some useful information that helps to understand the production systems.

The use of on-site developed bioenergetics models allows optimization of feed input. Optimal feeding in conjunction with improved feeding management practices, as those applied in this study, could lead to better economical performance and lower nutrient wastes from shrimp aquaculture. Bioenergetics models are important tools that can help to optimize feed input and reduce feed wastage in shrimp production. However, the constant monitoring and evaluation of the production system are critical factors that can not be replaced by any mechanical or mathematical system. Data generated by growth and feed requirement models can be used to compare with current performance in a shrimp farm (eg., weekly growth, FCR). This information could be used to adjust production conditions and to improve feeding and husbandry practices to reach biologically achievable goals. However, it must be clear that there is not a unique bioenergetics model than can be applied to every production condition. Maximum production and optimal growth of an animal species are factors that depend on the genetic, diet, environmental conditions (eg., temperature), and animals health conditions, among others. Hence, nutrient requirements should be determined for specific values of performance, feed composition and life stage. Therefore, these models will be useful only when they have been developed and adjusted to the specific biotic and environmental conditions of the production operation.

Results obtained in this study with the development and experimental application of a bioenergetics model support the principle that shrimp feed input should not be based on constant nutrient requirements referred as a dietary level of the nutrient, as traditionally has been used, but as a daily quantitative requirement that can be supplied with diets with variable levels of nutrient and energy. Results of the proposed bioenergetics model are encouraging and can be a valuable tool to help refine nutrient inputs (feeding) on farms.

VI. LITERATURE CITED

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VII. APPENDIX

Appendix: Example and calculations of feed allowance using a bioenergetics model based on digestible energy (DE) or digestible protein (DP) of the diet for 2-g shrimp having a growth rate of 0.5 g/week.

Based on DE		
Size (2g)	2	
DE maintenance (cal/shrimp/day)	82.2	41.1 cal/g x 2 g
Growth (g)/week	0.5	assumed
Growth (g/day)	0.071429	0.5/7days
Body energy= Kcal/kg	1124.7	From equation 2, y (Kcal/kg)=1831 $(kg)^{0.07842}$
DE growth (cal/shrimp/day)	275.6	(Growth/day x body energy)/energy deposition efficiency (slope of equation 5)
DE (maint.+growth)	357.8	
DE feed (cal/g DM)	3000	assumed
Feed allowance	0.119	DE (maint.+ growth)/DE feed
FCR	1.86: 1	
Based on DP		
Size (g)	2	
DP maintenance (g/sh/day)	0.0056	2.82 x 2/1000
Growth (g/sh/week)	0.5	assumed
Growth (g/sh/day)	0.071429	0.5/7days
Body protein= g/100 g	17.2	From equation 1, y(g CP/100 g)=24 $(kg)^{0.05336}$
DP growth (g/sh/day)	0.02279	(Growth/day x body protein)/protein deposition efficiency (slope of equation 7)
DP maint+growth (g/sh/day)	0.02843	
DP feed (g DP/100 g)	25	assumed
Feed allowance	0.114	DP (maint.+ growth)/DP feed
FCR	1.77:1	