DAILY DIGESTIBLE PROTEIN AND ENERGY REQUIREMENTS FOR

GROWTH AND MAINTENANCE OF SUB-ADULT PACIFIC WHITE SHRIMP

(*LITOPENAEUS VANNAMEI*)

A Dissertation

by

ANTHONY JOSEPH SICCARDI III

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2006

Major Subject: Nutrition

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ABSTRACT

Daily Digestible Protein and Energy Requirements for Growth and Maintenance

of Sub-adult Pacific White Shrimp (*Litopenaeus vannamei*). (August 2006)

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This study utilized two diets (25 and 35% crude protein) fed at 10 different rates to produce differences in shrimp specific growth rate which were regressed against daily digestible protein (DP) and digestible energy (DE) intake to estimate daily DP and DE requirements for sub-adult *L. vannamei*. Apparent DP and DE requirement for maximum growth decreased throughout the 7-week trial as shrimp size increased. Mean apparent daily DP requirement for 7.69 to 13.08-g *L. vannamei* fed the 25% protein diet was 6.31 g DP kg^{-1} BW d^{-1} while the 35% protein diet produced a mean apparent daily DP requirement of 8.00 g DP kg^{-1} BW d^{-1} for 8.11- to 13.79-g *L. vannamei*. Maintenance requirements were estimated by regressing DP feed allowances back to zero weight-gain and were 1.03 g DP kg^{-1} BW d^{-1} for shrimp fed the 25% protein diet and 1.87 g DP kg^{-1} BW d^{-1} for shrimp fed the 35% protein diet. Mean apparent daily DE requirement for shrimp fed the 25% protein diet was 402.62 kJ DE kg^{-1} BW d^{-1} while the 35% protein diet produced an apparent daily DE requirement of 334.72 kJ DE kg^{-1} BW d^{-1}. Mean apparent daily DE maintenance requirements for shrimp fed the 25% protein

diet was 66.23 kJ DE kg^{-1} BW d^{-1} while the requirement was 78.82 kJ DE kg^{-1} BW d^{-1} for shrimp fed the 35% protein diet. Daily DP and DE requirements were also determined by regressing whole-body protein or energy change against daily DP and DE intake and were similar to those values obtained by regressing change in body weight against daily DP and DE intake. Another component of this project involved evaluating 32 different feedstuffs for dry matter, protein and energy digestibility coefficients. Fish meal apparent crude protein digestibility coefficients as a group were higher than all other ingredient classifications except purified ingredients. Protein in 48% soybean meal and 90% isolated soybean protein were significantly more digestible than protein found in fish, animal and marine meals tested. This data will improve the quality and reduce the cost of commercial shrimp feeds.

DEDICATION

To my parents

for their unconditional

support throughout my education

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CHAPTER I

INTRODUCTION

Economic and environmental benefit of properly formulated feeds

The gradual erosion in shrimp prices has forced US shrimp farmers to reduce production costs to remain economically solvent. Cost reduction may be realized by increasing stocking densities in ponds and raceways to intensive or even super intensive levels. Such intensification places the nutritional burden on supplemented feed as opposed to natural productivity. To sustain optimum growth these feeds must contain the proper balance of energy, protein, minerals and vitamins while preserving the cost efficiencies realized through intensification. US shrimp farmers therefore rely on feed formulators to reduce feed costs, which currently account for the majority of production costs (Akiyama et al., 1992), while maintaining optimal shrimp growth. Feed formulators in turn look to researchers to provide them with optimal nutrient levels to meet their challenges. Of particular interest is protein which accounts for the majority of shrimp feed content and expense (Shiau et al., 1992; Cordova-Murueta and Garcia-Carreno, 2002). Dietary optimization of protein may lead to a reduction in feed costs, helping to reduce production expenditures and increase profits. A concomitant benefit

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This dissertation follows the style and format of the journal Aquaculture.

of protein optimization may be realized by reducing feeds' significant contribution of enriching nutrients in aquaculture effluent. Aquaculture effluent can produce negative environmental impacts (Boyd and Clay, 1998) and has forced US farmers to meet acceptable pollutant levels in discharge which in some ways has slowed expansion of the industry (Lawrence et al., 2001). Velasco et al. (1999) demonstrated the correlation between dietary protein and the accumulation of inorganic nitrogen in culture water. They also observed that diets which maximize protein utilization for growth as opposed to energy needs may lead to the reduction of nitrogenous compounds in aquaculture effluent. If shrimp farming is to remain one of the fastest growing segments of US aquaculture these dietary issues must be properly addressed.

Estimated protein and energy requirements of *L. vannamei*

Dietary protein requirements have been estimated by feeding trials in which graded levels of protein are fed to apparent satiation or in excess, to determine growth response (typically, weight gain) under controlled or observed environmental conditions. Results have suggested protein requirements of juvenile *L. vannamei* range from an as-fed dietary inclusion level of 15%, with a energy to protein (E:P) ratio of 119.58 kJ g^{-1} protein (Aranyakananda, 1995), to approximately 30% of diet, with a dietary E:P ratio of 41.86 kJ g^{-1} protein (Cousin et al., 1991), to greater than 36% of diet (Smith et al., 1985) and even greater than 40% of diet (Colvin and Brand, 1977). These variations are not surprising considering that protein requirements can vary with age, size, physiological

status, growth rate and dietary characteristics such as E:P ratio (Colvin and Brand, 1977; Bhaskar and Ali, 1984; Akiyama, 1991; Guillaume, 1997; Pedrazzoli et al., 1998).

Despite the suggestion of Andrews et al. (1972) that one of the most important requirements for formulating a suitable diet for shrimp was to determine the balance between dietary energy and protein, few such studies have addressed this issue for *L. vannamei* (Dokken, 1987; Aranyakananda, 1995; Rosas et al., 2001b; Cuzon et al., 2004). In one of the most comprehensive studies to date, Rosas et al. (2001b) determined the optimal (E:P) ratio for *L. vannamei* was 28 kJ g^{-1} (33-44% protein and 6-23% carbohydrate) for juvenile shrimp weighing less than 1 gram and 28-38 kJ g^{-1} (33-44% protein and 6-23% carbohydrate) for those shrimp greater than 1 gram. This study, however, did not quantify daily protein requirement for maintenance and/or maximum weight gain as the feeds were provided in excess. Cuzon et al. (2004) determined an optimal E:P ratio of 42 kJ DE g^{-1} for 7-8 g *L. vannamei* by feeding diets with constant protein, constant energy or constant P/E ratio and suggested maximum growth for a biomass of 100 g shrimp could be achieved by feeding a daily intake of 1.2 g DP and 140 kJ digestible energy (DE). Although daily protein and energy requirements were determined it is unclear if these results were obtained through studies involving incremental feed rates or estimated from *ad-libitum* feeding trials as experimental methods were not provided. Feeding method is important as in some cases, feed intake by shrimp could potentially be increased to negate the effect of a low protein diet and lead to substantial variation in dietary E:P requirement (Kureshy and Davis, 2002).

Kureshy and Davis (2002) estimated the protein requirement for both maintenance and maximum weight gain of juvenile and sub-adult *L. vannamei* by using feeds formulated to contain 16, 32, and 48% crude protein (CP) with calculated E:P ratios of 105.06, 52.61, and 37.5 kJ g^{-1} protein. Juvenile maintenance protein requirement was estimated between 1.8 and 3.8 g crude protein per kilogram of body weight per day (g CP kg^{-1} BW d^{-1}) and between 1.5 and 2.1 g CP kg^{-1} BW d^{-1} for sub-adults. To achieve maximum weight gain juveniles required 46.4 g CP kg^{-1} BW d^{-1} when fed the 32% protein diet and 23.5 g CP kg^{-1} BW d^{-1} for sub-adults fed the same 32% protein diet. Feed efficiency (FE) increased with the CP level of the diet and decreased with increased feeding rates, which indicates the importance of incremental compared to *ad-libitum* feeding strategies for determination of protein requirements. Although the authors determined a daily protein requirement, no attempt was made to determine the daily energy requirement.

Factorial-design, apparent-requirement trials

Although incremental feeding rate studies (i.e. factorial design) have been employed for years to determine both protein and energy requirements in terrestrial animals as well as fish (Pfeffer and Pieper, 1979; Gatlin et al., 1986; Shearer, 1995), few studies exist for shrimp. Factorial modeling relies upon the assumption that a growing shrimp's energy and protein needs are the sum of the requirement for growth and maintenance. Using the respective partial efficiencies of utilization, dietary feed intake can be calculated which allows energy and protein requirements to be expressed in terms

of absolute daily feed intake per unit weight and weight gain as opposed to being expressed as a percentage of the diet. Another advantage to this method is it allows for the determination of allometric equations to estimate energy requirements below and at maintenance; energy requirements above maintenance; protein requirements below and at maintenance; and protein requirements above maintenance.

A simple factorial model to determine a dietary requirement can be written as: $R = M*BW^b + G*\text{growth}$, where R = requirement, M = dietary energy or protein utilization efficiency for maintenance, BW^b = metabolic body weight (with weight typically measured in grams), G = dietary energy or protein utilization efficiency for growth, and growth is the rate of growth in grams per day. This model can be rewritten to determine dietary feed intake as follows: $I = G + M + E$, where I = ingested energy or protein, G = growth, M = metabolizable energy or protein and E = endogenous loss (Brett and Groves, 1979). Growth requirement (G) is determined through the composition of body mass added during growth, endogenous loss (E) is measured by calculating the body-stores decline during starvation, while digestible energy values are commonly used in place of metabolizable energy values due to the difficulty required in obtaining them (Pfeffer and Pieper 1979; NRC, 1980; Lovell 1989). This model allows for the determination of an energy budget at a point in time or for any phase of the life cycle which provides greater insight than results commonly obtained from growth trials.

Factors affecting growth response

Abiotic factors

Since the majority of nutrient-requirement studies involve measuring a growth response, particular attention must be taken to control abiotic factors (i.e. dissolved oxygen, salinity, temperature, etc.) which can affect energetic requirements. Dissolved oxygen (DO) is a limiting factor which reduces growth through its effect on metabolism. While *L. vannamei* appears to be able to withstand lower DO concentrations than *P. japonicus* (Egusa, 1961), *P. monodon* (Liao and Chen, 1994), *P. setiferus* and *P. schmitti* (Rosas et al. 1997); they still must be maintained at DO concentrations above 2 mg L^{-1} to avoid significant reductions in growth (Seidman and Lawrence, 1985).

Temperature is also a modifier of energy flow and has a significant effect on feeding rate, growth and an organism's overall activity (Wyban et al., 1995; Ponce-Palafox et al., 1997). Feeding rate and growth are positively correlated to increases in temperature between 23 and 30°C; however, the effect is less pronounced as shrimp weight increases (Wyban et al., 1995). Optimum temperature for *L. vannamei* growth appears to decrease as shrimp size increases, producing an optimum temperature >30°C for small shrimp (3.9 g), 30°C for medium shrimp (10.8 g) and 27°C for large shrimp (> 16 g) suggesting the importance of uniform stocking weight and predetermined experimental growth ranges (Wyban et al., 1995). Experimental temperature should also be standardized among various nutrient-requirement studies, as differences in growth responses mediated by temperature effects are complicated and can't be explained by a simple linear model (Wyban et al., 1995).

While growth rates are typically more affected by temperature than salinity, the interaction of these two abiotic variables can have a significant effect on *L. vannamei* growth (Ponce-Palafox et al., 1997). Salinity had a significant effect on growth when temperatures were high (35°C) but had a minimal effect on growth between 25-45‰ (test range 20-50‰) provided temperatures were maintained within *L. vannamei's* optimum range (Ponce-Palafox et al., 1997). Salinity effect on *L. vannamei* growth was more pronounced when tested over a larger range (5-49‰), producing significantly greater final weights at 5 and 15‰ (Bray et al., 1994). The actual mechanism producing the growth effect (i.e. salinity effect on metabolism) was investigated by Rosas at al. (2001a) by examining the interaction of salinity and dietary carbohydrates as well as the interaction between salinity, dietary carbohydrates and dietary protein. Metabolic efficiency was modulated through salinity effect on both dietary protein and dietary carbohydrate metabolism as Rosas et al. (2001a) observed maximum growth for shrimp fed a low carbohydrate (1%), high protein (50%) diet maintained at a salinity of 15‰ but depressed growth when maintained at a salinity of 40‰. This suggests the importance of appropriate dietary P/E ratios.

Inorganic compounds

The level of inorganic nutrients in shrimp systems is greatly influenced by stocking density, feed consumption, and feed and water quality management practices (Velasco et al., 1998). These inorganic compounds need to be maintained at acceptable levels as ammonia (Shilo and Rimon 1982) nitrite (Solbe 1978) and sulfide (Ram et al., 1981) build-up in shrimp culture systems have been shown to reduce growth and

survival. These inorganic nutrients also can reduce feeding response (Ram et al., 1981; Shilo and Rimon 1982) and increase the incidence of disease. To assure these compounds do not interfere with the experiment, ammonia-N should be maintained below 2.37 mg L^{-1} (0.09 mg L^{-1} for NH sub(3)-N) (Chen and Lin 1991), nitrite levels below 2.04 mg L^{-1} (Chen and Lin 1991), nitrate below 25 mg L^{-1} (Chen and Lei 1990) and sulfide should be as close to undetectable as possible. Controlling these factors will assure differences in growth can be attributed to dietary effects and allow for the proper nutrient determination.

Experimental design

Experimental design also can contribute to differences in growth rates which can have an effect on apparent requirements. Dokken (1987) reported *L. vannamei* fed four times per day had faster growth rates than those fed the same ration size two times per day. Lawrence et al. (unpublished results) determined feed utilization increased when ingestion rate, feeding frequency and daily ration size increased, suggesting differences in nutrient requirements may be achieved depending on how feed is presented to juvenile *L. vannamei*. Arayankanada (1995) also suggested feeding frequency could affect nutrient requirements and concluded the low (15%) dietary protein requirement obtained could be attributed to higher feeding frequency (15 feedings per day). This is not surprising since Beseres et al. (2005) observed gut passage times of less than one hour in shrimp.

Dietary energy considerations

Carbohydrates and lipids are commonly added to diets as energy sources in an attempt to spare the use of protein for energy. Although not dietarily essential, carbohydrates are typically added as they are the most economical source of dietary energy. Simple carbohydrates such as glucose are poorly utilized by shrimp (Andrews et al., 1972; Deshimaru and Yone, 1978; Alava and Pascual, 1987) and may even reduce survival rates (Shiau and Peng, 1992) necessitating the addition of more complex carbohydrates such as starch. Lipids also may be utilized as an energy source as they provide a concentrated source of energy as well as essential fatty acids but can have an adverse effect on growth when supplemented at high dietary levels (Dokken, 1987). Dokken (1987) reported weight loss in *L. vannamei* fed high (13.8-18.8%) dietary lipid levels and suggested the optimum range was between 5 to 10% of the diet.

Dietary energy levels also have been shown to affect the determination of protein requirements (Sedgwick, 1979; Shiau and Chou, 1991). Optimum growth for *P. merguiensis* was achieved feeding either a diet with 42% protein and 18.4 kJ g^{-1} or 36% protein and 12.1 kJ g^{-1} (Sedgwick, 1979). Similar results were determined for *P. monodon* fed a 40% protein 16.3 kJ g^{-1} diet (Shiau et al., 1991) and a 36% protein 13.8 kJ g^{-1} diet (Shiau and Chou, 1991). Dietary energy levels may also affect feed consumption. Davis and Arnold (1993) reported an inverse relationship between digestibility coefficients and consumption which suggests consumption could be energy-related. If this is correct, diets high in energy may limit consumption, reducing the amount of protein, minerals, vitamins, etc. consumed which may lead to reduced growth.

Conversely, increased consumption of diets which are low in energy will increase vitamin and mineral intake which may adversely affect growth if the nutrient is toxic when ingested in higher levels per day.

Dietary protein considerations

Cruz-Ricque et al. (1987) showed squid protein fraction significantly improved the growth rate of *L. vannamei* even at supplementation levels as low as 1.5%. Since all diets were well balanced and included all known nutrients they concluded squid protein fraction contains an unknown growth factor and should be included in all dietary formulations. Cordova-Murueta and Garcia-Carreno 2002 determined *L. vannamei* fed diets containing either 3% fish or krill hydrolysate grew significantly better than shrimp fed 9 or 15% of the same protein hydrolysate and concluded protein supplements must meet specific requirements to be properly assimilated from the diet. Growth effects were also witnessed when only 2% fish meal was exchanged for the same amount of krill meal despite the fact krill meal protein is usually less digestible and has a lower amino acid contribution than fish meal (Lopez et al., 1998). The authors suggested this growth effect may be attributed to krill's ability to increase feed attractibility, increase feed consumption and/or it may contain unknown growth factors.

Ingestion and attractability

Ingestion rates and attractibility add another level of complexity for studies involving nutrient requirements as both have been shown to affect growth (Lawrence and Castille, 1993; Smith et al., 2005) but are subjective in measurement (Cam et al., 1995; Smith et al., 2005). Differences in growth of *L. vannamei* fed diets with identical

nutritional values supplemented with either fish, krill or *Artemia* meals was partially

explained by differences in consumption rates (Lawrence and Castille, 1993) while

Smith et al. (2005) showed *P. monodon* exhibited significantly greater preference for

and grew 20% faster on feeds which contained crustacean or krill meal. From these

studies it is apparent even small dietary adjustments can have significant effects on

growth which suggests the importance of proper dietary formulation or the use of a

standard reference protein when determining nutrient requirements. Although protein

and energy requirements are crucial for developing a true least-cost least-polluting diet,

they must be combined with accurate digestible protein and energy data for ingredients

commonly used in the aquaculture industry.

Digestion in *Litopenaeus vannamei*

The gut in *L. vannamei* is basically a simple tube which runs the length of the

body from the mouth to the anus at the end of the last somite. Enzyme secretion is

limited to the midgut which is comprised of a large number of simple, fragile tubules.

Dietary proteins are digested by proteinases such as trypsins and chymotrypins (Lan and

Pan, 1993; Chevalier and Wormhoudt, 1998), lipids by lipase and esterase activity while

alpha-amylase and alpha-glucosidase are secreted to digest carbohydrates (Chevalier and

Wormhoudt, 1998) under slightly-basic (pH ~8) conditions (Garcia-Carreno et al.,

1997). Once digested, nutrients are absorbed in the midgut and fecal formation and

defecation takes place in the hindgut. This digestive scheme allows *L. vannamei* to be

highly effective at digesting protein (Akiyama et al., 1989; Aquacop, 1989) even though it lacks pepsin and an acidic stomach.

Brief overview of digestibility terminology

Feed digestibility is a term used to describe only the portion of feed which is absorbed by the organism. The portion of protein and energy lost by the gut during ingestion and digestion are subtracted from the calculation and are commonly referred to as metabolic fecal nitrogen losses (MFN) and metabolic fecal energy (MFE) losses. Since it is difficult to determine these losses with any degree of accuracy using empirical methods (Lee and Lawrence 1997) most nutritionists determine apparent digestibility. While apparent digestibility also describes the proportion of absorbed feed, it does not subtract losses associated with MFN and MFE as it is based on the difference between the amount of feed ingested and the amount of feces. Although not a true measurement of digestibility, apparent digestibility still provides an accurate estimate of the ingredients or feeds digestibility especially when one considers that MFN has been shown to have only a minor influence on fecal protein analysis (Forster and Gabbott, 1971; Colvin 1976).

Factors affecting apparent digestibility in crustaceans

Studies have been undertaken to determine the effect of species (Lee, 1970; Akiyama, 1988; Lemos et al., 2000), age (Smith et al., 1985), environmental factors (Coelho 1984; Seidman and Lawrence 1985) stressors (Cordova-Murueta et al., 2004)

and diet (Cordova-Murueta and Garcia-Carreno, 2002) on apparent digestibility. Lemos et al., (2000) showed clear differences in proteinase patterns between adult *Farfantepenaeus californiensis*, *F. paulensis*, *L. schmitti* and *L. vannamei* and suggested protein digestion may be species-specific. Lee (1970) reported minor differences in apparent dry matter digestibility (ADMD) for *Penaeus monodon*, *P. japonicus*, *P. semisulcatus*, and *Metapenaeus monoceros* while Akiyama (1988) found differences in apparent lipid and apparent carbohydrate digestibility in *L. vannamei*, *P. monodon* and *P. japonicus* fed a soybean-meal-based diet. These data suggest there are differences in digestibility among even closely related crustacean species and strengthen the argument for comprehensive digestibility studies for each species under consideration.

Smith et al. (1985) reported protein digestibility for small *L. vannamei* (average weight 4g) was strongly correlated with dietary protein level; however, no correlation existed for the 9.8 and 20.8 g shrimp. The authors also determined there was no correlation between any size class and either lipid or total diet digestibility. Coelho (1984) determined salinity had a minimal effect on digestibility provided the test diets had a protein content greater then 20% while Seidman and Lawrence (1985) showed feed digestibility in *L. vannamei* was not affected even when shrimp were exposed to dissolved oxygen concentrations of 1 mg L^{-1}. Cordova-Murueta et al (2004) exposed *L. vannamei* to alimentary stress by shifting from a 45% to a 35% protein feed and by physically manipulating the shrimp during weighing. Decreased trypsin and chymotrypsin activity in feces and mid-gut gland was observed in both treatments, with a greater effect being attributed to physical manipulation. For these reasons it appears

digestibility trials should be performed under "normal" environmental conditions using >8g *L. vannamei* which have been acclimated to the test diets and culture system.

Effects of dietary composition on digestion have been verified by Cordova-Murueta and Garcia-Carreno (2002), who determined both *in vitro* and *in vivo* digestibility were affected by not only the source of the protein supplement but also by the quantity in the diet. Akiyama et al (1989) suggested digestibility diets should be formulated entirely of the feedstuff being evaluated to eliminate any associative effects of the constituents of the diet however; he suggested the lower apparent dry matter digestibility values obtained in his study may be related to using nutritionally incomplete diets. Other authors have suggested reference diets should be used as production diets rarely are composed of a single ingredient (Davis and Arnold, 1993). No matter which method is chosen, differences can be expected as neither experimental design requires test diets which have a constant protein quantity. While ingredient digestibility values can provide valuable information for a feed formulator, one must take into account all factors which can affect its measurement and remember it is an apparent not a true digestibility value.

Brief evaluation of *in vivo* and *in vitro* digestibility methods

Lee and Lawrence (1997) suggested *in vitro* assays should be utilized by crustacean nutritionists based on preliminary evidence which showed *in vitro* assays produced the same general pattern of apparent digestibility as those previously reported in *in vivo* studies. Since this recommendation many studies have focused on comparing

in vivo and *in vitro* digestibility methods as *in vitro* methods are fast, cost effective and use only small amounts of raw materials (Ezquerra et al., 1997; Ezquerra et al., 1998; Lemos et al., 2000; Cordova-Murueta and Garcia-Carreno, 2002). Traditionally *in vitro* methods have relied on chemical analysis such as Kjeldahl analysis and determination of amino acid composition (Anderson et al., 1993). Since these methods involve the use of harsher chemical reactions than naturally occur in the digestive process, they typically release more nutrients than are available to the animal which produces inaccurate results (Anderson et al., 1993). A better method has been proposed by Ezquerra et al. (1997) who obtained strong correlations between *in vitro* and *in vivo* digestibility using a pH-stat assay. Unfortunately, this correlation was only valid when protein was compared according to their origin (i.e. animal or plant) as samples containing both animal and plant proteins yielded only an approximate estimate of protein digestibility based on *in vivo* estimates (Ezquerra et al., 1997). While Ezquerra et al. (1998) also determined the pH-drop method showed a significant correlation to *in vivo* digestibility, the correlation was low and the method is constrained by the same limitations described for the pH-stat assay. *In vitro* assays are also hindered by their inability to determine digestibility for any nutrient besides protein. Apparent dry matter, lipid, and energy digestibility values have been heavily utilized by the poultry industry to formulate least-cost environmentally sound diets and this information should also be valuable to crustacean nutritionists. While *in vitro* methods have improved greatly since 1997, it still appears they are not able to replace *in vivo* apparent digestibility trials, especially when one wishes to determine more than apparent protein digestibility.

Current *in vivo* methods utilized to determine apparent digestibility coefficients

To determine the apparent digestibility of nutrients (i.e. protein and energy) researchers typically utilize an *in vivo* digestibility method. Digestibility studies involving *L. vannamei* have utilized the indirect chromic oxide method (Akiyama et al., 1989; Davis and Arnold, 1993; Davis and Arnold, 1995; Davis et al., 2002), the indirect ytterbium acetate method (Smith and Tabrett, 2004), the indirect titanium dioxide method (Smith and Tabrett, 2004) as well as the gravimetric method (Smith and Tabrett, 2004). Although good results can be achieved using the gravimetric method (Smith and Tabrett, 2004) its use has been curtailed due to the labor involved in the full recovery of the uneaten food and feces. Of the indirect markers chromic oxide is the most widely used for studies involving *L. vannamei* (Smith et al., 1985, Akiyama et al., 1989, Davis and Arnold, 1993; Davis and Arnold, 1995; Davis et al., 2002). To produce valid results the method relies upon the following assumptions: 1) the marker must pass through the gut at the same rate as the feed, 2) the marker must not be lost from the feces or absorbed from the gut of the shrimp, 3) the marker must be completely physiologically inert, and 4) the ratio of nutrient to marker in the feed is the same as that ingested by the shrimp. While studies have questioned the validity of the chromic oxide method for lobster *Homarus* sp. (Bordner et al., 1983; Leavitt 1985), freshwater crayfish *Procambarus clarkii* (Brown et al., 1986) and caridean shrimp *Pandalus serratus*, *Palaemon platyceros* (Forster and Gabbot, 1971), it appears the assumptions are valid for studies involving penaeid shrimp (Smith and Tabrett, 2004). Deering et al. (1996) showed that chromic oxide, acid insoluble ash and ytterbium acetate produced

equivalent apparent protein digestibility values. This data suggests chromic oxide is as physiologically inert as acid insoluble ash and is a valid inert marker for digestibility studies. Akiyama et al (1989) reported chromic oxide levels were homogeneous in *L. vannamei* feces and achieved a low standard deviation between replicates which suggested the reproducibility of the indirect chromic oxide method. Smith and Tabrett (2004) determined that a maximum of 3.4%, but possibly less than 1% of chromic oxide was absorbed by the shrimp which would have a maximum underestimating effect of only 0.2% on apparent CP digestibility assuming the feed had an apparent dry matter digestibility of 80%. This absorption is most likely much lower as the authors attribute most of the absorption to radioactivity which adhered to the shrimp during feeding. The validity of chromic oxide as a marker was further strengthened by Fenucci et al. (1982) who determined there was no significant leaching or bacterial degradation loss of protein, carbohydrate or chromic oxide from feed and feces during six hours of submersion. These studies provide compelling evidence that chromic oxide is inert, passes uniformly through the gut, is minimally absorbed and is not significantly lost from feed and feces which suggest chromic oxide may be used with accuracy for apparent digestibility studies involving penaeid shrimp.

Current status of *in vivo* apparent digestibility coefficients for *L. vannamei*

Protein digestibility has been determined for many commonly used ingredients included in *L. vannamei* diets and have been tested both singly (Akiyama 1988; Akiyama et al., 1989; Fox et al., 1995) and mixed with a reference diet (Davis and

Arnold, 1993; Davis and Arnold, 1995). Energy digestibility values for ingredients

utilized in *L. vannamei* diets, however, are sparse (Davis and Arnold, 1993; Davis and

Arnold, 1995). To date the only published energy digestibility values for ingredients

used in *L. vannamei* diets are for steam-cracked corn, corn flour, milo, Nutribinder™,

rice flour, whole wheat and wheat starch (Davis and Arnold, 1993; Davis and Arnold,

1995). Utilization of currently available data will not allow for the formulation of a

least-polluting diet based on DE values and in some cases DP values.

Importance of apparent digestibility in least-cost formulations and the environment

       The Pacific white shrimp, *L. vannamei*, is one of the most commonly cultured

shrimp worldwide. While efficient culture techniques have reduced the cost of *L.

vannamei* culture additional savings may be realized by optimizing feed formulations as

feed is a major part of production costs (Akiyama et al., 1992 and Sarac et al., 1993).

Today's feed formulations are based upon data derived from studies which measured

growth parameters for cultured *L. vannamei*. Diets are formulated to be "least cost" by

adjusting protein sources while maintaining gross protein requirements which have been

shown to produce optimal growth. Formulations which rely solely on gross dietary

composition, as opposed to digestible composition, can produce a feed which is over-

formulated increasing both costs and pollutant levels as protein is the most expensive

component in feeds (Cordova-Murueta and Garcia-Carreno, 2002; Shiau, 1992) and can

lead to the accumulation of inorganic nitrogen in culture water (Velasco et al 1999).

While Lee and Lawrence (1997) suggested in 1997 that environmental regulations may

have a greater role in digestibility research than economical considerations few studies have focused on digestibility for either reason (Cuzon et al., 2004)

One only needs to look at the poultry industry to realize that a more cost efficient, and environmentally sound, feed can be formulated based on the digestibility (i.e. nutrient availability) of ingredients utilized in the diet. This formulation method allows ingredients to be selected to meet both the nutritional as well as economical requirements of the least-cost diet under consideration. Knowledge of digestibility coefficients of ingredients also allows for an added measure of quality assurance as digestibility of ingredients can vary considerable depending upon their overall freshness and previous treatment (Garcia-Carreno, 1998).

Objectives

The objectives of the current study were:

1) Simultaneously determine apparent daily DP and DE requirements for sub-adult *L. vannammei* under laboratory conditions.

2) Estimate the apparent DP and DE requirements for maintenance of four size classes (approximately 5.5, 7.5, 15.5 and 18.5 g) of *L. vannamei* by utilizing the comparative slaughter technique.

3) Determine apparent dry matter, protein and energy digestibility for ingredients used in formulating *L. vannamei* diets.

CHAPTER II

DIGESTIBLE PROTEIN AND ENERGY REQUIREMENTS FOR GROWTH

AND MAINTENANCE OF SUB-ADULT PACIFIC WHITE SHRIMP

Litopenaeus vannamei

Introduction

Although shrimp farming remains one of the fastest growing segments of US

aquaculture, worth an estimated 6 billion US dollars (USD) per year, its growth in the

US also has been associated with negative environmental impacts (Boyd and Clay,

1998). These environmental impacts have forced US farmers to meet acceptable

pollutant levels in discharge which in some ways has slowed expansion of the industry

(Lawrence et al., 2001). Feeds can contribute a significant amount of enriching nutrients

in effluent that could necessitate the formulation of "environmentally friendly" or "least

polluting" feeds to help meet environmental standards. Velasco et al. (1999)

demonstrated the correlation between dietary protein and the accumulation of inorganic

nitrogen in culture water. They also observed that diets which maximize protein

utilization for growth as opposed to energy needs may lead to the reduction of

nitrogenous compounds in aquaculture effluent.

Protein levels in feed also must be optimized to reduce production costs as protein

accounts for the majority of feed content and expense (Shiau et al., 1992; Cordova-

Murueta, 2002) and feed costs currently account for the majority of production costs

(Akiyama et al., 1992). Shrimp farmers also have begun to increase stocking densities in ponds and raceways to intensive or even super intensive levels to deal with the reduction in shrimp prices. Such intensification places the nutritional burden on supplemented feed as opposed to natural productivity and forces nutritionists to formulate feeds to contain the proper balance of energy, protein, minerals and vitamins while preserving the cost efficiencies realized through intensification. Feed formulators in turn look to researchers to provide them with optimal nutrient levels to meet these challenges.

Dietary protein requirements have been estimated by feeding trials in which graded levels of protein are fed to apparent satiation or in excess, to determine growth response (typically, weight gain) under controlled or observed environmental conditions. Results have suggested protein requirements of juvenile *L. vannamei* range from an as-fed dietary inclusion level of 15%, with a energy to protein (E:P) ratio of 119.58 kJ g^{-1} protein (Aranyakananda, 1995), to approximately 30% of diet, with a dietary E:P ratio of 41.86 kJ g^{-1} protein (Cousin et al., 1991), to greater than 36% of diet (Smith et al., 1985) and even greater than 40% of diet (Colvin and Brand, 1977). These variations are not surprising considering that protein requirements can vary with age, size, physiological status, growth rate and dietary characteristics such as E:P ratio (Colvin and Brand, 1977; Bhaskar and Ali, 1984; Akiyama, 1991; Guillaume, 1997; Pedrazzoli et al., 1998) and protein sources. Differences also may arise as these studies utilized an *ad-libitum* feeding method which could allow shrimp to increase their feed intake to negate the effect of a low protein diet and lead to substantial variation in dietary E:P requirement (Kureshy and Davis, 2002). Kureshy and Davis (2002) estimated the protein

requirement for both maintenance and maximum weight gain of juvenile and sub-adult *L. vannamei* by using feeds formulated to contain 16, 32, and 48% crude protein (CP) with calculated E:P ratios of 105.06, 52.61, and 37.25 kJ g^{-1} protein. Juvenile maintenance protein requirement was estimated between 1.8 and 3.8 g dietary protein per kg of body weight per day (g CP kg^{-1} BW d^{-1}) and between 1.5 and 2.1 g CP kg^{-1} BW d^{-1} for sub-adults. To achieve maximum weight gain juveniles required 46.4 g CP kg^{-1} BW d^{-1} when fed the 32% protein diet and 23.5 g CP kg^{-1} BW d^{-1} for sub-adults fed the same 32% protein diet. Feed efficiency (FE) increased with the CP level of the diet and decreased with increased feeding rates. This indicates the importance of incremental compared to *ad-libitum* feeding strategies for determination of protein requirements. Although the authors determined a daily protein requirement, no attempt was made to determine the daily energy requirement.

Although incremental feeding rate studies (i.e. factorial design) have been used to determine both protein and energy requirements for years in terrestrial animals as well as fish (Pfeffer and Pieper, 1979; Gatlin et al., 1986; Shearer, 1995), few studies exist for shrimp. Factorial modeling relies upon the assumption a growing shrimp's energy and protein need is the sum of the requirement for growth and maintenance. Using the respective partial efficiencies of utilization dietary feed intake can be calculated which allows energy and protein requirements to be expressed in terms of absolute daily feed intake per unit weight and weight gain as opposed to being expressed as a percentage of the diet. The objective of this study was to simultaneously determine apparent daily

digestible protein and digestible energy requirements of sub-adult *L. vannamei* under laboratory conditions.

Materials and methods

Starvation trial

Source of shrimp

Specific-pathogen-free *L. vannamei* postlarvae from four different maturation cycles were obtained from The Oceanic Institute (Kailua-Kona, HI) and stocked outdoors into 2.44-m diameter fiberglass tanks. Postlarvae were fed a commercial postlarval feed (Rangen 45/10; Rangen Inc., Buhl, ID) four times daily. Postlarvae were moved indoors 1 week prior to stocking to allow acclimation to laboratory conditions $(30.1 \pm 0.5^{o}C, 32.2 \pm 0.4‰)$ and to achieve proper weight (mean $\pm$ s.d., g) for stocking $(5.51 \pm 0.33, 7.19 \pm 0.32, 14.10 \pm 0.59, 16.59 \pm 1.02)$.

Experimental system and design

The experimental system for this study consisted of 400 tanks (19-L volume, bottom surface area 0.09 m^2) connected to a semi-closed (8% new water daily) recirculating seawater system. Seawater was pumped through a sand filter, biological filter, 50-µm cartridge filter, heat exchanger and ultraviolet disinfection unit to achieve a recirculating rate of 0.6 L min^{-1} $tank^{-1}$ (1,440% exchange $tank^{-1}$ day^{-1}). A light:dark photoperiod of 12:12 h was provided by supplemental compact fluorescent lighting. At the start of the experiment 100 shrimp from each size class were blotted dry, weighed and stocked individually into each tank. Shrimp were monitored daily for molting

activity as well as to assess mortality. Temperature, salinity and dissolved oxygen (DO) were monitored daily using a YSI 85® Meter (YSI Inc., Yellow Springs, OH). Ammonia-nitrogen, NO_2-N, NO_3-N, and pH were monitored weekly using methods adapted from those of Spotte (1979a,b) and Solarzano (1969), Spotte (1979a,b) and Mullen and Riley (1955), Spotte (1979a,b) and Strickland and Parsons (1972), and a Brinkman Metrohm® pH meter, respectively.

Sample collection and analyses

Ten shrimp from each size class were removed on a weekly basis, enumerated, blotted dry and individually weighed. Shrimp were then individually wrapped, labeled and frozen (-84^oC) until subsequent body composition analysis. Prior to compositional analysis shrimp were individually lyophilized, finely ground with a coffee grinder (Hamilton Beach/Proctor Silex, Inc., Racine, WI) to pass through a 20-mesh screen and analyzed for percent dry matter (AOAC, 1990). Protein (AOAC Method 990.3; FP-528 Nitrogen/Protein Determinator; Leco Corporation, St. Joseph, MI), energy (model 1241 adiabatic bomb calorimeter; Parr Instrument Co., Moline, IL) and ash (AOAC, 1990) were then determined for each lyophilized sample and reported on a dry-matter basis. Immediately prior to stocking, a representative sample of 10 shrimp per size class was individually processed as described above to determine initial body composition.

Statistical analysis

Allometric equations for the four different shrimp size classes were obtained by applying linear regression analysis to logarithmic transformations of the data to obtain allometric functions for ash, energy, moisture and protein. Energy and protein losses per

day per shrimp were calculated for each weight class and plotted against the weights of

the shrimp which was taken as the geometric mean between the initial and final weights

after 28 days of starvation. Allometric equations were then developed to predict the

daily loss of energy (kJ shrimp^{-1} day^{-1}) and protein (g shrimp^{-1} day^{-1}).

Preliminary evaluation of experimental diets

Experimental diets

Thirteen semi-purified diets were manufactured (cold extrusion via Hobart

mixer) at the Texas Agriculture Experimental Station (TAES) Shrimp Mariculture

Project (Port Aransas, TX). Ingredient compositions of the semi-purified basal diets are

shown in Table 1. All ingredients except alginate and sodium metaphosphate were

mixed in a food mixer (Model A-200, Hobart Corporation, Troy, OH) for 40 min. In a

separate bowl, alginate and sodium metaphosphate were added to deionized water (400

ml kg^{-1}) and mixed using a hand mixer (Sunbeam Products Inc., Milford, MA) for

approximately 45 seconds. This mixture was then added to the dry ingredients and

mixed an additional minute to achieve a mash consistency appropriate for extrusion.

Extrusion was accomplished using a meat chopper attachment (Model A-200, Hobart

Corporation, Troy, OH) fitted with a 3-mm die. Moist feed strands were dried on wire

racks in a forced air oven at 35 ^{o}C to a moisture content of 8-10%. Dry feed strands

were ground using a mortar and pestle to provide a particle size ranging from 2-4 mm

and stored at 4 ^{o}C until used.

Table 1. Composition of the thirteen preliminary diets.

Ingredient	Diet ID												
	100	101	102	103	104	105	106	107	108	109	110	111	112
	Inclusion level (g kg^{-1})												
Alginate[5]	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Calcium Carbonate[2]	14.6	14.6	14.6	14.6	14.6	14.6	14.6	14.6	14.6	14.6	14.6	13.0	12.3
Cellulose[4]	20.0	53.6	13.8	13.8	93.7	53.6	53.6	13.8	13.8	53.6	13.8	53.6	13.8
Cholesterol[2]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Diatomaceous Earth[4]		33.8			74.5	33.8	33.8			33.8		33.8	
Dicalcium Phosphorus[2]	65.6	65.6	65.6	65.6	65.6	65.6	65.6	65.6	65.6	65.6	65.6	22.8	24.6
Fish Meal[6]	150.0	136.1	136.1	136.1	136.1	150.0	136.1	136.1	136.1	136.1	136.1	136.1	136.1
Isolated Soy[1]	79.4	111.1			111.1	79.4	111.1			111.1		111.1	
KCL[3]	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5
Krill[1]	105.0	90.7	90.7	90.7	90.7	105.0	90.7	90.7	90.7	90.7	90.7	90.7	90.7
Methionine[2]			0.4	1.4				0.4	0.4		0.4		0.4
Mineral-Vitamin Premix[1]	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	1.8	1.8	2.7	2.7
MgO[3]	17.3	17.3	17.3	17.3	17.3	17.3	11.6	11.6	17.3	17.3	17.3	17.3	17.3
Phospholipid[1]	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0	42.0
Sodium Metaphosphate[5]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Soybean Oil[7]			14.3	14.3				14.3	14.3		14.3		14.3
Squid[1]	150.0	136.1	136.1	136.1	136.1	150.0	136.1	136.1	136.1	136.1	136.1	136.1	136.1
Vitamin C[1]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.34	0.5	0.5	0.5	0.5
Vitamin-Mineral Premix[1]	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	2.3	1.5	1.5	2.3	2.3
Wheat Starch[2]	300.1	242.8	412.9	411.9	162.4	232.5	248.6	418.6	413.1	244.5	414.6	287.1	456.1

[1]Zeigler Brothers, Gardners, PA, USA.
[2]MP Biomedicals, Cleveland, OH, USA.
[3]Fisher Scientific, Fair Lawn, NJ, USA.

Table 1. Continued

Diet ID:
100 – Shrimp Mariculture Project (A.L.L.) Reference Diet
101 – 35% Crude Protein Diet
102 – 25% Crude Protein Diet
103 – Diet 102 with Methionine Increased to 0.85%
104 – Diet 101 with Ash and Fiber Increased to 24 and 10%, respectively
105 – Diet 101 with Squid Meal : Krill Meal : Fish Meal Ratio Adjusted to 15 : 10.5 : 15
106 – Diet 101 with 1/3 Less MgO
107 – Diet 102 with 1/3 Less MgO
108 – Diet 102 with 1/3 Less Vitamin C
109 – Diet 101 with 1/3 Less Vitamins and Minerals
110 – Diet 102 with 1/3 Less Vitamins and Minerals
111 – Diet 101 with 1/3 Less Ca : P
112 – Diet 102 with 1/3 Less Ca : P

Source of shrimp

Specific-pathogen-free *L. vannamei* postlarvae were obtained from The Oceanic

Institute (Kailua-Kona, HI) and stocked into 8.00-m diameter outdoor fiberglass tanks.

Postlarvae were fed live *Artemia sp.* nauplii and a commercial postlarval feed (Rangen

45/10; Rangen Inc., Buhl, ID) twice and 12 times daily, respectively. Postlarvae were

held approximately 8 weeks to allow for acclimation to laboratory conditions (30.4 ± 0.3

^{o}C, 32.6 ± 0.3‰) and to achieve proper weight for stocking (5.22 g ± 0.44).

Experimental system and design

The experimental system for this study consisted of 400 tanks (19-L volume,

bottom surface area 0.09 m^2) connected to a semi-closed (8% new water daily)

recirculating seawater system. Seawater was pumped through a sand filter, biological

filter, 50-µm cartridge filter, heat exchanger and ultraviolet disinfection unit to achieve a

recirculating rate of 0.6 L min^{-1} tank^{-1} ($1,440$% exchange tank^{-1} day^{-1}). A light:dark

photoperiod of 12:12 h was provided by supplemental compact fluorescent lighting. At

the start of the experiment shrimp were blotted dry, weighed and stocked individually

into each tank. Twenty shrimp were randomly assigned to each diet listed in Table 1.

Uneaten feed, feces, molts, and dead shrimp in each tank were removed daily prior to

filling (0.4 g feed shrimp^{-1} day^{-1}) wheel-type automatic feeders (set to deliver 15

feedings day^{-1} tank^{-1}) with the appropriate experimental feed. Temperature, salinity and

dissolved oxygen (DO) were monitored daily using a YSI 85® Meter (YSI Inc., Yellow

Springs, OH). Ammonia-nitrogen, NO_2-N, NO_3-N, and pH were monitored weekly

using methods adapted from those of Spotte (1979a,b) and Solarzano (1969), Spotte

(1979a,b), Mullen and Riley (1955), Spotte (1979a,b) and Strickland and Parsons

(1972), and a Brinkman Metrohm® pH meter, respectively.

Termination of trial and statistical analysis

Experimental tanks used in the growth study were harvested after 7 weeks.

Shrimp where then enumerated, blotted dry and weighed individually by treatment tank.

Feed performance was evaluated by the following biometrics: ln final weight, ln weight

gain, percent survival, percent growth and instantaneous growth rate (IGR).

Instantaneous growth rate was calculated by the following equation: IGR = 100 ×

[ln(final weight/initial weight)]/duration of feeding trial in days (Cushing, 1968). Data

were statistically compared using SPSS by one-way ANOVA. Treatment means were

separated by the Student-Newman-Keuls test ($P<0.05$).

Growth and survival trial

Experimental diets

Two semi-purified nutritionally replete diets were manufactured (cold extrusion

via Hobart mixer) at TAES Shrimp Mariculture Project (Port Aransas, TX). Ingredient

composition of the 25% crude protein (15.89 kJ g^{-1}) and 35% crude protein (15.48 kJ g^{-1})

semi-purified basal diets are shown in Tables 2 and 3, respectively. All ingredients

except alginate and sodium metaphosphate were mixed in a food mixer (Model A-200,

Hobart Corporation, Troy, OH) for 40 min. In a separate bowl, alginate and sodium

metaphosphate were added to deionized water (400 ml kg^{-1}) and mixed using a hand

mixer (Sunbeam Products Inc., Milford, MA) for approximately 45 seconds. This

mixture was then added to the dry ingredients and mixed an additional minute to achieve

a mash consistency appropriate for extrusion. Extrusion was accomplished using a meat

chopper attachment (Model A-200, Hobart Corporation, Troy, OH) fitted with a 3 mm

die. Moist feed strands were dried on wire racks in a forced air oven at $35\,^{\circ}C$ to a

moisture content of 8-10%. Dry feed strands were ground using a mortar and pestle to

provide a particle size ranging from 2-4 mm and stored at $4\,^{\circ}C$ until used.

Table 2. Composition of the 25% crude protein, 15.89 kJ g^{-1} diet.

Ingredient	Inclusion level (g kg^{-1})	Ingredient	Inclusion level (g kg^{-1})
Alginate[5]	20.00	Mineral-Vitamin Premix[1,A]	2.70
Calcium Carbonate[2]	14.60	MgO[3]	17.30
Cellulose[4]	13.83	Phospholipid[1]	42.00
Cholesterol[2]	2.00	Sodium Metaphosphate[3]	10.00
Dicalcium Phosphorus[2]	65.60	Soybean Oil[7]	14.33
Fish Meal[6]	136.11	Squid[1]	136.11
KCl[3]	18.50	Vitamin C[1]	0.50
Krill[1]	90.74	Vitamin-Mineral Premix[1,B]	2.30
Methionine[2]	0.43	Wheat Starch[2]	412.95
Ash (g kg^{-1})	181.14[a]	Energy (kJ kg^{-1})	15899[a]
Dry Matter (g kg^{-1})	904.4[a]	DCP (g kg^{-1})	208.4[a]
Crude Lipid (g kg^{-1})	9.01[a]	DE (kJ kg^{-1})	13347[a]
Crude Protein (g kg^{-1})	250.7[a]	PE ratio (g/kJ)[*]	1.56[a]

[1]Zeigler Brothers, Gardners, PA, USA.
[2]MP Biomedicals, Cleveland, OH, USA.
[3]Fisher Scientific, Fair Lawn, NJ, USA.
[4]Sigma, St. Louis, MO, USA.
[5]Keltone HV Alginate, NutraSweet-Kelco Company, Chicago, IL, USA.
[6]Omega Protein Corporation Inc., Houston, TX, USA.
[7]The J. M. Smucker Company, Orrville, OH, USA.
[A]See Appendix A for composition.
[B]See Appendix B for composition.
*Calculation based on digestible energy and protein.
[a]Calculated on an as-fed basis.

Table 3. Composition of the 35% crude protein, 15.48 kJ g^{-1} diet.

Ingredient	Inclusion level (g kg^{-1})	Ingredient	Inclusion level (g kg^{-1})
Alginate[5]	20.00	Krill[1]	90.74
Calcium Carbonate[2]	14.60	Mineral-Vitamin Premix[1,A]	2.70
Cellulose[4]	53.69	MgO[3]	17.30
Cholesterol[2]	2.00	Phospholipid[1]	42.00
Diatomaceous Earth[4]	33.84	Sodium Metaphosphate[3]	10.00
Dicalcium Phosphorus[2]	65.60	Squid[1]	136.11
Fish Meal[6]	136.11	Vitamin C[1]	0.50
Isolated Soy (90%)[1]	111.12	Vitamin-Mineral Premix[1,B]	2.30
KCl[3]	18.50	Wheat Starch[2]	242.88
Ash (g kg^{-1})	217.13[a]	Energy (kJ kg^{-1})	15480[a]
Dry Matter (g kg^{-1})	906.7[a]	DCP (g kg^{-1})	318.0[a]
Crude Lipid (g kg^{-1})	7.68[a]	DE (kJ kg^{-1})	13221[a]
Crude Protein (g kg^{-1})	352.6[a]	PE ratio (g/kJ)[*]	2.40[a]

[1]Zeigler Brothers, Gardners, PA, USA.
[2]MP Biomedicals, Cleveland, OH, USA.
[3]Fisher Scientific, Fair Lawn, NJ, USA.
[4]Sigma, St. Louis, MO, USA.
[5]Keltone HV Alginate, NutraSweet-Kelco Company, Chicago, IL.
[6]Omega Protein Corporation Inc., Houston, TX, USA.
[A]See Appendix A for composition.
[B]See Appendix B for composition.
*Calculation based on digestible energy and protein.
[a]Calculated on an as-fed basis.

Source of shrimp

Specific-pathogen-free *L. vannamei* postlarvae were obtained from The Oceanic

Institute (Kailua-Kona, HI) and stocked into 2.44-m diameter fiberglass tanks.

Postlarvae were fed live *Artemia sp.* nauplii and a commercial postlarval feed (Rangen

45/10; Rangen Inc., Buhl, ID) twice and 12 times daily, respectively. Postlarvae were

held approximately 8 weeks to allow for acclimation to laboratory conditions (30.1 ± 0.5

°C, 32.2 ± 0.4‰) and to achieve proper weight for stocking (5.47 g ± 0.29).

Experimental system and design

The experimental system for this study consisted of 400 tanks (19 L volume, bottom surface area 0.09 m^2) connected to a semi-closed (8% new water daily) recirculating seawater system. Seawater was pumped through a sand filter, biological filter, 50-µm cartridge filter, heat exchanger and ultraviolet disinfection unit to achieve a recirculating rate of 0.6 L min^{-1} $tank^{-1}$ (1,440% exchange $tank^{-1}$ day^{-1}). A light:dark photoperiod of 12:12 h was provided by supplemental compact fluorescent lighting. At the start of the experiment shrimp were blotted dry, weighed and stocked individually into each tank. Twenty shrimp were randomly assigned to each constant feed rate (Table 4) for both semi-purified diets. Uneaten feed, feces, molts, and dead shrimp in each tank were removed daily prior to filling wheel-type automatic feeders (set to deliver 15 feedings day^{-1} $tank^{-1}$) with the appropriate experimental feed. Temperature, salinity and dissolved oxygen (DO) were monitored daily using a YSI 85® Meter (YSI Inc., Yellow Springs, OH). Ammonia-nitrogen, NO_2-N, NO_3-N, and pH were monitored weekly using methods adapted from those of Spotte (1979a,b) and Solarzano (1969), Spotte (1979a,b) and Mullen and Riley (1955), Spotte (1979a,b) and Strickland and Parsons (1972), and a Brinkman Metrohm® pH meter, respectively.

Table 4. Feed rates in grams of feed per shrimp per day and per week.

Treatment	Grams of feed per shrimp	
	Per day	Per week
1	0.046	0.322
2	0.073	0.511
3	0.117	0.819
4	0.187	1.309
5	0.300	2.100
6	0.479	3.353
7	0.767	5.369
8	1.227	8.589
9	1.963	13.741
10	3.141	21.987

Sample collection and analyses

To access growth rate shrimp were individually weighed each week throughout the trial. After 7 weeks shrimp were harvested, enumerated, blotted dry and weighed individually by treatment tank. Shrimp were then individually wrapped, labeled and frozen (-84°C) until subsequent body composition analysis. Prior to compositional analysis shrimp were individually lyophilized, finely ground with a coffee grinder (Hamilton Beach/Proctor Silex, Inc., Racine, WI) to pass through a 20-mesh screen and analyzed for percent dry matter (AOAC, 1990). Protein (AOAC Method 990.3; FP-528 Nitrogen/Protein Determinator; Leco Corporation, St. Joseph, MI), energy (model 1241 adiabatic bomb calorimeter; Parr Instrument Co., Moline, IL) and ash (AOAC, 1990) were then determined for each lyophilized sample and reported on a dry-matter basis. Immediately prior to stocking a representative sample of 20 shrimp were individually processed as described above to determine initial body composition.

Statistical analysis

To determine daily protein and energy requirements, shrimp specific growth rate and body composition data were regressed against protein and energy intake by using broken-line regression (Robbins et al., 1979). Maintenance energy and protein requirements were determined by regressing the growth rate back to zero.

Results

Starvation trial

Water quality

Mean ($\pm$ standard deviation) NH_3-N, NO_2-N, NO_3-N and pH were 0.05 ± 0.02 mg L^{-1}, 0.09 ± 0.02 mg L^{-1}, 1.48 ± 1.11 mg L^{-1}, and 8.03 ± 0.02, respectively. Mean ($\pm$ standard deviation) temperature, salinity, and DO were $30.2 \pm 0.47\,^{\circ}C$, 30.5 ± 0.6‰, and 5.99 ± 0.44 mg L^{-1}, respectively.

Allometric equations for whole-body composition

The absolute composition (i.e. g shrimp^{-1} or kJ shrimp^{-1}) of *L. vannamei* changed in a linear fashion as initial shrimp weight increased (Table 5) allowing allometric equations to be fitted for energy, protein, ash and dry matter:

Energy (kJ shrimp^{-1}) = 4.49 * W(g)$^{1.076}$ ($R^2 = 0.988$) (Equation 1)

Protein (g shrimp^{-1}) = 0.157 * W(g)$^{1.103}$ ($R^2 = 0.993$) (Equation 2)

Ash (g shrimp^{-1}) = 0.034 * W(g)$^{0.921}$ ($R^2 = 0.967$) (Equation 3)

Dry matter (g shrimp^{-1}) = 0.236 * W(g)$^{1.047}$ ($R^2 = 0.991$) (Equation 4)

Similar linear increases in *L. vannamei* composition were determined when the initial body composition was analyzed on a wet-weight basis (Table 6) which allowed allometric equations to be fitted for protein:

Protein (%) = 15.67 * W(g)$^{0.103}$ (R^2 = 0.995)   (Equation 5)

Linear increases in body composition were not determined for *L. vannamei* on a dry-matter basis (Table 7).

Allometric equations for daily protein and energy loss

   Daily loss of energy and protein was calculated for each weight class after 28 days of starvation by plotting nutrient losses against *L. vannamei* weight. Shrimp weight (W) was calculated as the geometric mean between the initial (T=0) and final (T=28) shrimp weights. To express the results as metabolic body weight the results were fitted as log-log functions and then transformed to the allometric relationship by taking the antilog. The daily loss of energy and protein per *L. vannamei* can be described by the following allometric functions, respectively:

Daily energy loss per shrimp (kJ shrimp^{-1} day^{-1}): 0.155 * W(g)$^{0.87}$ (Equation 6)

Daily protein loss per shrimp (g shrimp^{-1} day^{-1}): 0.0045 * W(g)$^{0.92}$ (Equation 7)

The above equations can be utilized to express the metabolic weights for energy and protein through the following expressions:

Energy: (g)$^{0.87}$ (Equation 8)

Protein: (g)$^{0.92}$ (Equation 9)

Table 5. Effect of starvation on absolute body composition of four different size classes of *L. vannamei*[1].

	Time in days at slaughter					% Gain (Loss)
	0	7	14	21	28	Over 28 Days
Mean Shrimp Wt. (g)	5.51±0.327	5.05±0.347	4.68±0.320	4.53±0.216	4.67±0.348	(15.24)
Ash (g shrimp^{-1})	0.17±0.017	0.16±0.020	0.14±0.023	0.14±0.012	0.16±0.036	(5.88)
Dry Matter (g shrimp^{-1})	1.38±0.143	1.10±0.094	0.81±0.076	0.66±0.095	0.70±0.102	(49.27)
Energy (kJ shrimp^{-1})	27.65±3.025	20.04±1.857	13.85±1.125	11.09±1.665	11.00±1.635	(60.21)
Protein (g shrimp^{-1})	1.02±0.107	0.85±0.078	0.59±0.059	0.45±0.086	0.43±0.045	(57.84)
Mean Shrimp Wt. (g)	7.19±0.320	6.81±0.342	6.49±0.484	6.09±0.280	6.16±0.239	(14.32)
Ash (g shrimp^{-1})	0.20±0.015	0.19±0.014	0.20±0.029	0.20±0.016	0.20±0.024	0.00
Dry Matter (g shrimp^{-1})	1.87±0.148	1.57±0.106	1.31±0.177	0.97±0.077	0.93±0.082	(50.26)
Energy (kJ shrimp^{-1})	37.95±3.522	29.75±2.167	22.93±3.250	15.52±1.736	14.94±1.539	(60.63)
Protein (g shrimp^{-1})	1.38±0.097	1.24±0.073	1.00±0.142	0.67±0.083	0.64±0.062	(53.62)
Mean Shrimp Wt. (g)	14.10±0.589	14.03±0.550	12.71±0.655	12.54±0.700	12.13±0.716	(13.97)
Ash (g shrimp^{-1})	0.38±0.022	0.40±0.016	0.36±0.034	0.40±0.025	0.42±0.033	9.52
Dry Matter (g shrimp^{-1})	3.76±0.185	3.38±0.169	2.62±0.194	2.31±0.192	2.08±0.187	(44.68)
Energy (kJ shrimp^{-1})	77.65±4.916	66.32±4.158	48.83±4.409	41.04±3.970	35.44±3.824	(54.36)
Protein (g shrimp^{-1})	2.89±0.129	2.75±0.161	2.11±0.179	1.75±0.206	1.50±0.192	(48.09)
Mean Shrimp Wt. (g)	16.59±1.016	16.17±0.842	14.97±0.679	14.73±1.008	13.95±1.049	(15.91)
Ash (g shrimp^{-1})	0.47±0.030	0.47±0.045	0.46±0.024	0.46±0.047	0.43±0.075	(8.51)
Dry Matter (g shrimp^{-1})	4.44±0.246	3.86±0.308	3.22±0.197	2.79±0.276	2.35±0.234	(47.07)
Energy (kJ shrimp^{-1})	91.88±5.372	76.40±7.091	61.55±4.184	51.34±5.464	41.88±3.999	(54.41)
Protein (g shrimp^{-1})	3.49±0.189	3.17±0.254	2.60±0.179	2.20±0.244	1.75±0.169	(49.86)

[1]Means of 10 shrimp ± standard deviation.

Table 6. Effect of starvation on body composition (wet-weight basis) of four different size classes of *L. vannamei*[1].

| | Time in days at slaughter | | | | | % Gain (Loss) |
	0	7	14	21	28	Over 28 Days
Mean Shrimp Wt. (g)	5.51±0.327	5.05±0.347	4.68±0.320	4.53±0.216	4.67±0.348	(20.69)
Ash (%)[2]	3.14±0.351	3.14±0.260	3.07±0.334	3.05±0.197	3.35±0.592	6.69
Moisture (%)	74.54±1.483	78.15±0.813	82.61±0.946	85.31±1.612	85.03±1.291	14.07
Energy (kJ g^{-1})[2]	5.10±0.326	3.97±0.200	2.97±0.163	2.42±0.271	2.34±0.209	(54.10)
Protein (%)[2]	18.73±1.084	16.89±0.866	12.60±0.739	9.82±1.573	9.22±0.427	(50.77)
Mean Shrimp Wt. (g)	7.19±0.320	6.81±0.342	6.49±0.484	6.09±0.280	6.16±0.239	(14.32)
Ash (%)[2]	2.74±0.141	2.85±0.193	3.07±0.278	3.25±0.228	3.20±0.307	16.79
Moisture (%)	73.97±1.606	76.89±1.108	79.93±1.430	84.05±1.171	84.86±1.119	14.72
Energy (kJ g^{-1})[2]	5.27±0.380	4.35±0.238	3.51±0.284	2.55±0.263	2.42±0.221	(53.97)
Protein (%)[2]	19.16±0.996	18.18±0.666	15.40±1.195	11.09±1.345	10.35±0.847	(45.98)
Mean Shrimp Wt. (g)	14.10±0.589	14.03±0.550	12.71±0.655	12.54±0.700	12.13±0.716	(13.97)
Ash (%)[2]	2.69±0.091	2.88±0.084	2.84±0.182	3.23±0.208	3.46±0.185	28.62
Moisture (%)	73.29±0.800	75.89±0.813	79.35±0.651	81.56±0.714	82.87±1.123	13.07
Energy (kJ g^{-1})[2]	5.52±0.255	4.72±0.225	3.84±0.184	3.26±0.179	2.92±0.263	(46.97)
Protein (%)[2]	20.50±0.638	19.55±0.623	16.57±0.693	13.94±1.048	12.40±1.364	(39.51)
Mean Shrimp Wt. (g)	16.59±1.016	16.17±0.842	14.97±0.679	14.73±1.008	13.95±1.049	(15.91)
Ash (%)[2]	2.83±0.076	2.91±0.229	2.89±0.160	3.12±0.216	3.04±0.353	7.42
Moisture (%)	73.18±0.678	76.17±0.815	78.51±0.778	81.10±0.780	83.13±0.860	13.60
Energy (kJ g^{-1})[2]	5.52±0.217	4.72±0.238	4.10±0.179	3.47±0.184	3.01±0.175	(45.45)
Protein (%)[2]	21.02±0.529	19.57±0.770	17.36±0.766	14.92±0.919	12.52±0.656	(40.44)

[1]Means of 10 shrimp ± standard deviation.
[2]Results expressed on a wet-weight basis.

Table 7. Effect of starvation on body composition (dry-matter basis) of four different size classes of *L. vannamei*[1].

| | \multicolumn{5}{c}{Time in days at slaughter} | % Gain (Loss) |
	0	7	14	21	28	Over 28 Days
Mean Shrimp Wt. (g)	5.51±0.327	5.05±0.347	4.68±0.320	4.53±0.216	4.67±0.348	(15.24)
Ash (%)[2]	12.34±1.253	14.39±1.361	17.66±1.647	20.88±1.645	22.28±1.906	80.55
Moisture (%)	74.54±1.483	78.15±0.813	82.61±0.946	85.31±1.612	85.03±1.291	14.07
Energy (kJ g^{-1})[2]	19.99±3.037	18.15±0.69	17.02±0.598	16.61±0.744	15.69±0.439	(21.55)
Protein (%)[2]	73.62±1.739	77.28±2.298	72.48±1.682	66.64±5.069	61.74±3.443	(16.14)
Mean Shrimp Wt. (g)	7.19±0.320	6.81±0.342	6.49±0.484	6.09±0.280	6.16±0.239	(14.32)
Ash (%)[2]	10.56±0.575	12.36±1.139	15.31±0.900	20.48±2.276	21.13±1.335	100.00
Moisture (%)	73.97±1.606	76.89±1.108	79.93±1.430	84.05±1.171	84.86±1.119	14.72
Energy (kJ g^{-1})[2]	20.25±0.456	18.91±0.380	17.53±0.393	15.98±0.648	16.02±0.615	(20.87)
Protein (%)[2]	73.68±1.180	78.73±1.427	76.71±1.376	69.31±3.735	68.34±2.361	(7.24)
Mean Shrimp Wt. (g)	14.10±0.589	14.03±0.550	12.71±0.655	12.54±0.700	12.13±0.716	(13.97)
Ash (%)[2]	10.08±0.212	11.97±0.618	13.75±0.966	17.53±1.410	20.27±1.848	101.09
Moisture (%)	73.29±0.800	75.89±0.813	79.35±0.651	81.56±0.714	82.87±1.123	13.07
Energy (kJ g^{-1})[2]	20.58±0.418	19.58±0.418	18.57±0.506	17.69±0.351	17.02±0.523	(17.28)
Protein (%)[2]	76.77±0.821	81.08±1.432	80.23±1.222	75.46±2.985	72.19±3.566	(5.97)
Mean Shrimp Wt. (g)	16.59±1.016	16.17±0.842	14.97±0.679	14.73±1.008	13.95±1.049	(15.91)
Ash (%)[2]	10.55±0.308	12.21±0.952	13.46±0.675	16.55±1.241	18.02±1.806	70.80
Moisture (%)	73.18±0.678	76.17±0.815	78.51±0.778	81.10±0.780	83.13±0.860	13.60
Energy (kJ g^{-1})[2]	20.66±0.364	19.79±0.472	19.12±0.271	18.40±0.414	17.82±0.430	(13.77)
Protein (%)[2]	78.42±1.678	82.12±1.319	80.77±2.039	78.91±2.441	74.21±1.156	(5.37)

[1]Means of 10 shrimp ± standard deviation.
[2]Results expressed on a dry-matter basis.

Preliminary evaluation of experimental diets

Water quality

Mean (± standard deviation) NH_3-N, NO_2-N, NO_3-N and pH were 0.12 ± 0.03 mg L^{-1}, 0.22 ± 0.07 mg L^{-1}, 5.48 ± 1.09 mg L^{-1}, and 8.01 ± 0.1, respectively. Mean (± standard deviation) temperature, salinity, and DO were $30.3 \pm 0.33\,^{o}C$, $31.6 \pm 0.5‰$, and 5.99 ± 0.21 mg L^{-1}, respectively.

Growth and survival of L. vannamei fed preliminary diets

Results from the preliminary growth trial are summarized in Table 8. Diet 100 (A.L.L. reference diet) produced significantly ($P<0.05$) greater responses than all other diets for the measured growth metrics (ln final weight gain, ln weight gain, IGR, and percent growth). Survival was high (95-100%) for all dietary treatments and was not significantly different among treatments ($P>0.05$). No significant differences were determined between the 35 and 25% crude protein diets (diets 101 and 102, respectively) or between these diets and Zeigler 35% Hi-density or Rangen 45/10 commercial feeds (diets 114 and 113, respectively). Dietary adjustments (+/- 33%) of magnesium oxide (diets 106 and 107), vitamin and mineral premixes (diets 109 and 110) or the Ca:P ratio (diets 111 and 112) had no significant effect on measured growth metrics for either the basal 35 or 25% crude protein diets. Adjustment of the methionine content to 0.85% (diet 103) or removal of 33% vitamin C (diet 108) from the 25% crude protein diet also had no significant effect on measured growth matrices. Similarly, adjustment of the ash and fiber contents (diet 104) or the squid meal:krill meal:fish meal ratio (diet 105) in the 35% crude protein diet had no significant effect on measured growth metrics.

Table 8. Response of *L. vannamei* fed preliminary diets.[1,2]

Diet	ln Final Weight (g)	ln Weight Gain (g)	IGR	Growth (%)	Survival (%)
100	2.54[b](0.13)	2.26[b](0.35)	3.82[b](0.64)	384[b](70)	100[a](0)
101	2.32[a](0.19)	2.03[a](0.23)	3.31[a](0.40)	306[a](69)	100[a](0)
102	2.31[a](0.14)	2.03[a](0.19)	3.44[a](0.42)	332[a](78)	100[a](0)
103	2.31[a](0.15)	2.02[a](0.19)	3.33[a](0.40)	311[a](68)	100[a](0)
104	2.21[a](0.13)	1.90[a](0.17)	3.18[a](0.36)	285[a](60)	95[a](8.43)
105	2.30[a](0.15)	2.00[a](0.20)	3.26[a](0.40)	298[a](70)	95[a](8.43)
106	2.35[a](0.16)	2.01[a](0.28)	3.30[a](0.44)	307[a](72)	95[a](8.43)
107	2.34[a](0.21)	1.90[a](0.27)	3.04[a](0.44)	266[a](67)	100[a](0)
108	2.31[a](0.22)	2.06[a](0.22)	3.35[a](0.41)	313[a](67)	100[a](0)
109	2.23[a](0.19)	1.93[a](0.23)	3.19[a](0.39)	287[a](65)	95[a](8.43)
110	2.31[a](0.21)	2.02[a](0.26)	3.35[a](0.49)	318[a](94)	95[a](8.43)
111	2.27[a](0.14)	1.97[a](0.19)	3.26[a](0.42)	300[a](69)	100[a](0)
112	2.29[a](0.15)	1.99[a](0.22)	3.22[a](0.47)	293[a](76)	100[a](0)
113	2.32[a](0.15)	2.04[a](0.22)	3.38[a](0.43)	320[a](78)	100[a](0)
114	2.21[a](0.17)	1.89[a](0.22)	3.19[a](0.39)	287[a](63)	100[a](0)

[1]Means of 20 replicates (SD)
[2]Means with similar superscripts in same column are not statistically different ($P>0.05$)

Diet ID:
100 – Shrimp Mariculture Project (A.L.L.) Reference Diet
101 – 35% Crude Protein Diet
102 – 25% Crude Protein Diet
103 – Diet 102 with Methionine Increased to 0.85%
104 – Diet 101 with Ash and Fiber Increased to 24 and 10%, respectively
105 – Diet 101 with Squid Meal : Krill Meal : Fish Meal Ratio Adjusted to 15 : 10.5 : 15
106 – Diet 101 with 1/3 Less MgO
107 – Diet 102 with 1/3 Less MgO
108 – Diet 102 with 1/3 Less Vitamin C
109 – Diet 101 with 1/3 Less Vitamins and Minerals
110 – Diet 102 with 1/3 Less Vitamins and Minerals
111 – Diet 101 with 1/3 Less Ca : P
112 – Diet 102 with 1/3 Less Ca : P
113 – Rangen 45/15 Commercial Feed
114 – Zeigler 35% Hi-Density Commercial Feed

Growth and survival trial

Water quality

Mean (± standard deviation) NH_3-N, NO_2-N, NO_3-N and pH were 0.09 ± 0.01 mg L^{-1}, 0.11 ± 0.03 mg L^{-1}, 3.74 ± 1.12 mg L^{-1}, and 8.01 ± 0.2, respectively. Mean (± standard deviation) temperature, salinity, and DO were $30.5 \pm 0.41\,^{o}C$, 30.8 ± 0.4‰, and 5.97 ± 0.39 mg L^{-1}, respectively.

Apparent consumption

To determine apparent consumption, feed rate was regressed against maximum weight gain for the 25 and 35% protein diets (Figures 1 and 2; respectively). Apparent consumption for the shrimp fed the 25% protein diet was estimated to be 0.32 g feed day^{-1} $shrimp^{-1}$ and was obtained from the regression equation: apparent consumption = $1383.57 + 42921.56x$ with a correlation coefficient (C) of 15287.77 as follows: apparent consumption = $(C - B_o)/B_1$. Apparent consumption for the 35% protein diet was estimated to be 0.31 g feed day^{-1} $shrimp^{-1}$ and was obtained from the regression equation: apparent consumption = $415.17 + 50039.30x$ with an correlation coefficient (C) of 16189.82 as described above.

Conversion efficiencies

Protein and energy conversion efficiencies increased with feed rate until shrimp received 0.063 g DP $shrimp^{-1}$ day^{-1} (4.00 kJ DE $shrimp^{-1}$ day^{-1}) of the 25% protein diet (Table 9) or 0.095 g DP $shrimp^{-1}$ day^{-1} (3.96 kJ DE $shrimp^{-1}$ day^{-1}) of the 35% protein diet (Table 10); then progressively decreased as feed rates increased. Protein conversion efficiency for maximum growth was more efficient for the 25% protein diet than the

35% protein diet; however, energy conversion efficiency for maximum growth was less

efficient. Digestible protein and digestible energy feed rates which provided the most

efficient protein and energy conversion also provided the best feed conversion ratio

(FCR) and feed efficiency ratio (FER) for the 35% protein diet (Table 11); however, the

best FCR and FER was obtained for the 25% protein diet when shrimp were fed 0.039 g

DP shrimp^{-1} day^{-1} (2.49 kJ DE shrimp^{-1} day^{-1}; Table 12). Similar decreases in FCR and

FER were witnessed for both diets as feed rates increased above those which provided

the best growth.
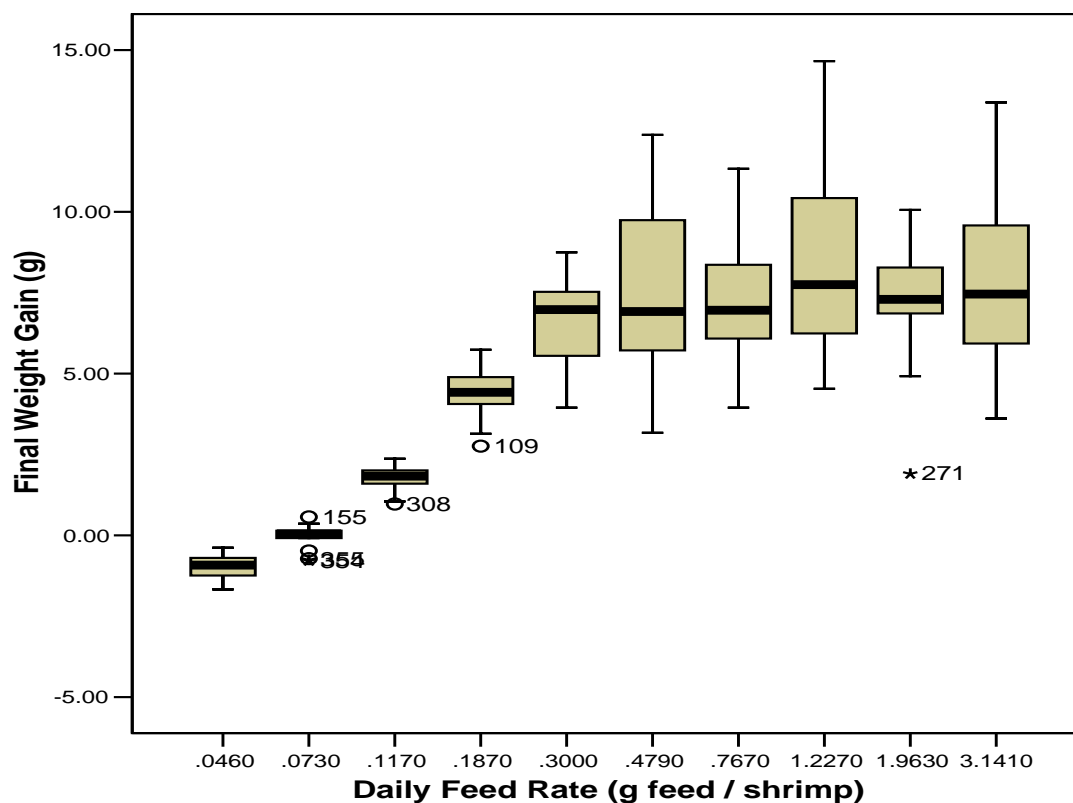
Figure 1. Weight gain (g) vs. daily feed rate (g feed/shrimp) for *Litopenaeus vannamei* fed a 25% protein, 15.89 kJ g^{-1} diet.
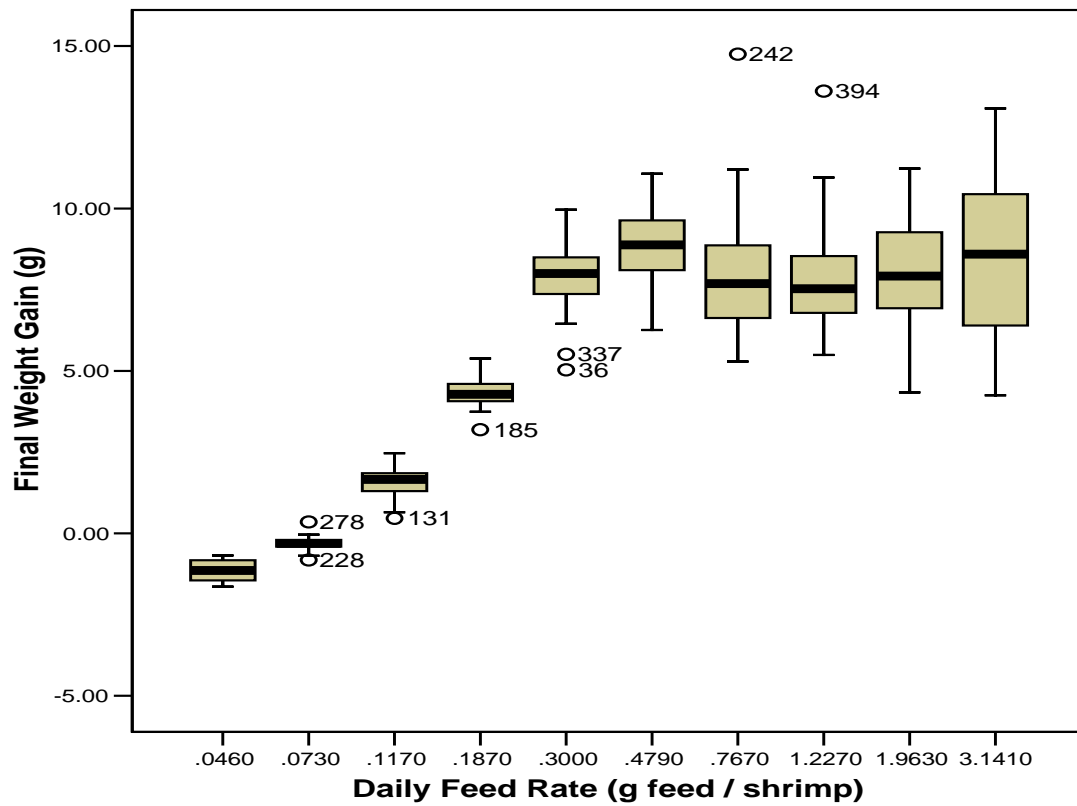
Figure 2. Weight gain (g) vs. daily feed rate (g feed/shrimp) for *Litopenaeus vannamei* fed a 35% protein, 15.48 kJ g^{-1} diet.

Table 9. Energy and protein conversion efficiencies of *L. vannamei* fed incremental levels of a 25% crude protein, 15.89 kJ g^{-1} diet[1].

| Daily Feeding Rate | | Energy Conversion | Protein Conversion |
Protein (g DP shrimp^{-1})	Energy (kJ DE shrimp^{-1})	Efficiency[2]	Efficiency[3]
0.000	0.00	0	0
0.010	0.615	-45.32	-96.09
0.015	0.974	-16.30	-25.53
0.024	1.564	0.60	12.80
0.039	2.497	10.86	34.61
0.063	4.008	12.63	35.95
0.100	6.401	9.60	26.36
0.160	10.250	5.72	15.68
0.256	16.397	4.13	11.41
0.409	26.233	2.33	6.38
0.655	41.978	1.48	4.06

[1]Results based on 20 shrimp.
[2]Final body protein-initial body protein x 100 (total protein fed)$^{-1}$.
[3]Final body energy-initial body energy x 100 (total energy fed)$^{-1}$.

Table 10. Energy and protein conversion efficiencies of *L. vannamei* fed incremental levels of a 35% crude protein, 15.48 kJ g^{-1} diet[1].

Daily Feeding Rate		Energy Conversion Efficiency[2]	Protein Conversion Efficiency[3]
Protein (g DP shrimp^{-1})	Energy (kJ DE shrimp^{-1})		
0.000	0.000	0	0
0.015	0.606	-50.29	-77.00
0.023	0.966	-20.06	-25.21
0.037	1.548	-2.33	3.76
0.059	2.472	11.77	25.78
0.095	3.966	15.45	30.64
0.152	6.334	11.81	22.55
0.244	10.142	6.31	12.32
0.390	16.225	4.05	7.73
0.624	25.957	2.75	5.14
0.999	41.534	1.74	3.31

[1]Results based on 20 shrimp.
[2]Final body protein-initial body protein x 100 (total protein fed)$^{-1}$.
[3]Final body energy-initial body energy x 100 (total energy fed)$^{-1}$.

Table 11. Growth and survival estimates of *L. vannamei* fed incremental levels of a 35% crude protein, 15.48 kJ g^{-1} diet over 49 days.[1]

Daily Feeding Rate		Final Mean Weight (g)	Grams Week	Survival (%)	FCR[2]	FER[3]
Protein (g DP shrimp^{-1})	Energy (kJ DE shrimp^{-1})					
0.000	0.000	4.31±0.279[4]	-0.29±0.040[4]	N/A	0	0
0.015	0.606	4.28±0.368	-0.17±0.050	65.0	-2.10	-0.52
0.023	0.966	5.05±0.304	-0.04±0.036	95.0	-15.34	-0.09
0.037	1.548	6.65±0.487	0.22±0.074	85.0	4.46	0.27
0.059	2.472	9.73±0.482	0.61±0.068	100	2.15	0.47
0.095	3.966	13.34±1.230	1.11±0.164	100	1.93	0.53
0.152	6.334	14.27±1.337	1.26±0.179	95.0	2.72	0.38
0.244	10.142	13.65±2.325	1.17±0.331	95.0	4.88	0.22
0.390	16.225	13.44±2.009	1.14±0.271	95.0	7.87	0.13
0.624	25.957	13.55±1.544	1.14±0.231	85.0	12.60	0.08
0.999	41.534	14.32±2.502	1.23±0.352	90.0	19.49	0.06

[1]Means based on 20 shrimp ± standard deviation.
[2]Feed conversion ratio = dry weight feed (wet weight gain)$^{-1}$.
[3]Feed efficiency ratio = wet weight gain (dry weight feed)$^{-1}$.
[4]Measured at 28 days.

Table 12. Growth and survival estimates of *L. vannamei* fed incremental levels of a 25% crude protein, 15.89 kJ g^{-1} diet over 49 days.[1]

Daily Feeding Rate		Final Mean Weight (g)	Grams Week	Survival (%)	FCR[2]	FER[3]
Protein (g DP shrimp^{-1})	Energy (kJ DE shrimp^{-1})					
0.000	0.000	4.31±0.279[4]	-0.29±0.040[4]	N/A	0	0
0.010	0.615	4.54±0.375	-0.14±0.050	80.0	-2.70	-0.43
0.015	0.974	5.57±0.366	-0.01±0.052	100	29.18	-0.01
0.024	1.564	7.11±0.408	0.25±0.053	90.0	3.38	0.31
0.039	2.497	9.91±0.694	0.64±0.107	90.0	2.11	0.49
0.063	4.008	12.10±1.434	0.94±0.208	95.0	2.35	0.45
0.100	6.401	13.06±2.530	1.08±0.366	100	3.49	0.32
0.160	10.250	12.45±1.742	1.01±0.245	95.0	5.63	0.19
0.256	16.397	13.84±2.801	1.19±0.393	100	7.99	0.14
0.409	26.233	12.67±1.856	1.03±0.261	90.0	15.26	0.07
0.655	41.978	13.17±2.763	1.10±0.385	90.0	22.69	0.05

[1]Means based on 20 shrimp ± standard deviation.
[2]Feed conversion ratio = dry weight feed (wet weight gain)$^{-1}$.
[3]Feed efficiency ratio = wet weight gain (dry weight feed)$^{-1}$.
[4]Measured at 28 days.

Apparent daily digestible protein requirements for growth and maintenance

Daily digestible protein (DP) requirements for shrimp fed increasing feed rates of the 25% crude protein diet produced linear increases in weight gain up to 0.065 g DP shrimp^{-1} day^{-1} for a 11.28 g shrimp to 0.069 g DP shrimp^{-1} day^{-1} for a 7.69 g shrimp (Table 13). Regression equations for the linear growth portion could best be described by the following equations, where y = growth response and x = feed rate in g DP: y = -1.246 + 97.885x (R^2 = 0.992) and y = -0.350 + 33.504x (R^2 = 0.989) for the 11.28 and 7.69 g shrimp, respectively. Maintenance DP requirements were between 0.009 g DP shrimp^{-1} day^{-1} for a 7.69 g shrimp to 0.014 g DP shrimp^{-1} day^{-1} for a 13.08-g shrimp and were determined by regressing DP feed rate back to zero weight gain.

Table 13. Weekly apparent protein requirements (g DP shrimp^{-1} day^{-1}) for maximum growth and maintenance of *L. vannamei* fed a 25% crude protein, 15.89 kJ g^{-1} diet obtained by regressing weight gain onto provided protein (g-DP protein shrimp^{-1} day^{-1}).

No. of	Protein Requirement (g DP Shrimp^{-1} Day^{-1})		
Weeks	Max. Wt. Gain	Maintenance	Mean Max. Wt. Shrimp (g)
2	0.069	0.009	7.69
3	0.067	0.009	9.07
4	0.066	0.010	10.23
5	0.065	0.012	11.28
6	0.067	0.012	12.36
7	0.066	0.014	13.08
Mean[1]	0.067±0.001	0.011±0.002	

[1]Mean protein requirements ± standard deviation.

Shrimp responded to increased feed rates by decreasing their percent body ash and moisture content while increasing protein content until the feed rate reached 0.063 g DP shrimp^{-1} day^{-1} (Table 14). Increases in feed rate above this level had minimal effect

on the rates of change for these body composition components. This plateau in body

protein allowed daily DP requirements for a 13.08-g shrimp to be estimated by

regressing net changes in body protein with increases in DP feeding rates (Table 15).

Net changes in body protein increased linearly ($R^2 = 0.971$) with increased DP feed rates

up to 0.066 g DP shrimp^{-1} day^{-1} and could best be described by the linear equation: y =

0.027 + 31.559x, where y = net change in body protein and x = feed rate in g DP.

Maintenance DP requirements were estimated at 0.014 g DP shrimp^{-1} day^{-1} for an 13.08-

g shrimp by regressing DP feed rate back to zero weight gain.

Daily DP requirements for shrimp fed increasing quantities of the 35% protein

diet were numerically higher than shrimp fed the 25% protein diet for the duration of the

trial. Daily DP requirements for shrimp fed the 35% protein diet were between 0.083 g

DP shrimp^{-1} day^{-1} for an 8.11-g shrimp to 0.098 g DP shrimp^{-1} day^{-1} for an 13.79 g

shrimp with the linear growth portion best described by the following equations, where y

= growth response and x = feed rate in g DP: y = -0.715 + 36.512x ($R^2 = 0.984$) and y =

-2.736 + 101.979 ($R^2 = 0.990$); respectively (Table 16). Regressing DP feed rates back

to zero weight gain produced maintenance DP requirements between 0.017 g DP shrimp^{-}

^{1} day^{-1} for an 8.11-g shrimp to 0.024 g DP shrimp^{-1} day^{-1} for an 13.79-g shrimp which

were higher than values obtained for shrimp fed the 25% protein diet.

Table 14. Body composition of *L. vannamei* fed incremental levels of a 25% crude protein, 15.89 kJ g^{-1} diet over 49 days.[1]

Daily Feeding Rate		Dry Matter	Energy	Protein	Ash
Protein (g DP shrimp^{-1})	Energy (kJ DE shrimp^{-1})	(g shrimp^{-1})	(kJ shrimp^{-1})[2]	(g shrimp^{-1})[2]	(g shrimp^{-1})[2]
0.000	0.00	0.613±0.070[3]	9.752±1.230[3]	0.416±0.062[3]	0.129±0.013[3]
0.010	0.615	0.675±0.110	11.413±1.820	0.475±0.082	0.134±0.022
0.015	0.974	1.041±0.096	18.388±1.876	0.789±0.080	0.163±0.011
0.024	1.564	1.539±0.117	28.208±2.476	1.202±0.108	0.223±0.010
0.039	2.497	2.280±0.144	43.484±2.928	1.813±0.123	0.296±0.031
0.063	4.008	2.935±0.334	57.182±6.656	2.343±0.249	0.366±0.054
0.100	6.401	3.254±0.634	63.500±12.899	2.569±0.508	0.378±0.056
0.160	10.250	3.126±0.501	61.847±10.853	2.495±0.411	0.344±0.049
0.256	16.397	3.448±0.658	67.136±12.355	2.738±0.508	0.424±0.093
0.409	26.233	3.196±0.571	63249±11.945	2.557±0.462	0.355±0.061
0.655	41.978	3.234±0.628	63.877±12.568	2.585±0.507	0.398±0.105
Initial		1.329±0.122	25.451±2.489	0.957±0.071	0.160±0.019

[1]Means of 20 shrimp ± standard deviation.
[2]Results expressed on a dry-matter basis.
[3]Results based on 28 days of starvation.

Table 15. Apparent protein requirement (DP shrimp^{-1} day^{-1}) for maximum growth and maintenance of *L. vannamei* fed a 25% crude protein, 15.89 kJ g^{-1} diet obtained by regressing change in body protein onto provided protein (g-DP protein shrimp^{-1} day^{-1}).

No. of	Protein Requirement (g DP Shrimp^{-1} Day^{-1})		
Weeks	Max. Wt. Gain	Maintenance	Mean Max. Wt. Shrimp (g)
7	0.066	0.014	13.08

Table 16. Weekly apparent protein requirements (g DP shrimp^{-1} day^{-1}) for maximum growth and maintenance of *L. vannamei* fed a 35% crude protein, 15.48 kJ g^{-1} diet obtained by regressing weight gain onto provided protein (g-DP protein shrimp^{-1} day^{-1}).

No. of	Protein Requirement (g DP Shrimp^{-1} Day^{-1})		
Weeks	Max. Wt. Gain	Maintenance	Mean Max. Wt. Shrimp (g)
2	0.083	0.017	8.11
3	0.083	0.021	9.60
4	0.086	0.021	10.92
5	0.095	0.021	12.08
6	0.096	0.023	13.01
7	0.098	0.024	13.79
Mean[1]	0.090±0.007	0.021±0.002	

[1]Mean protein requirements ± standard deviation.

As feed rate increased from 0.015 to 0.095 g DP shrimp^{-1} day^{-1} shrimp responded by decreasing their percent body moisture and ash while increasing percent protein (Table 17). Shrimp fed more than 0.095 g DP shrimp^{-1} day^{-1} had minimal increases in percent ash, moisture and protein which allowed changes in net body protein to be regressed with increases in DP feeding rates to estimate daily DP requirements for the 13.79 g shrimp (Table 18). Increased DP feed rates produced linear ($R^2 = 0.995$) increases in body protein up to 0.098 g DP shrimp^{-1} day^{-1} and could best be described by the linear equation: $y = 0.083 + 24.729x$, where y = net change in body protein and x = feed rate in g DP. Maintenance DP requirements were estimated at 0.030 g DP shrimp^{-1}

day^{-1} for the 13.79-g shrimp by regressing DP feed rate back to zero weight gain.

Apparent daily digestible energy requirements for growth and maintenance

Daily DE requirements for shrimp fed at increasing rates with the 25% crude protein diet produced linear increases in weight gain up to 4.171 kJ DE shrimp^{-1} day^{-1} for a 11.28-g shrimp, to 4.422 kJ DE shrimp^{-1} day^{-1} for a 7.69-g shrimp (Table 19). Linear growth portions can best be described by the following regression equations, where y = growth response and x = feed rate in kJ DE: y = -1.246 + 6386.905x (R^2= 0.992) and y = -0.350 + 2186.113x (R^2 = 0.989) for the 11.28 and 7.96 g shrimp, respectively. Regressing DE feed rate back to zero weight gain resulted in maintenance DE requirements between 0.564 kJ DE shrimp^{-1} day^{-1} for an 9.07-g shrimp to 0.887 kJ DE shrimp^{-1} day^{-1} for an 13.08-g shrimp.

Shrimp which were fed increasing quantities of feed experienced a decrease in percent body moisture and ash and an increase in energy (kJ g^{-1}) until the feed rate reached 4.008 kJ DE shrimp^{-1} day^{-1} (Table 14). Feed rates above 4.008 kJ DE shrimp^{-1} day^{-1} had a minimal effect on body energy which allowed changes in net body energy to be regressed with increases in DE feeding rates to estimate daily DE requirements for the 13.08-g shrimp (Table 20). Increased DE feed rates produced linear (R^2 = 0.971) increases in body energy up to 4.330 kJ DE shrimp^{-1} day^{-1} and could best be described by the linear equation: y = 1383.568 + 12152.384x, where y = net change in body energy and x = feed rate in kJ DE. Maintenance DE requirements were estimated at 0.891 kJ DE shrimp^{-1} day^{-1} for the 13.08-g shrimp by regressing DE feed rate back to zero weight gain.

Table 17. Body composition of *L. vannamei* fed incremental levels of a 35% crude protein, 15.48 kJ g^{-1} diet over 49 days[1].

Daily Feeding Rate		Dry Matter	Energy	Protein	Ash
Protein (g DP shrimp^{-1})	Energy (kJ DE shrimp^{-1})	(g shrimp^{-1})	(kJ shrimp^{-1})[2]	(g shrimp^{-1})[2]	(g shrimp^{-1})[2]
0.000	0.000	0.613±0.070[3]	9.752±1.230[3]	0.416±0.062[3]	0.129±0.013[3]
0.015	0.606	0.595±0.071	10.112±1.673	0.406±0.062	0.132±0.016
0.023	0.966	0.943±0.076	16.551±1.451	0.700±0.069	0.156±0.011
0.037	1.548	1.401±0.104	25.593±2.100	1.094±0.100	0.217±0.025
0.059	2.472	2.321±0.549	44.358±10.890	1.851±0.424	0.320±0.073
0.095	3.966	3.214±0.365	62.818±7.623	2.606±0.316	0.370±0.044
0.152	6.334	3.584±0.296	70.558±5.619	2.884±0.223	0.428±0.055
0.244	10.142	3.286±0.592	64.400±11.451	2.651±0.489	0.393±0.069
0.390	16.225	3.309±0.473	65.383±9.275	2.657±0.383	0.405±0.080
0.624	25.957	3.468±0.409	68.579±8.317	2.761±0.326	0.405±0.050
0.999	41.534	3.519±0.573	70.023±11.878	2.847±0.488	0.390±0.065
Initial		1.329±0.122	25.451±2.489	0.957±0.071	0.160±0.019

[1]Means of 20 shrimp ± standard deviation.
[2]Results expressed on a dry-matter basis.
[3]Results based on 28 days of starvation.

Table 18. Apparent protein requirement (g DP shrimp^{-1} day^{-1}) for maximum growth and maintenance of *L. vannamei* fed a 35% crude protein, 15.48 kJ g^{-1} diet obtained by regressing change in body protein onto provided protein (g-DP protein shrimp^{-1} day^{-1}).

No. of	Protein Requirement (g DP Shrimp^{-1} Day^{-1})		Mean Max. Wt. Shrimp (g)
Weeks	Max. Wt. Gain	Maintenance	
7	0.098	0.030	13.79

Table 19. Weekly apparent energy requirements (kJ DE shrimp^{-1} day^{-1}) for maximum growth and maintenance of *L. vannamei* fed a 25% crude protein, 15.89 kJ g^{-1} diet obtained by regressing weight gain onto provided energy (kJ shrimp^{-1} day^{-1}).

No. of	Energy Requirement (kJ DE Shrimp^{-1} Day^{-1})		Mean Max. Wt. Shrimp (g)
Weeks	Max. Wt. Gain	Maintenance	
2	4.426	0.606	7.69
3	4.276	0.564	9.07
4	4.250	0.644	10.23
5	4.171	0.736	11.28
6	4.292	0.782	12.36
7	4.238	0.887	13.08
Mean[1]	4.275±0.083	0.703±0.121	

[1]Mean energy requirements ± standard deviation.

Table 20. Apparent energy requirement (DE shrimp^{-1} day^{-1}) for maximum growth and maintenance of *L. vannamei* fed a 25% crude protein, 15.89 kJ g^{-1} diet obtained by regressing change in body energy onto provided energy (kJ DE shrimp^{-1} day^{-1}).

No. of	Energy Requirement (kJ DE Shrimp^{-1} Day^{-1})		Mean Max. Wt. Shrimp (g)
Weeks	Max. Wt. Gain	Maintenance	
7	4.330	0.891	13.08

Daily DE requirements for shrimp fed the 35% protein diet were between 3.460 kJ DE shrimp^{-1} day^{-1} for an 9.60-g shrimp to 4.091 kJ DE shrimp^{-1} day^{-1} for an 13.79-g shrimp with the linear growth portion best described by the following equations, where y = growth response and x = feed rate in kJ DE: -1.367 + 6036.066x (R^2 = 0.989) and y = -2.736 + 10260.754x (R^2 = 0.990); respectively (Table 21). Regressing DE feed intake

back to zero weight gain produced maintenance DE requirements between 0.740 kJ DE

shrimp^{-1} day^{-1} for an 8.11-g shrimp to 1.012 kJ DE shrimp^{-1} day^{-1} for an 13.79-g shrimp

which were higher than values obtained for the shrimp fed the 25% protein diet.

Table 21. Weekly apparent energy requirements (kJ DE shrimp^{-1} day^{-1}) for maximum growth and maintenance of *L. vannamei* fed a 35% crude protein, 15.48 kJ g^{-1} diet obtained by regressing weight gain onto provided energy (kJ shrimp^{-1} day^{-1}).

No. of Weeks	Energy Requirement (kJ DE Shrimp^{-1} Day^{-1})		Mean Max. Wt. Shrimp (g)
	Max. Wt. Gain	Maintenance	
2	3.476	0.740	8.11
3	3.460	0.861	9.60
4	3.581	0.878	10.92
5	3.974	0.882	12.08
6	4.012	0.945	13.01
7	4.091	1.012	13.79
Mean[1]	3.765±0.288	0.886±0.092	

[1]Mean energy requirements ± standard deviation.

Percent body ash and moisture content decreased while energy content (kJ g^{-1})

increased as feed rate increased to 3.966 kJ DE shrimp^{-1} day^{-1} (Table 17). Feed rates

between 3.966 kJ DE shrimp^{-1} day^{-1} and 41.534 kJ DE shrimp^{-1} day^{-1} only had a minimal

effect on body energy which allowed daily DE requirements to be estimated for the

13.79-g shrimp by regressing net changes in body energy with increases in DE feeding

rates (Table 22). Net changes in body energy increased linearly ($R^2 = 0.995$) with

increased DE feed rates up to 4.167 kJ DE shrimp^{-1} day^{-1} and could best be described by

the linear equation: $y = 918.932 + 13323.856x$, where y = net body change in energy and

x = feed rate in kJ DE. Maintenance DE requirements were estimated at 1.096 kJ DE

shrimp^{-1} day^{-1} for the 13.79 g shrimp by regressing DE feed rate back to zero weight gain.

Table 22. Apparent energy requirement (kJ DE shrimp^{-1} day^{-1}) for maximum growth and maintenance of *L. vannamei* fed a 35% crude protein, 15.48 kJ g^{-1} diet obtained by regressing change in body energy onto provided energy (kJ DE shrimp^{-1} day^{-1}).

No. of	Energy Requirement (kJ DE Shrimp^{-1} Day^{-1})		Mean Max. Wt. Shrimp (g)
Weeks	Max. Wt. Gain	Maintenance	
7	4.167	1.096	13.79

Discussion

Starvation trial

Water quality

To assure these inorganic compounds do no interfere with the experiment, ammonia-N should be maintained below 2.37 mg L^{-1} (0.09 mg L^{-1} for NH sub(3)-N (Chen and Lin 1991), nitrite levels below 2.04 mg L^{-1} (Chen and Lin, 1991) and nitrate should be below 25 mg L^{-1} (Chen and Lei, 1990). Values obtained during the experiment were well below these recommendations which suggest shrimp were maintained under optimal water quality parameters for the duration of the 4-week trial.

Allometric equations for whole-body composition

The absolute energy, protein, ash and dry matter content of shrimp increased in a linear fashion with growth (5.51 to 16.59 g) (equations 1, 2, 3 and 4, respectively), while only protein content (g 100 g^{-1} live weight) increased linearly (equation 5) with growth on a wet-weight basis. While no studies exist for shrimp, allometric equations determined for carp (Pfeffer and Potthast, 1977), trout (Pfeffer and Potthast, 1977) and

gilthead seabream (Lupatsch et al., 1998) suggest protein content per kg live weight does not change significantly with fish size, while lipid content increases. Shrimp have a limited ability to store lipid outside their hepatopancreas while fish and higher animals have the ability to deposit lipid stores in various tissues as they reach maturity. This physiological difference may explain why a linear increase in protein deposition (equation 5) was witnessed as shrimp growth increased; however, it is not currently known what effect sexual maturity may have on the linearity of the protein increase relative to body composition.

Allometric equations for daily protein and energy loss

The relationship between energy changes and body weight (equation 6) for *L. vannamei* maintained at 30$^{\text{o}}$C could best be described when shrimp body weight was raised to the power 0.87 (equation 8). No studies involving shrimp have utilized energy loss during starvation to determine the exponent b of metabolic body weight (a x BW(g)$^{\text{b}}$) which is proportional to the maintenance requirement for energy; however metabolic-body weight relationships are commonly determined for fish (Cui and Liu, 1990; Cho 1992; Lupatsch et al., 1998; Lupatsch et al., 2003). The exponent most commonly referenced in fish studies is 0.80 (Brett and Groves, 1979); however values range from 0.824 in trout (Cho 1992) to 0.86 in African catfish (Hogendoorn, 1983). The value obtained for shrimp in this study (0.87) is close to the values obtained by Cui and Liu (1990) for 6 different teleost species (0.855) and by Hogendoorn (1983) for African catfish (0.86); however, the daily energy loss during starvation for *L. vannamei* was approximately six times greater when compared to African catfish when referring to

a common metabolic weight of $(kg)^{0.86}$. This high energy loss may be attributed to the energetic inefficiency of utilizing body protein as energy, opposed to lipid reserves in fish, the loss of energy (~1.4 kJ per cast; Read and Caulton, 1980) attributed to the export of the cast during the molt cycle as well as from ejection of an peritrophic membrane by unfed shrimp (unpublished data, in Cordova-Murueta et al., 2003). These physiological differences may help explain why *L. vannamei* is unable to survive starvation periods greater than 28 days while studies utilizing catfish typically withhold feeding for periods of 56 days without significant mortality (Gatlin et al., 1986).

The relationship between protein changes and body weight (equation 7) for *L. vannamei* maintained at $30^{o}C$ could best be described when shrimp body weight was raised to the power 0.92 (equation 9). While few studies have been undertaken to determine this value in fish (Beck, 1987; Lupatsch et al., 1998) no studies exist for shrimp. The values commonly reported for fish are between 0.70 for gilthead seabream (Lupatsch et al., 1998) to 0.739 for trout (Beck, 1987) while the value obtained in this study suggests shrimp have a higher metabolic body weight for protein which may be attributed to their limited ability to store lipid and carbohydrate. The difference between the exponents for protein and energy loss in shrimp (0.92, 0.87; respectively) are closer than those obtained in most fish studies (0.70, 0.80; respectively) which further suggests proteins importance to supply the energy requirements to a starving shrimp.

Preliminary evaluation of experimental diets

Water quality

Values of pertinent water quality characteristics obtained during the experiment were well within acceptable levels which suggest shrimp were maintained under optimal water quality parameters for the duration of the 6-week trial.

Growth and survival of L. vannamei fed preliminary diets

The lack of a significant difference between the 25 and 35% crude protein diets and the commercial diets suggests the diets may be used in the requirement study described below to provide estimates for daily protein and energy requirements which are applicable to commercial feeds under the same experimental conditions. Similarly, the lack of a significant growth effect when the 25 and 35% diets were adjusted for magnesium, vitamins, minerals, vitamin C, calcium and phosphorus suggests these nutrients are neither limiting or in excess to a point which would affect growth and the determination of daily requirements. While the significant difference in growth matrices between the A.L.L. reference diet and the commercial diets precluded its use in the requirement study, this diet was selected for use in the digestibility trials due to its proven performance and high attractibility.

Growth and survival trial

Water quality

Values of pertinent water quality characteristics obtained during this experiment also were well within acceptable levels suggesting shrimp were maintained under optimal water quality parameters for the duration of the 7-week trial.

Apparent consumption

It has been suggested that digestible energy content is a major factor which controls feed intake in fish (Page and Andrews, 1973; Boonyaratpalin 1978; Wilson et al., 1985; Gatlin et al, 1986; Boujard and Medale 1994; Kentouri et al., 1995; Paspatis and Boujard, 1996; Lupatsch et al., 1998; Lupatsch et al., 2001) as well as shrimp (Davis et al., 1995). Similar apparent consumption between the 25 and 35% protein diets (0.32 and 0.31 g-feed day^{-1} shrimp^{-1}; Figures 1 and 2, respectively) appears to suggest *L. vannamei* regulates their feed intake to meet an energy requirement as opposed to a protein requirement originally suggested by Kureshy and Davis (2002). This apparent ability to consume a diet to meet an energy requirement may explain the conflicting results in *ad-libitum* feeding studies which attempted to determine an optimum protein requirement (as well as other dietary components) for *L. vannamei* as well as other species of shrimp. *Ad-libitum* requirement studies which utilized diets low in digestible energy would be consumed at a greater level than those diets with higher levels of digestible energy leading to different apparent dietary requirements. Since few, if any, of these requirement studies cited dietary digestible energy content or measured dietary consumption there exists the possibility that many nutrient requirement studies may need to be reevaluated to determine their accuracy.

Ad-libitum studies which cite dietary digestible energy also need to be aware there is the potential for a reduction in feed intake by dietary constituents other than energy. Page and Andrews (1973) witnessed a reduced feeding rate in channel catfish which were fed diets with increasing fiber levels while Bromley and Adkins (1984)

witnessed a similar reduction in feed intake in rainbow trout fed diets containing over 30% cellulose. These findings suggest an organism can only increase feed intake until they reach a gut/digestive capacity at which point they can no longer increase intake to compensate for a lower energy diet. *Ad-libitum* feeding studies utilizing *L. vannamei* suggest dietary levels of ash and fiber can be increased to 25 and 15 %, respectively, without a significant reduction in growth; however, growth was significantly reduced in a study involving 45.4% dietary ash (unpublished results, Texas A&M Shrimp Mariculture Facility). Until the ability to easily determine apparent consumption has been perfected, dietary studies involving *L. vannamei* should report dietary digestible energy as well as utilize factorial modeling to determine an apparent consumption to allow more uniform comparisons between different research efforts.

Conversion efficiencies

Protein conversion efficiency for maximum growth was more efficient in the 25% protein diet (35.9%; Table 9) as opposed to the 35% protein diet (30.6%; Table 10); however, growth was lower in the lower-protein diet. This suggests shrimp which consumed the 25% protein diet lacked an adequate amount of ingested protein to produce the same growth rate as observed in the 35% protein diet but were able to efficiently utilize the available protein for growth, as opposed to energy, better than the shrimp fed the 35% protein diet. The partitioning of protein between growth and energy also can be examined through a comparison of the energy conversion efficiencies. Shrimp which consumed the 35% protein diet had a higher energy conversion efficiency (15.5%) as compared to those which consumed the 25% protein diet (12.6%). This

further suggests the 35% protein diet was over-formulated in protein (i.e. limiting in non-protein energy) while the 25% protein diet was limited in protein due to an excess of non-protein energy. These findings also strengthen the argument *L. vannamei* eats to meet an energy requirement as the apparent consumption rate was ~10% of the dietary ration offered to the shrimp fed the highest feed ration. Based on the results from this study, it appears that to optimize protein utilization for growth, the digestible energy level of the 25% protein diet needs to be lowered to increase dietary intake or the digestible protein level in the 35% protein diet needs to be lowered while maintaining DE. The high level of ash (22%) and fiber (10%) already contained in these diets may reduce one's success in lowering dietary energy enough to increase consumption before the gut capacity of the shrimp is exceeded. Therefore it seems prudent to attempt to reduce the protein level in the 35% diet to reach an ideal protein/energy ratio where protein is maximally utilized for growth as opposed to energy.

The ability to optimize this ratio will depend greatly on the nutritional physiology of *L. vannamei* as previous studies have suggested *L. vannamei*, like other crustacea, prefers to utilize protein as an energy source even in the presence of adequate dietary non-protein energy (Scheer and Scheer 1951; Scheer et al., 1952; Huggins and Munday 1968; Lee and Lawrence 1997). These conclusions; however, are typically based on differences in weight gain, FCR and FER measurements for *L. vannamei* fed varying levels of protein without any reference to protein and energy conversion efficiencies. While this study clearly showed ingested protein was more efficiently utilized for growth for the 25% protein diet, better FCR and FER values were obtained

for the shrimp fed the 35% protein diet (Tables 12 and 11, respectively). This apparent anomaly occurs because shrimp are eating to meet an energy level and therefore can't ingest enough of the 25% protein diet to maximize their protein intake for growth while those which consume the 35% protein diet ingest excess protein which allows for maximum growth, increasing FCR and FER, but is also utilized for energy, reducing protein conversion efficiency. While *L. vannamei* can utilize dietary protein to meet energy requirements, it appears excess dietary protein is utilized not protein needed for growth. Studies which fail to take into account relative protein and energy efficiencies, dietary digestible protein and energy, as well as the fact shrimp may eat to an energy requirement, will conclude higher protein content is necessary to maximize growth, FCR and FER. When all factors are taken into close consideration it becomes readily apparent that optimization of the protein to energy ratio is necessary to reduce costs and nitrogenous waste through utilization of protein for growth as opposed to energy.

Apparent daily digestible protein requirements for maximum growth and maintenance

The mean apparent daily digestible protein requirement for 7.69 to 13.08 g *L. vannamei* fed the 25% protein diet was 0.067 g DP shrimp^{-1} day^{-1} (6.31 g DP kg^{-1} BW d^{-1}) while the 35% protein diet produced a mean apparent digestible protein requirement of 0.090 g DP shrimp^{-1} day^{-1} (8.00 g DP kg^{-1} BW d^{-1}) for 8.11 to 13.79 g *L. vannamei*. The difference in apparent protein requirement for maximum growth may be explained by the utilization of protein for energy in the 35% protein diet which produced an elevated apparent requirement due to the high metabolic cost to convert protein to energy.

Cuzon et al. (2004) recommended a daily intake of 1.2 g digestible protein for an

L. vannamei biomass of 100 g shrimp (12 g DP kg^{-1} BW d^{-1}) fed a diet with a P/E ratio

of 23 mg DP per kJ DE. This recommended DP feeding rate is approximately 47 and

33% greater than the daily DP values recommended in this study obtained from the 25

and 35% protein diets, respectively. Kureshy and Davis (2002) suggested 23.5 g CP kg^{-1}

BW d^{-1} (17.88 g DP kg^{-1} BW d^{-1}) of a 32% protein diet and 20.5 g CP kg^{-1} BW d^{-1}

(15.39 g DP kg^{-1} BW d^{-1}) of a 48% protein diet was necessary to produce maximum

growth of *L. vannamei* subadults. These apparent DP requirements are approximately

64 and 59% higher than the apparent DP requirement obtained for the 25% protein diet

and approximately 55 and 48% higher than the values obtained for the 35% protein diet

utilized in this study. This large apparent difference can be attributed to the significant

difference in feeding rates obtained between the two trials. Kureshy and Davis (2002)

reported a CP requirement which corresponded to a feeding rate of ~7% body weight

while the apparent feeding rates obtained in this study corresponded to ~2.5% body

weight. The difference in apparent protein requirement between the two studies may

also be attributed to shrimp in the current study were individually held and fed 15 times

per day while Kureshy and Davis (2002) placed eight shrimp per tank and fed them four

feedings per day. Lawrence et al. (unpublished results) determined feed utilization

increased when ingestion rate, feeding frequency and daily ration size increased

suggesting differences in nutrient requirements may be achieved depending on how feed

is introduced to subadult *L. vannamei*. Arayankanada (1995) also suggested feeding

frequency could affect nutrient requirements and concluded the low (15%) dietary

protein requirement obtained in his study could be attributed to higher feeding frequency (15 feedings per day). Furthermore, Kuresy and Davis (2002) adjusted the feeding rates after 2 weeks according to the biomass of each tank while the shrimp in the current study received a set amount of feed per day throughout the trial. Adjustment of the feeding rates 2 weeks prior to termination may have prevented shrimp from obtaining a weight consistent with available nutrients as it is unlikely, especially at the lowest feeding ration, that each shrimp obtained the same quantity of feed and grew at the same rate. Shrimp in the current study grew linearly ($R^2 = 0.992$ and $R^2 = 0.986$, for shrimp fed the 25 and 35% protein diets, respectively) over the course of the experiment which suggests they reached an apparent growth equilibrium with the provided nutrients until maximum growth was achieved.

While it is common to see variations in apparent requirements, the previous values reported for *L. vannamei* appear elevated especially when one considers Teshima et al. (2001) reported a protein requirement of 10 g per kg^{-1} BW d^{-1} to maintain maximum body protein retention in *Marsupenaeus japonicus*. *M. japonicus* is generally considered a shrimp species which requires more dietary protein than other shrimp species (Kanazawa, 1990). Dietary protein requirements for *M. japonicus* have been reported as high as 57% (Deshimaru and Yone, 1978), while the highest protein requirement reported for the more herbivorous *L. vannamei* was 40% (Colvin and Brand, 1977). Based solely on the feeding habit of *L. vannamei* one would expect the daily DP requirement (6.31 – 8.00 g DP kg^{-1} BW d^{-1}) to be lower than those obtained for the carnivorous *M. japonicus* (10 g DP kg^{-1} BW d^{-1}) which suggests the values obtained in

the current study may be closer to the true apparent requirement than those previously reported. Daily DP requirements obtained in this study are also in agreement with studies involving omnivorous fish. Gatlin et al. (1986) determined the protein requirement for maximum weight gain was 8.75 g DP kg^{-1} BW d^{-1} for channel catfish fed incremental feeding rates ranging from 0% to 5% body weight per day.

The apparent digestible protein requirement for maximum growth decreased throughout the 7 week trial from 8.97 g DP kg^{-1} BW d^{-1} at week 2 for 7.69 g shrimp to 5.04 g DP kg^{-1} BW d^{-1} at week 7 for 13.08 g shrimp fed the 25% protein diet. A similar reduction was witnessed for those shrimp which consumed the 35% protein diet as the apparent protein requirement decreased from 10.24 g DP kg^{-1} BW d^{-1} at week 2 for 8.11 g shrimp to 7.11 g DP kg^{-1} BW d^{-1} at week 7 for 13.79 g shrimp. Reduced protein requirements with age have been reported for *L. vannamei* (Colvin and Brand 1977; Akiyama et al., 1992; Pedrazzoli et al., 1998), *P. californiensis* (Colvin and Brand, 1977), *L. setiferus* (Garcia-Carreno 1998), *P. monodon* (Chen, 1998) and fish (Lupatsch et al., 1998). The reduction in apparent protein requirement has been attributed to a reduction in growth potential as shrimp get larger; however, maximum weekly growth throughout the 7 week trial was linear. This suggests the reduced apparent protein requirement may be partially explained by a reduction in protein digestibility as *L. vannamei* grows older (Table on p. 83). This reduction in apparent protein digestibility (Table on p. 83) combined with constant energy digestibility (Table on p. 83) suggests their ability to utilize carbohydrates and lipids as energy, as opposed to dietary protein, increases with age which may contribute to the reduction in apparent protein

requirement witnessed in this study. This reduction in apparent protein requirement ultimately will affect the digestible crude protein to digestible energy ratio and may need to be considered when formulating diets for "older" *L. vannamei*.

While Cuzon et al. (2004) reported that protein requirement was probably not as highly correlated to protein accretion as in vertebrates due to chitin synthesis, results from this study suggest the opposite. Daily digestible protein requirements for maximum growth for the 13.08 g shrimp fed the 25% protein diet obtained from growth and body compositional analysis were 0.066 g DP shrimp^{-1} day^{-1} and 0.066 g DP shrimp^{-1} day^{-1}, respectively. A perfect correlation between apparent daily digestible protein requirements was also obtained from growth and body compositional analysis (0.098 g DP shrimp^{-1} day^{-1} and 0.098 g DP shrimp^{-1} day^{-1}, respectively) for the 13.79 g shrimp fed the 35% protein diet. These results suggests daily digestible protein requirements for maximum growth can be estimated from the body compositional analysis of *L. vannamei* fed graded levels of feed and provide an important conformational metric when assessing apparent requirements.

The mean apparent daily digestible protein requirement for maintenance was 0.11 g DP shrimp^{-1} day^{-1} (1.03 g DP kg^{-1} BW d^{-1}) for 7.69 – 13.08 g *L. vannamei* fed the 25% protein diet and 0.021 g DP shrimp^{-1} day^{-1} (1.87 g DP kg^{-1} BW d^{-1}) for 8.11 – 13.79 g *L. vannamei* which consumed the 35% protein diet. The difference in apparent maintenance requirement between the two diets may be explained by the utilization of protein for energy in the 35% diet and by the higher growth rate achieved by *L. vannamei* which consumed the 35% protein diet. These factors will increase the

apparent requirement as there is a high metabolic cost to convert protein to energy (deamination of protein to be utilized as energy is energetically costly) and maintenance requirements for protein increase with increased shrimp weight.

The values obtained in this study are similar to apparent digestible protein maintenance requirements obtained by Kuresy and Davis (2002). Kuresy and Davis (2002) obtained daily CP maintenance values between $1.5 - 2.1$ g CP / kg^{-1} BW d^{-1} for subadult *L. vannamei* fed either 0.4, 0.6, 0.8, 1.0, 1.4, 1.8, 2.6 and 3.2 g of a 16% crude protein diet per shrimp per week or 0.4, 0.55, 0.7, 0.85, 1.0, 1.3, 1.6, and 1.9 g of a 32% crude protein diet per shrimp per week. The consistency between the results in the current study and those obtained by Kuresy and Davis (2002) for apparent maintenance requirement, but not apparent DP requirement for maximum growth, further strengthen the theory feeding level should not be adjusted based on a change in biomass but kept constant throughout the study by feeding a set level of diet per shrimp per day. It is not clear why Kureshy and Davis (2002) utilized two different experiments with two different feeding strategies to obtain apparent maintenance (g diet per shrimp per week) and apparent maximum weight gain (% diet per biomass adjusted biweekly) requirements as both values can be obtained from a single study. In one of the only other studies to determine an apparent DP maintenance requirement for shrimp, Teshima et al (2001) determined the apparent maintenance protein requirement was 1.09 g DP kg^{-1} BW d^{-1} for *M. japonicus* which is slightly higher than the apparent requirement obtained from the 25% protein diet but lower than the estimate obtained from the 35% protein diet. While this might suggest a similar metabolic rate among shrimp, Rosas et

al. (2001b) suggested *L. setiferus* might have a higher metabolic rate than *L. vannamei* juveniles based on a two fold increase in routine oxygen consumption and apparent heat increment of *L. setiferus* juveniles vs. *L. vannamei* juveniles. This two fold difference suggests *L. setiferus* may have twice the maintenance requirement of *L. vannamei*; however, research to determine the daily digestible maintenance requirement for *L. setiferus* has not yet been undertaken. Apparent DP maintenance values obtained in this study are also in agreement with DP maintenance requirements obtained for different fish species. McGoogan and Gatlin (1998) determined the maintenance DP requirement for juvenile red drum was $1.5 - 2.5$ g DP kg^{-1} BW d^{-1}, while Gatlin et al. (1986) determined the maintenance protein requirement was 1.32 g DP kg^{-1} BW d^{-1} for channel catfish. Apparent DP maintenance requirements estimated by regressing protein accretion back to zero displayed a high degree of correlation to those values obtained from growth data. Daily digestible protein requirements for maintenance obtained from growth and body compositional analysis were 0.014 g DP shrimp^{-1} day^{-1} and 0.014 g DP shrimp^{-1} day^{-1}, respectively for 13.08 g *L. vannamei*. A high correlation between apparent daily digestible maintenance protein requirements was also obtained from growth and body compositional analysis (0.024 g DP shrimp^{-1} day^{-1} and 0.030 g DP shrimp^{-1} day^{-1}, respectively) for 13.79 g shrimp fed the 35% protein diet. These results suggests daily digestible maintenance requirements can be estimated from body compositional analysis of *L. vannamei* fed graded levels of feed which helps to validate the results obtained from growth data.

Apparent daily digestible energy requirements for maximum growth and maintenance

Shrimp derive energy through the catabolism of feed and utilize the energy for maintenance, growth, reproduction and physical activity. Energy requirements have been estimated for fish since Ege and Krogh (1914) applied the principles of bioenergetics to fish; however, few studies have focused on daily digestible energy requirements in shrimp (Cuzon et al., 2004). In this study the mean apparent daily digestible energy requirement for 7.69 to 13.08 g *L. vannamei* fed the 25% protein diet was 4.275 kJ DE shrimp^{-1} day^{-1} (402.62 kJ DE kg^{-1} BW d^{-1}) while the 35% protein diet produced an apparent daily digestible energy requirement of 3.765 kJ DE shrimp^{-1} day^{-1} (334.72 kJ DE kg^{-1} BW d^{-1}) for 8.11 to 13.79 g *L. vannamei*. The lower apparent energy requirement for *L. vannamei* fed the 35% protein diet may by attributed to the lower efficiency of utilizing protein for energy. Rosas et al. (2002) determined that while *L. vannamei* are well adapted to live without starch, protein utilization as energy produces a substantial loss of energy through ammonia excretion.

Cuzon et al. (2004) suggested 140 kJ DE a day to be adequate for a biomass of 100 g shrimp which is equivalent to 1400 kJ DE a day for 1 kg shrimp. This recommended level is 71 and 76% higher than the value obtained in this study even though the energy retention in his study was higher (20%) than the maximum energy retention obtained for *L. vannamei* fed the 25 (~12%) and 35% (~15%) protein diets utilized in this study. While the reason for the large difference in apparent daily DE requirements for maximum growth is not known, the values obtained in the current study are similar to values reported for omnivorous fish while the values reported by Cuzon et

al (2004) are closer to values reported for carnivorous fish. Gatlin et al (1986) obtained an apparent daily DE requirement of 417.35 kJ energy kg^{-1} BW d^{-1} for channel catfish fed either a 25% crude protein (11.92 kJ/g) diet or a 35% crude protein (16.69 kJ/g) diet which is close to the values obtained in the current study for similarly omnivorous *L. vannamei*. McGoogan and Gatlin (1998) reported a DE requirement for maximum weight gain of 774 – 954 kJ DE kg^{-1} BW d^{-1} for the carnivorous red drum (Boothby and Avault 1971) which is closer, but still lower, than the energy requirement suggested by Cuzon et al (2004) for *L. vannamei*.

Apparent daily energy requirements for maximum growth decreased throughout the 7 week trial as shrimp size increased. Apparent daily DE requirements for maximum growth at week 2 for 7.69 g shrimp was 574.08 kJ DE kg^{-1} BW d^{-1} while the apparent requirement was only 322.79 kJ DE kg^{-1} BW d^{-1} at week 7 for 13.08 g *L. vannamei* which consumed the 25% protein diet. Apparent daily DE requirement also decreased for shrimp fed the 35% protein diet from 428.86 kJ DE kg^{-1} BW d^{-1} for 8.11 g *L. vannamei* at week 2 to 296.73 kJ DE kg^{-1} BW d^{-1} for 13.79 g shrimp at week 7. This reduction in apparent daily DE requirement may be attributed to *L. vannamei's* reduced apparent protein requirement with age due to a reduction in protein digestibility (Table on p. 83). A similar decrease in apparent daily energy requirement has not been reported for shrimp; therefore it is not possible to compare this event to other studies.

Apparent daily DE requirements for maximum weight gain obtained from whole body energy deposition were in agreement with those values obtained based on maximum weight gain. Apparent daily DE requirements based on energy deposition

was 4.330 kJ DE shrimp^{-1} day^{-1} while maximum weight gain produced an apparent

requirement of 4.238 kJ DE shrimp^{-1} day^{-1} for 13.08 g *L. vannamei* fed the 25% protein

diet. A high correlation was also obtained for 13.79 g shrimp which consumed the 35%

protein diet (4.167 kJ DE shrimp^{-1} day^{-1} and 4.091 kJ DE shrimp^{-1} day^{-1} for energy

deposition and maximum weight gain, respectively). McGoogan and Gatlin (1998)

witnessed a large difference in apparent DE requirement determined by maximum

weight gain and whole body energy gain and attributed the difference to a possible

increase in energy density with lipid deposition. As has been mentioned, shrimp have a

limited ability to store lipid and carbohydrate and therefore do not have the ability to

store excess energy, as lipid or carbohydrate reserves, as growth plateaus. This finding

once again helps to underscore this major physiological difference between fish and

shrimp and may help to explain the high degree of correlation between the apparent

requirements determined from both maximum growth and energy deposition.

Mean apparent daily DE maintenance requirements for 7.69 – 13.08 g *L.*

vannamei fed the 25% protein diet was 0.702 kJ DE shrimp^{-1} day^{-1} (66.232 kJ DE kg^{-1}

BW d^{-1}) while the requirement was 0.887 kJ DE shrimp^{-1} day^{-1} (7.698 kJ DE kg^{-1} BW d^{-1}

) for 8.07 – 13.79 g *L. vannamei* fed the 35% protein diet. The difference in apparent

maintenance requirements can partially be explained, as described for protein, by the

utilization of protein for energy in the 35% protein diet and by the higher growth rate

achieved by *L. vannamei* fed the 35% protein diet. There are few, if any, studies which

have reported apparent daily DE requirements for maintenance for any species of

shrimp; however, the values in the current study are in agreement with those values

obtained from fish. Gatlin et al. (1986) determined the apparent maintenance requirement for energy to be between 71.12 – 72.50 kJ energy kg^{-1} BW d^{-1} for channel catfish while McGoogan and Gatlin (1998) reported an apparent daily DE requirement between 57.99 – 93.01 kJ DE kg^{-1} BW d^{-1} for red drum fed a diet containing 36.5% DP and 14.2 kJ DE. The similarities between apparent daily DE maintenance requirements is not surprising as aquatic species tend to have on average a 10-fold lower basal metabolism than homeothermic vertebrates (Kleiber, 1965) due to the their ability to take advantage of the energetic benefits provided by their aquatic habitat.

Apparent daily DE requirements for maintenance obtained from whole-body energy deposition were in agreement with those values obtained based on maximum weight gain. Apparent daily DE requirements based on energy deposition was 0.891 kJ DE shrimp^{-1} day^{-1} while maximum weight gain produced an apparent requirement of 0.887 kJ DE shrimp^{-1} day^{-1} for 13.08 g *L. vannamei* fed the 25% protein diet. A high correlation was also obtained for 13.79 g shrimp which consumed the 35% protein diet (1.096 kJ DE shrimp^{-1} day^{-1} and 1.012 kJ DE shrimp^{-1} day^{-1} for maximum weight gain and energy deposition, respectively). This high degree of correlation between apparent daily maintenance requirements obtained from body accretion and maximum weight is in agreement with the high correlation obtained between the apparent daily requirements necessary to produce maximum growth in *L. vannamei*. Due to the sparse data concerning daily apparent maintenance energy for shrimp it is not possible to compare the current results with prior research efforts; however, it is suggested this agreement is due to *L. vannamei's* limited ability to store lipid and carbohydrate.

CHAPTER III

APPARENT DRY MATTER, PROTEIN AND ENERGY DIGESTIBILITY OF

INGREDIENTS FOR PACIFIC WHITE SHRIMP *Litopenaeus vannamei* DIETS

Introduction

Although *L. vannamei* has been cultivated commercially for years, few studies

have focused on determining the energy and protein availability for commonly utilized

dietary ingredients (Akiyama et al., 1989; Davis and Arnold, 1993; Davis and Arnold,

1995). While direct measurement of digestibility coefficients is difficult (Smith and

Tabrett, 2004), apparent digestibility may be determined by utilizing an *in vivo*

digestibility method such as the indirect chromic oxide method (Akiyama et al., 1989;

Davis and Arnold, 1993; Davis and Arnold, 1995, Davis et al., 2002), the indirect

ytterbium acetate method (Smith and Tabrett, 2004) or the indirect titanium dioxide

method (Smith and Tabrett, 2004). Studies involving *L. vannamei* typically have

utilized the chromic oxide method (Smith et al., 1985; Davis and Arnold, 1993) due to

its ability to produce consistent results (Akiyama et al., 1989; Smith and Tabrett, 2004).

Accurate digestible energy and digestible protein coefficients are necessary to

precisely formulate diets to meet nutritional requirements as well as to effectively allow

cost substitution of ingredients and reduce waste production. Commercial diets are

currently formulated based on data which was derived from pond and laboratory studies

which measured growth parameters with no knowledge of nutrient availability. Since

these formulations utilize gross dietary composition which produced "optimal" growth they can only be formulated "least cost" by adjusting protein sources while maintaining gross dietary requirements. Formulations which rely solely on gross dietary composition, as opposed to digestible composition, can produce a feed which is over-formulated increasing both costs and pollutant levels as protein is the most expensive component in feeds (Cordova-Murueta and Garcia-Carreno, 2002) and can lead to the accumulation of inorganic nitrogen in culture water (Velasco et al., 1999). While Lee and Lawrence (1997) suggested in 1997 that environmental regulations may have a greater role in digestibility research than economical considerations, few studies have focused on digestibility for either reason (Cuzon et al., 2004). This is surprising as feed is a major part of production costs (Akiyama et al., 1992; Sarac et al., 1993) and additional savings may be realized by optimizing feed formulations.

One only needs to look at the poultry industry to realize that a more cost efficient, and environmentally sound, feed can be formulated based on the digestibility (i.e. nutrient availability) of ingredients utilized in the diet. This formulation method allows ingredients to be selected to meet both the nutritional as well as economical requirements of the least-cost diet under consideration. Knowledge of digestibility coefficients of ingredients also allows for an added measure of quality assurance as digestibility of ingredients can vary considerable depending upon their overall freshness and previous treatment (Garcia-Carreno, 1998). Utilization of currently available data will not allow for the formulation of a least-polluting diet based on digestible energy values and in some cases digestible protein values. The objective of this study was to

determine apparent dry matter, protein and energy digestibility for select ingredients used in *L. vannamei* diets.

Materials and methods

In vivo experiments

Source of shrimp

Specific-pathogen-free *L. vannamei* postlarvae were obtained from the Oceanic Institute (Kailua-Kona, HI) and stocked into 2.44-m diameter fiberglass tanks. Postlarvae were fed live *Artemia sp.* nauplii and a commercial postlarval feed (Rangen 45/10; Rangen Inc., Buhl, ID) twice and 12 times daily, respectively. Postlarvae were held approximately 13 weeks to allow for acclimation to laboratory conditions ($30.1 \pm 0.5\,^{\circ}C$, $32.2 \pm 0.4‰$) and to achieve proper weight for the trial ($9.75\,g \pm 0.43$; 11.33 ± 0.61).

Experimental system

The experimental system consisted of 60 rectangular tanks (119 L volume; 0.3-m^2 bottom surface area) connected to a semi closed (10% new water daily) 43,000-L indoor recirculating system. Seawater was pumped through a sand filter to achieve a recirculating rate of $1.89\,L\,min^{-1}\,tank^{-1}$ ($2,400\%$ daily exchange $tank^{-1}\,day^{-1}$). A light:dark photoperiod of 12:12 h was provided by supplemental compact fluorescent lighting. Each tank was stocked with thirty 8-10 g *L. vannamei* to achieve a biomass of 270 ± 20 g. Temperature, salinity and dissolved oxygen (DO) were monitored daily using a YSI 85® Meter (YSI Inc., Yellow Springs, OH). Ammonia-nitrogen, NO_2-N, NO_3-N,

and pH were monitored weekly using methods adapted from those of Spotte (1979a,b)

and Solarzano (1969), Spotte (1979a,b), Mullen and Riley (1955), Spotte (1979a,b) and

Strickland and Parsons (1972), and a Brinkman Metrohm® pH meter, respectively.

Diet preparation and apparent digestibility determination

Apparent dry matter, protein and energy digestibility was determined for 32 feed

ingredients used to formulate *L. vannamei* diets (Table 23). The digestibility trial

followed the chromic oxide indicator method described by Cho et al. (1982). A 35%

crude protein, 8.41 kJ g-1 reference diet (Table 24) was mixed in bulk as a 35-kg batch

to assure uniformity between test diets. All ingredients for the reference diet, except

alginate and sodium metaphosphate, were mixed in a food mixer (Model L-800, Hobart

Corporation, Troy, OH) for 3 hours. One kg of reference diet and 32 test diets

comprised of 700 g kg^{-1} reference diet by weight and 300 g kg^{-1} test ingredient were

individually mixed in a food mixer (Model A-200, Hobart Corporation, Troy, OH) for

40 minutes. In a separate bowl, alginate and sodium metaphosphate were added to

deionized water (400 ml kg^{-1}) and mixed using a hand mixer (Sunbeam Products Inc.,

Milford, MA) for approximately 45 seconds. The alginate was then added to the dry

ingredients and mixed an additional minute to achieve a mash consistency appropriate

for extrusion. Extrusion was accomplished using a meat chopper attachment (Model A-

200, Hobart Corporation, Troy, OH) fitted with a 3-mm die. Moist feed strands were

dried on wire racks in a forced air oven at 35 $^{\circ}$C to a moisture content of 8-10%. Dry

feed strands were ground using a mortar and pestle to provide a particle size ranging

from 2-4 mm and stored at 4 $^{\circ}$C until used.

Table 23. Test ingredients used in the digestibility trials.

Trial No.	International Feed No.	Ingredients	Trial No.	International Feed No.	Ingredients
1		Blood Meal (Conventional)[1]	1		Fish Meal (Misc. Asian)[2]
1	5-00-381	Bood Meal (Spray Dried)[1]	1		Fish Meal (Misc. Peru)[2]
1	5-01-162	Casein[4]	1	5-14-503	Gelatin[4]
2	5-28-242	Corn Gluten[1]	2		Krill Meal[1]
2	5-01-663	Crab Meal[1]	2		Krill Flour[2]
1		Diatomaceous Earth[5]	1	5-03-798	Poultry By-product[1]
2	5-02-141	Distillers Grains[1]	2	5-04-612	Soybean Meal, 48% (Solvent Extract)[1]
1	5-03-795	Feather Meal[1]	2	5-04-597	Soybean Meal (Full Fat)[1]
1	5-01-985	Fish Meal (Anchovy)[1]	2	5-08-038	Soybean Meal (Isolated, 90%)[1]
1	5-01-985	Fish Meal (Anchovy-Peru)[2]	2		Squid (Liver Meal-Asian)[2]
1	5-02-000	Fish Meal (Herring)[1]	2		Squid (Muscle Meal)[1]
1		Fish Meal (Hoki-N. Zealand)[2]	2		Squid (Muscle Meal)[1]
1	5-01-985	Fish Meal (Mackerel-Chile)[2]	2		Squid (Whole)[1]
1	5-02-009	Fish Meal (Menhaden)[1]	2		Squid (Whole-Asian)[2]
1	5-02-009	Fish Meal (Menhaden)[1]	1		Wheat Gluten[4]
1	5-01-977	Fish Meal (Menhaden)[3]	1		Wheat Starch[4]

[1]Zeigler Brothers, Gardners, PA, USA.

[2]Evialis, Vannes Cedex, France.

[3]Omega Protein Corporation Inc., Houston, TX, USA.

[4]MP Biomedicals, Cleveland, OH, USA.

[5]Sigma, St. Louis, MO, USA.

[6]The J. M. Smucker Company, Orrville, OH, USA.

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Table 24. Composition of the 35% crude protein, 8.40 kJ g^{-1}, reference diet.

Ingredient	Inclusion level (g kg^{-1})	Ingredient	Inclusion level (g kg^{-1})
Alginate[5]	20.00	Krill[1]	105.00
Calcium Carbonate[2]	14.60	Mineral-Vitamin Premix[1,A]	2.70
Cellulose[4]	20.00	MgO[3]	17.30
Cholesterol[2]	2.00	Phospholipid[1]	42.00
Chromic Oxide[3]	10.00	Sodium Metaphosphate[3]	10.00
Dicalcium Phosphorus[2]	65.60	Squid[1]	150.00
Fish Meal[6]	150.00	Vitamin C[1]	0.50
Isolated Soy (90%)[1]	79.40	Vitamin-Mineral Premix[1,B]	2.30
KCl[3]	18.50	Wheat Starch[2]	290.10
Crude Protein (%)	35.00[*]	Energy, kcal g[-1]	2.01[*]
Digestible Protein (%)	31.63[*]	Digestible Energy, kcal g[-1]	1.72[*]
Ash (%)	17.01[*]	Lipid (%)	8.03[*]

[1]Zeigler Brothers, Gardners, PA, USA.
[2]MP Biomedicals, Cleveland, OH, USA.
[3]Fisher Scientific, Fair Lawn, NJ, USA.
[4]Sigma, St. Louis, MO, USA.
[5]Keltone HV Alginate, NutraSweet-Kelco Company, Chicago, IL.
[6]Omega Protein Corporation Inc., Houston, TX, USA.
[7]Ingredient composition of the premix.
[A]See Appendix A for composition.
[B]See Appendix B for composition.
[*]Calculated on an as-fed basis.

Sample collection

The first digestibility trial consisted of 19 test diets and the reference diet randomly assigned to 60 tanks providing three replicates per diet. Shrimp were acclimated to test diets and culture conditions 4 days prior to the start of fecal collections. At the start of each collection day tanks were siphoned of fecal material and shrimp molts. Shrimp were fed 0.2 g feed per shrimp per hour for 6 consecutive hours. Uneaten feed was removed from tanks prior to each feeding to minimize leaching losses. Fecal material was collected 1 h after each feeding by siphoning the feces onto a 42-µm

screen. Feces was rinsed with deionized water, transferred to individually labeled vials and frozen (-84^oC) until analysis. Feces from the first daily collection were discarded to minimize influence from previously eaten fecal material. Feces were collected for four consecutive days and pooled such that each replicate consisted of the feces from one tank collected over four consecutive days. The above procedure was then repeated for the remaining 13 test diets and the reference diet using 11.33 ± 0.61 g shrimp. To assess the effect of shrimp size on digestibility the above procedure was repeated for the reference diet using shrimp with mean weights of 8.65 ± 0.29, 13.14 ± 0.08, and 15.09 ± 0.08 g.

Analysis of feed and feces

Prior to compositional analysis, feed and fecal samples were lyophilized, ground into a fine powder using a mortal and pestle and analyzed for percent dry matter (AOAC, 1990). Protein (AOAC Method 990.3; FP-528 Nitrogen/Protein Determinator; Leco Corporation, St. Joseph, MI), energy (model 1241 adiabatic bomb calorimeter; Parr Instrument Co., Moline, IL) and chromic oxide (McGinnis and Kasting, 1964) were then determined for each lyophilized sample and reported on a dry-matter basis. Apparent digestibility coefficient (ADC) values for the test and reference diets were calculated by the following equation (Pond et al. 1995):

$$\text{ADC (\%)} = 100 - \frac{\%\text{ indicator in diet}}{\%\text{ indicator in feces}} \times \frac{\%\text{ nutrient in feces}}{\%\text{ nutrient in diet}} \times 100$$

where indicator is chromic oxide and nutrient is dry matter, protein, or energy. To determine the ADC for dry matter, protein and energy for the test ingredient the following equation was used (Bureau and Hua, 2006):

For all test ingredients:

$$ADC_{test\ ingredient} = ADC_{test\ diet} + [(ADC_{test\ diet} - ADC_{ref.\ diet}) \times (0.7 \times D_{ref}/0.3 \times D_{ingr})]$$

where D_{ref} = % nutrient (or kcal g^{-1} gross energy) of reference diet mash; D_{ingr} = % nutrient (or kcal g^{-1} gross energy) of test ingredient.

Statistical analysis

ADC values were subjected to analysis of variance using SPSS to determine if significant differences exist between the ingredients. Significant differences ($P<0.05$) were separated using the Bonferroni inequality to assure the experimentwise error rate was less than or equal to 0.05.

In vitro experiments

In vitro analysis of selected ingredients

Fourteen ingredients (conventional blood meal, spray dried blood meal, corn gluten, crab meal, distillers grains, feather meal, Anchovy fish meal, Herring fish meal, Menhaden fish meal, poultry by-product, 48% soybean meal, full fat soybean meal, squid muscle meal-Lima and squid muscle meal-Paita) were sent to Zeigler Brothers, Gardners, PA for *in vitro* ACPD analysis using either 0.20% or 0.0002% pepsin. ADC values were subjected to correlation analysis using SPSS to determine the strength of the linear relation between *in vivo* and *in vitro* crude protein digestibility coefficients.

Results

In vivo experiments

Water quality

To assure these inorganic compounds do not interfere with the experiment, ammonia-N should be maintained below 2.37 mg L^{-1} (0.09 mg L-1 for NH sub(3)-N (Chen and Lin 1991), nitrite levels below 2.04 mg L^{-1} (Chen and Lin 1991) and nitrate should be below 25 mg L^{-1} (Chen and Lei 1990). Values obtained during the experiment were well below these recommendations which suggest shrimp were maintained under optimal water quality parameters for the duration of the 4-week trial.

Weight-class effect on digestibility coefficients

Significant differences in apparent crude protein digestibility (ACPD) were observed for the five different weight classes of *L. vannamei* (Table 25). ACPD coefficients were significantly higher for the 8.56 g *L. vannamei* than all other weight classes. No significant differences in ACPD were determined among the three largest weight classes (11.33, 13.14, 15.09 g) while the second weight class (9.75 g) had an ACPD which was significantly higher than the two largest weight classes (13.14, 15.09 g) but not significantly different from the third weight class (11.33 g). No significant differences in apparent dry matter digestibility (ADMD) (range: 70.58-72.06%) or apparent energy digestibility (AED) (range: 84.30-86.00%) coefficients were observed between the five different weight classes.

Table 25. Effect of mean weight on *in vivo* digestibility in *L. vannamei* fed a 35% standard reference diet[1].

Mean Weight (g)[2]	ADMD (%)	ACPD (%)	AED (%)
8.56 ± 0.29^a	71.45 ± 2.36^f	90.38 ± 0.66^i	85.20 ± 0.79^j
9.75 ± 0.43^b	72.06 ± 0.88^f	89.10 ± 0.42^h	86.00 ± 0.19^j
11.33 ± 0.61^c	70.58 ± 2.35^f	88.44 ± 0.11^{gh}	85.48 ± 0.45^j
13.14 ± 0.08^d	71.58 ± 2.25^f	87.95 ± 0.34^g	85.85 ± 0.92^j
15.09 ± 0.08^e	70.77 ± 2.74^f	87.79 ± 0.25^g	84.30 ± 0.61^j

[1]Values are means ± sd and values with similar superscripts are not significantly different (P>0.05).
[2]Mean weight obtained at start of 4 day trial ± Standard Deviation (N = 3).

Apparent dry matter digestibility

Apparent digestibility coefficients for the ingredients are presented in Tables 26 and 27. ADMD values of ingredients ranged from 4.3% for diatomaceous earth to 96.5% for gelatin. Purified meals (range: 89.4-96.5%) had significantly higher ADMD than all other ingredients tested, with gelatin having the highest numerical ADMD. ADMD coefficients differed significantly between fish meals (range: 55.8-78.3%) and were inversely correlated to ash content (r=-0.89; P<0.0001). No significant differences in ADMD were noted between the two anchovy fish meals; however, there was a significant difference in ADMD between the three menhaden fish meals. Significant differences in ADMD coefficients also were observed for practical plant meals (range: 41.8 to 78.7%); however, the coefficients were not correlated to ingredient ash content (P>0.05). Corn gluten meal had the second lowest ash content of all ingredients (1.5%) but had an ADMD (41.8%) which was only significantly greater than diatomaceous earth (4.3%). Dry matter digestibility of practical animal meals (range: 57.0-63.9%) was less variable than ADMD of marine meals (range: 43.3-81.7%).

Table 26. Percent ash, dry matter protein content (DMPC), apparent dry matter digestibility (ADMD) and apparent crude protein digestibility (ACPD) of ingredients consumed by *L. vannamei*.*

Ingredient	Ash (%)	DMPC (%)	ADMD (%)	ACPD (%)
Blood Meal (Conventional)[1,C]	1.56 ± 0.09	97.6 ± 0.5	57.0 ± 3.8[l,m]	66.2 ± 1.6[l]
Blood Meal (Spray Dried)[1,C]	2.84 ± 0.01	99.1 ± 0.5	63.4 ± 4.5[h,i,j,k,l]	70.8 ± 1.8[k]
Casein[4,E]	0.73 ± 0.01	95.9 ± 0.2	89.5 ± 1.4[b]	96.4 ± 1.0[b]
Corn Gluten[1,D]	1.48 ± 0.02	71.6 ± 0.0	41.8 ± 1.0[o]	59.1 ± 1.9[m]
Crab Meal[1,B]	44.77 ± 0.59	33.3 ± 0.8	43.3 ± 1.4[n,o]	84.0 ± 1.9[f,g,,h]
Distillers Grains[1,D]	5.02 ± 0.13	30.4 ± 0.6	47.2 ± 3.7[n]	78.5 ± 1.4[i,j]
Feather Meal[1,C]	2.74 ± 0.04	86.7 ± 0.1	61.3 ± 0.9[j,k,l,m]	63.9 ± 0.7[l]
Fish Meal (Anchovy)[1,A]	14.99 ± 0.23	70.0 ± 0.8	78.3 ± 2.3[c,d]	87.9 ± 0.7[d,e,f]
Fish Meal (Anchovy-Peru)[2,A]	14.37 ± 0.16	74.4[5]	78.0 ± 1.0[c,d]	88.5 ± 2.4[d,e]
Fish Meal (Herring)[1,A]	12.21 ± 0.03	78.7 ± 0.6	72.7 ± 3.9[d,e,f,g]	90.1 ± 1.1[d,e]
Fish Meal (Hoki-New Zealand)[2,A]	17.76 ± 1.55	71.9[5]	67.1 ± 2.0[g,h,i,j,k]	88.1 ± 1.0[d,e,f]
Fish Meal (Mackerel-Chile)[2,A]	16.92 ± 0.00	74.7[5]	73.5 ± 3.9[d,e,f,g]	88.8 ± 2.8[d,e]
Fish Meal (Menhaden)[1,A]	20.09 ± 0.21	68.3 ± 0.2	68.1 ± 2.1[f,g,h,I,j]	89.0 ± 2.2[d,e]
Fish Meal (Menhaden)[1,A]	29.15 ± 0.35	61.8 ± 0.4	55.6 ± 3.7[m]	83.7 ± 0.7[g,h]
Fish Meal (Menhaden)[3,A]	21.25 ± 0.09	68.9 ± 0.6	60.2 ± 0.5[k,l,m]	83.2 ± 1.4[h]
Fish Meal (Misc. Species-Asian)[2,A]	22.66 ± 0.51	65.4[5]	55.8 ± 4.0[m]	78.6 ± 0.9[i,j]
Fish Meal (Misc. Species-Peru)[2,A]	16.17 ± 0.04	71.8[5]	70.7 ± 4.0[e,f,g]	87.6 ± 2.6[e,f,g]
Gelatin[4,E]	0.06 ± 0.00	112.4 ± 0.0	96.5 ± 1.9[a]	99.7 ± 1.9[a]
Krill Meal[1,B]	12.23 ± 0.25	70.2 ± 1.2	72.6 ± 0.2[d,e,f,g]	80.5 ± 1.1[i]
Krill Flour[2,B]	9.52 ± 0.05	62.8[5]	81.7 ± 1.0[c]	89.4 ± 1.1[d,e]
Poultry By-Product[1,C]	16.80 ± 0.07	68.3 ± 1.6	63.9 ± 3.9[h,i,j,k,l]	78.7 ± 1.7[i,j]
Soybean Meal (48% Solvent Extract)[1,D]	7.40 ± 0.12	51.6 ± 0.1	75.9 ± 1.6[c,d,e]	92.9 ± 0.3[b,c]
Soybean Meal (Full Fat)[1,D]	5.31 ± 0.08	42.5 ± 0.3	63.5 ± 2.2[h,i,j,k,l]	87.1 ± 1.8[e,f,g,h]
Soybean Meal (Isolated, 90%)[1,D]	4.65 ± 0.02	89.6 ± 0.3	78.7 ± 0.7[c,d]	93.7 ± 0.8[b,c]
Squid (Liver Meal-Asian)[2,D]	6.27 ± 0.15	53.5[5]	61.8 ± 3.3[i,j,k,l,m]	66.4 ± 1.9[l]
Squid (Muscle Meal-Lima)[1,D]	4.22 ± 0.59	91.4 ± 0.3	69.8 ± 4.6[e,f,g,h]	84.6 ± 2.4[f,g,h]

Table 26. Continued

Ingredient	Ash (%)	DMPC (%)	ADMD (%)	ACPD (%)
Squid (Muscle Meal-Paita)[1,D]	3.84 ± 0.03	90.1 ± 0.2	74.7 ± 1.4[d,e,f]	86.6 ± 0.8[e,f,g,h]
Squid (Whole)[1,D]	4.24 ± 0.05	88.9 ± 0.1	68.6 ± 1.0[f,g,h,i]	84.5 ± 1.9[f,g,h]
Squid (Whole, Asian)[2,D]	10.20 ± 0.08	73.0[5]	61.9 ± 0.8[i,j,k,l,m]	75.4 ± 0.9[j]
Wheat Gluten[4,E]	0.65 ± 0.00	83.7 ± 0.5	89.4 ± 1.0[b]	95.8 ± 0.6[b]

Values are means of three determinations ± s.d.

[*]Means with similar lowercase superscripts are not significantly different (P>0.05).

[1]Zeigler Brothers, Gardners, PA, USA.

[2]Evialis, Vannes Cedex, France.

[3]Omega Protein Corporation Inc., Houston, TX, USA.

[4]MP Biomedicals, Cleveland, OH, USA.

[5]Results provided by Evialis, Vannes Cedex, France.

[A]Fish Meals.

[B]Marine Meals.

[C]Practical Animal Meals.

[D]Practical Plant Meals.

[E]Purified Ingredients.

Table 27. Percent ash, dry matter energy content (DMEC), apparent dry matter digestibility (ADMD) and apparent energy digestibility (AED) of ingredients consumed by *L. vannamei*.*

Ingredient	Ash (%)	DMEC (kcal g^{-1})	ADMD (%)	AED (%)
Blood Meal (Conventional)[1,C]	1.56 ± 0.09	5.74 ± 0.05	57.0 ± 3.8[l,m]	72.2 ± 1.6[i,j]
Blood Meal (Spray Dried)[1,C]	2.84 ± 0.01	5.91 ± 0.06	63.4 ± 4.5[h,i,j,k,l]	75.1 ± 2.1[h,i]
Casein[4,E]	0.73 ± 0.01	5.74 ± 0.02	89.5 ± 1.4[b]	100.9 ± 1.8[a]
Corn Gluten[1,D]	1.48 ± 0.02	5.67 ± 0.00	41.8 ± 1.0[o]	65.4 ± 1.7[l]
Crab Meal[1,B]	44.77 ± 0.59	2.64 ± 0.04	43.3 ± 1.4[n,o]	80.6 ± 1.9[f,g]
Diatomaceous Earth[5,E]	99.23 ± 0.08	0.15 ± 0.00	4.3 ± 2.1[p]	80.6 ± 2.1[f,g]
Distillers Grains[1,D]	5.02 ± 0.13	5.33 ± 0.03	47.2 ± 3.7[n]	69.6 ± 1.4[j,k]
Feather Meal[1,C]	2.74 ± 0.04	5.91 ± 0.01	61.3 ± 0.9[j,k,l,m]	72.7 ± 0.2[i,j]
Fish Meal (Anchovy)[1,A]	14.99 ± 0.23	5.16 ± 0.01	78.3 ± 2.3[c,d]	89.5 ± 0.5[b]
Fish Meal (Anchovy-Peru)[2,A]	14.37 ± 0.16	4.77 ± 0.02	78.0 ± 1.0[c,d]	87.1 ± 2.1[b,c,d]
Fish Meal (Herring)[1,A]	12.21 ± 0.03	5.30 ± 0.01	72.7 ± 3.9[d,e,f,g]	89.4 ± 0.7[b]
Fish Meal (Hoki-New Zealand)[2,A]	17.76 ± 1.55	4.62 ± 0.03	67.1 ± 2.0[g,h,i,j,k]	88.8 ± 1.2[b,c]
Fish Meal (Mackerel-Chile)[2,A]	16.92 ± 0.00	4.54 ± 0.04	73.5 ± 3.9[d,e,f,g]	88.3 ± 2.1[b,c]
Fish Meal (Menhaden)[1,A]	20.09 ± 0.21	4.80 ± 0.03	68.1 ± 2.1[f,g,h,i,j]	88.4 ± 2.0[b,c]
Fish Meal (Menhaden)[1,A]	29.15 ± 0.35	4.42 ± 0.03	55.6 ± 3.7[m]	83.3 ± 1.2[c,d,e,f,g]
Fish Meal (Menhaden)[3,A]	21.25 ± 0.09	4.64 ± 0.01	60.2 ± 0.5[k,l,m]	86.7 ± 1.9[b,c,d,e]
Fish Meal (Misc. Species-Asian)[2,A]	22.66 ± 0.51	4.14 ± 0.02	55.8 ± 4.0[m]	81.3 ± 1.0[f,g]
Fish Meal (Misc. Species-Peru)[2,A]	16.17 ± 0.04	4.76 ± 0.01	70.7 ± 4.0[e,f,g]	87.3 ± 1.6[b,c,d]
Gelatin[4,E]	0.06 ± 0.00	5.14 ± 0.02	96.5 ± 1.9[a]	102.2 ± 2.0[a]
Krill Meal[1,B]	12.23 ± 0.25	5.19 ± 0.01	72.6 ± 0.2[d,e,f,g]	80.6 ± 0.9[f,g]
Krill Flour[2,B]	9.52 ± 0.05	5.47 ± 0.02	81.7 ± 1.0[c]	87.2 ± 0.6[b,c,d]
Poultry By-Product[1,C]	16.80 ± 0.07	4.94 ± 0.02	63.9 ± 3.9[h,i,j,k,l]	82.1 ± 1.3[d,e,f,g]
Soybean Meal (48% Solvent Extract)[1,D]	7.40 ± 0.12	4.42 ± 0.01	75.9 ± 1.6[c,d,e]	85.6 ± 0.7[b,c,d,e,f]
Soybean Meal (Full Fat)[1,D]	5.31 ± 0.08	5.56 ± 0.03	63.5 ± 2.2[h,i,j,k,l]	80.8 ± 1.9[f,g]
Soybean Meal (Isolated, 90%)[1,D]	4.65 ± 0.02	5.38 ± 0.01	78.7 ± 0.7[c,d]	95.0 ± 5.5[a,b]
Squid (Liver Meal-Asian)[2,B]	6.27 ± 0.15	5.33 ± 0.03	61.8 ± 3.3[i,j,k,l,m]	74.0 ± 1.5[i,j]

Table 27. Continued

Ingredient	Ash (%)	DMEC (kcal g^{-1})	ADMD (%)	AED (%)
Squid (Muscle Meal-Lima)[1,B]	4.22 ± 0.59	5.63 ± 0.02	69.8 ± 4.6[e,f,g,h]	81.8 ± 1.6[e,f,g]
Squid (Muscle Meal-Paita)[1,B]	3.84 ± 0.03	5.69 ± 0.01	74.7 ± 1.4[d,e,f]	84.1 ± 0.7[b,c,d,e,f]
Squid (Whole)[1,B]	4.24 ± 0.05	5.61 ± 0.01	68.6 ± 1.0[f,g,h,i]	67.6 ± 7.8[k,l]
Squid (Whole, Asian)[2,B]	10.20 ± 0.08	4.73 ± 0.01	61.9 ± 0.8[i,j,k,l,m]	78.5 ± 1.4[g,h]
Wheat Gluten[4,E]	0.65 ± 0.00	5.65 ± 0.01	89.4 ± 1.0[b]	99.5 ± 1.4[a]
Wheat Starch[4,E]	0.21 ± 0.01	4.17 ± 0.02	92.3 ± 2.3[a,b]	98.9 ± 0.9[a]

Values are means of three determinations ± s.d.

[*]Means with similar lowercase superscripts are not significantly different (P>0.05).

[1]Zeigler Brothers, Gardners, PA, USA.

[2]Evialis, Vannes Cedex, France.

[3]Omega Protein Corporation Inc., Houston, TX, USA.

[4]MP Biomedicals, Cleveland, OH, USA.

[5]Sigma, St. Louis, MO, USA.

[A]Fish Meals.

[B]Marine Meals.

[C]Practical Animal Meals.

[D]Practical Plant Meals.

[E]Purified Ingredients.

Apparent crude protein digestibility

Apparent crude protein digestibility (ACPD) coefficients ranged from 59.1% for corn gluten to 99.7% for gelatin. ACPD coefficients for all purified ingredients (range: 95.8-99.7%) were greater than 95%, with gelatin having the highest value with an ACPD of 99.7%. ACPD coefficients for fish meals (range: 78.6-90.1%) were lower than those obtained for purified ingredients but higher than all other ingredient classifications. Soybean products (90% protein isolate and 48% solvent extracted meal) had significantly higher ACPD than all other practical plant meals (range: 59.1-93.7%), fish meals, marine meals, and practical animal meals tested while corn gluten meal (59.1%) had the lowest ACPD of all ingredients. ACPD coefficients for all practical animal meals (range: 63.9-78.7%) were in the bottom third of all ingredients with feather meal, conventional blood meal and spray dried blood meal comprising three of the five lowest ACPD values. ACPD coefficients for marine meals were between 89.4% for krill flour to 66.4% for Asian squid liver meal. No significant differences in ACPD coefficients were observed between the two different squid muscle meals; however, there was a significant difference between the two different whole squid products. ACPD was not correlated to crude protein content (P>0.05), energy content (P>0.05) or ash content (P>0.05) of the ingredients.

Apparent energy digestibility

Apparent energy digestibility (AED) coefficients ranged from 65.4% for corn gluten to 102.2% for gelatin. As a group purified ingredients had the highest AED coefficients ranging from a low of 80.6% for diatomaceous earth to a high of 102.2% for

gelatin. Gelatin had the highest numerical AED but was not significantly different from casein, 90% isolated soybean protein, wheat gluten and wheat starch. AED values for menhaden fish meal (83.3) and Asian miscellaneous species fish meal (81.3) were lower than all other fish meal AED values (range: 81.3 to 89.5%) and corresponded to the two lowest ADMD coefficients in the group; however, variability in AED coefficients were smaller than for ADMD coefficients. AED values of practical plant meals (range: 65.4-89.5%) showed the greatest degree of variability. Corn gluten meal had a significantly lower AED (65.4%) than all ingredients while the AED for 90% isolated soybean protein (95.0%) was not significantly different than the highest numerical ingredient AED. Practical animal meals (range: 72.2-82.1%) as a group had the lowest average AED and ash content of all ingredient classifications. Poultry by-product meal, which had the highest ash content in the group, had a significantly higher AED coefficient than all other ingredients in the group. AED coefficients for marine meals ranged from a low of 67.6% for squid liver meal to 87.2% for krill flour. No significant differences were noted between the two squid muscle meals; however, there were significant differences in AED coefficients between the two whole squid products. Apparent energy digestibility was positively correlated (r=0.91 P<0.0001) to apparent crude protein digestibility. AED was not correlated to crude protein content (P>0.05), energy content (P>0.05) or ash content (P>0.05) of the ingredients.

In vitro experiments

In vitro determination of apparent crude protein coefficients

   In vitro ACPD values obtained using 0.20% pepsin ranged from 97.6% for

conventional blood meal to 76.6% for crab meal and between 98.3% for conventional

blood meal to 36.7% for feather meal using 0.0002% pepsin (Table 28). *In vivo* ACPD

coefficients were positively correlated (r=0.55; P<0.05) to *in vitro* values obtained from

using 0.0002% pepsin. No correlation (P>0.05) was observed between *in vivo* ACPD

coefficients and *in vitro* values obtained using 0.20% pepsin.

Table 28. A comparison of *in vivo* and pepsin digestibility in *L. vannamei*.

Ingredient	*In vivo* digestibility		0.20% Pepsin	0.0002% Pepsin
	ADMD[1]	ACPD[1]	ACPD[1]	ACPD[1]
Blood Meal (Conventional)[2]	57.0 ± 3.8	66.2 ± 1.6	97.6	98.3
Blood Meal (Spray Dried)[2]	63.4 ± 4.5	70.8 ± 1.8	93.3	96.1
Corn Gluten[2]	41.8 ± 1.0	59.1 ± 1.9	97.7	45.3
Crab Meal[2]	43.3 ± 1.4	84.0 ± 1.9	76.6	61.0
Distillers Grains[2]	47.2 ± 3.7	78.5 ± 1.4	79.3	41.7
Feather Meal[2]	61.3 ± 0.9	63.9 ± 0.7	86.9	36.7
Fish Meal (Anchovy)[2]	78.3 ± 2.3	87.9 ± 0.7	95.3	88.6
Fish Meal (Herring)[2]	72.7 ± 3.9	90.1 ± 1.1	94.3	85.4
Fish Meal (Menhaden)[2]	68.1 ± 2.1	89.0 ± 2.2	96.4	93.6
Poultry By-Product[2]	63.9 ± 3.9	78.7 ± 1.7	92.0	61.7
Soybean Meal (48%)[2]	75.9 ± 1.6	91.9 ± 0.3	92.9	83.6
Soybean Meal (Full Fat)[2]	63.5 ± 2.2	87.1 ± 1.8	95.2	89.6
Squid (Muscle Meal-Lima)[2]	69.8 ± 2.6	84.6 ± 2.4	97.4	81.9
Squid (Muscle Meal-Paita)[2]	74.7 ± 1.4	86.6 ± 0.8	96.1	82.9

[1]All values reported as percentage ± standard deviation, where applicable.
[2]Zeigler Brothers, Gardners, PA, USA.

Discussion

In vivo experiments

Water quality

Water quality values obtained during this experiment were well below the upper limited recommended for shrimp which suggesting the shrimp in this experiment were maintained under optimal water quality parameters for the duration of the fecal collection period.

Weight-class effect on digestibility coefficients

Significant differences in ACPD were determined for the five different weight classes of *L. vannamei* fed the standard reference diet. Smith et al. (1985) reported no differences in protein or feed digestibilities for *L. vannamei* between 10 and 15 grams fed identical diets containing 22, 30 and 38% protein. No significant differences in protein digestibility was witnessed in the current study for *L. vannamei* between 11.75 and 15.09 grams; however, significant differences in protein digestibility were determined between 9.75 and 15.09-g *L. vanammei* which is only slightly outside the size range reported by Smith et al. (1985). Fenucci et al. (1982) also determined there was no significant difference in ACPD between 7 and 14-g *L. vannamei* which spans a size range that produced significant differences in this study. Differences in ACPD reported in this study are similar to those reported for *L. setiferus* (Fenucci et al., 1982) and suggests *L. vannamei* utilizes protein more efficiently at sizes less than 9.75 g.

The significant differences in apparent crude protein digestibility may be attributed to increased statistical sensitivity due to the small standard deviation between

replicates as the numerical difference in ACPD between all size classes was only 2.59% (87.79% – 90.38%). Since all shrimp used in the trial were from the same "batch" it is not likely the differences in ACPD were genetically induced; however, the 13.14- and 15.09-g *L. vannamei* had been fed chromic oxide digestibility feeds for 4 and 5 weeks, respectively. Divakaran (2005) suggested the ~1% free chromium, as chromium salts, in chromic oxide could be absorbed by shrimp, therefore its possible the lower ACPD reported in this study may be caused by chromium affecting the gut either as an irritant or as a "mild" toxicant. However, this would not explain the lack of differences for ADMD and AED as these values should be equally affected by anything interfering with digestion. The significant difference in ACPD coefficients reported in this study had no effect on ingredient ACPD coefficients as there was no significant difference in ACPD between the 9.75- and 11.33-g shrimp used in the ingredient digestibility study.

Apparent dry matter digestibility

Apparent dry matter digestibility provides a good estimate of the degree to which an ingredient is digested and absorbed by the gut. ADMD values were between 41.8 and 96.8% and were highest for the purified ingredients. Significant differences in ADMD were not determined between high protein and high carbohydrate purified ingredients which suggests *L. vannamei* are able to utilize carbohydrates as efficiently as protein provided dietary levels are within reason. The difference in ADMD between diets high in carbohydrate and protein reported by Akiyama et al (1989) may have been caused by comparing the protein diets to the diet high in corn starch as corn products have produced low apparent digestibility coefficients (Davis et al., 1993; Tables 26 and 27).

Apparent dry matter digestibility for wheat starch in this study was only exceeded by the ADMD obtained for gelatin and is higher than all ADMD values obtained by Akiyama et al (1989) which suggests it is readily utilized by *L. vannamei* as a carbohydrate (energy) source.

Purified ingredients had the highest ADMD coefficients of all ingredients tested in the current study.   While other ingredients tend to vary between studies and batches, purified ingredients consistently have high dry matter, energy and protein digestibility coefficients (*L. vannamei*: Akiyama et al., 1989 and Tables 26 and 27; *P. monodon*: Shiau et al., 1992; *Palaemon serratus*: Forster and Gabbott, 1971; *Pandalus platyceros*: Forster and Gabbott, 1971; *Procambarus clarkia*: Brown et al., 1986). These ingredients, while not commonly used commercially due to their price, are important sources of energy and protein in purified and semi-purified research diets. The consistency in apparent digestibility between shrimp species may allow for the formulation of a universal reference digestibility diet which would allow better comparison of data between species as well as reduce variability between studies involving the same species.

Differences in ADMD between the fish meals can be attributed to the negative correlation observed between ash content and ADMD. This correlation makes fish meal one of the few ingredients which can be initially evaluated for apparent dry matter digestibility based on an easily measurable compositional metric. Differences in ash between the fish meals, however, can't be attributed to species differences as it is not known how the samples were processed. Fish meals which are made from whole fish

typically have less ash than those which are made after fillets are removed (Anderson et al., 1993). Despite these potential processing differences, ADMD values obtained for Menhaden fish meal were in agreement with those values previously obtained for *L. vannamei* (Akiyama et al., 1989) and *P. setiferus* (Brunson et al., 1997).

ADMD coefficients for soybean meal increased with the level of ingredient refinement and protein content from 63.5% for full fat soybean meal to 78.7% for 90% isolated soybean protein. Similar increases were determined by Akiyama et al (1989) who reported an ADMD of 55.9% for soybean meal and 84.1% for soy protein fed to *L. vannamei*. This increase in ADMD can be attributed to the highly digestible protein contained in these ingredients and suggests the lipid fraction is poorly digested in the full fat and 48% soybean meals. The poor utilization of the lipid fraction may be due to the high (>10%) dietary level of lipid in the digestibility diets as *L. vannamei* has been shown to poorly utilize dietary lipid above 10% (Dokken, 1987).

ADMD coefficients for squid also increased with the level of ingredient "refinement" from whole squid (61.9-68.6%) to squid muscle meal (69.8-74.7%). This increase in ADMD may be attributed to the increased level of highly digestible protein in the squid muscle meal. The significant difference in ADMD between the two krill meals may be due to the difference in particle size which would provided more surface area for digestive enzymes; however, the meals were obtained from two different sources and may have other compositional differences which affected the ADMD determination.

Apparent protein digestibility

Apparent protein digestibility coefficients from this study compare very favorably with previously reported data for *L. vannamei* (Akiyama et al., 1989) using a single feedstuff. The lack of differences between the studies suggests ACPD coefficients are minimally affected by nutrient associations (current study) or nutritionally incomplete diets (Akiyama et al., 1989), provided the respective experimental methods are followed. The minimal difference between the studies may also be attributed to the same extrusion process (cold extrusion using a Hobart mixer) as extrusion techniques have been shown to affect apparent digestibility coefficients (Davis and Arnold, 1995). Differences in apparent digestibility attributed to extrusion method are not universal for all ingredients (Davis and Arnold, 1995) which suggest the importance of utilizing a "reference extrusion method" to allow comparison between different studies.

Purified ingredients had the highest dry matter protein contents and ACPD coefficients and lowest ash content of all ingredients tested. While these ingredients are highly digestible their amino acid profiles are not well balanced. Wheat gluten is low in lysine and has been used in experiments to determine the lysine requirement for *L. vannamei* (Fox et al., 1995). Purified diets high in casein and gelatin have produced growth responses which are typically lower than those obtained when using practical plant and animal meals (D'Abramo and Castell, 1997). These deficiencies, combined with their high cost, have limited their use to purified and semi-purified research feeds

which can be supplemented with additionally expensive additives (i.e. crystalline amino acids) to produce an adequate research diet.

Fish meal ACPD coefficients as a group were higher than all other ingredient classifications except purified ingredients. The high ACPD obtained for fish meals, combined with their balanced essential amino acid profile, implies their relative importance in dietary formulations and helps to explain why fish meal substitution with animal and by-product meals is not always successful. The significant differences in digestibility between the different lots of menhaden fish meal suggest the importance of batch to batch digestibility screening of raw ingredients. The higher ash content of the low ACPD menhaden fish meal suggests a higher bone and scale content which is indicative of the use of low quality material left over after the fish was filleted. Differences in ash may be detected by performing routine compositional analysis on incoming ingredients; however, this type of screening will not detect differences in apparent digestibility caused by excessive heat treatment, freshness of the ingredient, or differences in the drying processes (Anderson et al., 1993). Differences in apparent digestibility between batches of ingredients are common (Lemos et al., 2000) and can lead to formulation errors which could reduced shrimp growth. Differences among fish meal ACPD coefficients may also be attributed to differences in chemical composition caused by processing (i.e. amount of lipid left in the meal), excessive heat treatment or from species differences (Anderson et al., 1993). Despite all these potential effects, fish meal ACPD coefficients obtained in this study were in agreement with those previously

obtained for *L. vannamei* (Akiyama et al, 1989), *Procambarus clarkia* (Reigh et al.,
1990) and *Penaeus setiferus* (Brunson et al., 1997).

The protein in both 48% and 90% soybean products was significantly more
digestible than the protein found in the fish, animal and marine meals tested. Similarly,
Ezquerra et al. (1997) determined plant proteins were more digestible than animal
proteins using an *in vitro* ACPD method involving *L. vannamei*, while Smith et al.
(1985) reported no difference in ACPD between plant and animal protein for both
medium and large *L. vannamei*. These results are in contrast to the significantly lower
ACPD coefficients obtained for plant meals versus fish, marine and animal meals for *P.
serratus* (Forster and Gabbot, 1971), *P. platyceros* (Forster and Gabbot, 1971) and *P.
stylirostris* (Fenucci et al., 1982) and imply the omnivorous nature of *L. vannamei*.
These results suggest the importance plant proteins may have in removing fish meals
from *L. vannamei* diets; however, high ACPD coefficients alone will not predict
ingredients' ability to support growth as plant proteins are typically low in the essential
amino acids lysine and methionine. Apparent digestibility coefficients for protein need
to be combined with apparent amino acid digestibility to allow effective substitution of
low priced plant ingredients with higher priced fish meals.

While ingredient ash content tended to be inversely related to ingredient protein
content a similar correlation was not determined between ash content and ACPD. Corn
gluten meal had the second lowest ash content of all ingredients tested but had the lowest
ACPD. These results demonstrate the difficulty in predicting apparent digestibility
coefficients even for plant ingredients which typically have greater compositional

consistency between batches than fish, marine and animal meals. While high ash content may reduce digestibility coefficients, digestibility is also affected by processing and anti-nutritional factors such as tannins, phytate and oligosaccharides.

Practical animal meals generally are high in protein and contain a balanced amino acid profile; however they are commonly affected by a lack of consistent quality from batch to batch due to differences in processing and the quality of raw ingredients. Therefore, it is hard to determine if the low ACPD coefficients obtained for practical animal meals is attributed to the terrestrial nature of the protein or because the ingredient quality of the waste by-products, obtained during the slaughter and processing of poultry and cattle, were low. Differences in blood meal ACPD coefficients may be attributed to the difference in processing temperatures as spray drying typically involves lower processing temperatures than those encountered during the conventional ring-drying process. High temperatures experienced in the drying process can damage amino acids (i.e., Mallard reaction, oxidative degradation, etc.) making them unavailable to the animal. The high percentage of protein (>97%) in blood meal makes it especially sensitive to heat which can lead to very significant reductions in apparent protein digestibility (Cho et al., 1982).

It is interesting to note that despite the commercial use of krill and squid muscle meals in Penaeid diets, previous ACPD coefficients have not been reported. The marine protein in both squid muscle meals produced ACPD coefficients which were statistically equivalent to those obtained by the fish meals with the highest ACPD coefficients. Similarly, the protein in krill flour was only significantly less digestible than the protein

contained in purified ingredients and was statistically equivalent to the fish meals with the highest ACPD coefficients. The use of these ingredients in commercial formulations demonstrates how growth data, not digestibility coefficients, are utilized to formulate diets. The ACPD for crab meal was higher than previous results obtained for *L. setiferus* (Brunson et al., 1997) and *Procambarus clarkia* (Reigh et al., 1990); however, one must interpret this coefficient with caution as the high chitin content of this meal will be included as protein overestimating the ingredients true protein content. Similarly, ACPD coefficients for squid liver meal need to be interpreted with care as the ingredient is typically blended with either highly digestible soy protein or the lower digestible potato protein. Apparent digestibility of squid liver meal may change depending on which ingredient is included which may explain the large difference in ACPD between the current study and the results reported for *Penaeus monodon* (Merican and Shim, 1995).

While the ACPD coefficients obtained in this study may not be completely applicable for other species of shrimp due to species specific protein digestion (Lemos et al., 2000), these values may still serve as an estimate, especially where species-specific data are not yet available.

Apparent energy digestibility

Purified ingredients were highly digested and produced the highest AED coefficients of all ingredient classifications. The AED coefficient obtained in this study for wheat gluten was numerically lower than that obtained for *L. setiferus* (Brunson et al., 1997). A similar AED value was originally obtained for wheat gluten using the same calculation method (Cho et al., 1982) utilized by Brunson et al. (1997). The wheat

gluten coefficient was reduced to 99.5% after recalculation using the formula suggested

by Bureau and Hua (2006) which has been shown to account for the mathematical errors

in the Cho et al (1982) calculation. Brunson et al. (1997) attributed the 106% AED to

associative effects among ingredients; however, digestibility coefficients in the current

study were adjusted on average 5% when recalculated by the method suggested by

Bureau and Hua (2006). The lack of apparent nutrient associations determined in this

study may be attributed to the use of the calculation method as the elevated AED

coefficient originally obtained in this study for wheat gluten most likely would have

been attributed to a nutrient interaction.

The high ADMD (92.3%) and AED (98.9%) for wheat starch reported in this

study helps to explain why starch may replace protein in diets without decreasing growth

(Cruz-Suarez et al., 1994) as well as why high levels of wheat starch were efficiently

utilized by *L. vannamei* (Cousin, 1995). Davis et al (1993) however, reported a

comparatively low ADMD (51%) and an AED (71%) for wheat starch fed to *L.

vannamei*. Since both studies utilized the same experimental and extrusion methods the

differences illustrate how apparent digestibility coefficients can vary for feed ingredients

based on the environmental, physiological, and dietary conditions under which the

measurements were made. The effect of these factors on carbohydrate digestion was

determined by Gaxiola et al. (2005) who reported *L. vannamei* hexokinase IV-like

specific activity was affected by synergistic effects between dietary carbohydrate,

salinity and moult stage. These complexities and interaction in *L. vannamei* digestion

make obtaining an absolute value nearly impossible; however, apparent digestibility

coefficients still allow for the determination of a defined range for each ingredient and provide a valuable measure to formulate cost-effective, environmentally-friendly feeds.

AED coefficients for the fish meals evaluated in this study were consistently high ranking them ahead of all other classifications except purified ingredients. The high AED suggests the lipid fractions, which are an excellent source of essential fatty acids, contained within the fish meals were highly digested even though dietary lipid exceeded 10% for many of the fish meal digestibility diets. This high digestibility also may help to explain why fish meal replacement using solvent-extracted soybean meals, which appeared to have a low lipid digestibility in this study, do not always produce equivalent growth responses in *L. vannamei* (Lim and Dominy, 1990).

The low AED for corn gluten meal was surprising as Davis and Arnold (1995) reported an increase in AED values with increased processing; however, the ADE in this study is higher than the value obtained for steam cracked corn (Davis and Arnold, 1993). Steam cracked corn had the lowest ADMD and AED of ingredients tested by Davis and Arnold (1993) while corn gluten meal had the second lowest ADMD, and lowest AED and ACPD of all ingredients tested in this study. Extruded corn products have produced high AED coefficients; however, this effect is attributed to the increased gelatinization which occurs during extrusion (Davis and Arnold, 1995). High AED coefficients were obtained for cooked corn using *Procambarus clarkii* (Brown et al., 1989) and *Macrobrachium rosenbergii* (Law et al., 1990); however, AED values for non cooked corn products for these species are not available for comparison.

AED coefficients for crab meal were much higher than those reported for

Penaeus setiferus and suggest *L. vannamei* has a much higher chitinase activity.

Chitinases permit the digestion of chitinous exoskeletons which account for the majority

of the ash in crab meal. Studies have shown *L. vannamei* has chitinase activity and is

effectively able to digest chitin (Lee and Lawrence, 1982). While *P. setiferus* also

possesses chitinase activity, its ability to digest chitin is limited to 25% of the available

chitin when dietary levels are in excess of 40 g kg^{-1} (Clark et al., 1993).

Apparent energy digestibility was positively correlated to apparent crude protein

digestibility which is not surprising as the majority of energy in the dietary ingredients

tested comes from protein. Additional ingredients which possess the majority of their

energy as carbohydrates need to be tested to assess *L. vannamei's* ability to utilize this

source of energy. It is unfortunate that no direct comparison of the energy digestibility

coefficients obtained in this study could be compared to previously reported data from *L.*

vannamei, or in most cases other species of shrimp, as no published reports exist for the

ingredients tested.

In vitro experiments

In vitro determination of apparent crude protein coefficients

In vitro ACPD coefficients were positively correlated (r=0.55; P<0.05) to *in vitro*

values obtained using 0.0002% pepsin. These preliminary results are promising as *in*

vitro determinations are fast, inexpensive and can be performed in settings not

appropriate for *in vivo* work. The *r* value obtained in this study is the same as that

obtained for the pH-drop method (r^2=0.55) but lower than that obtained for the pH-stat

correlated method (r^2=0.73 to 0.80; Ezquerra et al., 1998). Utilization of 0.0002%

pepsin tended to underestimate the digestibility of samples with high ash content and

overestimate the digestibility of poorly digested samples which also was witnessed in

samples analyzed using the pH-drop method (Ezquerra et al., 1998). The lack of

correlation using 0.20% pepsin is not surprising as *L. vannamei* does not possess this

enzyme in their digestive tract (Lee and Lawrence, 1982). While these preliminary

results suggest dilute concentrations of pepsin may be utilized to approximate *in vivo*

results, Lemos (2003) reported pepsin digestibility is not applicable for prepared feeds

and plant ingredients. Considerably more research needs to be undertaken before the

results can be used to supplement *in vivo* digestibility trials involving *L. vannamei*. *In*

vitro digestibility results will only be able to replace *in vivo* work if they can predict the

complex nature of shrimp digestion, which has been shown to be modulated by the

components in the feed producing 10-44% differences in ADC over a control (Cordova-

Murueta and Garcia-Carreno, 2002). While *in vitro* methods have improved greatly, it

still appears they are not able to replace *in vivo* apparent digestibility trials especially

when one wishes to determine more than apparent protein digestibility.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Summary

Similar apparent consumption between the 25 and 35% crude protein diets (0.32 and 0.31 g-feed day^{-1} shrimp^{-1}; Figures 1 and 2, respectively) appears to suggest that *L. vannamei* regulates feed intake to meet an energy requirement as opposed to a protein requirement. This apparent ability to consume a diet to meet an energy level may explain the conflicting results in *ad-libitum* feeding studies which have attempted to determine optimum dietary protein requirements (as well as other dietary components) for *L. vannamei* as well as other species of shrimp. *Ad-libitum* requirement studies which have utilized diets low in digestible energy would be consumed at a greater level than diets with higher levels of digestible energy leading to different apparent dietary requirements. Since few, if any, of these requirement studies have cited dietary digestible energy content or measured diet consumption, there exists the possibility that many nutrient requirement studies may need to be reevaluated to determine their accuracy.

In the present study, protein conversion efficiency for maximum growth was more efficient in shrimp fed the 25% protein diet (35.9%; Table 9) as opposed to the 35% protein diet (30.6%; Table 10), although, growth was lower for shrimp fed that diet. This suggests shrimp which consumed the 25% protein diet lacked an adequate amount

of ingested protein to produce the same growth rate as observed in those fed the 35%

protein diet but were able to more efficiently utilize the available protein for growth, as

opposed to energy compared to shrimp fed the 35% protein diet. Based on the results

from this study, it appears to optimize protein utilization for growth the digestible energy

(DE) level of the 25% protein diet would need to be lowered to increase dietary intake or

the digestible protein level in the 35% protein diet would need to be lowered while

maintaining the same DE. The high level of ash (22%) and fiber (10%) already

contained in these diets may reduce one's success in lowering dietary energy enough to

increase consumption before the gut capacity of the shrimp is exceeded. Therefore it

seems prudent to attempt to reduce the protein level in the 35% diet to reach an ideal

protein/energy ratio where protein is maximally utilized for growth as opposed to

energy.

Accurate digestible protein and energy requirements are needed to precisely

formulate diets to meet nutritional requirements as well as to effectively allow cost

substitution of ingredients and reduce waste production. While adequate dietary protein

requirements have been estimated, few studies have determined daily digestible protein

and energy requirements for *L. vannamei*. This study utilized two diets (25 and 35%

crude protein) fed at 10 different feed rates to produce differences in shrimp specific

growth rate which were regressed against daily digestible protein and energy intake to

estimate the daily digestible protein and energy requirements. The mean apparent daily

digestible protein requirement for 7.69 to 13.08 g *L. vannamei* fed the 25% protein diet

was 0.067 g DP shrimp^{-1} day^{-1} (6.31 g DP kg^{-1} BW d^{-1}) while the 35% protein diet

produced a mean apparent digestible protein requirement of 0.090 g DP shrimp^{-1} day^{-1} (8.00 g DP kg^{-1} BW d^{-1}) for 8.11 to 13.79 g *L. vannamei*. Maintenance requirements were estimated by regressing the digestible crude protein feed rates back to zero and was 0.11 g DP shrimp^{-1} day^{-1} (1.03 g DP kg^{-1} BW d^{-1}) for *L. vannamei* fed the 25% protein diet and 0.021 g DP shrimp^{-1} day^{-1} (1.87 g DP kg^{-1} BW d^{-1}) for *L. vannamei* which consumed the 35% protein diet. The mean apparent daily DE requirement for *L. vannamei* fed the 25% protein diet was 4.276 kJ DE shrimp^{-1} day^{-1} (402.6 kJ DE kg^{-1} BW d^{-1}) while the 35% protein diet produced an apparent daily DE requirement of 3.765 kJ DE shrimp^{-1} day^{-1} (334.7 kJ DE kg^{-1} BW d^{-1}) for *L. vannamei*. Mean apparent daily DE maintenance requirements for *L. vannamei* fed the 25% protein diet was 0.702 kJ DE shrimp^{-1} day^{-1} (66.2 kJ DE kg^{-1} BW d^{-1}) while the requirement was 0.887 kJ DE shrimp^{-1} day^{-1} (78.8 kJ DE kg^{-1} BW d^{-1}) for *L. vannamei* fed the 35% protein diet.

The apparent digestible protein requirement for maximum growth decreased throughout the 7-week trial from 8.97 g DP kg^{-1} BW d^{-1} at week 2 for 7.69 g shrimp to 5.04 g DP kg^{-1} BW d^{-1} at week 7 for 13.08 g shrimp fed the 25% protein diet. A similar reduction was witnessed for those shrimp which consumed the 35% protein diet as the apparent protein requirement decreased from 10.24 g DP kg^{-1} BW d^{-1} at week 2 for 8.11 g shrimp to 7.11 g DP kg^{-1} BW d^{-1} at week 7 for 13.79 g shrimp. Apparent daily energy requirements for maximum growth also decreased throughout the 7-week trial as shrimp size increased. Apparent daily DE requirements for maximum growth at week 2 for 7.69 g shrimp was 540.1 kJ DE kg^{-1} BW d^{-1} while the apparent requirement was only 322.8 kJ DE kg^{-1} BW d^{-1} at week 7 for 13.08 g *L. vannamei* which consumed the 25% protein

diet. Apparent daily DE requirement also decreased for shrimp fed the 35% protein diet from 428.9 kJ DE kg^{-1} BW d^{-1} for 8.11 g *L. vannamei* at week 2 to 296.7 kJ DE kg^{-1} BW d^{-1} for 13.79 g shrimp at week 7. The reduction in apparent protein requirement has been attributed to a reduction in growth potential as shrimp get larger; however, maximum weekly growth throughout the 7-week trial was linear. This suggests the reduced apparent protein requirement may be partially explained by a reduction in protein digestibility as *L. vannamei* grows older (Chapter III, Table 25). This reduction in apparent protein digestibility combined with constant energy digestibility (Chapter III, Table 25) suggests their ability to utilize carbohydrates and lipids as energy, as opposed to dietary protein, increases with age which may contribute to the reduction in apparent protein requirement witnessed in this study.

Daily digestible protein and energy requirements also were determined by regressing body compositional data against daily digestible protein and energy intake. Daily digestible protein requirements for maximum growth for the 13.08 g shrimp fed the 25% protein diet obtained from body compositional analysis was 0.066 g DP shrimp^{-1} day^{-1} and 0.098 g DP shrimp^{-1} day^{-1} for 13.79 g shrimp fed the 35% protein diet. Maintenance requirements were 0.014 g DP shrimp^{-1} day^{-1} for the 13.08 g shrimp fed the 25% protein diet and 0.030 g DP shrimp^{-1} day^{-1} for the 13.79 g shrimp fed the 35% protein diet. DE requirements for maximum weight gain obtained from energy deposition was 4.330 kJ DE shrimp^{-1} day^{-1} for 13.08 g *L. vannamei* fed the 25% protein diet and 4.167 kJ DE shrimp^{-1} day^{-1} for 13.79 g shrimp fed the 35% protein diet. Although protein and energy requirements are crucial for developing a true least-cost

least-polluting diet, they must be combined with accurate digestible protein and energy data for ingredients commonly used in the aquaculture industry.

Significant differences in apparent crude protein digestibility (ACPD) were determined for the five different weight classes of *L. vannamei* fed the standard reference diet. Differences in ACPD reported in this study are similar to those reported for *L. setiferus* (Fenucci et al., 1982) and suggests *L. vannamei* utilizes protein more efficiently at sizes less than 9.75 g. Apparent dry matter digestibility (ADMD) values were between 41.8 and 96.8% and were highest for the purified ingredients. Significant differences in ADMD were not determined between high protein and high carbohydrate purified ingredients which suggests *L. vannamei* are able to utilize carbohydrates as efficiently as protein provided dietary levels are within reason. ADMD coefficients for soybean meal increased with the level of ingredient refinement and protein content from 63.5% for full fat soybean meal to 78.7% for 90% isolated soybean protein. This increase in ADMD can be attributed to the highly digestible protein contained in these ingredients and suggests the lipid fraction is poorly digested in the full fat and 48% soybean meals. The poor utilization of the lipid fraction may be due to the high (>10%) dietary level of lipid in the digestibility diets as *L. vannamei* has been shown to poorly utilize dietary lipid above 10% (Dokken, 1987).

Apparent protein digestibility coefficients from this study compare very favorably with previously reported data for *L. vannamei* (Akiyama et al., 1989) using a single feedstuff. The lack of differences between the studies suggests ACPD coefficients are minimally affected by nutrient associations (current study) or

nutritionally incomplete diets (Akiyama et al., 1989) provided the respective

experimental methods are followed. Fish meal ACPD coefficients as a group were

higher than all other ingredient classifications except purified ingredients. The high

ACPD obtained for fish meals, combined with their balanced essential amino acid

profile, implies their relative importance in dietary formulations and helps to explain

why fish meal substitution with animal and by-product meals is not always successful.

The plant protein in both 48% and 90% crude protein soybean meal was significantly

more digestible than the protein found in the fish, animal and marine meals tested.

These results suggest the importance plant proteins may have in replacing fish meals

from *L. vannamei* diets; however high ACPD coefficients alone will not predict the

ingredients ability to support growth as plant proteins are typically low in the essential

amino acids lysine and methionine. Apparent digestibility coefficients for protein need

to be combined with apparent amino acid digestibility to allow effective substitution of

low priced plant ingredients for higher priced fish meals. It is interesting to note that

despite the commercial use of krill and squid muscle meals in penaeid diets, previous

ACPD coefficients have not been reported. The marine protein in both squid muscle

meals produced ACPD coefficients which were statistically equivalent to those obtained

by the fish meals with the highest ACPD coefficients. Similarly, the protein in krill flour

was only significantly less digestible than the protein contained in purified ingredients

and was statistically equivalent to the fish meals with the highest ACPD coefficients.

While the ACPD coefficients obtained in this study may not be completely applicable

for other species of shrimp due to species specific protein digestion (Lemos et al., 2000),

these values may still serve as an estimate, especially where species specific data is not yet available.

The high ADMD (92.3%) and AED (98.9%) for wheat starch reported in this study helps to explain why starch may replace protein in diets without decreasing growth (Cruz-Suarez et al., 1994) as well as why high levels of wheat starch were efficiently utilized by *L. vannamei* (Cousin 1995). Apparent energy digestibility (AED) coefficients for the fish meals evaluated in this study were consistently high ranking them ahead of all other classifications except purified ingredients. The high AED suggests the lipid fractions, which are an excellent source of essential fatty acids, contained within the fish meals were highly digested even though dietary lipid exceeded 10% for many of the fish meal digestibility diets. This high digestibility may also help to explain why fish meal replacement using solvent-extracted soybean meals, which appeared to have a low lipid digestibility in this study, do not always produce equivalent growth responses in *L. vannamei* (Lim and Dominy, 1990). The low AED for corn gluten meal was surprising as Davis and Arnold (1995) reported an increase in AED values with increased processing; however, the ADE in this study is higher than the value obtained for steam cracked corn (Davis and Arnold, 1993). Steam cracked corn had the lowest ADMD and AED of ingredients tested by Davis and Arnold (1993) while corn gluten meal had the second lowest ADMD, and lowest AED and ACPD of all ingredients tested in this study. Extruded corn products have produced high AED coefficients; however, this effect is attributed to the increased gelatinization which occurs during extrusion (Davis and Arnold, 1995).

In vivo ACPD coefficients were positively correlated (r=0.55; P<0.05) to *in vitro* values obtained using 0.0002% pepsin. Utilization of 0.0002% pepsin tended to underestimate the digestibility of samples with high ash content and overestimate the digestibility of poorly digested samples which was also witnessed in samples analyzed using the pH-drop method (Ezquerra et al., 1998). While these preliminary results suggest dilute concentrations of pepsin may be utilized to approximate *in vivo* results, Lemos (2003) reported pepsin digestibility is not applicable for prepared feeds and plant ingredients. Considerably more research needs to be undertaken before the results can be used to supplement *in vivo* digestibility trials involving *L. vannamei*.

Conclusions

- Similar apparent consumption between the 25 and 35% crude protein diets (0.32 and 0.31 g-feed day^{-1} shrimp^{-1}; Figures 1 and 2, respectively) appears to suggest *L. vannamei* regulates their feed intake to meet an energy requirement as opposed to a protein requirement.

- It appears neither diet utilized in this study was optimally balanced in terms of an ideal protein/energy ratio. This ratio needs to be optimized to allow protein to be maximally utilized for growth as opposed to energy.

- Daily digestible protein and energy requirements determined by regressing body compositional data against daily digestible protein and energy intake were very similar to those values obtained by regressing changes in growth against daily digestible values. This suggests growth changes in body composition are a valid method to estimate requirements.

- The apparent digestible protein and energy requirements for maximum growth decreased throughout the 7 week even though maximum weight gain was linear. This decrease in apparent requirement may be attributed to the change in molting frequency or the decrease in protein digestibility with age.

- Significant differences in ACPD were determined for the 5 different weight classes of *L. vannamei* fed the standard reference diet which suggests *L. vannamei* utilizes protein more efficiently at sizes less than 9.75 grams.

- Purified ingredients had the highest ADMD, ACPD and AED coefficients of all ingredients tested which suggests digestibility is greatly enhanced as the level of refinement increases.

- The high ACPD obtained for fish meals, combined with their balanced essential amino acid profile, implies their relative importance in dietary formulations and helps to explain why fish meal substitution with animal and by-product meals is not always successful.

- The plant protein in both 48% and 90% soybean meal was significantly more digestible than the protein found in the fish, animal and marine meals tested. These results suggest the importance plant proteins may have in removing fish meals from *L. vannamei* diets.

- *In vivo* ACPD coefficients were positively correlated (r=0.55; P<0.05) to *in vitro* values obtained using 0.0002% pepsin and suggests *in vitro* studies may someday approximate *in vivo* studies.

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APPENDIX A

COMPOSITION OF THE MINERAL-VITAMIN PREMIX

Table A-1. Composition of the mineral-vitamin premix.

Nutrient Name	Unit of Measure	Value	Nutrient Name	Unit of Measure	Value
Calcium	%	0.08	Phenylalanine	%	0.40
Phosphorus	%	1.08	Phenyl-Tyrosine	%	0.80
Sodium	%	38.90	Threonine	%	0.36
Potassium	%	1.20	Tryptophan	%	0.12
Magnesium	%	0.56	Valine	%	0.56
Iron	PPM	72	Retinol	IU/KG	600000
Zinc	PPM	46072	Cholecalciferol	IU/KG	500000
Manganese	PPM	1100	Tocopherol	MG/KG	40012
Copper	PPM	12024	Thiamine	MG/KG	7056
Arginine	%	0.56	Riboflavin	MG/KG	11001
Histidine	%	0.24	Pyridoxine	MG/KG	22003
Isoleucine	%	0.44	Niacin	MG/KG	22096
Leucine	%	0.96	Pantothenic Acid	MG/KG	8208
Lysine	%	0.41	Biotin	MG/KG	200
Methionine	%	0.16	Folic Acid	MG/KG	5000
Methionine/Cystine	%	0.32	Cyanocobalam	MG/KG	40

% = Percent.
PPM = Parts per million.
IU/KG = International units per kilogram.
MG/KG = Milligrams per kilogram.

APPENDIX B

COMPOSITION OF THE VITAMIN-MINERAL PREMIX

Table B-1. Composition of the vitamin-mineral premix.

Nutrient Name	Unit of Measure	Value	Nutrient Name	Unit of Measure	Value
Calcium	%	0.08	Phenylalanine	%	0.40
Phosphorus	%	1.08	Phenyl-Tyrosine	%	0.80
Sodium	%	38.90	Threonine	%	0.36
Potasium	%	1.20	Tryptophan	%	0.12
Magnesium	%	0.56	Valine	%	0.56
Iron	PPM	72	Retinol	IU/KG	1100000
Zinc	PPM	72	Cholecalciferol	IU/KG	500000
Manganese	PPM	5300	Tocopherol	MG/KG	40012
Copper	PPM	24	Thiamine	MG/KG	3556
Arginine	%	0.56	Riboflavin	MG/KG	5551
Histidine	%	0.24	Pyridoxine	MG/KG	11006
Isoleucine	%	0.44	Niacin	MG/KG	11096
Leucine	%	0.96	Pantothenic Acid	MG/KG	4108
Lysine	%	0.41	Biotin	MG/KG	100
Methionine	%	0.16	Folic Acid	MG/KG	2500
Methionine/Cystine	%	0.32	Cyanocobalam	MG/KG	20

% = Percent.
PPM = Parts per million.
IU/KG = International units per kilogram.
MG/KG = Milligrams per kilogram.

ADDITIONAL PROTEIN/ENERGY CALCULATIONS FROM CHAPTER II

Table C-1. Body composition of *L. vannamei* fed incremental levels of a 25% crude protein, 3.80 kcal g^{-1} diet over 49 days.[1]

Daily Feeding Rate		Moisture	Energy	Protein	Ash
Protein (g DP shrimp^{-1})	Energy (kcal DE shrimp^{-1})	(%)	(kcal g^{-1})[2]	(%)[2]	(%)[2]
0.000	0.000	85.79±1.144[3]	3.80±0.123[3]	67.74±3.461[3]	21.21±2.217[3]
0.010	0.147	84.04±1.500	4.04±0.108	70.22±2.350	19.81±1.553
0.015	0.233	79.98±1.105	4.22±0.101	75.70±2.335	16.40±0.917
0.024	0.374	77.91±0.840	4.38±0.100	78.04±1.758	14.97±1.017
0.039	0.597	76.01±0.914	4.56±0.104	79.51±1.737	13.00±0.861
0.063	0.958	74.82±0.982	4.66±0.104	79.93±1.531	12.36±0.916
0.100	1.530	74.05±0.986	4.66±0.119	78.97±2.294	11.38±0.671
0.160	2.450	74.07±0.976	4.72±0.103	79.81±2.133	11.48±0.670
0.256	3.919	74.30±0.870	4.66±0.101	79.47±1.898	11.75±0.984
0.409	6.270	74.29±1.844	4.72±0.117	79.97±1.217	11.92±1.187
0.655	10.033	74.44±0.606	4.72±0.068	79.94±1.929	11.95±1.043
Initial		73.98±1.225	4.58±0.073	72.28±3.214	12.06±1.118

[1]Means of 20 shrimp ± standard deviation.
[2]Results expressed on a dry matter basis.
[3]Results based on 28 days of starvation.

Table C-2. Body composition of *L. vannamei* fed incremental levels of a 25% crude protein, 3.80 kcal g^{-1} diet over 49 days.[1]

Daily Feeding Rate		Moisture	Energy	Protein	Ash
Protein (g DP shrimp^{-1})	Energy (kcal DE shrimp^{-1})	(%)	(kcal g^{-1})[2]	(%)[2]	(%)[2]
0.000	0.000	85.79±1.144[3]	0.54±0.048[3]	9.64±1.120[3]	3.00±0.296[3]
0.010	0.147	84.04±1.500	0.64±0.061	11.21±1.149	3.09±0.347
0.015	0.233	79.98±1.105	0.84±0.056	15.16±1.059	3.18±0.149
0.024	0.374	77.91±0.840	0.97±0.051	17.25±0.894	3.26±0.158
0.039	0.597	76.01±0.914	1.09±0.060	19.08±0.952	3.05±0.210
0.063	0.958	74.82±0.982	1.17±0.058	20.13±0.863	3.05±0.230
0.100	1.530	74.05±0.986	1.21±0.062	20.49±0.850	2.92±0.151
0.160	2.450	74.07±0.976	1.22±0.063	20.68±0.724	2.96±0.105
0.256	3.919	74.30±0.870	1.20±0.052	20.42±0.884	2.96±0.188
0.409	6.270	74.29±1.844	1.21±0.109	20.56±1.515	2.97±0.101
0.655	10.033	74.44±0.606	1.21±0.041	20.43±0.778	3.06±0.286
Initial		73.98±1.225	1.19±0.065	18.76±0.689	3.13±0.298

[1]Means of 20 shrimp ± standard deviation.
[2]Results expressed on a wet weight basis.
[3]Results based on 28 days of starvation.

Table C-3. Body composition of *L. vannamei* fed incremental levels of a 35% crude protein, 3.70 kcal g^{-1} diet over 49 days[1].

Daily Feeding Rate		Moisture	Energy	Protein	Ash
Protein (g DP shrimp^{-1})	Energy (kcal DE shrimp^{-1})	(%)	(kcal g^{-1})[2]	(%)[2]	(%)[2]
0.000	0.000	85.79±1.144[3]	3.80±0.123[3]	67.74±3.461[3]	21.21±2.217[3]
0.015	0.145	84.88±1.770	4.06±0.270	67.99±3.573	21.79±2.316
0.023	0.231	80.31±0.874	4.19±0.101	74.20±2.694	16.90±1.006
0.037	0.370	78.37±1.010	4.36±0.079	78.03±2.128	14.89±1.244
0.059	0.591	75.22±4.934	4.56±0.095	79.88±3.490	13.17±1.347
0.095	0.948	75.10±2.388	4.67±0.118	81.01±1.892	11.98±0.997
0.152	1.514	74.17±0.604	4.71±0.095	80.53±1.694	11.57±0.771
0.244	2.424	74.19±1.043	4.69±0.104	80.66±2.821	11.87±0.701
0.390	3.878	73.96±0.884	4.72±0.078	80.31±2.242	11.80±0.833
0.624	6.204	73.68±0.796	4.73±0.101	79.79±2.246	11.46±0.761
0.999	9.927	74.66±2.432	4.75±0.099	80.84±2.563	11.61±0.600
Initial		73.98±1.225	4.58±0.073	72.28±3.214	12.06±1.118

[1]Means of 20 shrimp ± standard deviation.
[2]Results expressed on a dry matter basis.
[3]Results based on 28 days of starvation.

Table C-4. Body composition of *L. vannamei* fed incremental levels of a 35% crude protein, 3.70 kcal g^{-1} diet over 49 days[1].

Daily Feeding Rate		Moisture	Energy	Protein	Ash
Protein (g DP shrimp^{-1})	Energy (kcal DE shrimp^{-1})	(%)	(kcal g^{-1})[2]	(%)[2]	(%)[2]
0.000	0.000	85.79±1.144[3]	0.54±0.048[3]	9.64±1.120[3]	3.00±0.296[3]
0.015	0.145	84.88±1.770	0.62±0.099	10.27±1.370	3.27±0.392
0.023	0.231	80.31±0.874	0.83±0.046	14.62±0.977	3.34±0.160
0.037	0.370	78.37±1.010	0.94±0.047	16.90±1.160	3.34±0.316
0.059	0.591	75.22±4.934	1.13±0.236	19.78±3.862	3.35±0.636
0.095	0.948	75.10±2.388	1.16±0.126	20.19±2.093	2.86±0.252
0.152	1.514	74.17±0.604	1.22±0.377	20.80±0.716	2.97±0.172
0.244	2.424	74.19±1.043	1.21±0.067	20.83±1.277	3.02±0.187
0.390	3.878	73.96±0.884	1.23±0.053	20.91±0.807	3.07±0.213
0.624	6.204	73.68±0.796	1.24±0.048	20.96±0.815	3.00±0.176
0.999	9.927	74.66±2.432	1.21±0.123	20.57±2.157	2.84±0.411
Initial		73.98±1.225	1.19±0.065	18.76±0.689	3.13±0.298

[1]Means of 20 shrimp ± standard deviation.
[2]Results expressed on a wet weight basis.
[3]Results based on 28 days of starvation.

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