

Provided for non-commercial research and educational use only.
Not for reproduction or distribution or commercial use.



This article was published in an Elsevier journal. The attached copy is furnished to the author for non-commercial research and education use, including for instruction at the author's institution, sharing with colleagues and providing to institution administration.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>

Variable feed allowance with constant protein input for the pacific white shrimp *Litopenaeus vannamei* reared under semi-intensive conditions in tanks and ponds

Jesus A. Venero, D. Allen Davis*, David B. Rouse

Department of Fisheries and Allied Aquacultures, Auburn University, 203 Swingle Hall, Auburn, AL 36849, USA

Received 1 November 2006; received in revised form 4 February 2007; accepted 5 February 2007

Abstract

Feed input may be reduced in shrimp aquaculture by increasing the protein density of the diet modifying the feeding rate so as to deliver the desired amount of nutrients. To evaluate this strategy, two parallel growth trials were conducted with juvenile *Litopenaeus vannamei*. In an outdoor tank trial, juvenile (0.57 ± 0.01 g, $n=30$) shrimp were reared for 56 days and fed two practical diets formulated to contain 30% or 40% crude protein (CP). Each diet was offered at three different feeding rates (50%, 75%, and 100% ration). At the end of the trial, final weight of shrimp offered the 30% CP diet ranged from 8.1 to 10.3 g, and 8.7–11.3 g for shrimp fed the 40% CP diet. Final weight, weight gain, FCR, and PCE was reduced significantly at every reduced feed ration (50 and 75%). There were no significant differences in final weight (10.3 g, mean weight) of shrimp offered the two treatments with similar protein inputs (30–100% and 40–75%). However, FCR was significantly lower for the 40–75% treatment (1.1 versus 1.4). To demonstrate the effect of variable feed allowance under practical pond conditions 12 ponds were stocked at 35 shrimp/m² (0.04 ± 0.04 g, $n=56$) and assigned three treatments (30–100%, 30–75%, and 40–75%). A fourth treatment (40–100%) was initiated two weeks later utilizing two additional ponds to allow observational data collection. At the end of the trial (114–121 days) the final weight (19.7–21.7 g), FCR (1.0) and survival (75–88%) between the treatments 30–100% and 30–75% were not significantly different. However, production was significantly higher for the treatment 30–100% than for the treatment 30–75% (6482 versus 5054 kg/ha). Based on observational data, yield was numerically higher for the 40–75 treatment than for the 30–75 treatment and it tended to be lower than that of the 30% CP diet offered at standard rate (30–100%). This study demonstrates that increasing the nutrient density (protein content) of the shrimp feed allows for a reduction of feed input without affecting the growth performance of shrimp in tanks. Results of the tank trial could not be verified through the pond trial due to the loss of some experimental units. As interactions of natural productivity and feed distribution may alter the results under pond production conditions further research in ponds is warranted.

© 2007 Elsevier B.V. All rights reserved.

Keywords: *Litopenaeus vannamei*; Feeding; High nutrient diets; Shrimp diets

1. Introduction

Commensurate with the growth of the crustaceans aquaculture industry there has been a shift toward high intensity systems and an increase in feed inputs (FAO, 2000; Tacon et al., 2000). Under current production

* Corresponding author. Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, AL 36849-5419, USA. Tel.: +1 334 844 9312.

E-mail address: davisda@auburn.edu (D.A. Davis).

conditions, feed represents one of the primary variable costs associated with the production of shrimp. At the same time the feed input produces wastes that can cause negative impacts to the environment if they are released to effluent waters (Persson, 1991; Cho and Bureau, 2001). The amount of these wastes is increased when shrimp farmers follow inappropriate feed management strategies and overestimate the required feed inputs. This excess of feed can cause deterioration of water quality that leads to poor growth and survival with a consequent reduction in production and economic return (Wyban et al., 1989). For these reasons there is an interest in optimizing feed input and feed management to improve economic return in shrimp farms and to reduce the potential of environmental impact.

In marine shrimp culture, optimal daily supply of protein is one of the most important components in a feed management program. Protein is one of the major nutrients for shrimp growth and represents one of the primary costs in a compound feed formulation (Tacon and Akiyama, 1997). Nitrogen, the product of protein metabolism when it is not deposited as growth is the nutrient that most contributes with eutrophication problems in marine and brackish water environments (Persson, 1991; Jackson et al., 2003).

In order to reduce the excretion of nitrogenous compounds and their associated problems, the amount of protein input in a shrimp farm should be optimized, based on the protein requirements of the species, and adjusted for availability (digestibility). Traditionally, to determine nutrient requirement of an animal species researchers have focused on manipulating the level of the major nutrients such as protein in the diet. However, it has been demonstrated that animals have a daily quantitative requirement that can be met with a variety of diets with different levels of the nutrients (Lawrence and Lee, 1997; McGoogan and Gatlin, 1998; Kureshy and Davis, 2002). Therefore, feed input will vary and should depend on the nutrient density of the diet. Based on this approach it has been recommended to increase the nutrient density of the diets to reduce feed input and associated problems (Cho and Bureau, 2001).

A direct advantage of feeding high-nutrient density diets at a reduced feeding input is an improvement in feed efficiency (FE) and feed conversion ratio (FCR). Since the FCR is the amount of feed offered to produce a unit of shrimp, a lower amount of feed and consequently lower FCR is expected when feeding a diet with higher concentration of nutrients to produce the same amount of growth.

As expected, the use of high nutrient density diets reduces the amount of feed necessary to reach the production goals. Although this practice has been recom-

mended and research has been conducted with some aquatic species (Watanabe et al., 1979; McGoogan and Gatlin, 1999; Cho et al., 2001; Cho and Lovell, 2002), its use and research has been more limited for shrimp, especially under pond semi-intensive production conditions. Consequently, the objective of this study was to evaluate the effect of adjusting the daily feed allowance to the protein requirements of *L. vannamei* when utilizing diets with two different protein levels.

2. Materials and methods

2.1. Study site, shrimp transport and acclimation

This research was conducted at the Claude Petet Mariculture Center, in Gulf Shores, Alabama, from May through mid September. Two experiments were conducted in parallel; one in plastic tanks that received water from a shrimp production pond and the other in replicated ponds under semi-intensive production conditions. For this work, approximately 1 million post-larva Pacific white shrimp *L. vannamei* (4.3 ± 2.3 mg, mean \pm standard deviation; $n=57$) were received from a commercial hatchery (GMSB Inc. hatchery, Key West, FL, USA). The post-larvae (PL) were shipped in styrofoam boxes that contained double plastics bags with about 6 l of 15 g/l salt water at 21 °C and 13.1 mg/l dissolved oxygen (DO) at an approximate density of 1500 PL/l. The bags were placed in a 940-l acclimation tank that was filled with 15 g/l sea water and allowed to float until the temperatures were equilibrated. The PL were released after the water in the bags and the acclimation tank were within 0.5 °C of each other. The PL were pooled together in the acclimation tank and newly hatched nauplii of *Artemia salina* (INVE Americas, Inc., Salt Lake City, UT, USA) were fed. After approximately 1 h, the PL were concentrated (50 l) in oxygenated water and quantified volumetrically (Hardin et al., 1985; Juarez et al., 1996). The average number of PL from three sub-samples (60 ml beaker) were determined. If the CV was >15% then additional sub-samples were counted. The average number of PL was then used to estimate the population in the 57-l container which was sub-divided into six groups of approximately the same number of PL and stocked in each of six nursery tanks.

2.2. Shrimp nursery

The nursery phase was carried out in six fiberglass tanks ($3.0 \times 1.5 \times 0.9$ m) that were located in a clear polyethylene plastic covered greenhouse. The nursery system was designed as a semi-closed recirculating

system containing six culture tanks, common biological filter, a rapid-rate sand filter (Model TR100, AREA, Homestead, FL, USA) and a circulation 2-hp high head pump (AREA, Homestead, FL, USA). PL were fed *Artemia* nauplii (~100 nauplii/PL) and a commercial PL feed (Zeigler Bros., Inc., Gardners, PA, USA) four times a day during the first three days (Table 1). From day four to day five equal proportion of the commercial PL feed and a crumbled commercial shrimp feed (Rangen® Inc., Buhl, ID, USA) were fed four times a day. Thereafter, four feedings per day of the crumbled feed (Rangen® Inc., Buhl, ID, USA) of various sizes were fed following the schedule shown in Table 1. Temperature, DO, pH, and salinity were monitored twice daily (at 0800 and 1600 h) using a YSI 556 DO meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonia-nitrogen (TAN) was measured every three days with a spectrophotometer (Spectronic 20 Genesys, Spectronic Instrument Inc. Rochester, NY, USA) following the Nesslerization method (APHA, 1989). Every week about 50% of the total water volume was exchanged with filtered sea water. Water quality parameters were as follows: temperature, 27.4 ± 1.55 °C; DO, 5.98 ± 1.67 mg/l; pH, 7.47 ± 0.33 ; salinity, 33.62 ± 0.33 g/l; and TAN, 0.81 ± 0.54 mg/l. The survival rate, FCR (dry weight of feed offered/wet weight gained), and final weight at the end of a 22-day nursery period were (mean \pm standard deviation) $67.48\% \pm 19$, 1.83 ± 0.79 , and 0.0413 ± 0.0091 g, respectively. At the conclusion of the nursery culture the juvenile shrimp were stocked into pond.

2.3. Tank trial

The tank trial was carried out over 56 days in an outdoor semi-closed recirculating system. It comprised

Table 1
Feeding regimen utilized during a 22-day nursery period of *Litopenaeus vannamei*

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

Feed rates as percentage of the biomass were based on an expected survival rate of 70% (during 22 days) and average shrimp weights.

^aINVE Americas, Inc., Salt Lake City, UT, USA.

^bPL Ready 50% Protein, Zeigler Bros., Inc., Gardners, PA, USA.

^c ^dRangen 45% Protein, Rangen Inc., Buhl, Idaho, USA.

Table 2

Composition (g/100 g as fed) of practical diets formulated to contain 30% CP–6% lipid and 40% CP–8% lipid

Ingredient*	D30	D40
Menhaden fish meal Select	7.5	9.99
Poultry by-product meal 62%	18.83	24.98
Milo	54.5	29.64
Soybean meal	14.5	30.7
Fish oil	1.07	2.37
Bentonite	1.5	1.5
Trace mineral premix	0.065	0.085
Vitamin premix	0.285	0.38
Copper sulfate	0.01	0.013
Stay C (30% active)	0.0057	0.008
Dicalcium phosphate (21% P)	1.583	0.583
Propionic acid (mold inhibitor)	0.15	0.15

Diets were commercially manufactured (Rangen® Inc., Angleton, TX).

*Information on the source and composition of the ingredients was not provided by the manufacturer.

six feeding treatments which were randomly assigned to 24 tanks (30 shrimps/tank) with four replicates per treatment. The feeding treatments were consisted of two practical diets (Tables 2 and 3) formulated to contain 30% and 40% crude protein, which were both assigned at three feeding rates: 100%, 75% and 50% of a standard feeding rate. The standard feeding rate was based on an expected feed conversion ratio (FCR) of 1.8 and a growth rate of 1 g/week, or 0.26 g/shrimp/day. The 40% CP diet fed at 75% feeding level (40–75%) matched the crude protein input of the control (30% CP diet at 100% ration). The diets had been prepared to the specified

Table 3

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

ADC	30% CP	40% CP
Crude protein	32.2	42.5
Crude fat	10.3	11.9
ADDM	60.8	63.8
ADP	69.3	76.9
ADE	66.7	75.4
Digestible protein (%)	22.3	31.1
Digestible energy (kcal/g diet)	3.12	3.66
kcal DE/g CP	9.96	9.04
kcal DE/g DP	13.99	11.76
DE fed at 100% rate (kcal/shrimp/day)	0.8	0.94
DE fed at 75% rate (kcal/shrimp/day)	0.6	0.71
DE fed at 50% rate (kcal/shrimp/day)	0.4	0.47
DP fed at 100% rate (g/shrimp/day)	0.053	0.079
DP fed at 75% rate (g/shrimp/day)	0.043	0.059
DP fed at 50% rate (g/shrimp/day)	0.029	0.039

formulation at a commercial feed mill using extrusion technologies (Rangen® Inc. Angleton, TX, USA).

Juvenile *L. vannamei* (0.57 ± 0.01 , $n=30$) used in the trial were selected and hand-sorted for size from production ponds. They were stocked into the tank system five days prior to the start of the growth trial, during which time they were fed two times daily with 0.06 g feed/shrimp/d. Thereafter, shrimp were fed the whole ration twice a day, at 0900 h and at 1500 h, half of the daily feed allowance at each feeding. Shrimp in each tank were counted every two weeks and the daily ration readjusted.

The semi-closed recirculating system consisted of 24 circular polyethylene tanks (0.85 m height \times 1.22 m upper diameter, 1.04 m lower diameter), a common reservoir with a biological filter, and a 1/3-hp circulation pump. The recirculation rate for each tank was set at 2.92 l/min ($n=3$). The reservoir received water from a shrimp production pond for 6 h each day at a flow rate of 3 l/min. Each tank was equipped with a center drain, a stand pipe of 3.2 cm diameter which was 75 cm long, screened and set to maintain a water depth of 61 cm or 570 l volume. Aeration was provided by two air stones connected to a common air supply from a 1hp regenerative blower (Sweetwater Aquaculture Inc. Lapwai, ID, USA).

Dissolved oxygen, pH and temperature were measured twice a day (at 0600 and 1600 h, which were the approximate times of minimum and maximum temperatures and DO, respectively). These parameters were determined with a YSI 556 DO meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Total ammonia-nitrogen and nitrite-nitrogen were determined once a week. Water samples were taken from the mid water column in the reservoir in two tanks selected at random. Total ammonia-nitrogen was measured with a spectrophotometer (Spectronic 20 Genesys, Spectronic Instrument Inc. Rochester, NY, USA) following the Nesslerization method (APHA, 1989). Nitrite-nitrogen was determined using a model PLN code test kit from LaMotte (Chestertown, MD, USA).

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

Samples were kept frozen in sealed plastic bags in a freezer at -15 °C until analyzed. Shrimp samples were

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

2.4. Pond trial

Juvenile (0.04 ± 0.04 g, $n=56$) Pacific white shrimp *L. vannamei* were reared in fourteen 0.1-ha ponds under semi-intensive conditions at a density of 35 shrimp/m² for 114–121 days and fed two practical diets of 30% and 40% crude protein (Rangen® Inc., Angleton, TX). Initially 12 ponds were stocked and assigned three experimental treatments with four replicates each. The 30% crude protein diet was fed at 75% and 100% of the feeding rate and the 40% crude protein diet was fed at 75%. The first treatment (30–100%) received a 30% CP feed at a typical feed rate (100% ration) to obtain an expected feed conversion rate (FCR) of 1.8 and a growth rate of 1 g/week. This feed rate was based in previous production records in the same experimental units with the same species (Zelaya, 2005) (Fig. 1). The second treatment (30–75%) was fed the 30% CP diet, but it was offered at 75% of the feed allowance of a typical ration. The third treatment (40–75%) consisted of feeding a 40% CP diet at 75% of the feed allowance. This matched the total crude protein inputs of treatment 1 (30–100%). Two weeks after the initial stocking of the 12 ponds, two additional ponds were added to the study. This allowed an observational review of the fourth treatment, which received the 40% CP diet fed at 100% ration.

Shrimp were fed twice a day (about 50% of total daily ration each time) spread uniformly throughout the pond area at approximately 0900 h and at 1600 h. All the ponds were fed equally during the first 18 days of culture (Fig. 1). During the first seven days, 1000 g of 35% CP feed (crumbles # 4, Rangen® Inc., Buhl, ID) was fed daily to each pond. From day 8 to day 18 shrimp were fed 1500 g per day of the same feed. Feeding the treatment diets started at day 19 at half of the total feed input per treatment and continued until day 22. The complete daily ration, for the assigned treatments was fed after day 23. The amount of daily feed input in each pond was calculated based on the ration for each treatment (75 or 100%) and adjusted weekly for mortalities (assuming an expected mortality of 30% over a 17-week growth-out period). Feeding ceased

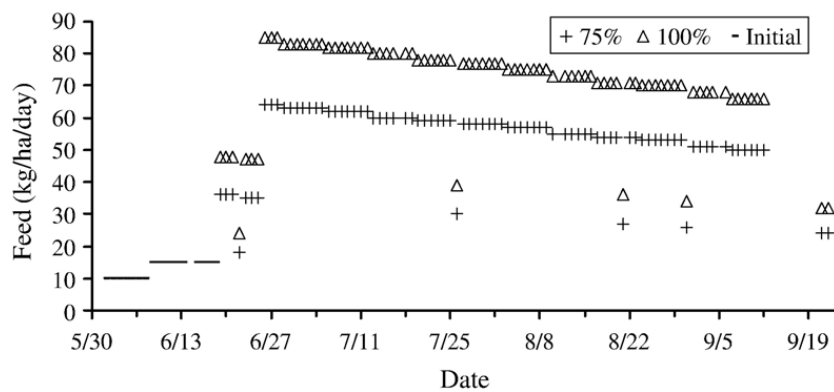


Fig. 1. Daily feed input (kg/ha/day) of *Litopenaeus vannamei* fed two practical diets (30% and 40% crude protein) at two feeding levels (75% and 100%) and raised in ponds at 35 shrimps/m².

9 days before harvesting due to the interruption of the electrical service caused by hurricane Ivan.

The ponds of approximately 1-m depth, were lined with 1.52-mm thick high-density polyethylene sheeting (Grundle Lining System, Inc., Houston Texas, USA), and had about 25-cm layer of sandy-loam soil. Each pond had a concrete catching basin and was drained through a 20-cm diameter screened standpipe located inside the catching basin. Pond preparation consisted of draining the water and letting the pond bottoms dry for several weeks. They were then tilled using a garden tiller set for a depth of 10–15 cm. This exposed the soil to the air and sunlight to improve oxidation and mineralization of the organic matter. Brackish (~24 g/l) water from the intracoastal waterway, a shipping channel that connects Bon Secour Bay with Wolf Bay in Gulf Shores and Orange Beach, was used to fill the ponds 2–3 weeks before stocking. The water was filtered through a 250- μ m nylon filter sock (Domestic Lace Mfg., Inc.) that prevented the introduction of potential predators. Liquid inorganic fertilizers (N–P–K) were applied at 303 ml of 10–34–0 and 1697 ml of 32–0–0 per pond that provided 5.73 kg N and 1.03 kg of P₂O₅ to stimulate plankton growth and natural productivity (Boyd and Tucker, 1998). Two weeks after the initial fertilization a second fertilization was applied if a secchi disk reading of 25–40 cm was not reached.

No water exchanges were carried out until the last 2 weeks of the pond production trial. Dissolved oxygen, pH and salinity, were measured three times a day (at 0600, 1200, and 2000 h). Total ammonia-nitrogen, nitrite-nitrogen, and secchi disk reading were determined once a week. Water samples were taken from the mid water column and taken to the lab in closed plastic 1-l containers. These water quality parameters were determined according to previously described procedures. Total phosphorus levels of the water were

determined two weeks before the end of the trial for the first three treatments. To determine total phosphorus, samples were digested using the potassium persulfate method (Boyd and Tucker, 1992) and analyzed to determine soluble ortho-phosphate using the ascorbic acid method (APHA, 1989).

Base aeration (7.5 kW/ha) was provided at night to keep the level of DO at 3 mg/l or higher using either a 1-hp spiral paddle wheel aerator (Little John Aerator, Southern Machine Welding Inc. Quinton, AL) or a 1-hp (11.2 A) or 2-hp aspirator (20 A) (Aire-O₂, Aeration Industries International, Inc. Minneapolis, MN, USA). Aeration was also provided during the day when the levels of DO fell below 3 mg/l (e.g., cloudy days or after a pond phytoplankton community had collapsed). Additional aeration (up to 30 hp/ha) was provided when the standard aeration was not enough to maintain the expected DO.

The mean weight of the shrimp were determined on a weekly basis starting two weeks after stocking. The first sample was taken by seine, thereafter ~80 shrimp per pond were captured by cast net (monofilament net, 1.22 m radius and 0.95 cm mesh). Pond harvest was initiated at day 107, but the activity had to be interrupted due to mandatory evacuation orders and the anticipated arrival of a hurricane. The rest of the ponds were harvested at days 114–115. One night before harvesting, approximately 70% of the water was drained. The following day, the remaining water was pumped out through a hydraulic fish pump with a 25-cm suction (Aqualife-Life pump, Magic Valley Heli-arc and Mfg., Twin Falls, Idaho, USA) placed in the catch basin. The shrimp were pumped from the pond basin to a dewatering tower, then to a harvest truck that was used to transport the shrimp to a cleaning table and weighing station. At harvest, mean weight and size distribution of 100 shrimps selected at random from each pond were determined. Average final weight,

percent survival, FCR, size distribution and yield were then determined.

2.5. Digestibility trial

In order to estimate protein digestibility of the test diets, a digestibility experiment was carried out. Chromic oxide was used as an inert marker to determine the digestibility coefficients. Six groups of 10 shrimp (~10.2 g mean weight) were stocked in six 60-l glass aquaria closed recirculating system. Half of the tanks were assigned a 30% CP practical diet while the other half was assigned a 40% CP diet ($n=3$, Tables 2 and 3). The practical diets were ground to a powder and then re-extruded after adding 0.5% of chromic oxide. Each ground diet was transferred to a food mixer (Hobart, Troy, OH, USA) where they were mixed for about 5 min. Boiling water was then added to the mixture while it was still mixing to attain a mash of consistency appropriate for pelleting. The mash was passed through a 3-mm die in a meat grinder (Hobart, Troy, OH, USA), and the pellets were dried at 65 °C overnight to reach a moisture content between 8 and 10%. Diets were ground and sieved to an appropriate size and stored in a -20 °C freezer until used.

Shrimp were allowed to adapt to the allocated diets for three days before starting the collection of feces. Prior to each feeding the tanks were cleaned. The shrimp were then offered an excess of feed. One hour after feeding, feces were collected by siphoning onto a 500 µm mesh screen. Only well formed and stable fecal filaments were collected to avoid contamination of the sample with food particles. Shrimp were offered five feedings per day, however, feces obtained after the first feeding were discarded. Collected feces were rinsed with distilled water and stored in sealed plastic containers and stored in a freezer. Samples were collected from each tank and were kept frozen until analyzed. Dry

matter, crude protein, and total energy were determined for the fecal and the diet samples according with procedures previously described. Chromic oxide was analyzed following the McGinnis and Kasting (1964) procedures. Apparent digestibility coefficients (ADC) of the dry matter, protein, and energy were calculated according to Maynard et al. (1979).

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

2.6. Statistical analysis

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

3. Results

3.1. Tank trial

Water quality parameters observed over the 56-day growth trial tanks are presented in Table 4. Water quality problems or diseases were not observed during the experiment and there were no significant differences in survival (Table 5). A two-way ANOVA was used to analyze the effect of feed rate (100, 75, and 50%) and

Table 4

Water quality of a tank recirculating system (tank trial) and a pond trial used to raise *Litopenaeus vannamei* juveniles fed diets with 30% or 40% protein at three different feeding levels (50, 75, and 100%)

Parameter	Tanks	Ponds			
		30–100%	30–75%	40–75%	40–100%
DO (mg/l)	6.9±1.9 (5.0–10.8)	5.9±2.8 (1.1–15.5)	6.1±2.6 (0.9–16.6)	5.9±2.8 (0.2–15.8)	6.2±2.6 (1.1–15.2)
pH	7.5±0.4 (5.9–8.1)	7.9±0.6 (5.9–9.5)	7.9±0.7 (5.7–9.6)	8.0±0.6 (5.9–9.8)	8.1±0.6 (6.3–9.3)
Temperature (°C)	27.0±1.9 (20.2–30.4)	30.2±1.8 (24.5–34.1)	30.3±1.9 (24.6–34.2)	30.1±1.80 (24.6–34.3)	30.3±1.8 (24.6–33.9)
Salinity (g/l)	18.6±1.7 (15.7–20.7)	18.9±2.5 (9.8–24.8)	18.4±2.7 (9.7–24.7)	18.9±2.5 (10.8–24.8)	14.0±1.5 (9.4–18.3)
TAN (mg/l)	0.73±0.18 (0.20–2.55)	1.25±0.28 (0–7.70)	1.08±0.27 (0–9.02)	1.07±0.29 (0–10.19)	1.01±0.31 (0–4.63)
Nitrite-N (mg/l)	1.92±0.43 (0–0.60)	0.07±0.13 (0–0.60)	0.08±0.18 (0–0.80)	0.07±0.18 (0–0.80)	0.04±0.09 (0–0.30)
Total P (mg/l)	–	0.43±0.06 (0.34–0.48)	0.61±0.16 (0.45–0.77)	0.88±0.21 (0.73–1.18)	–

Values are mean±standard deviation of daily and weekly determinations. Values on the side in parentheses represent minimum and maximum.

Table 5

Tank trial: final weight, weight gain, weight gain per week (WG/wk), and feed conversion ratio (FCR) of *L. vannamei* fed two diets (30 and 40% CP) at three different feeding levels (50, 75, and 100%) in a green-water recirculating system¹

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

¹Values are means of four replicates. Means in the same column with different superscripts are significantly different ($P \leq 0.05$).

²FCR=dry weight of feed offered/wet weight gained.

³PSE=pooled standard error.

dietary protein level (30 and 40% CP) and their possible interaction on final weight. Analyses indicated a significant effect for both feed rate ($P < 0.0001$) and dietary protein ($P = 0.0002$), but not for the interaction. At each protein level, growth decreased as the ration was reduced. For example, the lowest final weight and weight gain was observed for the 50% ration, both for the 30 and the 40% CP diet. Similarly, growth was reduced at any given feed input when the protein level was reduced. To further identify if the response was due to effect of treatments, the data was analyzed by one-way ANOVA and SNK means separation. Across all treatments, shrimp fed the 40% CP diet at 100% feeding level (40–100%) showed a significant higher final weight than the other treatments. The FCR of the 40–100% treatment was significantly higher than those from the 30–75% and the 40–75% treatments (Table 5).

Table 6

Tank trial: final body protein (% wet body weight), final body energy (kcal/g wet weight), protein conversion efficiency (PCE), and energy conversion efficiency (ECE) of *L. vannamei* fed two diets (30 and 40% CP) at three different feeding levels (50, 75, and 100%) in a green-water recirculating system¹

Protein (%)	Ration (%)	Body protein (% wet BW)	Body energy (kcal/g wet BW)	PCE ² (%)	ECE ³ (%)
30	100	18.3 ^a	1.23 ^a	48.8 ^c	21.2 ^b
	75	19.1 ^a	1.25 ^a	60.9 ^b	25.8 ^b
	50	17.1 ^a	1.16 ^a	78.2 ^a	33.0 ^a
40	100	18.9 ^a	1.26 ^a	41.8 ^c	24.7 ^b
	75	18.2 ^a	1.19 ^a	43.5 ^c	25.7 ^b
	50	18.0 ^a	1.23 ^a	58.6 ^b	32.9 ^a
P-value		0.4167	0.3428	<0.0001	<0.0001
PSE ⁴		0.6291	0.044	2.334	1.2409

¹Values are means of four replicates. Means in the same column with different superscripts are significantly different ($P \leq 0.05$).

²Protein conversion efficiency=dry weight protein gain \times 100/dry weight protein offered.

³Energy conversion efficiency=dry weight energy gain \times 100/dry weight energy offered.

⁴PSE=pooled standard error.

There were no significant differences between the final weight, PCE, and ECE of shrimp fed the 30% CP diet at 100% ration and the 40% CP diet at 75% (similar nitrogen input) (Tables 5 and 6). However, the feed conversion ratio (FCR) was significantly lower for the 40% CP at reduced feeding (75%) than the 30% CP diet at 100% feeding. Final weight was not significantly different among the two treatments (30% and 40% CP) fed at 50% of the ration, but they were significantly lower than the shrimp fed larger rations (75% and 100%).

In order to evaluate the influence of protein input, energy input, and feeding ration as a predictor of final weight, stepwise regression analyses was conducted. Results indicate that protein (total and digestible protein) input was the factor with the best fit to the model. This variable produced a significant regression ($P < 0.0001$) (Fig. 2) with a R^2 value of 0.87.

The final body protein and body energy of shrimp were not affected by the treatment levels (Table 6). Final body protein ranged between 17.5 and 19.1%. The PCE and ECE values ranged between 42 and 78% and between 21 and 33%, respectively. When feeding the 30% CP diet PCE increased as the ration was reduced. For the 40% CP diet the PCE was significantly higher at the lowest feeding rate (50%), but it was not significant different between the 75 and the 100% feeding rates. Energy conversion efficiency was significantly higher at the lowest ration (50%) for both levels of protein.

Values of digestible protein (DP), digestible energy (DE) and DE fed to the different treatments are presented in Table 2. The digestibility coefficients of the dry matter, protein, and energy were higher for the 40% CP diet than for the 30% CP diet (Table 7). The observed digestible energy/crude protein ratio (DE/CP)

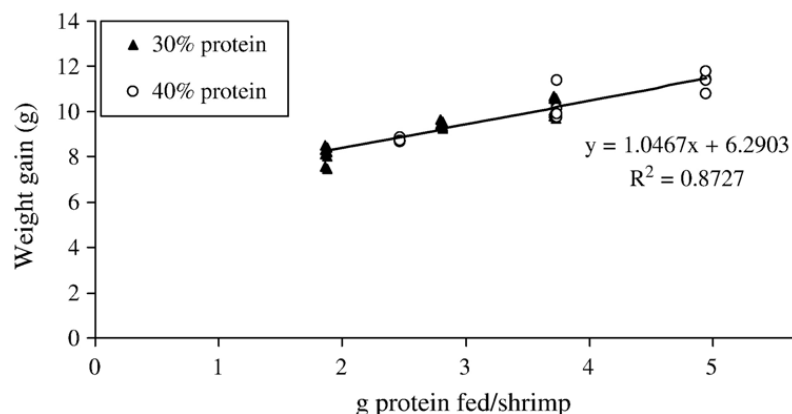


Fig. 2. Weight gain of *Litopenaeus vannamei* in response to varying levels of protein intake offered a 30 or a 40% CP diet at three different feeding rates (100, 75, and 50%) in a tank circulating system.

was 9.94 and 9.09 for the 30 and the 40% CP diet, respectively.

3.2. Pond trial

The observed water quality values for each treatment are summarized in Table 4. Average water quality parameters among all the experimental ponds were: DO, 6.0 mg/l; temperature, 30.2 °C; salinity, 18.2 mg/l; pH, 7.9; total ammonia-nitrogen, 1.10 mg/l; nitrite-nitrogen, 0.065 mg/l; and total phosphorus, 0.64 mg/l. The initial salinity for the first three treatments was 24.5 g/l. The two ponds that were stocked late (40–100%) had an initial salinity of 14 g/l. At the end of the experiment, salinity had been reduced through rainfall to about 11 g/l for all the

ponds. The observed values for water quality are typical for this pond system and provided adequate conditions for *L. vannamei* growth. Collapses of the phytoplankton population resulting in depressed oxygen levels were observed after 54 days of culture and were not related to the experimental treatments.

At day 106 (week 15) cast net samples from all ponds were taken. At this point there were no significant differences between mean weights of shrimp under the different treatments (Table 7, Fig. 3). By the end of the 107–114 day trial there were no significant differences between the final weight, FCRs and survival of shrimp under the 30–100% and the 30–75% treatments. However, production (yield, kg/ha) was significantly higher for the 30–100% than for the 30–75% treatment. Due to low oxygen and environmental constraints (Hurricane Ivan), two ponds were lost for the treatment 40–75%, hence these ponds were excluded from final analyses. Results from the two remaining ponds showed that production was numerically higher for the 40–75% treatment than for the 30–75% treatment, but it was lower than the control (30–100%). Using a generalized linear model and identifying the two ponds with high mortality as missing values, a *P* value of 0.0798 was observed for comparison of final yields.

The size and production distribution of the final shrimp samples were not significantly different between the two treatments that were analyzed statistically (30–100% and 30–75%) for all size ranges, except for the 31–35 shrimp/453 g size class (Fig. 4). The treatments offered 100% of the ration (30–100% and 40–100%) tended to have a higher proportion of the larger shrimp (16–20 shrimp/453 g) than those offered 75% of the ration (30–75% and 40–75%). When the feeding ration was reduced to 75%, especially for the 30% CP diet, a larger proportion of smaller shrimp (26–30 shrimp/

Table 7

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

Protein (%)	Ration (%)	FW ² (g)	WG/wk (g)	Yield (kg/ha)	FCR ⁴	Survival (%)
30	100	21.7 ^a	1.38 ^a	6482 ^a	1.0 ^a	88 ^a
30	75	19.7 ^a	1.28 ^a	5054 ^b	1.0 ^a	75 ^a
40	75	22.1 ^a	1.40 ^a	5660 ³	0.9 ³	82 ³
40	100	23.0 ³	1.79 ³	5408 ³	1.0 ³	71 ³
<i>P</i> -value		0.7591	0.3967	0.0374	0.7318	0.1136
PSE ⁵		1.0671	0.0635	378	0.0097	25.64

¹ Values are means of four replicates, except where indicated. Means in the same column with different letter superscripts are significantly different ($P \leq 0.05$).

² Based on samples taken with cast net on day 106.

³ Means of two replicates (values for this treatment were not included in the statistical analyses).

⁴ FCR = dry weight of feed offered/wet weight gained.

⁵ PSE = pooled standard error.

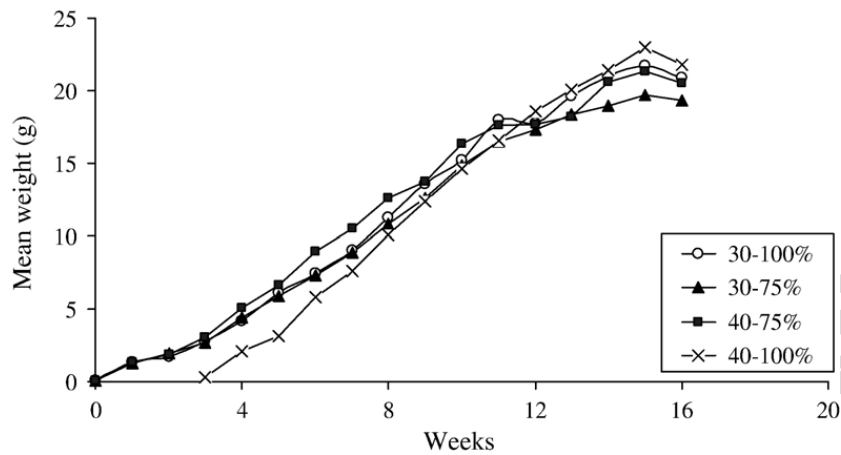


Fig. 3. Growth curve of *Litopenaeus vannamei* fed two practical diets (30% and 40% crude protein) at two feeding levels (75% and 100%) and raised in ponds at 35 shrimps/m² for 114–121 days.

453 g) was observed. However, for all the treatments, the largest proportion of shrimp was located in the count of 21–25/453 g (from 48% to 62%).

4. Discussion

This research was conducted using both tank and pond culture systems. The tanks received water from one of the production ponds to allow the incorporation of additional sources of food from the pond’s natural productivity (Leber and Pruder, 1988; Moss et al., 1992; Moss, 1995). As should be expected for this type of system, the tanks provided a more replicated environment than the ponds,

where the test variables were better controlled and isolated from the possible effect of uncontrollable factors. The pond trial, although more variable, provided useful information under production conditions. Research in ponds is encouraged because it provides information on the nutrient and feed management requirements under practical farming conditions (Lawrence and Lee, 1997). However, due to pond variation as well as uncontrolled factors, pond work is often less reliable.

The results of the tank trial demonstrated that by increasing the nutrient density of the diet (e.g., protein and energy) feed input can be reduced without affecting growth or net yield of shrimp. Shrimp offered a 40% CP

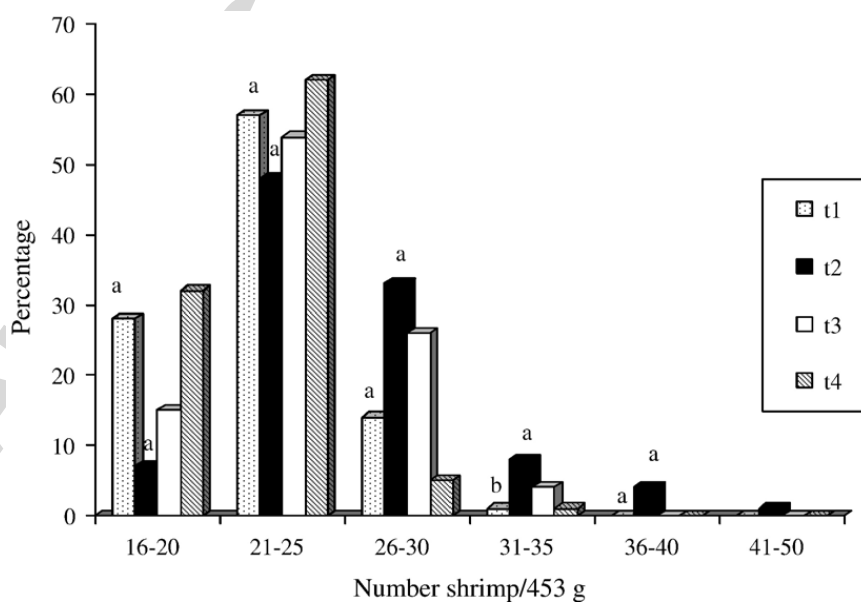


Fig. 4. Size distribution (number of shrimps/453 g) of *Litopenaeus vannamei* fed two diets (30% and 40% crude protein) at two feeding levels (75% and 100%) and raised in ponds at 35 shrimps/m². Within a size range means with different superscript letters are statistically different ($P \leq 0.05$).

diet at 75% of the ration had similar final weight as compared to shrimp fed a 30% CP diet at 100% of the ration. These two feed inputs delivered the same amount of crude protein and hence produced the same amount of growth. Since growth was not reduced it may be assumed that the diets were balanced for energy and other nutrients and were not limiting. Under similar approach Cho et al. (2001) did not find significant differences between weight gain of carp (*C. carpio*) fed a 35% protein diet to satiation and a 40% protein diet at 87.5% of the satiation rate that provided similar amounts of protein. However, they reported a significant reduction in weight gain when two 45% protein diets were fed at 77.8% of the satiation level. They observed that increasing the level of energy of the second 45% protein diet did not improve growth at 77.8% feeding, but the level of body fat in the fish was increased. Cho and Lovell (2002) found similar results in catfish (*I. punctatus*) reared in ponds and fed a 28%, a 32% and a 36% protein diet at 100, 87.5, and 77.8% of satiation, respectively. In shrimp, Kureshy and Davis (2002) observed significant differences between juvenile *L. vannamei* reared in tanks that were offered three diets (16, 32, and 48% crude protein) at the same nitrogen input. They reported that weight gain of shrimp fed the 16 and the 48% diets was significantly lower than shrimp fed the 32% diet. They concluded that the low nutrient level of the 16% protein diet restricted the amount of protein and energy that shrimp could consume since they would have to consume twice the amount of the diet in order to match the protein intake of the 32% protein diet. On the other hand, the low level of feeding of the 48% protein diet (66.7%), necessary to match the protein input of the 32% protein diet, probably limited the amount of energy and other essential nutrients available for shrimp growth. These results indicated that although various nutrient densities and feed inputs can be used they have their limits.

This study demonstrates that, as long as the level of energy is appropriate, the daily food allowance can be reduced by 25% without affecting shrimp growth if we substitute 30% for a 40% CP, diet. The levels of reduction of feeding reported in the literature for other species (Cho et al., 2001; Cho and Lovell, 2002) have been more limited than the values obtained in this research. There are several possible explanations to the observed differences between this research and the other studies: first the nature of the species studied; second the level of feeding of the lower protein diet (satiation versus below satiation); and third the capability of the species to utilize natural sources of food in the culture environment.

Although information from research conducted with fish can be applied to crustaceans, there are some

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

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

The third factor that can affect the effectiveness of a reduced feed input with increased nutrient density of the diets, is the capability of the species to utilize available natural sources of food in the culture environment. Aside from the improvement in FCR due to lower feed allowance (Cho et al., 2001; Cho and Lovell, 2002; Kureshy and Davis, 2002), additional sources of natural food in the culture environment can further improve apparent FCR as it provides additional sources of nutrients that allow optimal growth. In the previously reported research, the trials took place in clear water

tank systems (Cho et al., 2001; Kureshy and Davis, 2002) or with a species that cannot efficiently utilize natural productivity in a pond, such as catfish (Cho and Lovell, 2002). Although not evaluated, shrimp seem to have been able to benefit from those additional sources of nutrients (Cam et al., 1991; Burford et al., 2004).

For both the tank and the pond trials the values of FCR obtained were better than expected. The complete ration (100%) was calculated based in an expected FCR of 1.8:1. The highest FCR value obtained in this research was 1.41:1 in the tank system (for the 30–100% treatment) and 1.03:1 in the ponds (for the 30–75% and the 40–100% treatments). These improved FCRs are most likely the result of additional sources of nutrients going into the system, probably provided by natural productivity in the pond or from the green water provided to the tanks from the ponds. The contribution of natural productivity in shrimp ponds has been well documented. Lawrence and Lee (1997) pointed out that in spite of an increased use of feed in shrimp production, natural productivity still account for more than 25% of the nutrient intake of shrimp. Anderson et al. (1987) estimated that the contribution of feed to production of *L. vannamei* at levels below 5 mt/ha/crop, was only between 23 and 47%. At the same production level, Lawrence and Houston (1993) estimated that value to be 24 to 31% for *L. vannamei* and 17 to 23% for *L. setiferus*. For a production of about 4 mt/ha/crop the contribution of feed to *L. setiferus* production was estimated to be about 48% (Robertson et al., 1993). By using ^{15}N -enriched, Cam et al. (1991) found that *Marsupenaeus japonicus* retained between 4 and 10% of the ^{15}N -enriched that had been assimilated by the natural biota. By using the same technique Burford et al. (2002) reported that 15.2-g *Penaeus monodon* retained about 21% of the dietary ^{15}N -labeled (43.75% CP diet). Funge-Smith and Stewart (1996) estimated this value in 18 to 27% in intensive shrimp ponds.

Similarly, the contribution of natural productivity on shrimp growth in tank systems that received green water has been reported. Leber and Pruder (1988) and Moss et al. (1992) demonstrated that shrimp growth rates can be increased by 53%–89% over shrimp grown in clear well water when unfiltered water from an intensive shrimp pond is added. The enhancing materials were mainly microalgae and microbial–detrital aggregates of sizes between 0.5 and 5.0 μm . Moss (1995) suggested that shrimp could be able to consume directly suspended organic particles from the water column without being processed in a detrital food web. In this research, the results of the FCR, PCE, ECE, and the growth rate seem to indicate that natural productivity also improved shrimp growth and feed and nutrient utilization, as has been

reported from other investigators. For example the rate of growth in the tank system ranged between 1.22 and 1.35 g/week for the standard feed rate treatments (30–100% and 40–100%) (Table 5). In the pond trial these values increased to 1.38 to 1.79 g/week. Although the conditions of the experiments may have not been the same, Wasielesky et al. (2005) reported values of weekly growth lower than those obtained in this research when *L. vannamei* was raised in a filtered water system (0.94 g/week). Similarly, Moss et al. (1992) reported a growth rate of 0.97 g/week for *L. vannamei* raised in well water in contrast with 1.83 g/week when pond water was added to the tanks. Values of PCE and ECE observed in this research are higher than those commonly reported in the literature for aquatic species. Halver and Hardy (2002) reported an average value for PCE between 25 and 30%. The values of PCE in salmon culture has been improved to about 45% from 22 to 25% in the 1980s by feeding high energy density diets (Halver and Hardy, 2002). In this research the PCE values ranged between 42 and 78% probably due to the contribution of natural productivity.

As previously pointed out, the contribution of natural productivity in the tank system probably increased shrimp growth and improved FCR, PCE, and ECE as evidenced by the data. However, the levels of nutrients that shrimp obtained from these sources were not high enough to compensate for the reduced feed input. Regardless of dietary protein, reductions in feed inputs resulted in reductions in growth. Thus, feed inputs were more limiting than natural productivity. Final growth of shrimp offered the 30% CP diet at 50 and 75% ration was significantly lower than that of shrimp offered the 30% CP diet at 100% ration. Similarly, the PCE and ECE were significantly higher for shrimp fed either the 30 or the 40% CP diet at the lowest feeding rate (50%). For the 30% CP diet, PCE was significantly higher at each reduced feeding level (50 and 75%). The observed growth increase and protein and energy utilization of shrimp raised in green water or ponds may be due to an improvement in the quality of the food source and nutritional balance (e.g., essential amino acids, micro-nutrients) rather than the quantity of macronutrients (protein, carbohydrates, and lipids) provided by these sources (Burford et al., 2004).

To evaluate the effect of the tested variables and interaction among them, stepwise regression analyses and two-way ANOVA analyses were made. These analyses indicated that although energy input and dietary protein level had a significant effect on shrimp growth, these effects were not significant when either of these variables were included in a multiple regression analysis with protein input. Therefore, protein input was the most

important variable in shrimp growth and production. Shrimp growth responded linearly to increasing levels of dietary protein independently of the source of dietary protein. For example, shrimp offered the same amount of protein (30–100% and 40–75%), had non-significant differences in final weight, even though the two diets had different levels of protein. When both diets were offered at 100% of the feed rate, animals responded better to the 40% CP diet, probably because this diet provided more protein than the 30% CP. This effect should be expected since the 30–100% was selected to provide a sub-optimal level of feed input as discussed earlier.

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

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

With reference to the pond data, by the end of the 107–114 day trial there were no significant differences between the final weight, FCRs and survival of shrimp maintained on the 30–100% and 30–75% treatments. However reduction of the feed rate to 75% (30–75%) significantly reduced production as compared with the 30–100% treatment. Although the 40–75% treatment was not included in the statistical analyses due to loss of two ponds, results from the two remaining ponds

indicated that production was higher for the 40–75% treatment than for the 30–75% treatment, but it was lower than the 30–100% treatment. These results of shrimp reared on 30–100% and 40–75% treatments (iso-nitrogenous) tended to be different from those obtained for the same treatments in the tank trial. Since the level of protein allowance and energy were similar for both the tank and the pond trails, the observed differences in production could have been due to the effect of other variables, such as natural productivity or distribution of feed. In production conditions the higher feed input of the lower protein diet may have stimulated natural productivity possibly due to carbon enrichment. It is also possible that a larger amount of feed allowed a better distribution of the feed in the pond making it easier for shrimp to reach the feed, reducing competition. The combined effect of these variables may have affected production. Hence, further research under pond conditions is warranted to analyze these effects.

Under pond conditions, the size and production distribution of the final shrimp samples were not significantly different between the two treatments that were analyzed statistically (30–100% and 30–75%) for all size ranges, except for the count of 31–35 shrimp/453 g. However, only a small portion of the population fell within the 31–35 size-class (4.6 and 1.3% for the 30–100% and the 30–75%, respectively). Overall, more than half of the population for each feeding treatment was located in the 21–25 size class. A reduction of feed inputs to 75% for both protein levels (30–75% and 40%–75%) tended to produce a higher concentration of shrimp in the smaller size range, which is undesirable for shrimp production. However, shrimp in the size range of 26–30/453 g are still a commercial product of good quality. In addition, although yield was numerically lower under a reduced feeding level (75%) when feeding a high protein diet (40–75%), the levels of production (5660 kg/ha), FCR (0.92) and size distribution are still considered excellent values for commercial production of shrimp.

5. Conclusions

Under laboratory controlled conditions these studies demonstrate that shrimp have a daily requirement of protein that can be supplied with diets with different levels of protein. This is in agreement with findings previously reported in other studies with *L. vannamei* or for other species (Lawrence and Lee, 1997; McGoogan and Gatlin, 1998; Kureshy and Davis, 2002). Based on this, the use of diets with high nutrient density is encouraged for this semi-intensive type of system because a smaller quantity

of feed would be required to produce the same amount of growth (Cho and Bureau, 2001). Lower amounts of feed and better feed management would lead to higher efficiency of the use nutrients with a subsequent reduction in wastes from the feed. This may improve the economic return for the farm and reduce the potential of water pollution and environmental impact on ecosystems receiving the effluents.

Acknowledgments

This study was based on research conducted by J.A. Venero and presented in partial fulfillment of requirements for a Ph.D. degree at Auburn University. Special thanks to all the students of the Nutrition and Technology laboratory of the Department of Fisheries and Allied Aquacultures of Auburn University for all their help and technical support during the experiments and laboratory analyses. Also thanks to all the staff of the Alabama Marine Resources Division for allowing the use of their facilities during the experimental part of this study and for all their physical and logistic support given. Special thanks to Michael Grider for review of the manuscript.

References

- Anderson, R.K., Lawrence, A.L., Parker, P.L., 1987. A $^{13}\text{C}/^{12}\text{C}$ tracer study of the utilization of presented feed by a commercially important shrimp, *Penaeus vannamei*, in a pond grow-out system. *J. World Aquac. Soc.* 18, 148–155.
- Andrews, J.W., 1979. Some effects of feeding rate on growth, feed conversion and nutrient absorption of channel catfish. *Aquaculture* 16, 243–246.
- APHA (American Public Health Association), American Water Works Association, Water Pollution Control Association, 1989. Standard Methods for the Examination of Water and Waste Water, 17th edition. American Public Health Association, Washington, D.C., USA.
- Association of Official Analytical Chemists (AOAC), 1990. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th edition. Association of Official Analytical Chemists Inc., Arlington, Virginia.
- Boyd, C.E., Tucker, C.S., 1992. Water Quality and Pond Soil Analyses for Aquaculture. Alabama Agricultural Experiment Station, Auburn University, Auburn, AL, USA.
- Boyd, C.E., Tucker, C.S., 1998. Pond Aquaculture Water Quality Management. Kluwer Academics Publisher, Boston, Massachusetts, USA.
- Bureau, D.P., Azevedo, P.A., Tapia-Salazar, M., Cuzon, G., 2000. Pattern and cost of growth and nutrient deposition in fish and shrimp: potential implications and applications. In: Cruz Suarez, L.E., Riquemarie, D., Tapia-Salazar, M., Civera-Cerecedo, R. (Eds.), *Avances en Nutricion Acuicola, Memorias del V Simposium Internacional de Nutricion Acuicola*. Merida, Yucatan, Mexico, pp. 111–140.
- Burford, M.A., Preston, N.P., Glibert, P.M., Dennison, W.C., 2002. Tracing the fate of ^{15}N -enriched feed in an intensive shrimp system. *Aquaculture* 206, 199–216.
- Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero-exchange system. *Aquaculture* 23, 525–537.
- Cam, D., Rollet, P.-E., Mariotti, A., Guillaume, J., 1991. The relative contribution of natural productivity and formulated food in the nutrition of *Penaeus japonicus* reared in semi-intensive conditions. *Aquat. Living Resour.* 4, 175–180.
- Cho, C.Y., Bureau, D.P., 2001. A review of diet formulation strategies and feeding systems to reduce excretory and feed wastes in aquaculture. *Aquac. Res.* 32, 349–360.
- Cho, S.H., Lovell, R.T., 2002. Variable feed allowance with constant protein input for channel catfish (*Ictalurus punctatus*) cultured in ponds. *Aquaculture* 204, 101–112.
- Cho, S.H., Jo, J.-Y., Kim, D.S., 2001. Effects of variable feed allowance with constant energy and ratio of energy to protein in a diet for constant protein input on the growth of common carp (*Cyprinus carpio* L.). *Aquac. Res.* 32, 349–356.
- Cousin, M., Cuzon, G., Blanchet, E., Ruelle, F., AQUACOP, 1993. Protein requirements following an optimum dietary energy to protein ratio for *P. vannamei* juveniles. In: Kaushik, S.J., Luquet, P. (Eds.), *Fish Nutrition in Practice*. INRA, Paris, France, pp. 599–606.
- Cuzon, G., Guillaume, J., 1997. Energy and protein: energy ratio. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), *Crustacean Nutrition. Advances in World Aquaculture*, vol. 6. World Maric. Soc, Baton Rouge, LA, pp. 51–70.
- FAO, 2000. Yearbook of fishery statistics 1998. Aquaculture Production. FAO Statistics Series N. 154 and Fisheries Series N. 56, vol. 86/2. FAO, Rome. 82 pp.
- Funge-Smith, S.J., Stewart, J.A., 1996. Coastal aquaculture: identification of social, economic and environmental constraints to sustainability with reference to shrimp culture. Coastal Aquaculture and Environment: Strategies for Sustainability. ODA Research Project R6011. Institute of Aquaculture, University of Stirling, Stirling, Scotland.
- Halver, J.E., Hardy, R.W., 2002. Nutrient flow and retention. In: Halver, J.E., Hardy, R.W. (Eds.), *Fish Nutrition*, 3rd ed. Academic Press, Elsevier Science, San Diego, CA, pp. 755–770.
- Hardin, M.P., Aldrich, D.V., Chamberlain, G.W., 1985. Temperature and size effects on the accuracy of estimating postlarval shrimp populations. *Aquac. Eng.* 4, 85–92.
- Jackson, C., Preston, N., Peter, J.T., Burford, M., 2003. Nitrogen budget and effluent nitrogen components at an intensive shrimp farm. *Aquaculture* 218, 397–411.
- Johnsen, F., Hillestad, M., Austreng, E., 1991. High energy diets for Atlantic salmon. Effects on pollution. In: Kaushik, S.J., Luquet, P. (Eds.), *Fish Nutrition in Practice*. INRA, Paris, France, pp. 391–401.
- Juarez, L.M., Luxem, A.H., Rouse, D.B., 1996. Sampling shrimp populations in hatcheries. *J. World Aquac. Soc.* 27, 221–225.
- Kureshy, N., Davis, D.A., 2002. Protein requirement for maintenance and maximum weight gain for the Pacific white shrimp, *Litopenaeus vannamei*. *Aquaculture* 204, 125–143.
- Lawrence, A.L., Houston, D.M., 1993. Nutritional response of juvenile *Penaeus setiferus* and *Penaeus vannamei* to different quality feeds in the presence and absence of natural productivity. In: Collie, M.R., McVey, J.C. (Eds.), *Proceedings of the 20th US–Japan Joint Panel on Aquaculture*. Newport, Oregon, USA, pp. 113–124.
- Lawrence, A.L., Lee, P.G., 1997. Research in the Americas. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), *Crustacean Nutrition. Advances in World Aquaculture*, vol. 6. World Aquacult. Soc, Baton Rouge, LA, pp. 566–587.

- Leber, K.M., Pruder, G., 1988. Using experimental microcosms in shrimp research: the growth enhancing effect of shrimp pond water. *J. World Aquac. Soc.* 19, 197–203.
- Li, M., Lovell, R.T., 1992. Comparison of satiate feeding and restricted feeding of channel catfish with various concentration of dietary protein in production ponds. *Aquaculture* 103, 165–175.
- Lupatsch, I., Kissil, G.Wm., Sklan, D., Pfeffer, E., 1998. Energy and protein requirements for maintenance and growth in gilthead seabream (*Sparus aurata* L.). *Aquac. Nutr.* 4, 165–173.
- Ma, T.S., Zuazago, G., 1942. Micro-Kjeldahl Method for Organic Nitrogen. Academic Press, New York. 239 pp.
- Mangalik, A., 1986. Dietary Energy Requirements of Channel Catfish. Ph.D. dissertation, Auburn University, Auburn, AL, USA.
- Maynard, L.A., Loosli, J.K., Hintz, H.F., Warner, R.G., 1979. *Animal Nutrition*, 7th ed. McGraw-Hill, New York. 602 pp.
- McGinnis, A.J., Kasting, R., 1964. Colorimetric analysis of chromic oxide used to study food utilization by phytophagous insects. *Food Chem.* 12, 259–262.
- McGoogan, B.B., Gatlin, D.M., 1998. Metabolic requirements of red drum, *Sciaenops ocellatus*, for protein and energy, based on weight gain and body composition. *J. Nutr.* 128, 123–129.
- McGoogan, B.B., Gatlin, D.M., 1999. Dietary manipulations affecting growth and nitrogenous waste production of red drum, *Sciaenops ocellatus*—I. Effects of dietary protein and energy levels. *Aquaculture* 178, 333–348.
- Minton, R.V., 1978. Responses of Channel Catfish Fed Diets of Two Nutrient Concentrations at Three Rates in Ponds. Master's Thesis, Auburn University, Auburn, AL, USA.
- Moss, S.M., 1995. Production of growth-enhancing particles in a plastic-lined shrimp pond. *Aquaculture* 132, 253–260.
- Moss, S.M., Pruder, G., Leber, K.M., Wyban, J.A., 1992. The relative enhancement of *Penaues vannamei* growth by selected fractions of shrimp pond water. *Aquaculture* 101, 229–239.
- Munsiri, P., Lovell, R.T., 1993. Comparison of satiate and restricted feeding of channel catfish with diets of varying protein quality in production ponds. *J. World Aquac. Soc.* 24, 459–465.
- National Research Council (NRC), 1993. *Nutrient Requirements of Fish*. National Academy Press, Washington, DC. 114 pp.
- Persson, G., 1991. Eutrophication resulting from salmonid fish culture in fresh and salt waters. Scandinavian experience. In: Cowey, C.B., Cho, C.Y. (Eds.), *Nutritional Strategies and Aquaculture Waste. Proceedings of the First International Symposium on nutritional Strategies in Management of Aquaculture Waste*. Fish Nutrition Research Laboratory, University of Guelph, Canada, pp. 163–185.
- Robertson, L., Lawrence, A.L., Castle, F.L., 1993. Effect of feed quality on growth of Gulf of Mexico white shrimp, *Penaues setiferus* in pond pens. *Tex. J. Sci.* 45, 69–76.
- Sargent, J.R., Tocher, D.R., Bell, J.G., 2002. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), *Fish Nutrition*, Third edition. Academic Press, San Diego CA, pp. 182–257.
- Shearer, K.D., 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. *Aquaculture* 119, 63–88.
- Steel, R.G.D., Torrie, J.H., 1980. *Principles and Procedures of Statistics: A Biometrical Approach*. McGraw-Hill, New York. 633 pp.
- Steffens, W., 1996. Protein sparing effect and nutritive significance of lipid supplementation in carp diets. *Arch. Anim. Nutr.* 49, 93–98.
- Tacon, A.G.J., Akiyama, A.G.J., 1997. Feed ingredients. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), *Crustacean Nutrition, Advances in World Aquaculture*, vol. 6. World Maric. Soc, Baton Rouge, LA, pp. 411–472.
- Tacon, A.G.J., Dominy, W.G., Pruder, G.D., 2000. Tendencias y retos globales de los alimentos para el camaron. In: Ricque-Marie, D., Cruz Suarez, L.E. (Eds.), *Avances en Nutricion Acuicola IV, Memorias del IV Simposium Internacional de Nutricion Acuicola*. Merida, Yucatan, Mexico, pp. 1–26.
- Wasielesky, W., Browdy, C.L., Atwood, H., Stokes, A., 2005. Effect of dietary protein on food consumption of white shrimp *Litopenaeus vannamei*. Abstracts America Aquaculture. World Aquacult. Soc. Baton Rouge, LA, USA, p. 476.
- Watanabe, T., Takeuchi, T., Ogino, C., 1979. Studies on the sparing effect of lipids on dietary protein in rainbow trout (*Salmo gairdneri*). In: Halver, J.E., Tiems, K. (Eds.), *Proceedings of the World Symposium on Finfish Nutrition and Fishfeed Technology I*. Schriften der Bundesforschungsanstalt fur Fischerei, Hamburg, Germany, pp. 113–125.
- Wyban, J.A., Pruder, G.D., Leber, K.M., 1989. Paddlewheel effects on shrimp growth, production and crop value in commercial earthen ponds. *J. World Aquac. Soc.* 20, 18–23.
- Zelaya, O., 2005. An Evaluation of Nursery Techniques and Feed Management During Culture of Marine Shrimp *Litopenaeus vannamei*. Ph.D. Dissertation, Auburn University, Auburn, AL, USA.