FEEDING RATE AND STOCKING DENSITY IN SEMI-INTENSIVE *Litopenaeus Vannamei* CULTURE WITH MODERATE PERIODIC FERTILIZATION

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ABSTRACT

We tested a 50% reduction in feeding rate of *Litopenaeus vannamei* at stocking densities of 15 to 35 post-larvae/ m^2 in conjunction with periodic pond fertilization with 35 kg/ha of urea and 12 kg/ha of triple super phosphate. Two commercial diets with 35% protein foods were fed for 84 days. Survival was over 90% in all experimental units. Best feed conversion rates (1.46 to 1.75) and greatest weight gains (0.70 to 0.81 g/week) were obtained at 15 post-larvae/m^2. Results showed that feeding Tables of food manufacturers are well exceeded when used together with moderate periodic fertilization and that stocking density is highly significant ($p < 0.01$).

Keywords: *litopenaeus vannamei*, semi-intensive culture, diets, feed conversion rates, urea, triple superphosphate.

INTRODUCTION

Shrimp aquaculture in Mexico has grown dramatically over the last 20 years, from 5,000 up to 130, 000 tons per annum (CONAPESCA 2010). Currently, the states with largest shrimp aquaculture are Sonora (62%) and Sinaloa (29%). Some 98% of production is from semi-intensive ponds on land adjacent to salt marshes or brackish lagoons with large capacity pumps for daily water recycling. The main cultivated species is the Pacific white shrimp *Litopenaeus vannamei* of which Mexico is one of the world's major producers.

Diet, fertilization and stocking density are factors that greatly affect production and costs of shrimp farming. The task of determining a proper diet is a very important one and may require many empirical trials or to perform controlled statistical experiments. Usually, each food manufacturer provides feeding Tables for use with its products but these Tables recommend feeding rates based only on shrimp size and biomass and do not adequately take into account natural nutrients in the water and the nutrients produced by fertilization and can thus lead to overfeeding and degradation of water quality (Millamena, 1990). On the other hand, a fertilization regime supports the natural productivity and maintains a high biomass of organisms consumed by shrimp (Weigel, 1994).

Diverse experimental results for *Litopenaeus vannamei* have been reported in the literature. Martinez-Cordova *et al*. (1998) tested the use of manufacturer's feeding tables, with and without natural food supplementation, and the use of feed trays adjustable to consumption. Their results showed that the best gains in weight were obtained with the use of feed trays and feeding Tables supplemented with natural food, and like Casillas-Hernandez *et al*. (2006) found no statistical significant difference for feed conversion rates between these two methods. Kureshy and Davis (2002) analyzed weight gains and feed conversion rates with diets containing 16, 32 and 48% protein, finding that the best diets were those with 32 and 48% protein content. Robertson *et al*. (1993) evaluated the effects of feeding time and frequency, finding that instantaneous growth rates were improved significantly with daytime feeding and switching frequency from 1 to 4 times per day. FAO (2012) states that shrimp natural habitats have temperatures above 20°C throughout the year so these temperatures are good for growth. Walker *et al*. (2009) analyzed the effect of salinity on growth rate at temperatures near 28°C finding no significant difference in growth rate at levels of 10 and 28 ppt. Teichert-Coddington and Rodriguez (1995) found that at stocking densities of 7.5 post-larvae/m^2 the production in the dry season does not differ significantly with a 50% reduction of manufacturer's recommendations and that in the wet season the recommendations can potentially be reduced but not to 50%. Green *et al*. (1997) investigated, at an expected 8 shrimp/m^2 survival density, reductions of the levels of the diets recommended by manufacturers in 50 and 75%; the best feed conversion rate was obtained with a 30% protein diet reduced to 50%. Nunes *et al*. (2006) tested the effect of temporal feeding restrictions (28 days) at a 36 shrimp/m^2 density finding no significant differences in final body weight with rate restricted feeding up to 50%. Esparza-Leal *et al*. (2010), analyzed the effect of stocking density under low salinity, and found that the lesser the density the higher the weight gain. Previous experiments under saltwater and freshwater gave the same results, in all of them the optimum density was the lowest density tested in the experiments.

In this paper we tested reductions of feeding tables to 50% and proved that, under a moderate periodic fertilization regime, results of Teichert-Coddington and Rodriguez (1995) and Green *et al*. (1997) can be extrapolated to higher stocking densities and protein contents, and that results of Nunes *et al*. (2006) can be valid for longer periods.

MATERIALS AND METHODS

The experiment described in this paper was performed in the farm “El Remolino” (The Whirl), located in Sinaloa, Mexico, in the dry season, beginning in late October when ambient temperatures were expected to be close to the optimum temperatures for growth. We used two 35% protein foods of leading brands that from previous experiments were known to be statistically similar, manufacturer’s feeding Tables (without and with 50% reductions) with periodic fertilization to induce natural food supplementation, three daily rations of equal size and stocking densities of 15 to 35 post-larvae/m^2.

The farm coordinates are 105° 45' 00’’ west longitude and 22° 46' 10" north latitude. The farm is bordered on the west by 1200 m of an estuary and a marsh and is about 40 km from the coastline. In this geographical area the climate is warm sub-tropical with two rainy periods. The first one, with frequent rainfall, occurs in the summer (June-October) and the other, with occasional rainfall, in winter (December-February). The salinity of the estuary, and hence of the water in the ponds, depends on the rainfall, resulting in minimum values around 15 parts per thousand (ppt) in the rainy season and maximum of 90 in the dry season. The maximum temperatures (38°C) occur on August afternoons and the minimum (18°C) on February early mornings.

The experiment was conducted at three of the acclimation tanks of the farm. These tanks are 20 meters long by two wide and depth between 1.2 and 1.4 m. The slope in the bottom facilitates drainage. All tanks were provided with additional aeration equipment. Each tank was divided into four experimental units (cages) of 10 m^2 surface and a minimum capacity of 13, 250 l using three wooden frames covered with 2 mm mesh. Tank divisions gave a total of 12 experimental units.

The experiment was a 3x2 factorial with:
Factor-1: Stocking density (3 levels: 15, 25 and 35 post-larvae/m^2)
Factor-2: Food reduction (2 levels: 0 and 50%)

The treatments were randomly assigned to each experimental unit and tanks were stocked using 0.5-1 g post-larvae obtained from a pre-nursery where they had stayed for 32 days since their arrival.

The minimum water level in tanks was 1 m. Daily water exchanges were of 5 to 15% and once a week, when the sampling was performed, the tank bottoms were partially cleaned by removing debris with suction equipment. The water exchange procedure was to lower the level by the percentage set by opening the drain and raising the level using reservoirs water. In the experimental units assigned with stocking densities of 35 post-larvae/ m^2, additional aeration was connected from 22:00 to 05:00 h in order to provide a suitable environment preventing oxygen levels to fall below 2 ppm. Shrimps were fed at 08:00, 16:00 and 20:00 h and water fertilization was done twice a week with 35 kg/ha of urea and 12 kg/ha of triple super phosphate.

Sampling was performed weekly in at least 50% of the organisms of each experimental unit. Sampling was done with a fishnet mesh of 2 mm. Recorded data was total weight, number of shrimps, average weight, molting, survival, and direct observations on the state and behavior of the shrimp. Every day at 0600 h temperature and oxygen were monitored and at 1800 h temperature, oxygen, salinity and turbidity.

Statistical analysis consisted of ANOVA, ANCOVA, and multiple comparison tests of means of Bonferroni-Dunn, Tukey and Scheffe. All were conducted using SAS program, version 9.2 (SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSIONS

The weekly average weights observed in the experimental units are shown in Figures 1 and 2. Figure-1 includes weights of shrimp fed with commercial food CF1, and Figure-2 the weights of shrimp fed with commercial food CF2.

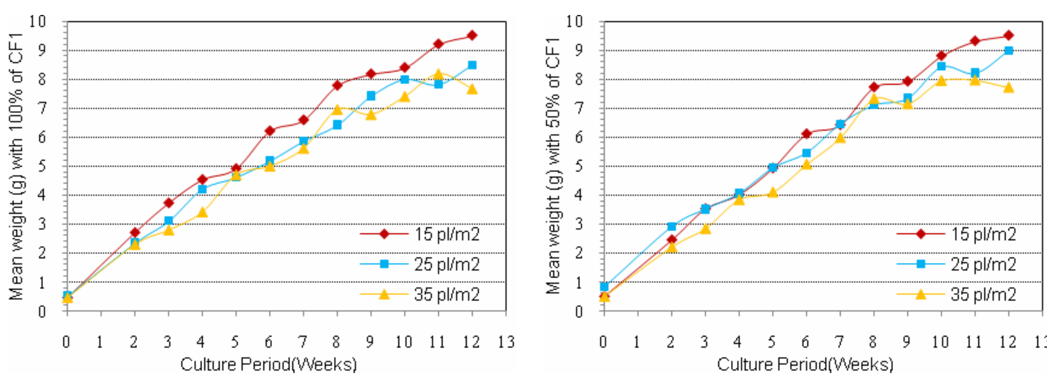

Figure-1. Mean weekly weights of shrimp fed with commercial food CF1, two ration sizes (100 and 50%) and three stocking densities (15, 25 and 35 post-larvae/m^2).

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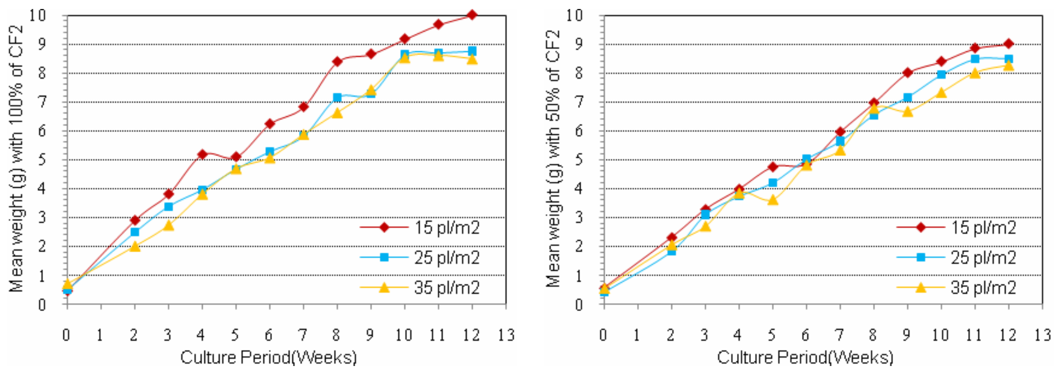

Figure-2. Mean weekly weights of shrimp fed with commercial food CF2, two ration sizes (100 and 50%) and three stocking densities (15, 25 and 35 post-larvae/m^2).

The weekly average values of the four environmental measured variables (temperature, oxygen, salinity and turbidity) are found in Figure-3.

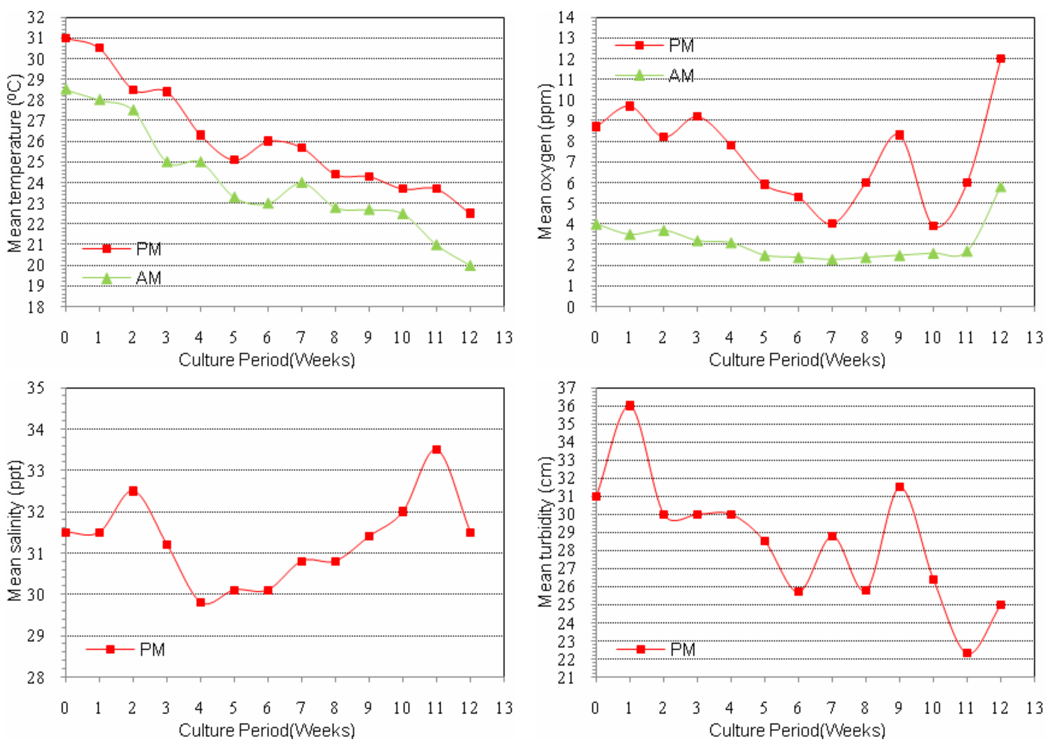

Figure-3. Mean weekly values of water variables monitored daily at 0600 and 1800 h.

From the growth curves, it can be observed that from late December (week 8), when the temperature dropped to levels below 24°C, the growth rates decreased. After 84 days, as the growth observed in most of the experimental units in the last three weeks was not the desirable (stagnant growth) and environmental conditions did not seem to improve (on the contrary, predictions were that low temperatures will continue and salinity would increase), it was decided to terminate the experiment.

Analyzing the results, this experiment can be considered successful because in less than three months of fattening, commercial sizes were attained in all experimental units. The growth rates obtained, between 0.60 and 0.81 g/week, were similar to the historic ones of 0.6-0.8 g/week observed in these periods (October to January). Respect to survival rates, they were between 90 and 94%, which are very good. With regard to environmental conditions, because of the usual rains in November and December, salinity values were between 29 and 33 ppt which were ideal for the growth of shrimp. The maximum temperature, 31°C, was recorded in October afternoons at the beginning of the experiment, and the trend was downwards to 20°C in the early hours of January. Turbidity was maintained between 22 and 36 cm of the Secchi disc. Oxygen was maintained between 2 and 4 ppm in the mornings and between 4 and 10 ppm in the afternoons. Table-1 resumes mean weekly growth rates, mean weight gains, feed conversion rates and yields resulting from the experiment.

Table-1. Mean weekly growth rates, mean weight gains, feed conversion rates and yields.

Variable	Reduction	Stock density (post-larvae/m^2)		
		15	25	35
Mean weekly growth rates (g)	0%	0.78±0.03	0.68±0.02	0.62±0.03
	50%	0.73±0.32	0.68±0.01	0.62±0.02
Mean weight gains (g)	0%	9.37±0.43	8.13±0.19	7.49±0.41
	50%	8.72±0.38	8.13±0.08	7.47±0.33
Feed conversion rates	0%	2.71±0.10	3.00±0.21	3.27±0.21
	50%	1.48±0.02	1.53±0.04	1.65±0.14
Yields (t/ha)	0%	1.35±0.06	1.98±0.04	2.59±0.20
	50%	1.28±.062	2.03±0.11	2.57±.18

An Analysis of Variance (ANOVA) was performed to the means of the weight gains. Its results are presented in Table-2, which shows that no significant differences were found between amounts of food, and that stocking densities were highly significant ($p < 0.01$).

Table-2. Results of 1st ANOVA performed on mean weight gains ($R^2 = 0.8889$).

Source of variance	DF	Sum of	Mean	F	Pr > F
		Squares	Square	Value	
Reduction	1	0.1496	0.1496	1.35	0.2901
Density	2	4.9123	2.4561	22.09	0.0017
Reduction*Density	2	0.2733	0.1366	1.23	0.3570
Error	6	0.6671	0.1112		

In spite that, as was mentioned before, the two 35% protein foods used in the experiment were known to be statistically similar from previous experiments, to dissipate any doubt on statistical significances obtained in Table-2, another ANOVA was performed to the means of the weight gains, this time using the food types as logical blocks. Its results are given in Table-3 and are similar to those obtained with no blocks.

Table-3. Results of 2nd ANOVA performed on mean weight gains (R^2=0.9116).

Source of Variance	DF	Sum of	Mean	F	Pr > F
		Squares	Square	Value	
Block	1	0.1365	0.1365	1.29	0.3081
Reduction	1	0.1496	0.1496	1.41	0.2884
Density	2	4.9123	2.4561	23.15	0.0030
Reduction*Density	2	0.2733	0.1366	1.29	0.3539
Error	5	0.5306	0.1061		

Also, an Analysis of Covariance (ANCOVA) was performed to the means of the final weights with the mean initial weights as covariate to remove the effect (if any) of these on the final weight. Its results are given in Table-4 and are similar to the two ANOVA results for the means of the weight gains.

Table-4. Results of ANCOVA performed on mean final weights ($R^2 = 0.9241$).

Source of Variance	DF	Sum of Squares	Mean Square	F Value	Pr > F
Block	1	0.1261	0.1261	1.15	0.3443
Reduction	1	0.0954	0.0954	0.87	0.4041
Density	2	4.4941	2.2471	20.46	0.0079
Reduction*Density	2	0.2395	0.1198	1.09	0.4188
Initial weight	1	0.3976	0.3976	3.62	0.1298
Error	4	0.4393	0.1098		

A substantial part of this experiment was to assess the feeding tables provided by the food manufacturers. Based on previous observations in the farm, it was thought that, at stocking densities of 15 or more post-larvae/m^2 the same growth would be achieved with much less food than the recommended by such Tables when used together with moderate periodic fertilization. As no statistical significant differences were found between manufacturer's feeding Tables without and with 50% reductions, it can be concluded that it is possible to substantially reduce the amount of food recommended by the manufacturers, resulting in very similar yields at much less cost.

The feed conversion rates (see Table-1), which measure the amount of food necessary to provide a unit of biomass, ranged between 1.46 and 1.75 for feeding Tables reduced in 50% and between 2.63 and 3.42 for feeding Tables with no reductions. Conversion rates obtained using the Tables at 100% were too high for this type of crop where expected values are between 1.5 and 2.5 (FAO 2012).

The other goal of the experiment was to analyze the effect of stocking density on growth. In this case, the ANOVA and ANCOVA results (see Tables 2-4) indicate that stocking densities are highly significant ($p < 0.01$). The highest growth rates (see Table-1), between 0.70 and 0.81 g/week, occurred at lower densities (15 post-larvae /m^2) while the lowest, between 0.60 and 0.65 g/week, occurred at higher densities (35 post-larvae/m^2). This means a difference of approximately 0.1 g/week, which accumulated over time, in large quantities can make a considerable difference in biomass and therefore in sales value.

Bonferroni-Dunn, Tukey and Scheffe multiple-comparison tests were applied to the means of the weight gains under the different stocking densities. The results of the three tests showed that, at $p < 0.05$, stocking densities of 25 and 35 post-larvae /m^2 are not significantly different and that stocking density of 15 post-larvae /m^2 is significantly different to 25 and 35.

With regard to yields (see Table-1), these ranged from 1.2 to 2.7 t/ha which are very good for semi-intensive crops where yields per crop are usually between 0.5 and 2 t/ha (FAO 2012).

Results of this experiment that used moderate periodic fertilization, extrapolate previous results of other authors which did not use periodic fertilization to: higher stocking densities, higher protein content foods and longer periods. It should be noted that the results obtained in this experiment at stocking densities of 35 post-larvae /m^2, which belong to the range of intensive crops, cannot be extrapolated directly to conditions of semi-intensive culture in ponds, since these usually do not have additional aeration.

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