Effect of Diet Protein on Food Conversion and Nitrogen Discharge during Semi-Intensive Production of *Penaeus vannamei* during the Wet Season

Interim Work Plan, Honduras Study 1 (Part I)

David R. Teichert-Coddington, Bartholomew Green, Claude E. Boyd
Department of Fisheries and Allied Aquacultures
Auburn University
Auburn, U.S.A.

John L. Harvin, Rigoberto Rodriguez
Grupo Granjas Marinas, Sn. Bernardo, SA
Choluteca, Honduras

Delia Martinez and Eneida Ramírez
Labroratorio de Calidad de Agua
La Lujosa, Choluteca, Honduras

Introduction

Prior research demonstrated that shrimp production was similar at protein levels ranging from 20 to 40%, when shrimp were stocked at densities ranging from 5 to 11/m^2 (Teichert-Coddington and Rodriguez, 1995). Many producers feed diets containing protein levels of 27% and higher during at least a part of the growing season. It appears that economic efficiency of production could be improved by reducing protein levels in the diet.

The reduction of feeding rates, particularly during the dry season may also improve production efficiency. Dry season production of shrimp juveniles stocked at 7.5/m^2 was not significantly affected by a 50% reduction in the conventional feeding rate (Teichert-Coddington and Rodriguez, 1994). Wet season production was not significantly impacted by reducing the feeding rate by 50%, but feeding efficiency was improved.

These data indicate that both protein content of feed and rates of feeding could be reduced during at least part of the year without reducing shrimp yields. However, it is possible that a comparatively high protein diet fed at a comparatively low rate might result in higher feed conversion than a low protein diet fed at a higher rate.

Phytoplankton blooms in riverine estuaries of southern Honduras appear to be limited by nitrogen. Blooms can result in anoxic conditions at night when respiration by a large plankton biomass consumes oxygen. Highly fertile estuarine water is, therefore, inadequate for replacing water in production ponds.

Nutrient discharge from farms contributes to estuarine fertility. Chemical budget studies of large commercial ponds indicated that nitrogen discharge increased with both feeding rate and diet protein level (Teichert-Coddington et al., 1996). Better feed conversion should result in less nitrogen discharge as well as decreased operating costs. The objective of this experiment was to evaluate the effect of diet protein level and feeding rate on yield, food conversion, and nitrogen effluent during the production of *Penaeus vannamei* at semi-intensive stocking levels.

Materials and Methods

A completely randomized design with a 2 X 2 factorial arrangement was used to test two feeding rates at two levels of protein. Feeding rates were 50 or 75% of the standard feeding curve, and crude protein content was 30 or 20% of the feed (Table 1). Each treatment was replicated. The feeding curve was described by the following equation:

$$Y = 10^{-0.899 - 0.561 \mathrm{Log}_{10}(X)}$$

where,

Y = % of biomass; and

X = mean weight of shrimp (g).

Equal quantities of feed were added to replicate ponds within a treatment based on the average weight of shrimp from the three replicates.

Post larval *Penaeus vannamei*, spawned in a hatchery, were stocked at $24/m^2$ (240,000/ha) in earthen ponds ranging in area from 0.7 to 2.0 ha. It was assumed that the survival rate was 30% due to Taura Syndrome, and that most of the mortality (75%) would occur during the first five weeks.

Water samples for chemical analyses were taken from supply canals and from water discharged during weekly water exchanges. Salinity, Secchi disk, total nitrogen, nitrate nitrogen, total ammonia, BOD_2, and pH were determined weekly. Total phosphorus and filterable phosphate were determined every two weeks, and total alkalinity and primary productivity (free-water method) were determined three times during the experiment. Temperature and dissolved oxygen were determined daily with a YSI meter. Total settleable solids were measured at harvest. Nutrients discharged during harvest were determined from a weighted mean of concentrations determined from water sampled at 100%, 10%, and 0% of pond volume.

Water was exchanged once a week at a nominal rate of 10% of pond volume assuming a depth of 0.9 m. Later measurements demonstrated that mean pond depth actually varied from 0.9 m, hence the percentage of water exchange also varied. Water exchange was accomplished by first draining and then refilling the pond. If morning dissolved oxygen concentration was lower than 2.5 mg/l, then 5% of pond volume was exchanged. Records were kept of exchange events. Total material exchange in the pond during weekly water exchange was calculated by subtracting the mass discharge from the mass intake.

A chemical budget excluding the soils component was made for nitrogen. The general balance equation was:

$$S_{in} + F_{in} + PWV_{in} + WE_{in} = S_{out} + PWV_{out} + WE_{out} \pm Ø≠$$

where,

S = shrimp;

F = feed;

PWV = pond water volume; and

WE = water exchange.

Feed was analyzed for nitrogen content; nitrogen composition of shrimp was 25.5% of dry matter (Boyd and Teichert-Coddington, 1995).

Shrimp were harvested by completely draining ponds on 6 December 1995, 118 days after stocking. Total weight of shrimp was measured for each pond. Mean shrimp weight for each pond was determined by counting and weighing 400 shrimp each at the beginning, middle, and end of harvest. Shrimp were weighed in groups of 100.

Data were analyzed by 2-factor ANOVA where protein content of feed and feeding rate were factors. Survival and water exchange data were arcsine transformed before statistical analysis. Material exchange by water was calculated by subtracting the mass of nutrient discharged from the mass of nutrient input. Treatment differences were declared significant at alpha = 0.05.

Results

Mean treatment pond area was 1.6 ha. Mean treatment pond volumes and weekly water exchange rates were not significantly different. Pond volumes ranged from 0.9 to 1.1 ha-m and weekly water exchange was 59 to 74% of pond volume (Table 2).

Protein level in the diet had no significant effect on gross shrimp yield, mean shrimp size, or feed conversion (Table 2). Survival was significantly

Table 1. Shrimp diet containing 20% or 30% crude protein.

Ingredient	20% Protein	30% Protein
White Corn	42.5	14.0
Wheat Midds	23.9	33.5
Soy 48.5%	10.8	26.1
Fish Meal 67%	9.0	15.0
Calcium Carbonate	3.9	2.9
Meat & Bone Meal	2.0	2.0
Rice Bran	5.9	4.5
Maxi-Bond	2.0	2.0
Total	100.0	100.0

higher in low protein treatments than in high protein treatments although mean differences were low. Mean survival was 22.2 and 19.3% for low and high protein treatments, respectively.

Feeding rate had no significant effect on gross yield, mean size, or survival; however, the mean feed conversion ratio (FCR) was significantly higher for the high feeding rate than for the low feeding rate treatment. No interaction was detected between protein level and feeding rate for yield, mean shrimp size, or FCR.

Nutrient concentrations of intake and discharge water are summarized in Table 3. Differences among treatments were not detected among mean discharge concentrations.

Mean material exchange via water in the pond was negative (greater mass discharge than mass intake) in all treatments for total nitrogen, total phosphorus, chlorophyll-*a*, and BOD_2 (Table 4). Greater masses of total ammonia nitrogen and nitrates were taken into the pond than were discharged, and mean weekly filterable phosphate exchange ranged from -9 to + 73 g/ha. Mean differences among treatments were not significant with one exception—significantly greater quantities of filterable phosphate were discharged by the high feeding rate than by the low feeding rate treatments. Mean discharge of total nitrogen for the high protein treatment was more than twice that for the low protein treatments; however, the differences were not significant because of high within-treatment variation.

Table 2. Mean production (± SD) of *P. vannamei* stocked 24 post larvae/m^2 and fed high or low rates of feed containing 20% or 30% crude protein.

Variable	20%; Low	20%; High	30%; Low	30%; High	Effects		
					Protein	*Rate*	*Interaction*
Gross Yield (kg/ha)	597 ± 148.5	687 ± 52.5	560 ± 119.3	668 ± 74.0	ns	ns	ns
Mean Size (g)	12.6 ± 0.96	12.0 ± 1.48	12.6 ± 0.12	13.9 ± 0.90	ns	ns	ns
Survival	20 ± 2.6	24 ± 2.3	20 ± 2.0	19 ± 0.6	*	ns	*
FCR	2.50 ± 0.47	3.04 ± 0.29	2.58 ± 0.30	3.66 ± 0.23	ns	**	ns
Water Exchange (%/wk)	59 ± 51	71 ± 65	62 ± 51	74 ± 50	ns	ns	ns

** Differences were highly significant (P < 0.01).
* Differences were significant (P < 0.05).
ns: Differences were not significant (P > 0.05).

Table 3. Mean nutrient concentrations of intake and discharge water from ponds stocked with *P. vannamei* fed high or low rates of feed containing 20% or 30% crude protein.

Variable	Intake	Discharge				Effects		
		20%; Low	*20%; High*	*30%; Low*	*30%; High*	*Protein*	*Rate*	*Interaction*
Total Nitrogen (mg/l)	0.82 ± 0.04	0.92 ± 0.37	0.93 ± 0.18	1.32 ± 0.58	1.13 ± 0.26	ns	ns	ns
Total Ammonia-N (mg/l)	0.14 ± 0.022	0.07 ± 0.013	0.07 ± 0.020	0.07 ± 0.013	0.07 ± 0.002	ns	ns	ns
Nitrate+Nitrite-N (mg/l)	0.040 ± 0.018	0.004 ± 0.004	0.004 ± 0.002	0.003 ± 0.002	0.004 ± 0.001	ns	ns	ns
Total Phosphorus (mg/l)	0.21 ± 0.007	0.22 ± 0.013	0.23 ± 0.025	0.24 ± 0.056	0.26 ± 0.007	ns	ns	ns
Filterable Phosphate (mg/l)	0.105 ± 0.016	0.09 ± .026	0.09 ± 0.018	0.08 ± 0.004	0.10 ± 0.020	ns	ns	ns
Chlorophyll-*a* (µg/l)	39.2 ± 5.0	57.7 ± 25.9	51.0 ± 16.4	92.1 ± 60.6	65.6 ± 12.3	ns	ns	ns
BOD_2 (mg/l)	2.8 ± 0.5	4.8 ± 1.9	3.9 ± 0.6	5.1 ± 2.0	4.8 ± 0.4	ns	ns	ns

ns: Differences were not significant (P > 0.05).

More total nitrogen was introduced into ponds by shrimp, water, and feed than was removed from ponds as harvested shrimp and discharge water (Table 5). The mean treatment difference between input and output nitrogen for the high feeding rate was significantly higher than for the low feeding rate.

Discussion

This experiment was designed to evaluate effects on shrimp yield, FCR, and nitrogen effluents of feeding a low-protein diet at a relatively high rate and feeding a high-protein diet at a relatively low rate. The feeding curve, used as a reference in this study, ranged from 10.8 to 2.5% of biomass per day for shrimp weighing from 1 to 18 g. Previous reasearch had demonstrated that this rate of feeding was too high for semi-intensive shrimp culture, and resulted in high FCRs, particularly during the dry, cool season (Teichert-Coddington and Rodriguez, 1994). The standard feeding rate was consequently reduced to 75% of the curve. This reduced rate constituted the high feeding rate treatment used in this experiment.

Some producers claimed that FCRs could be improved by feeding at very low rates if the feed contained a relatively high level of protein. This theory implies that protein quality may impact production if protein quantity applied as feed is low. Prior research had demonstrated no significant effect on shrimp production or FCRs if the protein level was increased from 20 to 40% at stocking rates ranging from 5 to 11/m² (Teichert-Coddington and Rodriguez, 1995). FCRs were as high as 2.56 in the experiment and were considered by some producers too high for the high-protein diet to be effective. The current experiment was designed to evaluate this assumption by testing for interaction between feeding rate and protein level. The low feeding rate used in the current experiment had been tested in a prior study and appeared to be insufficient for optimum yield (Teichert-Coddington and Rodriguez, 1994).

There were no significant treatment differences for production variables, indicating that neither a high feeding rate nor a high protein level in the diet affected production. There was no interaction between feeding rate and protein level, indicating that a high protein diet fed at a low rate did not influence production any more than a low protein diet at a low rate.

Shrimp yields were not significantly higher at the high feeding rate; and FCRs were high, especially with the high feeding rate. These results indicated that shrimp were overfed. Indeed, overfeeding resulted for three reasons. First, with reference to the feeding curve, shrimp were overfed during weeks two through six, because the computer-generated feeding schedule did not take into account mortality which should have been 53% by week five. The schedule was corrected by week seven. Second, stocking of the ponds was delayed by six weeks, so almost half the study took place during the cool season of the year, when growth is historically one half or less of warm season growth (Teichert-Coddington et al., 1994). Dry,

Table 4. Mean treatment nutrient exchange (± SD) per weekly water exchange event with respect to pond area or pond volume. Nutrient exchange was calculated by subtracting nutrient discharge from nutrient intake. Values in parentheses are negative (greater nutrient discharge than nutrient intake).

Variable	% Protein; Feeding Rate				Effects		
	20%; Low	*20%; High*	*30%; Low*	*30%; High*	*Protein*	*Rate*	*Interaction*
Total N (g/ha)	(1100) ± 1613	(887) ± 1014	(2611) ± 1771	(2527) ± 1252	ns	ns	ns
Total Ammonia - N (g/ha)	245 ± 123	130 ± 133	274 ± 159	243 ± 358	ns	ns	ns
Nitrate + Nitrite - N (g/ha)	73 ± 36	105 ± 33	85 ± 56	117 ± 47	ns	ns	ns
Total Phosphorus (g/ha)	(12) ± 156	(25) ± 117	(25) ± 206	(132) ± 18	ns	ns	ns
Filterable Phosphate-P (g/ha)	23 ± 60	(9) ± 27	73 ± 27	(13) ± 30	ns	*	ns
Chlorophyll-*a* (g/ha)	(91) ± 105	(23) ± 76	(239) ± 203	(99) ± 9	ns	ns	ns
BOD_2 (kg/ha)	(9.2) ± 6.1	(5.9) ± 2.4	(10.8) ± 4.2	(10.7) ± 2.8	ns	ns	ns

* Differences were significant ($P < 0.05$).
ns Differences were not significant ($P > 0.05$).

Table 5. Mean difference (± SD) between nitrogen input and measurable nitrogen output during 118 d of shrimp culture in earthen ponds. Values in parentheses are negative.

Variable	% Protein; Feeding Rate				Effects		
	20%; Low	20%; High	30%; Low	30%; High	*Protein*	*Rate*	*Interaction*
Input Shrimp (kg/ha)	0.53 ± 0.29	0.53 ± 0.21	0.47 ± 0.15	0.60 ± 0.26	ns	ns	ns
Input Water (kg/ha)	43.1 ± 29.2	42.6 ± 29.6	43.2 ± 30.0	42.7 ± 30.0	ns	ns	ns
Input Feed (kg/ha)	44.2 ± 1.6	63.9 ± 8.4	66.7 ± 13.6	113.5 ± 7.0	**	**	*
Total Input (kg/ha)	87.9 ± 28.8	107.1 ± 21.8	110.3 ± 30.3	156.8 ± 35.1	ns	ns	ns
Output Shrimp (kg/ha)	17.0 ± 4.3	19.6 ± 1.5	16.0 ± 3.4	19.1 ± 2.1	ns	ns	ns
Output Water (kg/ha)	55.8 ± 25.5	53.2 ± 20.4	73.6 ± 30.0	71.4 ± 40.6	ns	ns	ns
Total Output (kg/ha)	72.9 ± 27.3	72.8 ± 19.4	89.6 ± 28.8	90.5 ± 42.4	ns	ns	ns
Input - Output (kg/ha)	15.0 ± 22.1	34.3 ± 9.2	20.7 ± 31.1	66.3 ± 14.8	ns	*	ns

* Differences were significant ($P < 0.05$).
** Differences were highly significant ($P < 0.01$).
ns Differences were not significant ($P > 0.05$).

cool season feeding rates should be further reduced (Teichert-Coddington and Rodriguez, 1994) because shrimp consume less feed. Production during this study was about half of expected yields, partly because of the cool season effects. Third, mortality from Taura Syndrome was projected to be 70%, but actual mortality averaged 79%, so shrimp biomass was overestimated when calculating feed inputs.

Overfeeding of shrimp violated the low feeding rate treatment designed to underfeed shrimp. Conclusions, therefore, cannot be drawn about the effects of a high-protein diet at a very low feeding rate. Otherwise, these results supported earlier conclusions that a higher protein level in the diet did not result in higher yields or better FCR (Teichert-Coddington and Rodriguez, 1995). Hopkins et al. (1994) arrived at similar conclusions when cultivating shrimp at high densities with a 20 or 40% protein diet in ponds without water exchange.

Ponds had a net discharge of organic material, measured as total nitrogen, total phosphorus, chlorophyll-*a*, and BOD_2, and a net consumption of inorganic nitrogen. There was a net discharge of filterable phosphate from the high feeding rate treatments and a net accumulation of filterable phosphate in the low feeding rate treatment. These results are very similar to those reported by Teichert-Coddington et al. (1996), where inorganic nitrogen and phosphorus from estuarine intake water were converted to organic matter within the pond. Inorganic nitrogen and phosphorus tend to accumulate in the estuaries probably because sediments, suspended due to high tidal action, limit primary productivity by blocking sunlight. Sediment precipitation in water supply canals and ponds thereby allowed phytoplankton to bloom.

Teichert-Coddington et al. (1996) reported that nitrogen discharge from commercial shrimp ponds increased with higher FCRs. In this study, significant treatment differences were not detected for net discharge of nitrogen (Table 4) although there were higher inputs of nitrogen with the high protein diet supplied at the high feeding rate. Nitrogen that was not detected in water discharge or shrimp harvest was significantly greater at the high feeding rate (Table 5). Unobserved nitrogen averaged 17 and 37% for low and high feeding rates, respectively. This is higher than the 1% value formerly reported for semi-intensive, commercial ponds in Honduras (Teichert-Coddington et al., 1996). Higher proportions of input nitrogen not detected in water discharge or shrimp harvest were reported for intensively managed shrimp ponds. Briggs and Funge-Smith (1994) determined that 44% of input nitrogen was undetected in discharge water or shrimp flesh from ponds in Thailand, and Hopkins et al. (1993) reported rates ranging from 9 to 67% for ponds in South Carolina. Both of the intensively managed pond studies demonstrated accumulation of nitrogen in soils and sludge, but could not otherwise account for 3 to 42% of total nitrogen input. Undetected nitrogen was assumed lost to denitrification. Research has not determined if significant amounts of nitrogen in shrimp ponds are fixed by algae, but nitrogen fixation may be important to the nitrogen budget of low-input shrimp ponds.

Anticipated Benefits

Results from this experiment have corroborated results from past studies in Choluteca. We can consequently make recommendations with more confidence with respect to reducing feeding rates and dietary protein levels. As feeding becomes more efficient, the impacts of shrimp farming on estuarine environment should become less.

Acknowledgments

Jaime López assisted in the laboratory. This study was made possible through collaboration of the Dirección General de Pesca y Acuicultura, Secretaría de Recursos Naturales, Government of Honduras, and shrimp producers of the Honduran National Association of Aquaculturists (ANDAH).

Literature Cited

Boyd, C.E. and D. Teichert-Coddington, 1995. Dry matter, ash, and elemental composition of pond-cultured *Penaeus vannamei* and *P. stylirostris*. Journal of the World Aquaculture Society, 26(1):88-92.

Briggs, M.R.P. and S.J. Funge-Smith, 1994. A nutrient budget of some intensive marine shrimp ponds in Thailand. Aquaculture and Fisheries Management, 25:789-811.

Hopkins, J.S., R.D.I. Hamilton, P.A. Sandifer, C.L. Browdy, and A.D. Stokes, 1993. Effect of water exchange rate on production, water quality, effluent characteristics and nitrogen budgets of intensive shrimp ponds. Journal of the World Aquaculture Society, 24(3):304-320.

Hopkins, S.J., C.L. Browdy, P.A. Sandifer, and A.D. Stokes, 1994. Effect of two feed protein levels and two feed rates, stocking density combinations on water quality and production in intensive shrimp ponds which do not utilize water exchange. Abstract presented at Annual Meeting of the World Aquaculture Society in January 14-18, New Orleans, Louisiana, USA, p. 30.

Teichert-Coddington, D.R., D. Martinez, and E. Ramírez, 1996. Characterization of shrimp farm effluents in Honduras and chemical budgets of selected nutrients. In: H. Egna, B. Goetze, D. Burke, M. McNamara, and D. Clair (Editors), Thirteenth Annual Technical Report, Pond Dynamics/Aquaculture Collaborative Research Program, Office of International Research and Development, Oregon State University, Corvallis, OR, USA, pp. 70-84.

Teichert-Coddington, D.R. and R. Rodriguez, 1994. Inorganic fertilization and feed reduction in commercial production of *Penaeus vannamei* during wet and dry Seasons in Honduras. In: H.S. Egna, J. Bowman, B. Goetze, and

N. Weidner (Editors), Twelfth Annual Technical Report, Pond Dynamics/ Aquaculture Collaborative Research Program, International Research and Development, Oregon State University, Corvallis, OR, USA, pp. 136-146.

Teichert-Coddington, D.R. and R. Rodriguez, 1995. Semi-intensive commercial grow-out of *Penaeus vannamei* fed diets containing differing levels of crude protein during wet and dry seasons in Honduras. Journal of the World Aquaculture Society, 26(1):72-79.

Teichert-Coddington, D.R., R. Rodriguez, and W. Toyofuku, 1994. Cause of cyclical variation in Honduran shrimp production. World Aquaculture, 25(1):57-61.