Effect of Diet Protein on Semi-Intensive Production of *Penaeus vannamei* during the Rainy Season

Interim Work Plan, Honduras Study (Part Ia)

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Introduction

Shrimp culture in Honduras is practiced at the semi-intensive level, where final stocking rates vary from 5 to 11 shrimp m^{-2}, daily water exchange rates average 10% of pond volume, and formulated rations contain 20 to 40% protein. A number of studies have been conducted to determine the effect of dietary protein level and feeding rate on production of *Penaeus vannamei*. Teichert-Coddington and Rodriguez (1995) reported no significant differences in yield for *P. vannamei* in ponds stocked with 5 and 11 shrimp m^{-2} and offered a 20% or 40% protein commercial ration. A prior feed trial in Choluteca with a stocking density of 7.5 shrimp m^{-2} demonstrated that production during the dry season was not significantly affected by a 50% reduction in the feeding rate. Wet season production was significantly impacted by the 50% reduction in feeding, but feeding efficiency was improved. These results indicated that excessive feed was applied regularly during the dry season and that wet season feed rates could be reduced (although not by as much as 50%). Practical rainy season feeding rates approximated the rates indicated by the theoretical feeding curve.

Thus, it appears that shrimp yields do not improve when feed protein is increased from 20 to 40% of the ration. In fact preliminary data indicate that shrimp production during the rainy season is not affected by feed with a protein content range of 16 to 30%. The objective of this experiment was to determine the effect of dietary protein on shrimp growth and yield.

Methods and Materials

Sixteen 2-ha (± 0.08 ha SD) ponds located on a commercial shrimp farm located on a riverine estuary of the Gulf of Fonseca, Honduras were used for this randomized design study. Four diets were tested containing 12, 16, 20 or 30% crude protein (Table 1). Shrimp were fed six days per week beginning on 10 September 1996. Feed rate for each treatment was 75% of an empirically derived feeding curve for *P. vannamei*:

$$\mathrm{Log}_{10} y = -0.899 - 0.56\mathrm{Log}_{10} x$$

where y is the feed rate as a percent of biomass and x is the mean shrimp weight in grams. Feed was offered once daily. Feed rate was calculated for individual ponds and then averaged by treatment, so that all ponds within a treatment received the same quantity of feed. Feed rate was adjusted weekly, based on samples to monitor shrimp growth, taken weekly by cast net from each pond population. The weight of feed offered

Table 1. Composition of shrimp diets formulated to contain 12 to 30% crude protein.

Ingredient	**Formulated Ration**			
	12% Protein	*16% Protein*	*20% Protein*	*30% Protein*
Soybean Meal (48.5%)	10.0	7.7	17.8	26.1
Ground Wheat	84.4	76.5	66.2	
Fish Meal (67%)		7.5	7.5	15.0
Meat and Bone Meal		1.0	1.0	2.0
Wheat Midds				33.5
White Corn				14.0
Rice Semolina				4.5
Fish Oil		2.0	2.0	
Palm Oil	2.7			
$CaCO_3$	2.9	3.3	3.5	2.9
Bentonite		2.0	2.0	
Maxi-bond				2.0
Total	100.0	100.0	100.0	100.0

divided by the gross yield of whole shrimp was used to calculate the feed conversion ratio (FCR).

Ponds were stocked with hatchery-spawned, post-larval (PL) *P. vannamei* at 250,000 PL ha^{-1} (25 PL m^{-2}) on 15 August 1996. A survival rate of 25% was assumed, to account for Taura Syndrome effects on hatchery-produced larvae; most mortality was assumed to occur during the first month following stocking. Shrimp were harvested 110 days after stocking by completely draining ponds. The total weight of shrimp was obtained for each pond and mean individual weight was determined by weighing a sample of 300 shrimp per pond.

No water was exchanged during the first three weeks of culture. Starting with week four, water was exchanged at 20% of pond volume once weekly. In addition if the early morning dissolved oxygen concentration was ≤ 2.5 mg l^{-1}, then 5% of the pond volume was exchanged. For all water exchanges, water was discharged first and then ponds were refilled. All water exchange events were recorded. Total material exchange per pond during weekly water exchange was calculated by subtracting mass discharge from mass intake.

Water quality variables in each pond were measured upon initiation of the experiment. Beginning with week four, at the initiation of water exchange, discharge and intake water quality was monitored weekly. Intake water was sampled from supply canals, while discharge water was sampled from each pond's outfall. Initial pond water and replacement water samples were obtained with a column sampler. Water samples were analyzed for pH (measured potentiometrically), nitrate-nitrogen (measured by cadmium reduction) (Parsons et al., 1992), total ammonia-nitrogen (Parsons et al., 1992), soluble reactive phosphorus (SRP) (Grasshoff et al., 1983), chlorophyll *a* (Parsons et al., 1992), total alkalinity (measured by titration to pH 4.5 endpoint), salinity, and BOD_2 at ambient temperature. Total nitrogen and total phosphorus were determined by nitrate and phosphate analysis, respectively, after simultaneous persulfate oxidation (Grasshoff et al., 1983).

Data were analyzed by ANOVA (Haycock et al., 1992). Percent data were arcsine transformed prior to analysis. Differences were declared significant at alpha level 0.05.

Results

Shrimp survival, which was much lower than the rate anticipated to account for Taura Syndrome, averaged 5% among all treatments (Table 2); survival did not differ significantly among treatments. Feed

Table 2. Mean production (± SD) of *Penaeus vannamei* in 16 two-ha earthen ponds during a 110-day rainy season study. Post larval shrimp were stocked at 25 PL m^{-2}. Four levels of dietary protein were tested.

Variable	Formulated Ration			
	12% Protein	*16% Protein*	*20% Protein*	*30% Protein*
Gross Yield (kg ha^{-1})	186.0 ± 41.2[a]	223.0 ± 43.6[a]	277.0 ± 98.6[a]	235.0 ± 83.4[a]
Mean Weight (g $shrimp^{-1}$)	13.7 ± 1.18[a]	13.7 ± 0.77[a]	14.1 ± 1.25[a]	13.8 ± 1.38[a]
Survival (%)	5.3 ± 0.14[a]	6.6 ± 0.07[a]	7.8 ± 0.21[a]	6.3 ± 0.19[a]
FCR	6.1 ± 1.41[a]	4.9 ± 0.98[a]	4.1 ± 1.26[a]	5.0 ± 1.48[a]
Total Feed Offered (kg ha^{-1})	1088.0 ± 10.1[a]	1065.0 ± 45.3[a]	1045.0 ± 42.1[a]	1081.0 ± 37.7[a]

[a] Means with the same superscript designation are not significantly different ($P > 0.05$). Horizontal comparison only.

protein level did not significantly affect gross yield, mean shrimp weight, or FCR (Table 2), nor did it affect shrimp growth (Figure 1). Similar quantities of feed were offered in all treatments, however the total quantity of nitrogen added increased significantly with feed protein content.

Total nitrogen, total ammonia-nitrogen, total phosphorus, soluble reactive phosphorus, and chlorophyll *a* concentrations of pond intake and discharge water did not differ significantly (Table 3). Intake water had significantly greater oxidized nitrogen and significantly lower BOD_2 concentrations than discharge water (Table 3). There were no treatment differences among discharge water nutrient concentration variables (Table 3). No significant relationship was detected between total quantity of feed added per pond and concentration of any nutrient in discharge water.

The mean volume discharged from ponds per exchange event did not differ significantly among treatments; the global mean volume discharged was 2,624 m^3. Mean material exchange was negative (i.e., net discharge) for total nitrogen, total phosphorus, soluble reactive phosphorus

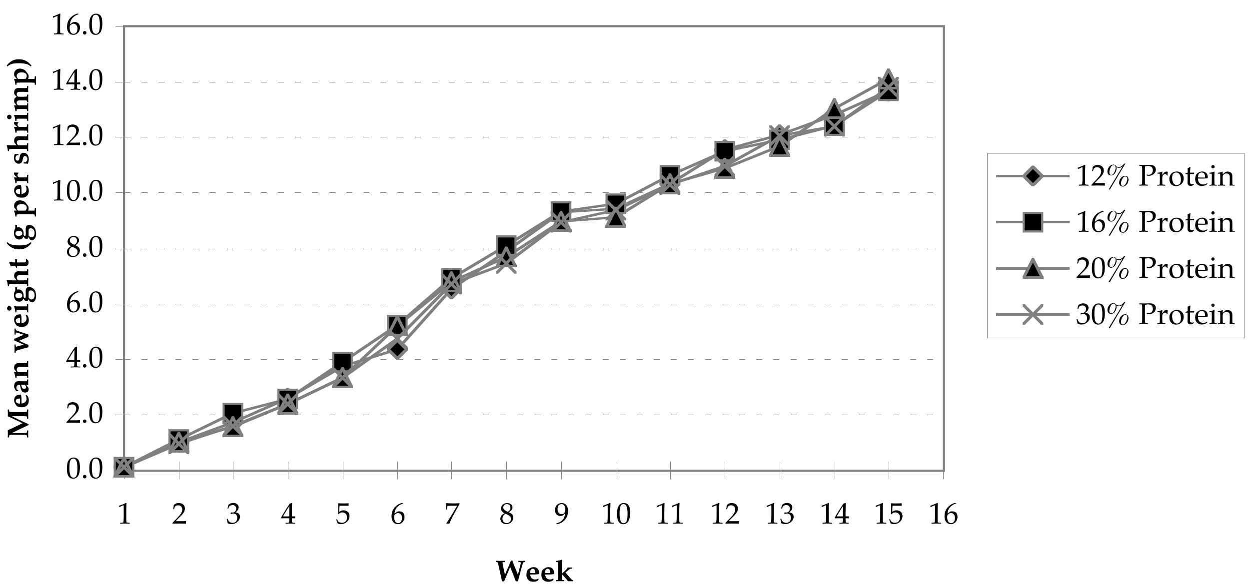

Figure 1. Growth of *Penaeus vannamei* stocked in two-ha earthen ponds at 25 post larvae m^{-2} and fed one of four dietary protein level feeds during a 110-d grow-out in Honduras.

Table 3. Mean nutrient concentrations (± SD) of intake and discharge water from 16 two-ha earthen ponds stocked with *Penaeus vannamei* (25 m^{-2}) during a rainy season study that tested four levels of dietary protein.

Variable	Intake Water	Discharge Water			
		12% Protein	*16% Protein*	*20% Protein*	*30% Protein*
Total Nitrogen (mg l^{-1})	0.78 ± 0.015^{a}	1.38 ± 0.463^{a}	1.26 ± 0.398^{a}	1.43 ± 0.515^{a}	1.66 ± 0.511^{a}
Total Ammonia-N (mg l^{-1} NH_3-N)	0.08 ± 0.044^{a}	0.06 ± 0.014^{a}	0.06 ± 0.025^{a}	0.05 ± 0.013^{a}	0.05 ± 0.016^{a}
Oxidized N (mg l^{-1} NO_2-NO_3-N)	0.042 ± 0.035^{a}	0.003 ± 0.001^{b}	0.002 ± 0.001^{b}	0.005 ± 0.008^{b}	0.006 ± 0.008^{b}
Total Phosphorus (mg l^{-1})	0.20 ± 0.035^{a}	0.24 ± 0.035^{a}	0.29 ± 0.023^{a}	0.24 ± 0.062^{a}	0.30 ± 0.089^{a}
Soluble Reactive Phosphate (mg l^{-1} PO_4-P)	0.11 ± 0.027^{a}	0.11 ± 0.046^{a}	0.15 ± 0.014^{a}	0.10 ± 0.065^{a}	0.14 ± 0.112^{a}
Chlorophyll *a* (mg m^{-3})	33.0 ± 3.80^{a}	59.5 ± 40.06^{a}	47.5 ± 32.77^{a}	67.6 ± 37.85^{a}	95.2 ± 50.88^{a}
BOD_2 (mg l^{-1})	2.9 ± 0.46^{b}	5.8 ± 1.78^{a}	5.5 ± 1.76^{a}	6.4 ± 2.08^{a}	6.8 ± 1.92^{a}

[a,b] Means with the same superscript designation are not significantly different ($P > 0.05$). Horizontal comparions only.

Table 4. Mean net nutrient exchange (intake minus discharge) per water exchange event (± SD) in 16 two-ha earthen ponds stocked with *Penaeus vannamei* (25 m^{-2}) during a 110-d rainy season study that tested four levels of dietary protein. Parentheses indicate negative values.

Variable	**Formulated Ration**			
	12% Protein	*16% Protein*	*20% Protein*	*30% Protein*
Total Nitrogen (kg)	(1.61) ± 1.110[a]	(1.25) ± 0.935[a]	(1.90) ± 1.415[a]	(2.51) ± 1.408[a]
Total Ammonia-N (g)	16.1 ± 41.87[a]	15.5 ± 59.81[a]	60.6 ± 30.03[a]	45.9 ± 37.40[a]
NO_3-NO_2-N (g)	85.2 ± 8.43[a]	82.3 ± 2.10[a]	79.2 ± 24.38[a]	73.6 ± 20.04[a]
Total Phosphorus (g)	(118) ± 79.6[a]	(229) ± 52.1[a]	(123) ± 175.9[a]	(279) ± 212.3[a]
Soluble Reactive Phosphorus (g)	(7.3) ± 118.02[a]	(113.1) ± 37.11[a]	31.6 ± 193.08[a]	(85.2) ± 287.68[a]
Chlorophyll *a* (g)	(68.9) ± 99.46[a]	(37.1) ± 78.50[a]	(99.4) ± 103.82[a]	(169.9) ± 137.67[a]
BOD_2 (kg)	(7.4) ± 4.24[a]	(6.4) ± 4.14[a]	(9.8) ± 5.72[a]	(10.8) ± 5.19[a]

[a] Means with the same superscript designation are not significantly different ($P > 0.05$).

(with the exception of the 20% protein treatment where more SRP was taken in than discharged), and chlorophyll *a*. Total amounts of ammonia-nitrogen and nitrate-nitrite-nitrogen taken into ponds were greater than amounts discharged (Table 4). There were no significant differences in material exchange among treatments.

Discussion

The unexpectedly high shrimp mortality made it impossible to determine the effect of dietary protein level on shrimp production. Effects of dietary protein content should become apparent only when the shrimp biomass has attained the critical standing crop and the biomass of natural food organisms in the pond is no longer adequate to support rapid shrimp growth. Shrimp yields in this experiment were 30 to 44% of rainy season yields reported for Taura Syndrome-affected ponds in Honduras (Teichert-Coddington et al., 1996; Teichert-Coddington et al., 1997).

The question of whether dietary protein level can be reduced below 20% for semi-intensively managed shrimp ponds in Honduras remains unanswered. Results of other research conducted in Honduras indicate that 30% protein feed did not result in increased shrimp production compared to 20% protein feed (Green et al., 1997; Teichert-Coddington et al., 1997). Shrimp yield and growth were similar when shrimp were stocked in ponds at 5 to 11 *P. vannamei* m^{-2} and offered a 20 or 40% protein formulated ration (Teichert-Coddington and Rodriguez, 1995). No significant difference in shrimp yield was reported when a 29 or 37% protein feed was offered to *P. vannamei* stocked in earthen ponds at four to eight shrimp m^{-2} (Teichert-Coddington and Arrue, 1988). *Penaeus vannamei* growth in ponds stocked with seven to nine shrimp m^{-2} did not differ significantly among the following treatments: no external inputs, cow manure, cow manure and chemical fertilizer, or 20% protein feed (Lee and Shlesser, 1984). Since it is indicated that feed protein content could be reduced to below 20% for semi-intensive *P. vannamei* culture, this study should be repeated using post larval *P. vannamei* with demonstrated and/or improved survivorship, such as wild-caught *P. vannamei*.

Feed conversion ratios were very high for this experiment because of overfeeding. The computer-generated feed curve incorporated the expected mortality (75% of stocked animals), but because mortality exceeded anticipated levels by 27%, feed inputs were overestimated. While it is impossible to make inferences regarding treatment effects on FCR, these results clearly demonstrate the difficulty in achieving efficient feed management in ponds affected by Taura Syndrome.

No treatment differences were detected in total nitrogen concentrations in pond effluents during weekly water exchange events despite nitrogen additions (in the form of feed) that increased significantly with increased feed protein level. However, the observed mean discharge of nitrogen was higher with the 20 and 30% protein feeds compared with the lower protein feeds. Previous studies have reported that weekly exchange events discharged organically-rich water—measured as total nitrogen, total phosphorus, chlorophyll *a*, and BOD_2—into estuaries (Green et al., 1997; Teichert-Coddington et al., 1996; Teichert-Coddington et al., 1997). Inorganic nitrogen and phosphorus entering ponds was also converted to organic matter that was discharged into estuaries.

Anticipated Benefits

This study was designed to determine the effect of feed protein level on growth and yield of semi-intensively cultured *P. vannamei* in Honduras, with the goal of reducing nitrogen inputs as feed to ponds. However, because shrimp survival was only 20% of that expected, it was impossible to evaluate treatment effects. This experiment should be repeated.

Acknowledgments

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Literature Cited

Grasshoff, K., M. Ehrhardt, and K. Kremling, 1983. Methods of Seawater Analysis. Verlag Chemie, Weinheim, 419 pp.

Green, B.W., D.T. Teichert-Coddington, C.E. Boyd, J.L. Harvin, H. Corrales, R. Zelaya,

D. Martinez, and E. Ramírez, 1997. Effect of diet protein on food conversion and nitrogen discharge during semi-intensive production of *Penaeus vannamei* during the dry season. In: D. Burke, B. Goetze, D. Clair, and H. Egna (Editors), Fourteenth Annual Technical Report, Pond Dynamics/ Aquaculture CRSP, Office of International Research and Development, Oregon State University, Corvallis, Oregon, pp. 77-86.

Haycock, K., J. Roth, J. Gagnon, W.R. Finzer, and C. Soper, 1992. StatView 4.0. Abacus Concepts, Inc., Berkeley, 466 pp.

Lee, C.S. and R.A. Shlesser, 1984. Production of *Penaeus vannamei* in cattle manure-enriched ecosystems in Hawaii. Journal of the World Mariculture Society, 15:52-60.

Parsons, T.R., Y. Maita, and C.M. Lalli, 1992. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, New York, 173 pp.

Teichert-Coddington, D.R. and M. Arrue, 1988. Effectos de dietas de proteinas y densidades de siembra sobre la produccion de *Penaeus vannamei* en estanques de tierra. Revista Latinoamericana de Acuicultura, 35:29-33.

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, 26:72-79.

Teichert-Coddington, D.R., W. Toyofuku, J. Harvin, and R. Rodriguez, 1996. Relationships among stocking density, survival and yield in ponds affected by the Taura Syndrome during wet and dry seasons in Honduras. In: H. Egna, B. Goetze, D. Burke, M. McNamara, and D. Clair (Editors), Thirteenth Annual Technical Report. Pond Dynamics/ Aquaculture CRSP, Office of International Research and Development, Oregon State University, Corvallis, Oregon, pp. 85-94.

Teichert-Coddington, D.R., B. Green, C.E. Boyd, J.L. Harvin, R. Rodriguez, D. Martinez, and E. Ramírez, 1997. Effect of diet protein on food conversion and nitrogen discharge during semi-intensive production of *Penaeus vannamei* during the wet season. In: D. Burke, B. Goetze, D. Clair, and H. Egna (Editors), Fourteenth Annual Technical Report, Pond Dynamics/Aquaculture CRSP, Office of International Research and Development, Oregon State University, Corvallis, Oregon, pp. 71-77.