The effect of dietary protein on the growth
and survival of the shrimp, *Penaeus monodon* in outdoor tanks

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Abstract

The comparative effect of reducing the protein content of formulated feed on the growth and survival of black tiger shrimp, *Penaeus monodon*, and on water quality was tested in outdoor tanks. Three diets, 300, 350 and 400 g kg^{-1} crude protein (CP), were fed to *P. monodon* (3.1 g animals, 25 animals m^{-2}) in each of 8 replicated outdoor 2500 l tanks in an 8 week trial. There was no statistical difference ($P > 0.05$) in shrimp growth rate (1.34 to 1.50 g week^{-1}), survival, or final biomass between the treatments. However, when tanks with lower survival were removed from the analysis (<60% and <80% were tested), shrimp growth rate was statistically higher ($P < 0.05$) in the 350 and 400 g kg^{-1} CP diets than in the 300 g kg^{-1} CP diet treatment. There were no differences in the nutritional condition of shrimp between treatments, as determined by moisture and protein content, and lipid content of the digestive gland. Using ^{15}N-nitrogen isotope tracers, it was determined that shrimp were consuming natural biota, although these were unlikely to have contributed substantially to their nutrition. Total nitrogen (TN) concentrations in the water column increased over the eight week experiment and were statistically different ($P < 0.001$) between treatments (3.60, 5.17 and 6.45 mg L^{-1} in the 300, 350 and 400 g kg^{-1} CP treatments respectively). Concentrations of dissolved organic nitrogen (DON) were also statistically different between treatments and made up 35 to 40% of the TN in the water column. Concentrations of total ammoniacal nitrogen (TAN) and oxides of nitrogen, and fluorescence were not statistically different between treatments but there was a trend of higher concentrations in treatments with higher protein levels. There was no difference in sediment nutrients between treatments. This study has shown that there is scope to reduce the protein content of *P. monodon* diets but only by 5 to 10%. However, further validation of these results in commercial ponds is needed. Reducing the feed protein content may result in cost savings and also has the advantage of improving water quality and reducing nitrogen discharge.

Introduction

The black tiger shrimp, *Penaeus monodon* is the most widely cultured species in the eastern hemisphere. In intensive systems, *P. monodon* is typically fed a high protein,

fishmeal-based diet. The use of such feed formulations is supported by the results of laboratory studies showing that the optimal dietary crude protein content for *P. monodon* diets is between 350 and 460 g kg^{-1} (Guillaume, 1997). However, laboratory studies do not take into account the contribution of natural feed to the nutrition of the animals (Tacon 1996). *P. monodon* is one of the most omnivorous of shrimp species, and has been shown to consume plant and detrital material (Bombeo-Tuburan *et al*. 1993; Focken *et al*. 1998). In intensive shrimp systems, detrital material is abundant and may provide an additional food source for shrimp.

The natural biota has been shown to contribute substantially to the nutrition of other shrimp species in pond and tank systems. In semi-intensive *Litopenaeus vannamei* and *P. setiferus* ponds, 52% of the dietary nitrogen was supplied by the pond biota (Parker *et al*., 1989, 1991). In outdoor tanks with *L. vannamei* stocked at 50 animals m^{-2}, 31% of the nitrogen requirements were derived from tank biota (Epp *et al*. 2002). There was a correlation between percentage of living carbon in shrimp ponds and *L. vannamei* growth rates in tanks (Leber & Pruder 1988; Moss & Pruder 1995). In intensive tank systems, the presence of structures with epiphytic growth increased *L. vannamei* production (Bratvold & Browdy 2001). Reducing the CP level in *L. vannamei* diets from 40 to 20% in ponds did not reduce shrimp production (Teichert-Coddington & Rodríguez 1995; Hopkins *et al*. 1995), and farms growing *L. vannamei* intensively have fed diets with reduced CP content in recent years (McIntosh & Avnimelech 2001). This evidence points to natural biota contributing substantially to shrimp nutrition in pond systems.

There is considerable feed wastage in intensive shrimp ponds where the protein level of the feed has not been adjusted to allow for the contribution of natural biota. This results in only 15 to 30% of the nitrogen added as feed being retained in the shrimp biomass at harvest (Funge-Smith & Briggs 1998; Martin *et al*. 1998; Jackson *et al*. 2003). This may be due to suboptimal feed formulations and water stability, and feeding strategies. Feed wastage is expensive for farmers who spend up to 50% of their variable costs on feed (Lawrence & Lee 1997). Additionally, waste nutrients can have detrimental effects on water quality, and ultimately on shrimp health and growth. If pond water is discharged, nutrients and suspended solids can impact on the health of aquatic ecosystems (Naylor *et al*. 1998*;* Smith *et al*. 1999; McKinnon *et al*. 2002),

and in some places incur penalties from environmental protection authorities (Preston *et al*. 2001).

This study aimed to reduce dietary protein levels to determine the comparative effect on *P. monodon* growth and survival, and water quality.

Methods

Experimental design

The experiment comprised an 8-week growth assay using *P. monodon.* Non-selectively bred animals, which had not been screened for health status, were used. These animals were collected from one commercial pond. There were three diet-based treatments varying only in their CP content (300, 350 and 400 g kg^{-1} CP). A balanced block design was used, with four blocks of six circular 2500 L tanks. Treatments were randomly assigned to two tanks within each block. The blocking design was used to determine and account for any position effect within the tank array.

Each tank contained a 50 mm deep layer of sediment taken from the bottom of a shrimp pond. A sample of sediment was taken for nutrient analysis. Approximately 3 weeks prior to the start of the experiment, 20% of the tank volume was filled with water drawn from a single commercial shrimp production pond, and the remainder was sand-filtered oceanic water. Airlift pumps were used to aerate the tanks, and to create a circulating current sweeping feed waste and other detritus into the centre of the tank. To encourage the growth of microalgae, tank water was fertilized with 0.01 mg P L^{-1} as dihydrogen potassium orthophosphate and 0.1 mg N L^{-1} as ammonium chloride. The tanks were initially stocked with 10 x 10 g *P. esculentus* shrimp to help establish a benthic microbial community in the sediment. These shrimp were trapped and removed from the tanks just prior to the start of the experiment.

P. monodon were obtained from a commercial shrimp farm then weighed and distributed so that each tank contained 75 shrimp with a similar mean ($\pm$ s.d.) weight and size distribution (3.11 $\pm$ 0.43 g). To prevent shrimp from escaping from the

tanks, each tank was covered with a monofilament net (12 mm mesh size). Tanks were fertilized again with nitrogen and phosphorus as described above.

The experiment was carried out from January to March 2001. Water exchanges were conducted as required to prevent algal blooms from becoming unstable. Between 10 and 30% of the volume of the tanks was removed over the course of each week, and oceanic water was used to refill the tanks. When tank salinities increased above 36 mg L^{-1}, freshwater was used to replace water lost through evaporation. The tank walls were cleaned twice weekly to remove barnacles, filamentous algae and other organisms.

At the conclusion of the experiment, the tanks were drained to a depth of 20 cm above the sediment. Sediment cores (37 mm dia.) were taken at 20 random sites in each tank ensuring that disturbance to the sediment was minimized. The water above the cores was decanted off and cores were then combined and mixed. A subsample was taken for porewater ammonium analysis by centrifuging at 5000 rpm for 15 min at 4°C, decanting the supernatant and filtering it through a cellulose acetate filter (0.45 μm) before freezing. A subsample for total Kjeldahl nitrogen (TkN) and phosphorus (P) analyses, and dry weight:wet weight ratio was also taken and frozen.

After coring, shrimp in each tank were trapped, counted, weighed and checked for visual signs of health status. The criteria used were: gill fouling; exoskeletal fouling; exoskeletal lesions; short antennae; antennal palp damage and melanization; uropod damage and melanization; tail rot blisters; body turgor; and shrimp colour.

Feeds and feeding

Diets were formulated to contain 300, 350 and 400 g kg^{-1} CP on an as-used basis (Table 1). Sufficient water was added to dry ingredients to form a soft dough. The dough was extruded through a 3 mm die, using the mincer attachment on a commercial food mixer (Hobart Corporation, OH, USA), spread onto mesh trays, steamed in a commercial atmospheric food steamer for 5 min and air dried at 40°C for 36 h. The dried strands were broken into pellets of about 8 mm in length and stored at -4°C until used.

The shrimp were fed three times daily at 0600, 1200 and 1900 h. A previous study in these tanks showed that three times daily feeding of shrimp of the same size as our study, and grown under similar conditions using a 400 g kg^{-1} CP commercial feed resulted in the same shrimp growth rate as six times a day (Smith *et al*. 2002). Rations were equally divided onto two feed trays (30 cm dia.) at each feed. The trays were removed and checked for uneaten food prior to the next feed and the ration adjusted accordingly to minimize feed wastage. All uneaten food was returned to the tank before the feed tray was used again.

[Insert Table 1]

Water quality sampling

Water temperature, salinity, pH, dissolved oxygen (DO) and turbidity were measured in all tanks with a datalogger (Yeokal model 611) at 0500 and 1400 h each day. Duplicate water samples (for fluorescence measurements and TN analyses) were taken from just below the water surface in all tanks twice weekly at 1400 h. Soluble nutrients and TP were sampled once a week using the same procedure. Subsamples for TN and phosphorus analysis were frozen until analyzed. Subsamples for analysis of TAN, nitrite and oxides of nitrogen, were filtered through prefilters (glass fibre) and disposable filters (cellulose acetate, 0.45 μm pore size) within 1 h of collection, then frozen until analyzed. The fluorescence of water samples (as indication of phytoplankton biomass) was determined using a field fluorometer (Turner Designs Model 10-AU). When fluorescence readings were offscale, samples were diluted 1:10 with filtered water just prior to reading. A subsample of water samples from a range of days and tanks (n = 24) was filtered onto GF/F glass fibre filters for chlorophyll *a* analysis. Filters were extracted into 100% acetone using a sonicator, and the absorbance of samples diluted to 90% acetone was measured spectrophotometrically (Jeffrey & Welshmeyer 1997).

Role of natural biota in shrimp nutrition

To ascertain the proportion of natural biota consumed by *P. monodon*, 0.04 g ^{15}N-nitrogen enriched ammonium chloride was added to each tank 48 h prior to

harvesting. Shrimp were harvested and 10 animals from each tank were placed in seawater for a few hours to allow gut evacuation. Animals were then sacrificed and frozen. After thawing, shrimp were blotted dry, weighed, freeze dried, reweighed to determine the moisture content, and finely ground in a water-cooled Knifetec grinder (Tekator, Sweden). $^{15}N/^{14}N$-nitrogen ratios were determined by mass spectroscopy.

Feed and shrimp analyses

Proximate analysis of diets was carried out as follows: DM from weight change following heating in a thermogravimetric analyzer (Leco TGA-601) at 105°C to a constant weight; ash from weight change following burning in a thermogravimetric analyzer (Leco TGA-601) at 600°C to constant weight; CP (CP = %N x 6.25) by the Dumas combustion method, calibrated using aspartic acid (Elementor Rapion Analyzer, AOAC 1999); total lipid by a chloroform-methanol extraction (Folch *et al*. 1957); and gross energy by isothermal bomb calorimetry (Leco AC200) calibrated with benzoic acid.

DM loss (water stability) and nitrogen leaching rate of the three diets were determined (Smith *et al*. 2002). For water stability estimation, weighed feed pellets (ca. 1 g) were immersed in jars containing 70 mL of seawater and maintained at 28°C in a shaking water bath (oscillating at 40 rpm) for periods of 2, 4 and 6 h. At the end of the immersion period, pellets were rinsed, dried at 105°C overnight, cooled in a vacuum desiccator and weighed to determine the DM loss. The leaching rate of TkN from feed pellets was determined from 2 g samples placed in 80 mL of distilled water for periods of 0.5, 1, 2, 3, 4, 6, 9 and 12 h. The nitrogen leaching from a commercial *P. monodon* diet (= reference) was also measured as a comparison. At the end of each leaching period, water was filtered through a glass fibre filter (Whatman GF/C), digested using a Kjeldahl method, followed by distillation of liberated ammonia into 2% boric acid, and titration with hydrochloric acid to pH 5.0.

The same sample of 10 shrimp taken from each tank for $^{15}N/^{14}N$ analyses was used for TkN analyses, using the same method as for the diet analysis. A second sample of seven shrimp from each tank was taken to determine the lipid content of the digestive gland. Shrimp were initially frozen then partially thawed to allow rapid dissection of the intact digestive gland. The digestive glands from each tank were pooled and

weighed, then freeze-dried and ground. Total lipid in the digestive gland was measured using the same method as for the diets.

Water and sediment analyses

Water samples for nutrient analyses were thawed and mixed. TAN was analyzed using the phenate method 4500-NH3 F and filterable reactive phosphorus by the ascorbic acid method 4500-P E. (American Public Health Association 1995). Nitrate was analyzed using a spongy cadmium method (Jones 1983), and nitrite by removing the cadmium reduction step of the spongy cadmium method. TN, dissolved nitrogen (DN) and TP were measured using a modified version of the simultaneous persulfate oxidation method (Hosomi & Sudo 1986). DON was calculated by subtracting nitrate, nitrite and TAN from DN.

Pore water extracted from sediments was analyzed for TAN using the method described above. Sediment cores for TN and TP were freeze-dried, ground and digested using a kjeldahl digestion procedure. TkN and TP analysis were analyzed using methods 4500-NH$_3$ H and 4500-P respectively (American Public Health Association, 1998) on a segmented flow instrument (SKALAR).

Statistical analyses

Prior to starting the experiment, historical data on the variability of mean growth rates of shrimp grown in this outdoor tank array was analyzed to ensure that there was sufficient replication to provide adequate statistical power (80%) in the experimental design (Smith *et al*., 2002).

At the conclusion of the experiment, differences across treatments in growth rate, shrimp composition, apparent feed intake and survival were tested using an analysis of variance that accounted for the randomized block design of the experiment. Statistical analyses were conducted using S-PLUS and SAS (PROC-MIXED) statistical software. The number of surviving shrimp was used as a weighting factor for analysis of final shrimp weight, final biomass, growth rate and feed input. Differences between treatment effects were examined *a-posteriorly* using Fischer's protected '*t*' test (Snedecor & Cochran, 1989). Analyses for final shrimp weight, final

biomass, growth rate and feed input were repeated excluding data from tanks where survival was lower, i.e. < 80% and < 60% were arbitrarily chosen.

Prior to statistical analysis of water quality data, nutrient and fluorescence data were log-transformed. Water quality parameters were tested using an analysis of variance that accounted for the randomized block design of the experiment and the repeated nature of the measures. Statistical analyses were conducted using SAS (PROC-MIXED) statistical software.

Results

Diet analyses

The diets used in the three treatments in this experiment had a CP content (by analysis) of 318, 368, and 421 g kg^{-1} on a DM basis (Table 1). This was equivalent to 294, 344, and 394 g kg^{-1} on an 'as fed' basis, close to the formulated levels of 300, 350 and 400 g kg^{-1}. Total lipid content varied from 118 to 123 g kg^{-1} and gross energy ranged from 20 to 21 MJ kg^{-1}. Ash was more variable ranging from 108 to 153 g kg^{-1}. Water stability (DM retained) of all the diets over 2h was > 90%, decreasing to 91, 89 and 87% over 4 h for 300, 350 and 400 g kg^{-1} CP diets respectively, with little further change at 6 h.

Nitrogen leaching, expressed as a percentage of the original nitrogen content of the diets, was fastest for all diets over the first 2 h after which it began to plateau (Fig. 1). The % nitrogen lost from leaching increased with CP in the diet: 14.5, 17.5 and 20.6% nitrogen was lost from 300, 350 and 400 g kg^{-1} CP diets respectively over 12 h. This compares with 18% nitrogen lost from the commercial diet over the same period.

[Insert Figure 1]

Effects of treatments on shrimp

There was no significant difference in shrimp survival between treatments with mean survival (± s.e.) of 82.8 ± 5.93, 77.7 ± 5.93, and 72.1 ± 5.93% in the 300, 350 and 400 g kg^{-1} CP treatments respectively after 8 weeks (Table 2). CP level had no significant effect on final shrimp weight, final biomass or growth rate when all tanks

were included (Table 2). When the tanks with lower survival, i.e. two scenarios; less than 80%, and less than 60%, were removed from the analysis, there were significantly lower ($P < 0.05$) growth rates and final shrimp weights in the 300 g kg^{-1} CP treatment than for the higher CP treatments.

[Insert Table 2]

There was no significant difference in feed input between treatments irrespective of whether all tanks or only tanks with survival >60% were included (Table 2). In tanks with >80% survival there was significantly less feed input in the 300 g kg^{-1} CP treatment than the higher CP treatments. The moisture and protein content, and digestive gland lipid content of the shrimp were measured as indicators of nutritional condition (Table 3). There was no significant difference ($P > 0.05$) between treatments. There were also no consistent differences in visual health status of surviving shrimp between treatments or tanks at the end of the experiment.

The stable isotope, ^{15}N-nitrogen was added to the tanks as a method of determining the contribution of natural biota to shrimp nutrition in the different treatments. Previous studies have shown that natural biota were labelled with ^{15}N-nitrogen within 1 to 2 h (Burford, 2000). Our study showed that 10.9 ± 0.86, 10.2 ± 1.03 and $9.3 \pm 1.01\%$ of the ^{15}N-nitrogen added as ammonium to the 300, 350 and 400 g kg^{-1} CP treatment tanks respectively was retained by the shrimp over 2 d with no statistical differences between treatments.

[Insert Table 3]

Water and sediment quality

Mean water temperatures during the experiment ranged from 26.6°C at 0500 h to 29.4°C at 1400 h with no statistical differences between treatments (Table 4). There was also no statistical difference in pH at 0500 h (7.61, 7.59 and 7.67 in 300, 350 and 400 g kg^{-1} CP treatments respectively); at 1400 h, pH was statistically higher ($P < 0.05$) for the 400 g kg^{-1} CP treatment (8.08) than the 300 g kg^{-1} (7.93). Oxygen concentrations were statistically lower at 0500 h for the 400 g kg^{-1} CP treatment (4.63 mg L^{-1}) than the other two treatments (4.90 and 4.80 mg L^{-1} in the 300 and 350 g kg^{-1}

CP treatments respectively). The reverse was true at 1400 h where oxygen concentrations were statistically higher for the 400 g kg^{-1} CP treatment than for the other two. Temperature, pH and dissolved oxygen all changed significantly ($P < 0.005$) with time, but only pH showed a trend of decreasing in all treatments over the course of the experiment. Mean salinity was 32.4 mg L^{-1}.

[Insert Table 4]

Mean TN concentrations increased significantly ($P \leq 0.0001$) from 3.60 mg L^{-1} to 6.45 mg L^{-1} with increasing CP in the diets (Table 5). DON concentrations were significantly lower ($P \leq 0.01$) for the 300 g kg^{-1} CP treatment (1.44 mg L^{-1}) than for the other two treatments (1.87 mg L^{-1} for the 350 g kg^{-1} CP and 2.22 mg L^{-1} for the 400 g kg^{-1} CP treatments). Most of the dissolved inorganic nitrogen was TAN. There were no significant differences in TAN, fluorescence and oxides of nitrogen between treatments ($P > 0.01$) (Table 5). However, there was a trend of higher nutrient concentrations and fluorescence with higher CP treatments. Mean fluorescence units of 0.46, 0.82 and 0.95 for 300, 350 and 400 g kg^{-1} CP treatments respectively were equivalent to chlorophyll *a* concentrations of 88, 197 and 236 µg L^{-1} respectively (R^2 = 0.86). Nutrient concentrations and fluorescence increased significantly over time ($P \leq 0.0001$) across all treatments (Fig. 2). There was an interaction between time and treatment. TP and filterable reactive phosphate showed a trend of increasing with CP level (Table 5). Most of the TP was in particulate form.

[Insert Figure 2]

There were no statistical differences in TkN and TP concentrations in the sediment between treatments (Table 5). Mean TkN concentrations ranged from 1480 to 1760 mg kg^{-1} and TP ranged from 1000 to 1130 mg kg^{-1}. Concentrations of porewater TAN ranged from 19.46 to 21.89 mg N L^{-1} and there was no statistical difference between treatments.

[Insert Table 5]

A nitrogen budget was constructed for the three treatments to compare inputs and outputs to the tanks over 8 weeks (Table 6). Over 90% of the N input was feed in all treatments. Between 17 and 25% of the total N inputs was harvested as shrimp, 29 to 36% of the N was present in the discharge water and 18 to 27% was present as accumulated N in the sludge. In the 300 g kg^{-1} CP treatment, 81% of the N inputs were accounted for in shrimp, discharge water and sediment, compared with 75% for the 350 g kg^{-1} CP treatment and 71% for the 400 g kg^{-1} CP treatment.

[Insert Table 6]

Discussion

Our study has shown that there is scope to reduce the CP content of *P. monodon* feeds below 400 g kg^{-1} (on an 'as fed' basis) without negatively affecting growth. A CP content of 400 g kg^{-1} is typical of many commercial feeds for 3 to 15 g size *P. monodon* (D. Smith, unpubl. data). However, the reduction in growth rates of *P. monodon* fed the lowest CP diet (300 g kg^{-1} CP), when tanks with lower survival were removed from the analysis, suggests that natural biota were only minor contributors to the metabolizable protein and energy intake of the animals. Additionally, the ^{15}N-tracer study showed that natural feed contributed in a minor way to the nutrition of shrimp irrespective of treatment, with 10% of the ^{15}N-nitrogen that was present in the tank biota being incorporated into shrimp in 48 h.

In contrast, natural abundance stable isotopes have shown that natural biota contribute substantially (31 to 52% of nitrogen) to the nutrition of *Litopenaeus vannamei* in ponds and outdoor tanks stocked at 20 to 50 animals m^{-2} (Parker *et al*. 1989, 1991; Epp, *et al*. 2002). Reducing the CP level in *L. vannamei* diets from 40 to 20% in ponds did not reduce shrimp production (Teichert-Coddington & Rodríguez 1995; Hopkins *et al*. 1995), and farms growing *L. vannamei* intensively have fed diets with reduced CP content in recent years (McIntosh & Avnimelech 2001). The proportion of the nutritional requirements contributed by the natural biota may vary between shrimp species based on their feeding behaviour or protein requirements. This may explain why there seems to be more scope to reduce protein levels in diets for *L. vannamei* than for *P. monodon*.

Only 17 to 25% of nitrogen input to the tanks became shrimp biomass at the end of the 8 week experiment. This is consistent with studies in conventional pond systems that have shown that only 15 to 30% of the dietary nitrogen added to ponds is retained by the shrimp at harvest (Funge-Smith & Briggs 1998; Martin *et al.* 1998; Jackson *et al.* 2003). The remainder enters the pond system as particulate and dissolved nutrients. As the CP content of the feed increased, so did the proportion of input nitrogen that was discharged via routine water exchanges or during harvest, ranging from 29 to 36%. This was the result of increasing concentrations of TN and TP in the water column with CP content of the diet.

The largest proportion of nitrogen in the water column in all treatments was particulate N (50%), followed by DON (34 to 40%). Phytoplankton biomass, as measured by fluorescence, was a major component of particulate N. The phytoplankton community was relatively stable throughout the experiment as shown by the low variability between tanks.

This study has shown that a substantial proportion of nitrogen in the feed leached out; 10% over 4 h from the 300 g kg^{-1} CP diet, increasing to 16% in 4 h with the 400 g kg^{-1} CP diet. These rates are consistent with commercial feeds. A previous study has shown that much of the N leached from feeds is DON (Burford & Williams 2001), and in our experiment, DON concentrations in tank water were correlated with dietary CP content. Therefore the source of the DON in the water column was principally the feed.

Ammonium concentrations in the water column also increased with CP content of diets although the increase was not significant. Ammonium is produced from shrimp gill excretion, sediment remineralization processes, and microbial processes in the water column. In ponds, sediment remineralization of pond detritus is the dominant source of ammonium in the water column (Burford & Longmore 2001). However in our tank system, there was no difference in ammonium levels in the sediment porewater suggesting that sediment processes were not the cause of differences in ammonium concentrations in the water column. Microbial processes in the water column can also produce ammonium (Burford & Glibert 1999), however there was no systematic increase in particulate nitrogen or fluorescence with increasing protein

level.   The most likely explanation for the increases in ammonium level in the water was an increase in ammonium excretion by shrimp consuming higher protein feed.

This study showed that the variance in water quality between replicate tanks within a treatment can be minimized by standardizing the tank inlet water and sediment source, water exchange rates, and initial shrimp biomass. The main differences between tanks were, therefore, the treatment effects, i.e. protein levels in the feed and the changes in shrimp biomass over time, as well as changes in phytoplankton species dominance over time. The reduced within-treatment variability and high replication mean that differences between treatments became more apparent. In contrast, few pond studies have demonstrated statistical differences in water quality between treatments (Martinez-Cordova *et al*. 1998; Cowan *et al*. 1999). Many previous studies on relationships between feeds, management practices and water quality have had insufficient replication and, as a result, have obtained inconclusive results (Smart *et al*. 1998). Conducting controlled experiments in commercial ponds is logistically difficult with the competing demands of production and experimentation. This study has shown that tanks provide a reasonable alternative for statistically significant experiments, however validation of the results in ponds is still warranted.

In conclusion, this study suggests that there is scope to reduce the protein level in *P. monodon* diets from 400 to 300-350 g kg^{-1}. However, further reductions are likely to affect growth rates. Reducing protein levels in diets had a measurable effect on improving water quality but not sediment quality. There is, therefore, benefit in trialling lower protein feeds in commercial ponds to improve production efficiency and reduce nutrient waste.

Acknowledgements

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Figure Legend

Figure 1. Cumulative percentage nitrogen leached from 300, 350 and 400 g kg^{-1} CP diets, and a commercial diet (Ref) over 12 h.

Figure 2. Mean ($\pm$ s.e.) of nutrient concentrations (mg L^{-1}) (TN, TAN, DON, nitrite and nitrate) and fluorescence in the diet treatments over the eight week growth trial.

Table 1: Formulation and chemical composition of experimental diets fed to

P. monodon in the three treatments.

Ingredients	Diets		
	300 g kg^{-1} CP	350 g kg^{-1} CP	400 g kg^{-1} CP
	Formulation (% as used)		
Fishmeal (720 g kg^{-1} CP, Peru)	10.1	17.5	24.9
Crustacean meal (380 g kg^{-1} CP, Chile)	10.0	10.0	10.0
Soybean meal (520 g kg^{-1} CP, solvent extr)	15.0	15.0	15.0
Squid meal (720 g kg^{-1} CP, Japan)	5.0	5.0	5.0
Cholesterol (100%)	0.1	0.1	0.1
Soya lecithin (700 g kg^{-1} total lipid)	1.2	1.2	1.2
Squid oil	5.7	4.9	4.0
Wheat flour	35.9	32.4	28.7
Gluten (wheat)	6.0	6.0	6.0
Banox E†	0.02	0.02	0.02
Aquabind® binder, Dupont	3.0	3.0	3.0
Carophyll Pink*	0.05	0.05	0.05
Choline Chloride (50%)	0.02	0.02	0.02
Vitamin C**	0.1	0.1	0.1
Vitamin premix***	0.2	0.2	0.2
Diatomaceous earth	7.6	4.5	1.6
Total	100	100	100

Analyte	*Composition as analyzed*		
Dry Matter (g kg^{-1} as used)	926	936	936
CP (g kg^{-1} DM)	318	368	421
Total lipid (g kg^{-1} DM)	123	122	118
Gross energy (MJ kg^{-1} DM)	19.6	20.1	20.7
Ash (g kg^{-1} DM)	153	132	108
Water Stability (% DM, 2 h)	93	92	90
Water Stability (% DM, 4 h)	91	89	87
Water Stability (% DM, 6 h)	91	89	86

† Blend of antioxidants (Alltech Biotechnology Pty Ltd)

* Carophyll Pink (80 g kg^{-1} astaxanthin), Roche Vitamins Australia.

** Stay C, 150 g kg^{-1} L-Ascorbyl-2-polyphosphate, Argent Laboratories.

*** As recommended by Conklin (1997).

Table 2: Mean (± s.e.) of shrimp weights, biomass, growth, feed input, food conversion ratio (FCR) and survival in the three treatments over the 8 week experiment. Statistical differences are denoted by superscripts. Treatments were analyzed statistically including and excluding tanks with survival lower than 60%. ns denotes no statistical difference. *$P < 0.05$. Note: FCR refers to biomass of shrimp produced/feed input, not feed intake.

	Treatments		
Attribute	300 g kg^{-1} CP	350 g kg^{-1} CP	400 g kg^{-1} CP
All tanks			
Initial shrimp weight (g)^{ns}	3.1 (0.17)	3.1 (0.17)	3.1 (0.17)
Final shrimp weight (g)^{ns}	13.9 (0.53)	14.9 (0.54)	15.1 (0.54)
Final biomass (g tank^{-1})^{ns}	880 (79.2)	919 (81.0)	873 (82.8)
Growth rate (g week^{-1})^{ns}	1.34 (0.049)	1.48 (0.049)	1.50 (0.050)
Feed input (kg tank^{-1})^{ns}	1.8 (0.09)	2.0 (0.09)	2.1 (0.09)
FCR^{ns}	2.9 (0.58)	3.3 (0.59)	3.9 (0.62)
Survival (%)^{ns}	82.8 (5.93)	77.7 (5.93)	72.1(5.93)
Number of tanks	8	8	8
Excluding tanks with survival < 80%			
Final shrimp weight (g)*	13.9^a (0.0.36)	15.3^b (0.40)	16.0^b (0.42)
Growth rate (g week^{-1})*	1.35^a (0.037)	1.51^b (0.042)	1.61^b (0.045)
Feed input (kg tank^{-1})*	1.9^a (0.08)	2.1^b (0.10)	2.3^b (0.10)
Number of tanks	4	4	4
Excluding tanks with survival < 60%			
Final shrimp weight (g)*	13.9^a (0.55)	14.9^b (0.55)	15.4^b (0.56)
Growth rate (g week^{-1})*	1.35^a (0.048)	1.48^b (0.050)	1.53^b (0.050)
Feed input (kg tank^{-1})^{ns}	1.8 (0.09)	2.0 (0.09)	2.1 (0.09)
Number of tanks	7	7	6

Table 3: Mean composition of whole shrimp and digestive gland in three treatments at the end of the 8 week growth trial. There was no statistical difference between treatments.

	Treatment			
Parameter	300 g kg^{-1} CP	350 g kg^{-1} CP	400 g kg^{-1} CP	± sem
Moisture content (% wet weight)	73.3	72.4	72.6	0.52
CP content (% wet weight)	19.5	20.2	20.3	0.40
Digestive gland lipid (g animal^{-1})	0.23	0.25	0.20	0.031

Table 4: Mean (± s.e.) levels of physical conditions in eight tanks in each of three treatments (300, 350, 400 g kg^{-1} CP in the feed) at 0500 h and 1400 h each day over the 8 week experiment. Statistical differences are denoted by superscripts. ns denotes no significant difference. na denotes not analyzed. *$P < 0.05$.
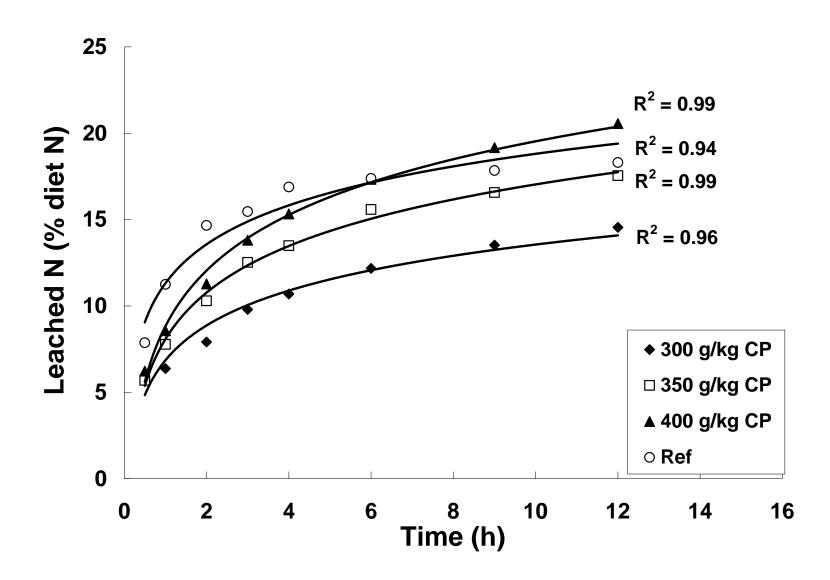
Parameter	Time	Treatments		
		300 g kg^{-1} CP	350 g kg^{-1} CP	400 g kg^{-1} CP
Temperature (°C)	0500 h^{ns}	26.6 (0.69)	26.6 (0.71)	26.6 (0.70)
	1400 h^{ns}	29.4 (0.82)	29.4 0.84)	29.4 (0.83)
pH	0500 h^{ns}	7.61 (0.095)	7.59 (0.113)	7.67 (0.113)
	1400 h	7.93 (0.071)^a	8.01 (0.088)^{ab}	8.08 (0.092)^b
Oxygen (mg L^{-1})	0500 h	4.90 (0.325)^a	4.80 (0.350)^a	4.63 (0.371)^b
	1400 h	6.96 (0.367)^a	7.36 (0.470)^a	7.63 (0.509)^b
Salinity (mg L^{-1})	1400 h	32.8 (1.75)^{na}	32.3 (2.43)^{na}	32.2 (2.66)^{na}

Table 5: Mean (± s.e.) of water column and sediment nutrients (mg L^{-1}, mg kg^{-1}), and fluorescence in eight tanks in each of three treatments (300, 350, 400 g kg^{-1} CP in the feed) over the 8 week experiment. Filt. reactive P = filterable reactive phosphate. Statistical differences are denoted by superscripts. Overall significant differences between means for the CP levels are indicated by; ***$P \leq 0.001$; **$P \leq 0.001$, $P \leq 0.01$; na = not analyzed. ns denotes no statistical difference, na denotes not analyzed statistically.

Parameter	Treatments		
	300 g kg^{-1} CP	350 g kg^{-1} CP	400 g kg^{-1} CP
Water column			
TN (mg L^{-1})***	3.60 (0.809)[a]	5.17 (1.251)[b]	6.45 (1.597)[c]
Oxides of nitrogen (mg L^{-1})[ns]	0.01 (0)	0.06 (0)	0.09 (0)
TAN (mg L^{-1})[ns]	0.35 (0.233)	0.60 (0.364)	0.97 (0.608)
DON (mg L^{-1})**	1.44 (0.237)[a]	1.87 (0.392)[b]	2.22 (0.477)[b]
TP (mg L^{-1})[na]	0.22 (0.046)	0.32 (0.060)	0.37 (0.074)
Filt. reactive P (mg L^{-1})[na]	0.006 (0.001)	0.013 (0.005)	0.018 (0.010)
Fluorescence[ns]	0.46 (0.124)	0.82 (0.279)	0.95 (0.314)
Sediment			
TkN (mg kg^{-1})[ns]	1760 (515)	1480 (276)	1500 (474)
TP (mg kg^{-1})[ns]	1040 (207)	1000 (154)	1130 (238)
Porewater TAN (mg L^{-1})[ns]	19.46 (2.933)	21.10 (2.935)	21.89 (4.716)

Table 6: N inputs (%) and outputs (% of inputs) for all tanks in the three treatments over the 8 week experiment.

	Treatments		
Parameter	300 g kg^{-1} CP	350 g kg^{-1} CP	400 g kg^{-1} CP
% of inputs			
Feed	91	93	94
Shrimp	6	5	4
Water	3	2	2
Total	**100**	**100**	**100**
Outputs as % of inputs			
Shrimp	25	21	17
Routine water exchange	11	13	14
Harvest water	18	20	22
Sludge	27	21	18
Total	**81**	**75**	**71**

Fig. 2