This is the peer reviewed version of the following article: Arnold, S., Smullen, R., Briggs, M., West, M. and Glencross, B. (2016), The combined effect of feed frequency and ration size of diets with and without microbial biomass on the growth and feed conversion of juvenile *Penaeus monodon*. *Aquacult Nutr*, 22: 1340–1347, which has been published in final form at https://doi.org/10.1111/anu.12338. This article may be used for non-commercial purposes in accordance With Wiley Terms and Conditions for self-archiving.

The combined effect of feed frequency and ration size on the growth and feed conversion of juvenile *Penaeus monodon*

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Keywords: Feed management, restricted ration, feed efficiency, satiation

Submitted to : Aquaculture (16th ISFNF Edition)

Abstract

Feed management strategies that maximise shrimp growth and optimise feed utilisation are critical to the cost effectiveness of production.. In this study, juvenile shrimp (~3 g) were cultured for six weeks in a laboratory based clear-water tank system. The experiment design was a three way factorial with two diets (Diet A – standard industry formulation, or Diet B – the same diet with 10% microbial biomass), two feed frequencies (twice or six times daily) and three rations (60%, 80% and 100% of satiation).. The results demonstrated clear growth benefits of feeding more than 2 times per day and feed efficiency benefits of a restricted ration. There was also a significant interaction between frequency and ration, which demonstrated that growth improved using 6 feeds compared with 2 feeds as ration amount decreased. The effects of frequency and ration were consistent for both diets; however, the addition of a microbial biomass provided significant growth improvements across all treatments. These outcomes define the gains produced by the combined effect of frequency and ration, and suggest a compromise between feed utilization and feeding effort for adoption in feed management strategies.

1. Introduction

Feed is the single largest cost to a shrimp farm and therefore feed management strategies that maximise shrimp growth but optimise feed utilization are critical to the cost effectiveness of the farm. In order to maximise productivity, it is important to know the optimal number of times to feed per day. It is also important to know how much feed to administer at any given feed event to maximise feed intake and minimise wastage. Therefore, another key component is the amount fed as a percentage of the estimated satiation level or biomass (ration).

There have been very few studies investigating optimal feed frequency for *Penaeus monodon*. In clear water systems, Josekutty and Jose (1996) fed 0.21g juvenile *P. monodon* 1, two, three and four times per day. The amount fed was 5% of the estimated biomass split across the different feed times. In that study, growth, survival and food consumption increased when feed offerings were increased from one to two and two to three times per day, but there was no difference between three and four times per day. In a green-water tank system, Smith et al. (2002) fed 5.5 g *Penaeus monodon* three, four, five and six times per day to satiation at each feed. They found no effect of feed frequency on shrimp growth or feed conversion over a 6 week period. In a more recent study, Hasan et al. (2012), reported improved growth and FCR when feeds were fed four or five times per day compared to three or six times per day in 0.5 ha ponds over 65 days culture. However, the true effect of feed frequency in that study was difficult to determine because of the different initial weights of the treatments (approximately 12 g for four and five feeds and 9 g for three and six feeds).

Variable results have also been reported for other penaeids. For *Penaeus vannamei*, improved growth has been reported for 0.24 g juveniles fed four or five times daily compared to only one, two or three times (Ye et al., 2005). Growth of 6.6 g animals was also improved when feed frequency increased from one to two and two to four times daily (Robertson et al., 1993). In contrast, no improvement in growth was reported for 0.19 to 0.6 g animals (Velasco et al., 1999), or 2.7 g *P. vannamei* juveniles (Carvalho and Nunes, 2006) when feed frequency was increased. For juvenile *Penaeus merguiensis*, Sedgwick (1979) reported improved growth and FCR when shrimp were fed four times daily in comparison with only one feed and suggested that further improvements might be achieved at even higher frequencies. For *Penaeus indicus*, improved growth and FCR has been reported for 1.56 g juveniles fed six and eight times daily compared with two and four times (Moradizadeh et al., 2011). Nair and Sridhar (1995) also reported improved growth for 0.13 g *P. indicus* fed four times daily compared to one, two or three times but no improvement in growth for either 1.5 g or 4.4 g juveniles.

While there is some evidence to suggest that higher feed frequencies, up to a certain point, can improve shrimp performance, there is little information on the effect of ration size in combination with different feed frequencies. Feed efficiency has been shown to improve by reducing the ration to 75% satiation for *P. monodon* (Glencross et al., 1999; Glencross et al., 2013) and *P. vannamei* (Venero et al., 2007), but at the expense of growth. However, when Sedgwick (1979) assessed the combined effect of four ration levels and two feed frequencies with juvenile *Penaeus merguiensis* (0.13 g), they observed an interaction between frequency and ration, which suggested that growth could be maintained at a restricted ration by increasing the feed frequency. Their results also demonstrated the feed efficiency benefits of restricted rations. Such feed efficiency benefits were also demonstrated in a study Nair and Sridhar (1995) for juvenile *P. indicus.* A basic understanding of the combined effect of feed frequency and ration size on growth and feed efficiency is required for *P. monodon* to assist the development of optimal feed management strategies.

Furthermore, as new diets and feed ingredients enter the market there is a need to reassess feeding strategies so that the nutritional benefits of such diets can be fully realised. The Commonwealth Scientific and Industrial Research Organisation (CSIRO) has developed a growth promoting microbial biomass based ingredient (Novacq™, CSIRO, Dutton Park, QLD, Australia) that, when included in shrimp diets, can increase growth in excess of 50% above that of a standard reference diet with the same nutritional specifications (Glencross et al., 2014). The inclusion of the microbial biomass in the diet has also led to significantly higher feed intake (Glencross et al., 2014) and therefore may require different feeding strategies for maximum realisation of its growth promoting benefits.

This study was designed to assess the combined effect of feed frequencies and ration size on the growth and feed efficiency of juvenile *P. monodon*. The study also aimed to investigate whether this new microbial biomass ingredient required different feeding strategies than those required for a specification consistent with current commercial diets used in Australia.

2. Methods

2.1 Experimental design

The experiment was designed to evaluate the effect of feed frequency and ration size on the growth, survival and feed conversion efficiency of juvenile black tiger shrimp, *Penaeus monodon*, when cultured for six weeks and fed either of two formulated diets. The first diet was formulated consistent with standard industry diets used in Australia, and the second was a similar formulation, but additionally it was supplemented with 10% microbial biomass (Novacq™). Within each diet treatment the following factorial array was applied to feed frequency and ration design:

- Two feed frequencies
  - Twice daily (0900h and 2100h)
  - Six times daily (0100h, 0500h, 0900h, 1300h, 1700h and 2100h)
- Three feed rations – 100%, 80% and 60% satiation.

The design had a total of 12 treatments with 5 replicates per treatment.

2.2 Experimental system and set up

One thousand shrimp of a wild-type genotype were collected from the grow-out pond of Truloff's Prawn Farm in Alberton, south east Queensland and transported to four indoor holding tanks (2,000L) at the Bribie Island Research Centre, Woorim, QLD. The shrimp were held in these tanks for 6 days and supplied with filtered seawater at a continuous rate of 5.0L min^{-1} with water temperature and salinity maintained at 29^{o}C and 38 g L^{-1}, respectively. The shrimp were fed twice daily on a commercial diet (Enhance™, Ridley Aqua-Feed Pty Ltd). Prior to stocking into the indoor experimental tanks, 40 shrimp were randomly selected and individually weighed to the nearest 0.01 g to estimate the mean and standard deviation. Eight shrimp were then stocked into each of the 60 x 100L experimental tanks based on being within ±1 standard deviation of the mean. The mean ± SEM initial weight across all tanks was 3.10 ± 0.02 g. Each of the experimental tanks was supplied with filtered seawater at a continuous rate of 0.6L min^{-1} and maintained at 29.10 ± 0.02^{o}C water temperature, 4.53 ± 0.02 mg L^{-1} dissolved oxygen, 38.7 ± 0.0 g L^{-1} salinity, and pH 8.2 ± 0.0.

2.3 Diet preparation

Two diets were used for the experiment (

Table 1); Diet A, formulated to be equivalent to a standard industry specification used in Australia and Diet B, the same diet but with 10% microbial biomass (supplied as Novacq™) included. Each diet was prepared by ensuring all ingredients were milled to <750 µm prior to mixing in an upright planetary mixer (Hobart, Sydney, NSW, Australia). Water was then added (approximately 30%) during the mixing to form a dough which was subsequently screw-pressed (Dolly, La Monferrina, Castell'Alfero, Italy) through a 2 mm die and cut to pellet lengths of about 6 mm. The pellets were then steamed for 3 min before being oven dried at 65^{o}C for 24 hrs. When not being used, all diets were stored at -20^{o}C.

2.4 Management

During the experiment the feed ration allocations were determined by feeding the 100% satiation treatments to marginal excess. Uneaten feed in these treatments was scored (counted) in each tank at 0800h, with the scoring used to estimate the amount of uneaten feed (number of pellets x average pellet weight) and to adjust the following days ration according to the feed intake score. The feed rates for the reduced ration treatments were then adjusted from the average feed amounts calculated for the 100% satiation treatments relative to each feed frequency (e.g. 80% and 60% at 2 feeds adjusted from 100% at 2 feeds, and 80% and 60% at 6 feeds adjusted from 100% at 6 feeds). All feed fed and uneaten was recorded to allow the estimation of total feed intake within each tank daily and over the experiment period.

Daily feed amounts were divided evenly between each ration. All feed rations were weighed individually to the nearest 0.1g and the amount of feed fed to each tank recorded. Feed rations that were required to be fed at 0100h, 0500h and 2100h were fed using a Fish Mate F14 automated fish feeder (Pet Mate, Surrey, England). All feed was loaded into the feeders at 1700h. The following morning, the automatic feeders were checked and any unfed rations recorded.

All uneaten feed and faeces were removed from the tanks daily by siphoning. The number of shrimp in each tank was recorded daily and moults were recorded and removed as soon as they were observed. Each tank was aerated with a single air diffuser and dissolved oxygen, pH and salinity were monitored 3 times weekly in each tank. Temperature was monitored 4 times weekly in each tank. Flow rates were checked and adjusted as required to maintain optimal water conditions.

After three and six weeks culture the shrimp were collected from each tank, blot dried on a cloth towel and individually weighed to a minimum of 0.01 g accuracy. The number of shrimp in each tank was recorded. The mean shrimp weight for each treatment at each assessment point was calculated from the mean tank weights, which were used as the replicate (n=5). Survival was calculated as the percentage of remaining shrimp in each tank from the number stocked. Feed conversion ratio (FCR) was calculated based on the cumulative feed intake (on an as fed basis) within each tank divided by the cumulative weight gain within each tank.

2.5 Chemical analysis

Diets and whole shrimp samples were analyzed for dry matter, ash, protein, total lipids and carbohydrates. Diet samples were also analyzed for gross energy content. Dry matter of the samples was calculated by gravimetric analysis of a milled sample following oven drying at 105^{o}C for 6 h. Protein levels were calculated from the determination of total nitrogen (N) by Elemental analyzer, based on N x 6.25. Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a muffle furnace at 550^{o}C for 12 h. The lipid content of the diets was determined gravimetrically following extraction of the lipids using the chloroform:methanol (2:1) method. Carbohydrates were estimated based on dry matter content of the feed minus the lipid, ash and protein contents. Gross energy was determined by ballistic bomb calorimetry. All methods were consistent with those recommended by AOAC (2005).

2.6 Statistical analysis

All statistical analyses were performed using R software (R Development Core Team, 2009). The growth, survival and FCR were analyzed by three-way ANOVA. The statistical model included diet, frequency and ration as the main effects and interaction terms. Where significant interactions were found, pair-wise comparisons were performed separately within each level of main effects using a Tukey’s test. Curve fittings of relationships were undertaken using the data analysis tools and graphics elements of Microsoft Excel (Microsoft Australia, North Ryde, NSW, Australia).

3. Results

3.1 Shrimp performance

After three weeks culture the mean survival of shrimp was high (97.3 ± 0.6%) and not significantly different between treatments (*P*>0.05) (

Table 2). The mean shrimp weight for each treatment ranged between 5.63 ± 0.06 g and 7.82 ± 0.36 g. The mean growth rate ranged between 0.81 ± 0.02 g shrimp^{-1} week^{-1} and 1.61 ± 0.11 g shrimp^{-1} week^{-1}.

After three weeks culture there was a significant main effect for diet, frequency and ration (P<0.001) but no significant interactions (P>0.05) (

Table 3). Shrimp growth rate (g shrimp^{-1} week^{-1}) was significantly greater (P<0.001) when fed Diet B (1.34 ± 0.04) compared to Diet A (1.09 ± 0.03)(

Table 4). Shrimp growth rate was significantly greater ($P<0.05$) when ration increased from 60% (1.05 ± 0.04) to 80% (1.25 ± 0.05) to 100% satiation (1.36 ± 0.05). Shrimp growth rate was also significantly greater ($P<0.001$) when the feed frequency was increased from 2 feeds (1.12 ± 0.04) to 6 feeds (1.32 ± 0.04) per day (

Table 4).

After six weeks culture the average survival of shrimp was high (95.4 ± 0.5%) and not significantly different between treatments ($P>0.05$) (

Table 5). The mean shrimp weight for each treatment ranged between 8.31 ± 0.11 g and 13.24 ± 0.72 g. The mean growth rate ranged between 0.85 ± 0.02 g shrimp^{-1} week^{-1} and 1.71 ± 0.12 g shrimp^{-1} week^{-1}.

There was a significant ($P<0.001$) main effect for diet, frequency and ration and a significant ($P<0.05$) frequency x ration interaction (

Table 6). When averaged across all feed frequency and ration treatments, shrimp growth rate (g $shrimp^{-1}$ $week^{-1}$) was significantly greater ($P<0.001$) when fed Diet B (1.49 ± 0.05) compared to Diet A (1.18 ± 0.03)(

Table 7), demonstrating that the addition of the microbial biomass (Novacq™) to the diet provided an overall 26% increase in growth.

When fed two times per day, shrimp growth rate was significantly greater (*P*<0.001) when ration increased from 60% (0.99 ± 0.05) to 80% satiation (1.31 ± 0.06) but there was no significant (*P*>0.05) improvement in growth when ration was increased from 80% to 100% satiation (1.46 ± 0.07)(

Table 7). This trend was also the same when shrimp were fed six times per day, with growth rates of 1.26 ± 0.03 (60%), 1.49 ± 0.07 (80%) and 1.49 ± 0.10 (100%).

Shrimp growth rate was also significantly greater (*P*<0.05) when the feed frequency was increased from two feeds to six feeds at rations of 60% (0.99 ± 0.05 and 1.26 ± 0.03) and 80% satiation (1.31 ± 0.06 and 1.49 ± 0.07), but not 100% satiation (1.46 ± 0.07 and 1.49 ± 0.10)(

Table 7). This interaction between feed frequency and ration is more clearly illustrated in

Figure 1. This plot shows that as ration increased from 60% up to 100% satiation, the growth benefits of six feeds compared to two feeds was reduced.

3.2 Feed utilization

After six weeks culture, the FCR averaged 1.32 ± 0.03 across all treatments (

Table 9). There was no significant ($P>0.05$) effect of diet on FCR, but there was a significant ($P<0.001$) frequency and ration effect (

Table 8). FCR decreased from 1.42 ± 0.04 when fed two feeds a day to 1.22 ± 0.04 when fed six feeds. There was also a significant decrease (*P*<0.001) in FCR when the ration was reduced from 100% satiation (1.52 ± 0.05) to 80% satiation (1.26 ± 0.04), but there was no significant difference (*P*>0.05) between a ration of 80% and 60% satiation (1.18 ± 0.04).

3.3 Composition analysis

There was no significant main effect (*P*>0.05) for diet or frequency on the composition of the shrimp as determined by 3-way ANOVA (

Table 10). However, there was a significant effect (*P*<0.001) of ration on ash composition. Shrimp fed a ration of 80% satiation had a significantly lower (*P*<0.01) ash composition (3.0 ± 0.13%) than shrimp fed a ration of 60% satiation (3.3 ± 0.09%).

4. Discussion

The present study found significant effects of feed frequency and ration on the growth and feed utilization of *Penaeus monodon*. Feeding shrimp six times per day relative to twice a day significantly improved shrimp growth and FCR when the ration was 80% or 60% of satiation. However, when the ration was 100% satiation, FCR was improved by feeding six times, but there was no difference in growth compared to feeding twice daily. The effect of frequency and ration was consistent for each of the two diets tested. The inclusion of the microbial biomass into the diet resulted in significantly enhanced shrimp growth in all treatments compared to the diet without. The main outcomes of the study demonstrate the significant influence that feed frequency and ration has on production efficiency and highlight the importance of understanding this relationship when developing feed management strategies.

4.1 Effects on growth performance

When only considering the treatments fed to 100% satiation, the growth of shrimp fed the commercially formulated diet in this study (range from 1.26 g to 1.32 g shrimp^{-1} week^{-1}) was well above that which is typical for this species in a clear-water tank system over this period, previously recorded to be 0.91 g shrimp^{-1} week^{-1} (Smith et al., 2007), or range from 0.87 g to 0.91 g shrimp^{-1} week^{-1} (Glencross et al., 2014). The growth rate of shrimp fed the diet with the microbial biomass (1.61 g to 1.71 g shrimp^{-1} week^{-1}) was also greater than that achieved with previous administration of this ingredient to animals of a similar size in the same system, which was 1.30 g week^{-1} (Glencross et al., 2014) and greater than that modelled for pond cultured *P. monodon* of the same size grown under the same temperature regimen, which was 1.32 g week^{-1} (Jackson and Wang, 1998). The improved growth may be attributed to the addition of krill meal to both diets and the use of stock that may have been genetically superior. Glencross et al., (2013) achieved growth rates of 2.56 g shrimp^{-1} week^{-1} in the same tank system with diets containing 10% krill meal and 10% Novacq when fed to eighth generation selected stocks.

There have been few studies investigating the effect of feed frequency on *P. monodon* with the results being quite varied. Smith et al., (2002) reported no difference in growth or feed efficiency of 5.5 g animals at higher feed frequencies whereas Josekutty and Jose (1996) reported improved growth and feed efficiency of smaller 0.21 g animals. The effect of feed frequency on growth of other penaeids is also quite varied. In enclosures within ponds, an improvement in growth as feed frequency increased was demonstrated for 6.6 g *P. vannamei* (Robertson et al., 1993) but not smaller 2.7 g *P. vannamei* (Carvalho and Nunes, 2006). Ye et al., 2005 demonstrated improved growth for 0.24 g juveniles in 2 t tanks whereas as Velasco et al., 1999 found no improvement in growth of 0.19 g to 0.6 g juveniles under laboratory conditions. Improved growth and FCR from higher feed frequencies was reported for juvenile *Penaeus merguiensis* (Sedgwick, 1979) and *Penaeus indicus* (Moradizadeh et al., 2011). Nair and Sridhar (1995) also reported improved growth for 0.13 g juvenile *P. indicus* but no improvement in growth for 1.5 g or 4.4 g juveniles.

The variation observed among previous studies could likely attributed to the use of different animal sizes, systems (clear water compared to green water culture) and diets. In addition, the present study demonstrated that ration can have a significant influence on the effect of feed frequency, and therefore how the specific ration used was calculated in previous studies could have also contributed to the varied results. Considering shrimp were fed to satiety in the study by Smith et al. (2002) the results are comparable to our results at 100% satiation whereby no difference was observed between two and six feeds. Smith et al. (2002) also reported FCR's of 2.0 and suggested that feed may have been offered in excess requirements. This is consistent with Carvalho and Nunes (2006) who reported FCR's of 1.98 to 3.49 and also demonstrated no effect of feed frequency. In that study, feed ration was adjusted weekly based on estimated biomass and considering the low survival achieved, feed may have been provided in excess. The authors therefore clearly stated that higher feed frequencies were not advantageous under their feeding protocol. These results suggest that growth can be maintained with fewer feed events, as long as feed is supplied in excess requirements. The trade off is that feed efficiency is compromised.

4.2 Effects on feed conversion ratio

Improved feed efficiency (reducing FCR) has been demonstrated by restricting the feed ration for *P. monodon* (Glencross et al., 1999; 2013) and *P. vannamei* (Venero et al., 2007) but at the expense of growth. A study by Glencross et al. (1999) on the evaluation of a purified research diet for *P. monodon* observed a significant improvement in FCR (1.58 cf. 2.08), with 25% feed restriction, but notably no significant effect on growth (0.60 cf. 0.63 g/wk) was observed. There was also significant variation among different diets (purified, commercial and practical) in that study with FCR's ranging from 2.08 to 3.40. Another study reported no significant effect on final weight by restricting the ration by 25% and even 50% of apparent satiation when fed to 9.1 g *P. vannamei* cultured for 4 weeks (Nunes et al., 2006). However, in the study of Nunes et al (2006) the initial weight of shrimp fed to 100% satiation was smaller than the restricted ration treatments and therefore the results difficult to interpret. The present study demonstrated that the feed efficiency benefits of restricted ration could be realised with minimal or no impact on growth by increasing the feed frequency. A comparable outcome was observed by Sedgwick (1979), who assessed the combined effect of four ration levels and two feed frequencies with juvenile *Penaeus merguiensis* (0.13 g). The study of Sedwick (1979) also demonstrated the combined benefit of higher feed frequency and restricted ration. The final weight and FCR of shrimp fed four times daily at 9.8% body weight was 1.29 g and 1.35 respectively. In comparison, shrimp fed once daily at 14.0% body weight grew to 1.17 g with an FCR of 2.41.

Our findings suggest that a compromise between feed utilization and feeding effort under the conditions tested. Notably, similar growth rates were achieved in the present study by feeding six times daily at 80% satiation compared with two times daily at 100%, and this was consistent for both diets. However, the former strategy also demonstrated improved FCR for both diets (26% for Diet A and 28% for Diet B). This outcome was similar to that reported by Sedgwick (1979) where growth and feed efficiency of juvenile *P. merguiensis* improved when fed four times daily on a lower ration compared to only being fed once daily. In adopting any of these strategies, a farmer would have to consider the balance between costs of extra labour to increase feed frequency against the reduced feed costs from improved FCR.

4.3 Conclusion

This study has defined some important boundaries of the combined effects of variables of feed frequency and ration allocation on the growth and feed utilization of 3.0 to 13.0 g *P. monodon*. Our results showed that the optimal ration was between 80% and 100%, but reducing ration below 80% satiation is likely to compromise growth. A high feed frequency of six times a day was beneficial under restricted ration but further improvements in efficiency may be possible by yet more frequent feeding, especially under restricted ration regimen. Further research is needed to assess a narrower range of ration levels (e.g. 100%, 90%, 80%) across a broader range of feed frequencies (2, 4 or 8 times a day) followed by confirmation or modification of the findings in green water tanks and/or commercial ponds. Furthermore, additional effort could be placed on exploring the effects of variation in initial shrimp size and also the effects of hypoxia on the responses to such feed management constraints.

Acknowledgements

This work was supported by funds from both Ridley Aquafeeds Pty Ltd and CSIRO. We acknowledge the support of Natalie Habilay and Kinam Salee in assisting with the maintenance of the animal trial.

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Table 1 Formulations and composition of experimental diets

	Diet A	Diet B
Formulation (%)		
Fish Meal (Anchovetta, 68% protein)[a]	40.00	40.00
Krill Meal (Qrill™)[a]	10.00	10.00
Gluten (wheat)[b]	7.00	7.00
Wheat Flour[b]	40.03	30.03
Lecithin[a]	1.00	1.00
Fish Oil[a]	1.50	1.50
Microbial biomass (Novacq™)[c]	-	10.00
Astaxanthin (Carophyll Pink™)[d]	0.05	0.05
Cholesterol[e]	0.10	0.10
Antioxidant (Banox E)[f]	0.02	0.02
Vitamin C (Stay C™)[d]	0.10	0.10
Vitamin premix[g]	0.20	0.20
Composition (%DM)		
Dry matter (% as is)	97.01	96.89
Protein	47.28	47.16
Lipid	9.77	7.80
Ash	8.35	14.71
Carbohydrate	34.60	30.33
Gross energy (MJ/kg DM)	20.95	19.33

[a] Ridley Aqua-Feed, Narangba, Qld, Australia.
[b] Manildra, Auburn, NSW, Australia.
[c] CSIRO, Bribie Island, Qld, Australia.
[d] DSM, Wagga Wagga, NSW, Australia.
[e] MP Bio, Aurora, OH, USA.
[f] BEC Feed Solutions, Carole Park, Qld, Australia.
[g] Rabar, Beaudesert, Qld, Australia.

Table 2 Mean (±SEM) growth and survival for *P. monodon* after three weeks culture fed two diets at two frequencies and three rations

Diet	A						B					
Frequency	2			6			2			6		
Ration	60	80	100	60	80	100	60	80	100	60	80	100
Initial weight (g shrimp^{-1})	3.19 (0.05)	3.09 (0.05)	3.11 (0.07)	3.13 (0.03)	3.06 0.07)	3.07 (0.06)	3.08 (0.05)	3.04 (0.03)	3.18 (0.04)	3.10 (0.02)	3.21 (0.04)	2.99 (0.07)
3 week weight (g shrimp^{-1})	5.62 (0.06)	6.13 (0.11)	6.74 (0.25)	6.40 (0.07)	6.68 (0.14)	6.79 (0.21)	6.16 (0.19)	6.87 (0.11)	7.34 (0.28)	6.93 (0.12)	7.67 (0.25)	7.82 (0.36)
Weight gain (g shrimp^{-1})	2.43 (0.06)	3.05 (0.12)	3.63 (0.22)	3.27 (0.06)	3.62 (0.08)	3.72 (0.18)	3.08 (0.14)	3.83 (0.10)	4.17 (0.25)	3.83 (0.10)	4.47 (0.26)	4.83 (0.34)
Growth rate (g shrimp^{-1} week^{-1})	0.81 (0.02)	1.02 (0.04)	1.21 (0.07)	1.09 (0.02)	1.21 (0.03)	1.24 (0.06)	1.03 (0.05)	1.28 (0.03)	1.39 (0.08)	1.28 (0.03)	1.49 (0.09)	1.61 (0.11)
Survival (%)	100.0 (0.0)	97.5 (2.5)	92.5 (5.0)	95.0 (3.1)	95.0 (3.1)	100.0 (0.0)	97.5 (2.5)	97.5 (2.5)	97.5 (2.5)	97.5 (2.5)	97.5 (2.5)	100.0 (0.0)

Table 3 Three way analysis of variance of the diet x frequency x ration effects on the growth rate of *P. monodon* after three weeks culture

	df	Sum Sq.	Mean Sq.	F Value	Pr (>F)
Diet	1	0.9321	0.9321	50.881	4.60E-09***
Frequency	1	0.5816	0.5816	31.75	9.02E-07***
Ration	2	0.9902	0.4951	27.025	1.37E-08***
Diet*Frequency	1	0.0137	0.0137	0.746	0.392
Diet*Ration	2	0.0172	0.0086	0.47	0.628
Frequency*Ration	2	0.0493	0.0247	1.347	0.270
Diet*Frequency*Ration	2	0.034	0.017	0.927	0.403
Residuals	48	0.8793	0.0183		

*Significant at $P<0.05$.

**Significant at $P<0.01$.

***Significant at $P<0.001$.

Table 4 Mean (±SEM) shrimp growth rate (g shrimp^{-1} week^{-1}) after three weeks culture fed two different diets at two feed frequencies and three rations

		Ration			
	Frequency	100%	80%	60%	Frequency mean
Diet A	2 Feeds	1.21 (0.07)	1.02 (0.04)	0.81 (0.02)	**1.01 (0.05)**
	6 Feeds	1.24 (0.06)	1.21 (0.03)	1.09 (0.02)	**1.18 (0.03)**
	Ration mean	**1.22 (0.04)**	**1.11 (0.04)**	**0.95 (0.05)**	**1.09 (0.03)**$^{\mathrm{B}}$
Diet B	2 Feeds	1.39 (0.08)	1.28 (0.03)	1.03 (0.05)	**1.23 (0.05)**
	6 Feeds	1.61 (0.11)	1.49 (0.09)	1.28 (0.03)	**1.46 (0.06)**
	Ration mean	**1.50 (0.08)**	**1.38 (0.06)**	**1.15 (0.05)**	**1.34 (0.04)**$^{\mathrm{A}}$
Combined	2 Feeds	1.30 (0.06)	1.15 (0.05)	0.92 (0.04)	**1.12 (0.04)**$^{\mathrm{b}}$
	6 Feeds	1.42 (0.09)	1.35 (0.06)	1.18 (0.04)	**1.32 (0.04)**$^{\mathrm{a}}$
	Ration mean	**1.36 (0.05)**$^{\underline{\mathrm{a}}}$	**1.25 (0.05)**$^{\underline{\mathrm{b}}}$	**1.05 (0.04)**$^{\underline{\mathrm{c}}}$	**1.22 (0.03)**

Values for each diet-frequency-ration combination are means (±SEM) of 5 replicate tanks.

Diet means with different superscripts (upper case) are significantly different (P<0.05).

Frequency means with different superscripts (lower case) are significantly different (P<0.05).

Ration means with different superscripts (underlined lower case) are significantly different (P<0.05).

Table 5 Mean (±SEM) growth and survival for *P. monodon* after six weeks culture fed two diets at two frequencies and three rations

Diet	A						B					
Frequency	2			6			2			6		
Ration	60	80	100	60	80	100	60	80	100	60	80	100
Initial weight (g shrimp^{-1})	3.19 (0.05)	3.09 (0.05)	3.11 (0.07)	3.13 (0.03)	3.06 0.07)	3.07 (0.06)	3.08 (0.05)	3.04 (0.03)	3.18 (0.04)	3.10 (0.02)	3.21 (0.04)	2.99 (0.07)
6 week weight (g shrimp^{-1})	8.31 (0.11)	9.91 (0.24)	11.01 (0.36)	10.23 (0.21)	10.87 (0.12)	10.64 (0.43)	9.89 (0.36)	11.88 (0.23)	12.85 (0.48)	11.07 (0.18)	13.23 (0.50)	13.24 (0.72)
Weight gain (g shrimp^{-1})	5.12 (0.11)	6.82 (0.26)	7.90 (0.35)	7.10 (0.19)	7.81 (0.12)	7.57 (0.40)	6.81 (0.33)	8.84 0.24)	9.67 (0.46)	7.96 (0.17)	10.03 (0.50)	10.25 (0.70)
Growth rate (g shrimp^{-1} week^{-1})	0.85 (0.02)	1.14 (0.04)	1.32 (0.06)	1.18 (0.03)	1.30 (0.02)	1.26 (0.07)	1.14 (0.05)	1.47 (0.04)	1.61 (0.08)	1.33 (0.03)	1.67 (0.08)	1.71 (0.12)
FCR (feed fed/gain)	1.34 (0.08)	1.28 (0.04)	1.62 (0.16)	0.98 (0.04)	1.20 (0.05)	1.44 (0.04)	1.30 (0.05)	1.44 (0.09)	1.57 (0.07)	1.10 (0.04)	1.13 (0.03)	1.45 (0.08)
Survival (%)	95.0 (3.06)	97.5 (2.50)	90.0 (7.29)	95.0 (3.06)	95.0 (3.06)	100.0 (0.0)	97.5 (2.50)	92.5 (5.0)	95.0 (3.06)	95.0 (3.06)	97.5 (2.5)	95.0 (5.0)

Table 6 Three way analysis of variance of the diet x frequency x ration effects on the growth rate of *P. monodon* after six weeks culture

	df	Sum Sq.	Mean Sq.	F Value	Pr (>F)
Diet	1	1.4634	1.4634	81.422	6.55E-12***
Frequency	1	0.3559	0.3559	19.802	5.10E-05***
Ration	2	1.3451	0.6725	37.419	1.61E-10***
Diet*Frequency	1	0.0009	0.0009	0.053	0.8194
Diet*Ration	2	0.0755	0.0378	2.101	0.1335
Frequency*Ration	2	0.1495	0.0747	4.158	0.0216*
Diet*Frequency*Ration	2	0.0532	0.0266	1.481	0.2376
Residuals	48	0.8627	0.018		

*Significant at $P<0.05$.

**Significant at $P<0.01$.

***Significant at $P<0.001$.

Table 7 Mean (±SEM) shrimp growth rate (g shrimp^{-1} week^{-1}) after six weeks culture fed two different diets at two feed frequencies and three rations

		Ration			
	Frequency	100%	80%	60%	Frequency mean
Diet A	2 Feeds	1.32 (0.06)	1.14 (0.04)	0.85 (0.02)	**1.10 (0.06)**
	6 Feeds	1.26 (0.07)	1.30 (0.02)	1.18 (0.03)	**1.25 (0.03)**
	Ration mean	**1.29 (0.04)**	**1.22 (0.04)**	**1.02 (0.06)**	**1.18 (0.03)**^{B}
Diet B	2 Feeds	1.61 (0.08)	1.47 (0.04)	1.14 (0.05)	**1.41 (0.06)**
	6 Feeds	1.71 (0.12)	1.67 (0.08)	1.33 (0.03)	**1.57 (0.06)**
	Ration mean	**1.66 (0.07)**	**1.57 (0.05)**	**1.23 (0.04)**	**1.49 (0.05)**^{A}
Combined	2 Feeds	1.46 (0.07)^{A}	1.31 (0.06)^{Ab}	0.99 (0.05)$^{B\underline{b}}$	**1.25 (0.05)**
	6 Feeds	1.49 (0.10)$^{\underline{A}}$	1.49 (0.07)$^{\underline{A}a}$	1.26 (0.03)$^{\underline{B}\underline{a}}$	**1.41 (0.05)**
	Ration mean	**1.47 (0.06)**	**1.40 (0.05)**	**1.13 (0.04)**	**1.33 (0.03)**

Values for each diet-frequency-ration combination are means (±SEM) of 5 replicate tanks.

Diet means with different superscripts (upper case) are significantly different (*P*<0.05).

There was a significant main effect of both frequency and ration and a significant frequency x ration interaction. Therefore significant differences between ration treatments within each frequency treatment, and frequency treatments within each ration treatment are indicated.

Ration means (within 2 feeds) with different superscripts (upper case) are significantly different (*P*<0.05).

Ration means (within 6 feeds) with different superscripts (underlined upper case) are significantly different (*P*<0.05).

Feed frequency means (within 80% ration) with different superscripts (lower case) are significantly different (*P*<0.05).

Feed frequency means (within 60% ration) with different superscripts (underlined lower case) are significantly different (*P*<0.05).

Table 8 Three way analysis of variance of the diet x frequency x ration effects on the FCR of *P. monodon* after six weeks culture

	df	Sum Sq.	Mean Sq.	F Value	Pr (>F)
Diet	1	0.0066	0.0066	0.247	0.621
Frequency	1	0.6370	0.6370	23.734	1.25E-05***
Ration	2	1.2757	1.2757	23.766	6.70E-08***
Diet*Frequency	1	0.0000	0.0000	0.000	0.994
Diet*Ration	2	0.0132	0.0066	0.246	0.783
Frequency*Ration	2	0.0370	0.0185	0.690	0.506
Diet*Frequency*Ration	2	0.1006	0.0503	1.874	0.165
Residuals	48	1.2883	0.0268		

*Significant at $P<0.05$.

**Significant at $P<0.01$.

***Significant at $P<0.001$.

Table 9 Mean (±SEM) after six weeks culture of *P. monodon* fed two different diets at two feed frequencies and three rations

		Ration			
	Frequency	100%	80%	60%	Frequency mean
Diet A	2 Feeds	1.62 (0.16)	1.28 (0.04)	1.34 (0.08)	**1.41 (0.07)**
	6 Feeds	1.44 (0.04)	1.20 (0.05)	0.98 (0.04)	**1.21 (0.06)**
	Ration mean	**1.53 (0.08)**	**1.24 (0.03)**	**1.16 (0.07)**	**1.31 (0.05)**
Diet B	2 Feeds	1.57 (0.07)	1.44 (0.09)	1.30 (0.05)	**1.43 (0.05)**
	6 Feeds	1.45 (0.08)	1.13 (0.03)	1.10 (0.04)	**1.23 (0.05)**
	Ration mean	**1.51 (0.05)**	**1.28 (0.07)**	**1.20 (0.04)**	**1.33 (0.04)**
Combined	2 Feeds	1.60 (0.08)	1.36 (0.05)	1.32 (0.05)	**1.42 (0.04)**^{a}
	6 Feeds	1.45 (0.04)	1.16 (0.03)	1.04 (0.03)	**1.22 (0.04)**^{b}
	Ration mean	**1.52 (0.05)**$^{\underline{a}}$	**1.26 (0.04)**$^{\underline{b}}$	**1.18 (0.04)**$^{\underline{b}}$	**1.32 (0.03)**

Values for each diet-frequency-ration combination are means (±SEM) of 5 replicate tanks.

Frequency means with different superscripts (lower case) are significantly different ($P<0.05$).

Ration means with different superscripts (underlined lower case) are significantly different ($P<0.05$).

Table 10 Composition of *P. monodon* after six weeks culture fed two different diets at two feed frequencies and three rations

Diet	A						B					
Frequency	2			6			2			6		
Ration	60	80	100	60	80	100	60	80	100	60	80	100
Dry matter	28.3	24.7	27.3	25.2	24.9	28.2	28.2	27.1	28.4	28.3	26.9	28.1
(%)	(0.8)	(2.9)	(1.1)	(2.0)	(1.7)	(1.1)	(0.5)	(1.5)	(0.6)	(0.7)	(1.1)	(0.8)
Protein (%)	20.4	17.5	19.1	17.6	17.4	19.6	20.0	19.2	20.0	20.2	19.0	19.2
	(0.6)	(2.0)	(0.7)	(1.5)	(1.3)	(0.8)	(0.5)	(1.0)	(0.7)	(0.4)	(1.1)	(0.5)
Lipid (%)	1.8	1.9	2.4	2.1	2.2	2.2	2.0	2.4	2.2	2.1	2.1	2.4
	(0.1)	(0.3)	(0.2)	(0.3)	(0.2)	(0.1)	(0.1)	(0.3)	(0.2)	(0.2)	(0.1)	(0.2)
Ash (%)	3.8	2.9	3.2	3.3	2.9	3.4	3.6	3.3	3.2	3.4	2.8	3.5
	(0.2)	(0.4)	(0.2)	(0.2)	(0.2)	(0.2)	(0.1)	(0.2)	(0.1)	(0.2)	(0.2)	(0.2)

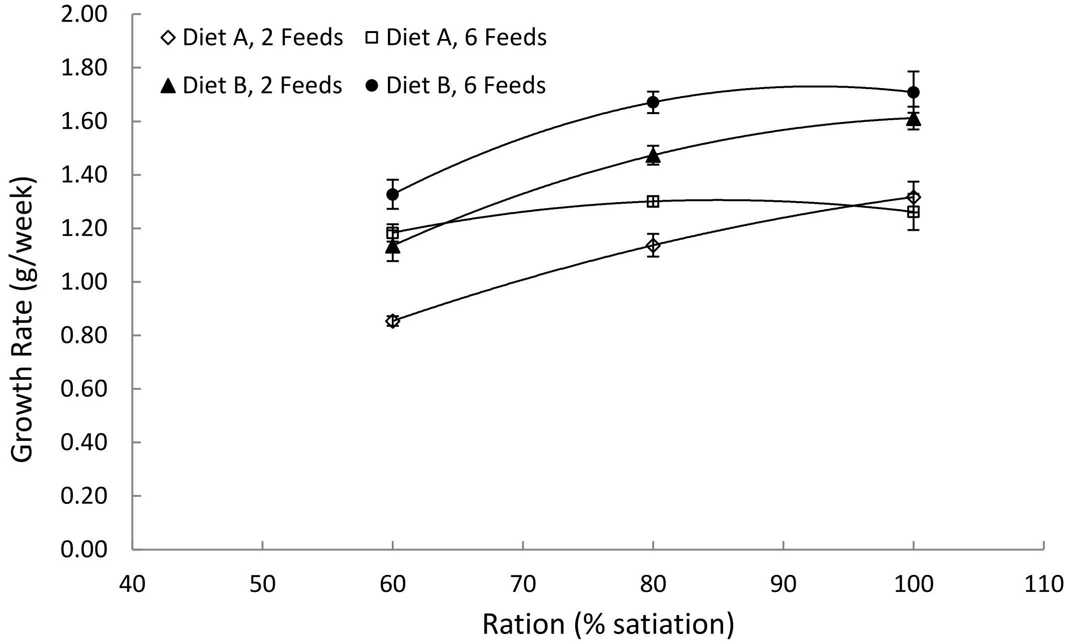

Figure 1 *P. monodon* growth rate after six weeks culture when fed two diets at two frequencies and three rations. Over the full range of rations, growth rates for shrimp from the different diet x feed frequency treatments were defined by the equations $y = -0.0001x^2 + 0.0321x - 0.6104$ (Diet A, 2 Feeds), $y = -0.0002x^2 + 0.0333x - 0.1089$ (Diet A, 6 Feeds), $y = -0.0002x^2 + 0.0517x - 1.0722$ (Diet B, 2 Feeds) and $y = -0.0004x^2 + 0.0708x - 1.5439$ (Diet B, 6 Feeds).