Introduction

The shrimp farming industry is big business especially in many tropical and sub-tropical countries (Shishehchian, 1999). *Penaeus monodon* is one of the most widely cultured species involved in this industry. The many costs involved in this industry as well as creation and maintenance of farming environment centre around the costs of formulating feed and labour associated with feeding. Optimising the feeding strategy is of great importance to intensive shrimp pond management (Smith, 2002) not just for cost reasons either. The shrimp farming industry is also facing increasing pressure to lower its environmental impact; the major concern in this area is the quality of water and nutrients from shrimp farms discharged into coastal waters. (Smith, 2002)

The technique for the commercial larval culture system of *P. monodon* was first developed in Taiwan in 1968 (Liao, 1996). Commercial production of formulated feeds started in 1977 (Liao, 1996) and a variety of studies have been done since then on formulated artificial diets for shrimp. Kurmaly etc al (1989) draws reference to the fact that a lot of successful culture techniques still depend on complex chains of live foods, which are difficult to maintain, and are of variable quality. Also feedstuffs such as shrimp meal, fish oil etc that are commonly used in mixed natural diets are high in acids which are susceptible to oxidation and degradation of water (Bautista, 1997). Artificial diet composition is therefore very important.

For farming of shrimp or prawns to be economic and effective an effective diet is essential. Studies on the diet for *Penaeus monodon* include research into vitamins and mineral supplements and their effect on growth and survival (Trino, 1995) essential vitamins in diets (Reddy, 1999) and even studies into detection and attraction of food by prawns to ensure they will be attracted to and eat feed that is fed to them, (Hindley, 1975). Recently studies have even been carried out on whether soybean can be used as an ingredient in shrimp feed partly to try and cut the cost of feed (Zamal, 2003). The most important thing that is assessed when looking at these various dietary supplements or ingredients and sources is the effect various diets have on the growth rates and survival and ensuring there is no significant decreases in their response to artificial diets.

The economic efficiency of a shrimp farm will be very concerned with the cost and nutritional quality of the feed. The culture and handling of live food for production of shrimp feed is expensive unpredictable and often does not provide all the essential vitamins for the larvae. (Gallardo, 2002). There is therefore a lot of interest in substituting natural diets for alternative lower cost ingredients in shrimp feeds while maintaining high levels of performance: good growth and good survivorship. Effort has been devoted to developing micro particulate diets that could meet the nutritional needs of larvae and juveniles (Gallardo, 2002). Studies on reference diets for *P. monodon* (Glencross & Smith etc al, 1999) help to evaluate the correct composition levels of various artificial feeds but even a good reference diets must be readily consumed by the animal and produce growth at least equal to a good commercial diet.

The aim of studying these results is to look at the comparative growth and survivorship of *P. monodon* fed using either a natural diet or artificial diet. The content of lipid, ash, proteins and energy were examined in each diet and the bacteria level in the water, which the *P.monodon* were fed in.

Results

The composition of the diet is very important. Data for the content of the natural and artificial diet of protein, lipid and ash were collected as well as energy content for both diets and analysed for differences after first determining if the data was normal data (using an Anderson darling test) with equal variance (using an ANOVA test) so that the appropriate test for differences in the data could be used. Test for differences were T-test for parametric data with equal or non-equal variances, Mann Whitney or Mood's Median test for non-parametric data depending on the results for A-squared and associated probability from the Anderson Darling test and either the F-test or Levene's test statistics for equal variance. Table 1 displays the results of content of the diets, the biochemical data.

Results of a parametric T-test on data assuming non-equal variance showed that there was a significant difference between the means of energy content of the natural diet and the artificial diet ($p<=0.05$). The energy content for the normal diet was lower (Estimate for difference -11.43). The T-test was used on the energy data as the test for normality showed natural diet A-squared (A^{2}) value was 0.41 with P value of 0.270 and the artificial diet A^2 value was 0.18 with associated P value of 0.884. Both probability values were greater than 0.05 thus normality of data was assumed. F-test statistic was 5.56 with a P-value of 0.018 ($p<=0.05$) so a T test without assuming equal variance was used

	Mean	Median	CI of difference	Test results
Energy content – Kjoules / g (10)				
Natural Diet	142.83 ± 3.25 (SD 4.54)	-	-19.45, -3.41	T = 3.11, P=0.009 to 12 df
Artificial Diet	154.26 ± 7.67 (SD 0.85)	-		
Protein content – by weight (10)				
Natural Diet	36.86 ± 1.71 (SD 2.39)	-	-0.89, 4.00	T = 1.34, P=0.197 of 17 df
Artificial Diet	35.30 ± 1.99 (SD 2.78)	-		
Lipid content – by weight (10)				
Natural Diet	SD (5.49)	23.41 (20.07 – 28.11) 95%CI	-15.19, -6.32	W = 60.0, P=<0.001 9 df
Artificial Diet	SD (3.61)	35.45 (31.49 – 36.99) 95%CI		
Ash content – by weight (10)				
Natural Diet	29.50 ± 3.15 (SD 4.41)	-	6.02, 12.92	T = 5.76, P=<0.001 to 18 df
Artificial Diet	20.03 ± 1.97 (SD 2.76)	-		

Table 1: Biochemical Data showing content levels of either artificial or natural diets that were fed to juvenile *P. monodon*. CI = Confidence Interval. Figures in brackets show the number of observations. T = T-Test statistic. P = associated probability. df = degrees of freedom. Energy content is shown in Kjoules/g. W = Mann Whitney test statistic. SD = Standard Deviation.

Significant differences were found between Ash content in the natural (A^2 0.34 and P-value 0.419) and artificial diets (A^2 0.32 and P-value 0.472). ANOVA test for equal variance F-test statistic was 0.39 with an associated P-value of 0.18 so a T-test assuming equal variance on normal data was used. The results showed a significant difference with the Ash content higher in the normal diet; the estimate for the difference was 9.47

Significant differences were also found between Lipid content in the natural (A^2 0.27 and P-value 0.600) and artificial diets (A^2 0.90 and P-value 0.013). Because one of the data sets (Artificial data, P-value <=0.05) was not normal the Levene's statistic result was used from the ANOVA test of equal variance to determine which test was appropriate for the data. Levene's statistic was 1.56 with an associated p-value of 0.228 showing equal variance on the data. A Mann Whitney test was therefore used and showed a significant difference between the medians (P<=0.05). Lipid content was significantly lower in the natural diet with an estimated difference between the medians of –11.91.

Protein content was very similar in the natural and artificial diet. Data for the natural diet was normally distributed (A2 = 0.19, P-value 0.874), as was the data for the artificial diet (A2 = 0.22, P-value 0.762). Since both probability values showed the data to be normal (>0.05) the F-test statistics were analysed from the ANOVA test to determine if the data was of equal variance. The F-test statistic of 5.56 with p-value 0.018 showed the data was not of equal variance (p<=0.05) so a T-test for normal data without assuming variance was used on the protein data. The results showed there was not a significant difference between the protein content in the natural diet and the artificial diet. The natural diet was slightly higher with the estimated difference 1.55.

	Mean	Median	CI of difference	Test results
Growth (mm/month) (30)				
Natural diet	10.05 ± 0.23 SD 0.60	-	-0.13, 0.63	T =1.33, P=0.19 for 58 df
Artificial diet	9.79 ± 0.32 SD 0.85	-		
Survivorship (15)				
Natural diet	SD 4.99	82.1% (78.86 – 84.88) 95%CI	-22.4, 4.0	Chi^2=10.8, P=0.001 for 9 df
Artificial diet	SD 18.86	65.6% (51.2 – 73.4) 95%CI		
Bacterial count (25)				
Natural diet	129.32 ± 4.79 SD 11.60	-	-37.76, -23.44	T = -8.59, P=<0.001 to 48 df
Artificial diet	159.92 ± 5.58 SD 13.52	-		“

Table 2: Biological data for the growth and survival of juvenile *P. monodon* fed on either artificial or natural DIETS. Rate of growth is shown in mm of the carapace length / month. Survivorship is shown as % of *P.monodon* surviving after 1 week from batch of 20 organisms. Bacterial count per ul of water in which *P. monodon* had fed on either of the two diets in a 24hour period is also shown. CI = Confidence Interval. Figures in brackets show the number of observations. T = T-Test statistic. P = associated probability. df = degrees of freedom. SD = Standard Deviation.

The biological data shows that *P.monodon* growth rates are not significantly different with prawns fed either the natural or artificial diet. Data for the natural diet was normally distributed (A2 = 0.56, P-value 0.135), as was the data for the artificial diet (A2 = 0.37, P-value 0.400). Since both probability values showed the data to be normal (>0.05) the F-test statistics were analysed from the ANOVA test to determine if the data was of equal variance. The F-test statistic of 1.96 with p-value 0.074 showed the data was of equal variance (p>0.05) so a T-test for normal data assuming variance was used. The results showed there was not a significant difference between the growths of the carapace measurements for prawns fed the natural diet or the artificial diet. The prawns fed the natural diet grew slightly larger with the estimate of difference between the means showing that prawns fed on the natural diet grew an estimate of 0.25mm larger than the prawns fed on the artificial diet but this is not a significant difference (p>0.05).

Data for survivorship however shows a very different picture. Although the prawn’s growth on the artificial diet was not significantly different to that of those fed the natural diet their chances of survival were significantly lower. Both sets of survivorship data were not normal. The survivorship rate data for the natural diet was normal ($p>0.05$) with an A2 result of 0.24 with associated probability of $p=0.725$. The survivorship rate data for the artificial diet was not normal however ($p<=0.05$) with an A2 result of 0.85 and p-value of 0.021. Levene's statistic results of 5.94 with P-value of 0.021 indicting non-equal variance ($p<=0.05$) meant that the non-parametric test used to examine differences in the data was Moods Median Test. The results of this test showed that there was a significant difference between survivorship of prawns fed on the artificial to the natural diet ($p<=0.05$) with 13.3% more of the prawns fed the artificial diet dying. The estimated difference between the medians was 13.3.

This may be connected to the bacteria count, which showed significant differences between water of the prawns fed the natural diet to those fed the artificial diet. Anderson darling test results showed that the data for the natural diet was normal (A2 0.44 and P-value 0.273) and the data for the artificial diet was normal (A2 0.18 and P-value 0.904). F-test statistic was 1.36 with an associated P-value of 0.458 so a T-test assuming equal variance ($P>0.05$) was used to determine the differences. Results showed that the bacteria count per ul of water in which prawns were fed for 24hrs for the natural diet was significantly lower than the bacteria count for the artificial diet water. The estimated difference was –30.60.

Discussion

Results showed there was no significant difference in growth rates between prawns fed the artificial diet and those fed the natural diet. This fits with the findings of other studies. Tacon et al (2002) found that although overall growth response of shrimp fed a control feed (natural diet) was higher than those animals fed with commercial artificial diet the differences were not significant. Our results match this.

The content levels of the diets differed in some cases significantly but overall the shrimp must be fairly happy with the diet since if they were not happy they would not grow. No significant difference in growth rates would indicate that the artificial diet was as acceptable to the prawns as the natural diet. Feeds must be nutritionally adequate and acceptable to shrimp in order for them to feed and show good growth and survival rates (Glencross, 1999).

Studies into diet content show that artificial feeds must contain at least 40% proteins and a substantial amount of lipids to sustain growth (Glencross, 1999). Since according to our results there was no significant difference in Protein content ($P>0.05$) the additional Energy content in the artificial diet must be coming from the higher amounts of lipid in the artificial diet. The estimated difference between lipid levels in the natural and artificial diet showed a significant difference. Ash content was significantly lower in the natural diet so this was possibly reduced to allow more lipids content in the artificial diet by weight. Lipids can provide essential nutrients to the prawn required for growth. Although prawn nutrition studies are ongoing it is widely accepted that protein content and other nutrients such as fatty acids are necessary for providing nutrients required for growth (Sarac, 1993)

The most startling result of our data was the survivorship figures. Despite the fact that overall growth showed no significant differences between prawns fed the natural diet to those fed the artificial diet the differences in survivorship were significant. An average of over 13% more prawns fed the artificial diet died than on the natural diet. There are a number of reasons why this might be the case. Some research shows that prawns obtain additional nutrients from natural food organisms present within the water column and/ or pond ecosystem (Leber, 1988). The quality of the water is obviously very important and the organisms that live in the water such as bacteria, algae, diatoms, flagellates, ciliates etc have important functions in removing and harnessing potentially toxic faecal wastes and metabolites (e.g. by nitrification) from the shrimp within the culture system (Tacon, 2002).

Our results showed that bacteria count in water where prawns were fed the artificial diet was significantly higher than bacteria in water where prawns were fed the natural diet. A higher level of bacteria in the water is therefore possibly contributing to the death of prawns fed the artificial diet. The higher levels of bacteria could be caused by the composition of the artificial diet since there are significant differences in lipid and ash it is most probably one of these. Smith et al (2002) recognised that feeding strategies have been found to influence water quality and shrimp health. The nutrient content of the feed will influence growth, survival and the amount of metabolic and excreted waste products entering the system. (Smith, 2002). Tacon et al (2002) surmised during studies of shrimp farms operating different pool systems that in closed water systems (zero-water exchange culture) that it was not surprising that many essential nutrients would be progressively depleted from the column. Also that other digestive/ excretory metabolites or feed contaminants could progressively accumulate to toxic levels. This would certainly compromise shrimp growth and /or survival.

The high lipid content in the artificial feed may contribute to the fouling of the water for prawns fed artificial feed. Bautista (1997) showed that fats in shrimp feed are susceptible to oxidation (rancidity) especially with higher levels of fatty acids. During process of fat oxidisation chemical degradation products are formed which in turn react with other ingredients in the diet like lipids and reduce their biological value and availability during digestion. Feeding any animal species with feeds containing oxidised fats may results in increased mortality. (Bautista, 1997)
Studies by Kurmaly et al (1988) further discussed the issue of water fouling and bacteria with attention to larvae. The survival of *P.monodon* larvae was significantly better when fed combinations of artificial and algal diets. The reason for this was not concluded but hypothesises that it could be due to combinations being more nutritionally balanced, more digestible or live algae functioning as a bio-filter removing toxic wastes from the culture water. In view of this it is possible that decrease or lack of live algae in our artificial diet is having similar effect opn the water. Certainly significantly more bacteria was found in the water where prawns were fed artificial diet. It has been shown in some experiments that nitrogenous excretion especially ammonia is predominant in prawns fed with artificial diet's Natural foods like algae contain high protein but contribute to low nitrogenous excretion hence posing less adverse effects on water quality. (Shishehchian, 1999)

In conclusion whilst our results show no significant change in growth between *P.monodon* fed natural and artificial diets since the survivorship is significantly lower in those fed the artificial diet we must conclude that more studies should be done to discover why before artificial diets are used exclusively in a farming environment. As an effective feed management approach a combination of natural food with a reasonable amount of artificial diet might be considered for an efficient system to regulate water quality (Shishehchian, 1999), which may be the reason for lower survivorship in *P.monodon* fed on an artificial diet.

References

Bautista. M.N. Subosa, P.F. 1997. Changes in shrimp feed quality and effects on growth and survival of *Penaeus monodon* juveniles. Aquaculture Vol 151. p121-129

Gallardo, P.P. Pedroza-Islas, R. Garcia-Galano, T. Pascual, C. Rosal, C. Sanchez, A. Gaxiola, G. 2002. Replacement of live food with a microbound diet in feeding *Litopenaeus setiferus* (Burkenroad) larvae. Aquaculture research. Vol 33. p 681-691.

Glencross, B.D. Smith, D.M. Thomas, M.R. Williams, K.C. 2002. The effects of dietary lipid and fatty-acid composition on the digestibility of lipids by the prawn, *Penaeus monodon*. Aquaculture Vol 205. p 157-169.

Glencross, B.D. Smith, D.M. Tonks, M.L. Tabrett, S.J. Williams, K.C. 1999. A reference diet for nutritional studies of the giant tiger prawn *Penaeus monodon*. Aquaculture Nutrition Vol 5. p 33-39

Kurmaly, D.A. Jones, A.B. East, J. 1989. Comparative Analysis of the Growth and Survival of *Penaeus monodon* (Fabricus) larvae, from Protozoea 1 to Postlarvae 1, on Live Feeds, Artificial Diets and on Combinations of Both. Aquaculture, 81. p 27-45

Laio, I, Chiu. Chien, Yew-Hu. 1995. The evolution of the Grass Prawn (*Penaeus monodon*) Hatchery Industry in Taiwan. Aquacultural Engineering. Vol 15. No 2 pp 111-132

Reddy, H.R.V. Ganapathi Naik, M. Annappaswamy, T.S. 1999. Evaluation of the dietry essentiality of vitamins for *Penaeus monodon*. Aquaculture Nutrition. Vol 5. p 267-275

Sarac, Z. Thaggard, H. Saunders, J. Gravel, M. Neill, A. Cowan, R.T. 1993. Observations on the chemical composition of some commercial prawn feeds and associated growth responses in *Penaeus monodon*. Aquaculture. Vol 115. p 97-110.

Shishehchian, F. Yusoff, F.M. Omar, H. Kamarudin, M.S. 1999. Nitrogenous Excretion of *Penaeus monodon* Postlarvae Fed with Different Diets. Mar Poll Bull Vol 39. Nos 1-12 p 224-227

Smith, D.M. Burford, M.A. Tabrett, S.J. Irvin, S.J. Eard, L. 2002. The effect of feeding frequency on water quality and growth of the black tiger shrimp (*Penaeus monodon*). Aquaculture Vol 207. p 125-136.

Tacon, A.G.J. Cody, J.J. Conquest, L.D. Divakaran, S. Forster, I.P. Decamp, O.E. 2002. Effect of culture system on nutrition and growth performance of Pacific white shrimp *Litopenaeus vannamei* (Boone) fed different diets. Aquaculture Nutrition. Vol 8. p 121-137

Zmal, H. and co-workers. 2003. Incorporation of local soybean meal in diets for *Penaeus monodon.* Postlarvae. Global Aquaculture Advocate Vol 6(2): 55.

Leber, K.M. Pruder, G.D. 1988. Using experimental microcosms in shrimp research: the growth-enhancing effect of shrimp pond water. Journal of World Aquacultural Society. Vol 19. p 197-203