Accepted refereed manuscript of:

Glencross B, Arnold A, Irvin S (2014) Bioactive factors in microbial biomass have the capacity to offset reductions in the level of protein in the diet of black tiger shrimp, *Penaeus monodon*, *Aquaculture*, 446, pp. 74-79.

DOI: 10.1016/j.aquaculture.2015.04.007

Bioactive factors in microbial biomass have the capacity to offset reductions in the level of protein in the diet of black tiger shrimp, *Penaeus monodon*

Brett Glencross[1,2], Stuart Arnold[1,3], Simon Irvin[1,3]

1. CSIRO Aquaculture Program, CSIRO Agriculture Flagship, GPO Box 2583, Brisbane, QLD 4001, Australia
2. CSIRO Aquaculture Program, CSIRO Agriculture Flagship, North St, Woorim, QLD 4710, Australia

(p) 61-7-3883-5926

(e) Brett.Glencross@csiro.au

Keywords : prawns, protein requirement, microbial floc, protein retention

Submitted to : Aquaculture

Highlights :

1. Optimal growth and feed efficiency by *Penaeus mondon* requires high levels of dietary protein.
2. Use of a microbial biomass can stimulate shrimp growth.
3. Dietary protein can be partially offset through the use of a microbial biomass.
4. Improved growth was underpinned by improved protein retention efficiencies.

Abstract

A factorial experiment was conducted with black tiger shrimp (*Penaeus monodon*) juveniles to determine the effects of varying protein inclusion in the diet and also varying inclusion of a microbial biomass on growth, feed and nutrient utilisation when fed in indoor laboratory conditions. Growth performance of the shrimp improved with increasing diet protein level. However, in the absence of the added microbial biomass this growth performance plateaued at the 480 g/kg protein level. The addition of the microbial biomass improved growth at each inclusion level of both protein and the microbial biomass. No plateau in growth was observed with the addition of the microbial biomass. Improvements in feed conversion were seen with increasing dietary protein levels and also the inclusion of the microbial biomass. Examination of the feed intake of each treatment supports that there was a combined effect of an increase in feed intake and improvements in feed conversion that contributed to the improvements in growth performance with the use of the microbial biomass, but that the increases in dietary protein level largely influenced growth through improvements in feed conversion.

1. Introduction

Protein inclusion in diets for shrimp continues to be the primary cost driver in formulations for these species. The requirements by the black tiger shrimp *Penaeus monodon* for protein were first examined by Bages and Sloane (1981), who studied protein requirements for post-larval shrimp (2 mg initial weight) using a factorial design with a series of protein levels matched against a series of starch levels. Growth in this experiment was observed to be strongly influenced by protein level of the diet but was largely unaffected by the starch content, with the best growth by those shrimp fed the highest protein level (550 g/kg). Subsequent to this Alava and Lim (1983) followed this work with a study that suggested that optimal protein requirements for juvenile (1.32 g initial weight) *P. monodon* were lower, between 400 and 450 g/kg. Bautista (1986) followed these earlier studies with another factorial study of protein by lipid by carbohydrate and was able to show that both protein and lipid had dominant effects on performance of juvenile shrimp (0.60 g initial weight). In that study the best growth was seen with 500 g/kg protein levels, though this was only marginally better than that achieved with a protein level of 400 g/kg. Collectively these studies suggest that the protein requirement for juvenile shrimp are between the 400 and 500 g/kg, and that animal size likely has an impact on the requirement.

Bioactive raw materials have been routinely used in shrimp diets for a long time (Cruz-Suarez et al., 1987; 2007). The recent development of and use of a range of microbial biomass products has also been reported to achieve significant improvements in growth performance in several shrimp species (Ju et al., 2008; 2009; Kuhn et al., 2008; 2009; Glencross et al., 2013; 2014; Emerenciano et al., 2014). The use of these microbial biomass products has also resulted in success in being able to off-set poorer performance due to a range of formulation changes, including the complete replacement of any fishery derived resources in the diet of shrimp (Glencross et al., 2014). However, whether similar such gains can be achieved against changes in not the raw materials, but to key dietary specifications has not been examined.

The present study therefore aimed to test the hypothesis, that the use of a microbial biomass can assist in ameliorating a decline in performance observed with a reduction in protein and that this would be achieved by improvements in the protein and energy utilisation by the shrimp fed the microbial biomass.

2. Materials and Methods

2.1 Study design

A factorial design experiment using three microbial biomass levels (0, 50 and 100 g/kg) x four protein levels (360, 420, 480 and 540 g/kg) was undertaken to examine the capacity of a microbial biomass with reported bioactive properties (Glencross et al., 2014) to offset changes in dietary protein in a clear-water tank experiment.

2.2 Diet manufacture

The details and composition of each of the key ingredients used in this study are presented in Table 1. Each diet was initially prepared milling all ingredients to <750 μm, prior to mixing in an upright planetary mixer (Hobart, Sydney, NSW, Australia). Water was then added during the mixing to form a dough which was subsequently screw-pressed (Dolly, La Monferrina, Castell'Alfero, Italy) through a 2mm die and cut to pellet lengths of about 6mm. The pellets were then steamed at 100ºC for 3 minutes before being oven dried at 60ºC for 24h. All diets were kept at -20ºC when not being fed. Formulations and origins of all ingredients in each diet are presented in Table 2.

2.3 Shrimp collection and trial management

Several hundred individuals (~3 g) of a wild-type genotype of black tiger shrimp (*Penaeus monodon*) were collected from a grow-out pond at Melivan Prawn Farm (Kurramine Beach, QLD, Australia) by cast-netting and were transferred to a holding tank (10,000 L) at the Bribie Island Research Centre (Woorim, QLD, Australia), where they were held pending allocation to trial tanks. During the holding period (~7 days) they were fed a standard commercial grower diet (Prawn Grower, Ridley Aquafeeds, Narangba, QLD, Australia).

Eight shrimp were then allocated to each of 48 x 100 L tanks in an indoor laboratory system. The mean initial weight across all tanks was 2.98 ± 0.09 g. Tanks of shrimp were maintained with flow-through seawater at a rate of 600 mL/min. Temperatures of each tank were maintained at 29.6 ± 0.71ºC and dissolved oxygen maintained at 6.2 ± 0.12 mg/L. Light was maintained on a 12:12 light:dark cycle. Shrimp were individually weighed at day 0, 21 (as an interim assessment point – data not shown) and again at day 42. The mean weight of each tank was determined at each assessment point to calculate the mean weight for each

treatment, with tanks used as the replicate (n = 4 per treatment). All shrimp remaining with each tank at the end of the experiment were pooled within each tank for carcass analysis. During this period the shrimp were manually fed the diets twice daily to marginal excess and the number of feed pellets remaining the following day counted and used to adjust the next day's ration (increase or decrease) according to the number of pellets counted, and also provide a quantitative assessment of the amount of uneaten feed. The uneaten feed was then siphoned from each tank daily after scoring. This method was also used to estimate as accurately as possible feed intake within each tank on each day (Smith et al., 2007; Glencross et al., 2013).

2.4 Chemical analysis

Diets and whole shrimp samples were analysed for dry matter, ash, nitrogen, total lipid, carbohydrate and gross energy content. Ingredient samples were analysed for amino acids. Dry matter of the samples was calculated by gravimetric analysis of a sample following oven drying at 105ºC for 24 h. Protein was calculated from the determination of total nitrogen by CHNOS auto-analyser, based on N x 6.25. Amino acid analysis involved the samples being hydrolyzed at 110ºC for 24 h in 6M HCl with 0.05% Phenol. Cystine was derivatized during hydrolysis by the addition of 0.05% 3-3-dithiodipropoinic acid. The acid hydrolysis destroyed tryptophan making it unable to be determined. Separation was by HPLC on a Hypersil AA-ODS 5μm column using an 1100 series Hewlett Packard HPLC system. Gross ash content was determined gravimetrically following loss of mass after combustion of a sample in a furnace at 550°C for 12 h. Total lipid content of the diets was determined gravimetrically following extraction of the lipids using chloroform:methanol (2:1). Carbohydrates were estimated based on the dry matter content of the feed minus the lipid, ash and protein. Gross energy was determined by ballistic bomb calorimetry. All methods were consistent with those recommended by AOAC (2005).

2.5 Nutrient and energy retention assessment

Protein, lipid and energy retention were determined based on the mass gain in each respective nutrient and E over the course of the growth study, against the respective consumption of each respective nutrient and energy. All values were calculated according to the following formula (Glencross et al., 2007):

$$Nitrogen/Energy\,Retention(\%) = \left(\frac{Nt - Ni}{Nc}\right) \times 100$$

Where Nt is the nutrient/energy content of the shrimp in a specific replicate at time t and Ni is the mean initial nutrient/energy content of the shrimp at the beginning of the study. Nc is the amount of nutrient/energy consumed by the shrimp from the time of initial assessment to time t.

2.7 Statistical analysis

All values are means ± standard deviations unless otherwise specified. Significant differences were determined using a two-way ANOVA followed by a Fishers LSD test with critical ranges were set at $P < 0.05$. These tests were undertaken using Statistica™ v6.0 (Statsoft®, Tulsa, OA, USA). Linear and curvilinear regression analysis and line fitting of those relationships was undertaken using the data analysis tools and graphics elements of Microsoft Excel.

3. Results

3.1 Growth and feed utilisation

Significant effects on the final weight and gain rate were identified for both protein and microbial biomass levels but not the interaction term (Table 3). In the absence of microbial biomass the increase in diet protein concentration resulted in a significant improvement in growth (as defined by final weight and gain rate) of those shrimp fed those diets with the protein concentration up to 480 g/kg (Table 4). The addition of either 50 or 100 g/kg of the microbial biomass also significantly improved the gain rate of shrimp at any of the protein levels examined (Figure 1).

Across all treatments a significant regression relationship (P=0.004) was observed between feed intake and weight gain (Figure 2). Significant effects were identified for both protein and microbial biomass levels and also the interaction term for feed intake (Table 3). With the addition of the microbial biomass there was an increase in feed intake within each of the different protein levels of the factorial array (Table 4). In those treatments without the addition of the microbial bioactive, regression analysis identified a marginal decrease in feed intake (P=0.092) with an increase in diet protein levels. The relationship was stronger, albeit still not significant when examined as a function of energy intake (P=0.082). By contrast, when the microbial biomass was added to the diets the relationships were not only weaker but there was also an increase in feed intake with increasing protein level at the 50 g/kg inclusion level and no effect on intake with changing protein level when the microbial biomass was included at 100 g/kg (Figure 2).

The combined effects of growth and feed intake produced an array of significant effects on the feed conversion ratio (FCR) by shrimp fed the different treatment diets (Table 4). Significant effects were identified for both protein and microbial biomass levels and also the interaction term for FCR (Table 3). In the absence of the microbial biomass the FCR significantly improved with increasing protein level in the diet. By contrast, with both the 50 g/kg and 100 g/kg inclusion levels of the microbial biomass, the lowest FCR was seen from shrimp fed the diets with second highest (treatment 48P5M) and third highest (treatment 42P10M) protein levels respectively. Within protein levels, the addition of the microbial biomass improved the FCR with the 50 g/kg inclusion level, but no significant improvements in FCR were noted with the 100 g/kg inclusion level.

3.2 Nutrient and energy retention

Nutrient and energy retention by shrimp was significantly affected by treatment. Significant effects on protein retention were identified for the microbial biomass levels and also the interaction term, but not for protein level (Table 5). In the absence of the microbial biomass protein retention was significantly improved with increasing protein level up to 480g/kg, but not with further increases in diet protein level (Figure 3). Regression analysis of this data set (diets without microbial biomass) produced a p-value of 0.052. With the addition of the 50 g/kg and 100 g/kg microbial biomass the best protein retention was seen with those diets containing the 420 and 480 g/kg protein levels respectively, but were not significantly different from each other across the protein range used in this study. Within each protein level in the factorial array the addition of the microbial biomass improved the protein retention, except for those diets with the highest protein levels (Table 5; Figure 3).

Significant effects on lipid retention were identified for the microbial biomass levels and also the interaction term, but not for protein level (Table 5). Lipid retention in the absence of the microbial bioactive was improved with increasing diet protein up to the 480 g/kg level, but then declined at the 540 g/kg level. When microbial biomass was included in the diet there was a significant improvement in the lipid retention at the lower diet protein levels, but not at the higher diet protein levels.

Energy retention was substantially lower than protein retention for all treatments (Table 5). For energy retention significant effects were identified for both the protein level and the microbial biomass levels and also the interaction term (Table 5). With increasing protein content of the diet there was a significant improvement in energy retention in the absence of the microbial biomass, but this was not observed when it was included at either 50 g/kg or 100 g/kg. Energy retention was improved with the addition of the microbial biomass within all protein levels except the highest.

4. Discussion

The development and use of microbial biomass derived bioactive products has recently been shown to produce results that can help sustain the complete replacement of both fishmeal and fish oil in shrimp diets (Glencross et al., 2014). The next logical evaluation in exploring the application of these products was to see what level of protein reduction could be offset with the use of these microbial biomass products. To examine this we used a factorial design to quantify the performance benefits that could be achieved from the concurrent use of a microbial biomass and varying protein levels and to explore the potential that this combination could have to ameliorate declines attributable to the reduction in dietary protein level.

4.1 Performance effects of protein and microbial biomass

The results from the present study support that dietary protein levels, in the absence of microbial biomass, for juvenile (~3g) *P. monodon,* are optimal at around the 480 g/kg DM level and growth performance plateaus above this protein level. Although when assessed using regression it could be argued that the growth continues to improve with increasing protein. In earlier studies it was identified with ~0.5 to 1g shrimp that an optimal protein requirement was between 435 and 489 g/kg DM (Alava and Lim, 1983; Bautista 1986). Similar to the present study, the work of Bautista (1986) also observed the best growth with protein levels at or above 500 g/kg, though this was only marginally higher than that achieved with a protein level of 400 g/kg. The present results are generally consistent with these earlier observations that this species (*P. monodon*) grows faster with a higher (>400 g/kg) protein diet.

The addition of the microbial biomass resulted in growth improvements with every increase in protein level in the diet. Increasing the inclusion of the microbial biomass stimulated faster growth at each protein level. At the highest protein level, the addition of 50 g/kg microbial biomass improved growth by 42% and with 100 g/kg inclusion growth was improved by 60%. Such growth improvements through the use of a microbial biomass are consistent with the gains reported in Glencross et al. (2014). The use of similar such microbial biomass products in shrimp feeds has been reported by other researchers with varying degrees of success (De Schryver et al., 2008; Ju et al., 2008; 2009; Kuhn et al., 2008; 2009). Ju et al. (2008), included a microbial biomass in diets at a 200 g/kg inclusion level and

observed an increase in growth rate of *L. vannamei* from 0.85 g/wk to 1.03 g/wk, a 21% increase in growth rate. However, what is critically important from the present study is the finding that the use of this microbial biomass can provide a clear mechanism to offset reductions in protein levels in the diet. For example by including 50 g/kg of the microbial biomass in a diet with 360 g/kg protein it is possible to achieve the same gain rates as that achieved with 480 g/kg protein and no microbial biomass.

A clear link was observed in the present experiment between feed intake and growth across all the treatments. However, separating a feed stimulant effect from a growth stimulating effect; which in turn demands greater feed intake, is not possible to determine from the present design. Earlier work by Glencross et al. (2013) implicated that it was a growth stimulation effect of a high-performance diet, which included a microbial biomass, which was driving growth and subsequently feed intake. However, that interpretation was based on a design where the feed was pair-fed and the growth of shrimp fed the high-performance diet achieved significant gains, despite that there was no difference in feed intake.

In the present study there were effects identified for both protein and microbial biomass levels and also the interaction between the two factors on feed intake by the shrimp. The addition of the microbial biomass increased feed intake within each of the different protein levels, where as the effect of protein levels within each of the other factors gave contrasting responses. In those treatments without the addition of the microbial bioactive there was a marginal decrease in feed intake with an increase in diet protein levels and this relationship was stronger when examined as a function of energy intake. This is consistent with what has been observed in terms of energetic responses to dietary energy concentrations in some fish studies (Einen and Roem, 1997; Glencross et al., 2008). However, when the microbial biomass was added to the diets there was a marginal increase in feed intake with increasing protein level. These observations are clearly what have contributed to the determination of an interaction effect of these factors on feed intake.

The observation that there was a significant improvement of feed conversion ratio (FCR) identified for both protein and microbial biomass levels and also the interaction term, supports that both factors are influencing growth independent of feed intake to some extent, though this is difficult to quantify from the present experimental design. This improvement in FCR is typical with what has been observed for both improving diet

specifications and the use of microbial biomass in other studies (Glencross et al., 1999; 2014; Ju et al., 2008; Kuhn et al., 2009).

4.2 Effect of microbial biomass on protein utilisation

Typically, shrimp studies do not usually report nutrient and/or energy utilisation parameters, but they are a commonly used assessment parameter in fish studies (Glencross et al., 2007; Glencross and Smith, 2010; Richard et al., 2010). There are a range of reasons why this might be the case, but one of the most obvious is the poorer capacity to accurately assess feed intake in shrimp compared to fish and the associated error that this has in estimating such nutrient retention parameters. So it is with acknowledgement of this limitation that the nutrient retention data is considered.

In the present study improvements in retention efficiency of protein were seen with increasing inclusion of the microbial biomass, but not with the increasing inclusion of protein, however a significant interaction term shows that the dietary protein level did have an effect subject to the presence of the microbial biomass (Table 5). A look at Figure 3 shows this clearly, where little effect of dietary protein on protein retention efficiency can be seen when microbial biomass is included, but when it is absent then improvements in protein retention occur with increasing protein level. Other data from the literature on this aspect of nutrition is scarce. However, it can be implied if a constant protein content of the shrimp is assumed and feed intake data is presented (or able to be calculated from growth and FCR data). In such cases, then it can also be realised that in most instances there is an increase in protein retention efficiency occurring with increasing protein levels (to the point of maximal weight gain) and also in some studies with the use of microbial biomass (Bages and Sloane, 1981; Alava and Lim, 1983; Kuhn et al., 2009; Anand et al., 2014).

The protein retention efficiency values observed in this study (range 5.4% to 24.0%) are substantially lower than what is typically observed in studies with fish (Dumas et al., 2007; Glencross et al., 2008). This is largely driven by the higher apparent FCR obtained in such shrimp studies. However, in pond or pond-like experimental systems FCR values closer to those more typically seen with fish have been reported (Smith et al., 2002; Tacon et al., 2002; Burford et al., 2004; Amaya et al., 2007). It has largely been assumed that under such pond systems that there is additional food intake that enhances production to result in these improved FCR values.

Data on lipid retention efficiency in aquaculture species is scarcer than that of protein retention, however in combination with protein it is this retention of lipid that constitutes the principle biomass gain that occurs in animals. In the present study lipid retention efficiency was similarly influenced as protein retention by the microbial biomass levels and the interaction with protein, and also not for protein level alone. In the absence of the microbial bioactive lipid retention efficiency was improved with increasing dietary protein up to the 480 g/kg level, but above that level the efficiency declined.

The combination of protein and lipid deposition by an animal can often be amortised in terms of the energetic contributions they represent. In the present study we have measured this in terms of energy retention. The energy retention produced significant effects for both the protein level and the microbial biomass levels and also the interaction between these two factors. In the present study this energy retention is mostly driven by the protein retention component, due to the very low levels of lipid found in the whole body analysis of the shrimp (~2% cf. protein at ~20%).

4.3 Conclusions

The findings of this study demonstrate that with the use of a microbial biomass product that not only it is possible to partially off-set the need for protein in diets for *P. monodon*, but that the use of this product can significantly improve growth performance of shrimp in excess of 50% of that achieved in the absence of this microbial biomass. These findings are a major progression in the sustainability of shrimp farming in that they demonstrate potential for reducing the need for key nutrient inputs to sustain productivity.

Acknowledgements

This work was funded by CSIRO's Food Futures Flagship. We acknowledge the technical support of Natalie Habilay, Nick Polymeris, Dylan Rylatt and Kinam Salee. Thanks to Greg Coman for constructive comment and editorial on an early draft.

References

AOAC (Association of Official Analytical Chemists). 2005. Official Methods of Analysis of the Association of Official Analytical Chemists. 15th edition. Association of Official Analytical Chemists. Washington, DC, USA.

Alava, V.R., Lim, C., 1983. The quantitative dietary protein requirement of *Penaeus monodon* juveniles in a controlled environment. Aquaculture 30, 53-61.

Amaya, E.A., Davis, D.A., Rouse, D.B., 2007. Replacement of fish meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under pond conditions. Aquaculture 262, 393-401.

Anand, P.S.S., Kohli, M.P.S., Kumar, S., Sundaray, J.K., Roy, S.D., Venkateshwarlu, G., Sinha, A., Pailan, G.H., 2014. Effect of dietary supplementation of biofloc on growth performance and digestive enzyme activities in *Penaeus monodon*. Aquaculture 418–419, 108–115.

Bages, M., Sloane, L., 1981. Effects of dietary protein and starch levels on growth and survival of *Penaeus monodon* (Fabricus) postlarvae. Aquaculture 25, 117-128.

Bautista, M.N., 1986. The response of *Penaeus monodon* juveniles to varying protein/energy ratios in test diets. Aquaculture 53, 229-242.

Burford, M.A., Thompson, P.J., McIntosh, R.P., Bauman, R.H., Pearson, D.C., 2004. The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity zero-exchange system. Aquaculture 232, 525-537.

Cruz-Suarez, L.E., Guillaume, J., Van Wormhoudt, A., 1987. Effect of various levels of squid protein on growth and some biochemical parameters of *Penaeus japonicus* juveniles. Bull. Japan. Soc. Sci. Fish. 53, 2083-2088.

Cruz-Suárez, L.E., Nieto-López, M., Guajardo-Barbosa, C., Tapia-Salazar, M., Scholz, U., Ricque-Marie, D., 2007. Replacement of fish meal with poultry by-product meal in practical

diets for *Litopenaeus vannamei*, and digestibility of the tested ingredients and diets. Aquaculture 272, 466-476.

Dumas, A., de Lange, C.F.M., France, J., Bureau, D.P., 2007. Quantitative description of body composition and rates of nutrient deposition in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 273, 165-181.

Einen, O., Roem, A., 1997. Dietary protein / energy ratios for Atlantic salmon in relation to fish size: growth, feed utilisation and slaughter quality. Aquaculture Nutrition 3, 115-126.

Emerenciano, M., Ballester, E.L.C., Cavalli, R.O., Wasielesky, W., 2011. Effect of biofloc technology (BFT) on the early postlarval stage of pink shrimp Farfantepenaeus paulensis: growth performance, floc composition and salinity stress tolerance. Aquaculture International 19, 891-901.

Glencross, B., Smith, D.M., 2010. Raw material evaluation strategies for shrimp diets - Optimizing raw material sustainability. In: The Shrimp Book (V. Alday and D.A. Davis, Eds.). Nottingham University Press. pp 938

Glencross, B.D., Smith, D.M., Tonks, M.L., Tabrett, S.M., Williams, K.C., 1999. A reference diet for nutritional studies of the prawn, *Penaeus monodon*. Aquaculture Nutrition 5, 33-40.

Glencross, B.D., Booth, M., Allan, G.L. 2007. A feed is only as good as its ingredients – A review of ingredient evaluation for aquaculture feeds. Aquaculture Nutrition 13, 17 – 34.

Glencross, B.D, Michael, R., Austen, K., Hauler, R., 2008. Productivity, carcass composition, waste output and sensory characteristics of large barramundi, *Lates calcarifer*, fed high-nutrient density diets. Aquaculture 284, 167-173.

Glencross, B.D., Tabrett, S.J., Irvin, S., Wade, N., Smith, D.M., Coman, G.E., Preston, N.P., 2013. An examination of the genotype by diet interaction effects in the Black Tiger shrimp,

Penaeus monodon. – why do selected shrimp grow faster ? Aquaculture Nutrition 19, 128-138.

Glencross, B.D., Irvin, S., Arnold, S.J., Bourne, N.B., Preston, N.P., 2014. Effective use of microbial biomass products to facilitate the complete replacement of fishery resources in diets for the black tiger shrimp, *Penaeus monodon*. Aquaculture 431, 12-19.

Ju, Z.Y., Forster, I., Conquest, L., Dominy, W., 2008. Enhanced growth effects on shrimp (*Litopenaeus vannamei*) from inclusion of whole shrimp floc or floc fractions to a formulated diet. Aquaculture Nutrition, 14, 533-543.

Ju, Z. Y., Forster, I.P., Dominy, W.G., 2009. Effects of supplementing two species of marine algae or their fraction to a formulated diet on growth, survival and composition of shrimp (*Litopenaeus vannamei*). Aquaculture, 292, 237-243.

Kuhn, D.D., Boardman, G.D., Lawrence, A.L., Marsh, L., Flick Jr. G.L., 2008. Use of microbial flocs generated from Tilapia effluent as a nutritional supplement for shrimp *Litopenaeus vannamei*, in recirculating aquaculture systems. Journal of the World Aquaculture Society, 39, 72-82.

Kuhn, D.D., Boardman, G.D., Lawrence, A.L., Marsh, L., Flick Jr. G.L., 2009. Microbial floc meal as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture, 296, 51-57.

Richard, L., Blanc, P.P., Rigolet, V., Kaushik, S.J., Geurden, I., 2010. Maintenance and growth requirements for nitrogen, lysine and methionine and their utilisation efficiencies in juvenile black tiger shrimp, *Penaeus monodon*, using a factorial approach. British Journal of Nutrition 103, 984-995.

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

Smith, D.M., Tabrett, S.J., Glencross, B.D. 2007. Growth response of the black tiger shrimp, *Penaeus monodon* fed diets containing different lupin cultivars. Aquaculture 269, 236-246.

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

Tables and Figures

Table 1. Composition of the key experimental ingredients (all values are g/kg dry basis - unless otherwise specified).

	Fishmeal	Gluten	Wheat	MB
Dry matter (g/kg)	912	904	900	917
Protein	753	807	129	42
Lipid	102	22	22	6
Ash	159	8	839	269
Carbohydrates	0	163	10	683
Energy (kJ/g DM)	21.5	22.1	18.4	13
Alanine	45	19	4	2
Arginine	40	26	6	1
Aspartic acid	66	25	7	4
Cysteine	9	20	1	0
Glutamate	92	299	40	3
Glycine	42	25	5	2
Histidine	23	13	1	0
Isoleucine	32	28	4	2
Leucine	55	53	9	2
Lysine	55	11	5	1
Methionine	23	15	2	1
Phenylalanine	29	43	6	1
Proline	30	115	25	6
Serine	30	40	6	2
Taurine	7	0	0	0
Threonine	32	21	5	3
Tyrosine	24	27	4	1
Valine	37	28	5	2

MB : Microbial biomasss

Table 2. Formulations and composition of diets from. Data are g/kg values unless otherwise stated.

	36P	42P	48P	54P	36P5M	42P5M	48P5M	54P5M	36P10M	42P10M	48P10M	54P10M
Raw material type												
Fishmeal [a]	300.0	400.0	500.0	610.0	300.0	400.0	500.0	610.0	300.0	400.0	500.0	610.0
Wheat gluten [b]	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0	70.0
Wheat flour [b]	600.3	500.3	400.3	290.3	550.3	450.3	350.3	240.3	500.3	400.3	300.3	190.3
Lecithin [a]	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Fish oil [a]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Microbial Biomass [a]	-	-	-	-	50.0	50.0	50.0	50.0	100.0	100.0	100.0	100.0
Astaxanthin [e]	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
BanoxE [d]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Cholesterol [c]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin C [e]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin Premix [f]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Diet composition - As measured												
Dry matter (% as fed)	95.5	94.9	95.7	96.4	95.7	95.8	95.7	96.8	96.0	95.7	96.8	96.8
Protein (% DM)	38.7	44.5	49.2	56.0	36.0	42.1	48.9	53.6	37.5	42.8	48.9	53.3
Lipid (%DM)	6.3	8.6	7.9	10.0	7.9	7.5	7.7	9.0	5.9	7.0	8.4	10.1
Ash (%DM)	16.3	5.2	6.5	8.0	8.2	9.6	11.0	12.8	11.4	12.6	14.9	16.3
Carbohydrates (%DM)	38.7	41.8	36.5	26.1	47.9	40.8	32.4	24.6	45.2	37.7	27.9	20.4
Gross Energy (%DM)	19.73	19.92	19.98	20.71	19.98	19.25	19.48	20.03	18.24	18.52	19.22	19.48

[a] Fish (Peruvian anchovetta) meal, Lecithin, Microbial bioactive: Novacq™ and Fish (Peruvian anchovetta) oil : Ridley Aquafeeds, Narangba, QLD, Australia. [b] Wheat gluten and flour : Manildra, Auburn, NSW, Australia. [c] Cholesterol : MP Bio, Aurora, OH, USA. [d] Banox-E™ : BEC Feed Solutions, Carole Park, QLD, Australia. [e] Astaxanthin (10%) as Carophyll Pink™ and Vitamin C as Stay C™: DSM, Wagga Wagga, NSW, Australia. [f] Vitamin and mineral premix : Rabar, Beaudesert, QLD, Australia; includes (IU/kg or g/kg of premix): Vitamin A, 2.5MIU; Vitamin D3, 1.25 MIU; Vitamin E, 100 g; Vitamin K3, 10 g; Vitamin B1, 25 g; Vitamin B2, 20 g; Vitamin B3, 100 g; Vitamin B5, 100; Vitamin B6, 30 g; Vitamin B9, 5; Vitamin B12, 0.05 g; Biotin, 1 g; Vitamin C, 250 g; Banox-E, 13 g.

Table 3 Two-way ANOVA of growth and feed utilisation parameters

	P-value			F-value			df		
Parameter	P	M	P x M	P	M	P x M	P	M	P x M
Final weight	0.002	0.000	0.624	6.27	37.52	0.74	3	2	6
Rate	0.002	0.000	0.604	6.20	39.20	0.76	3	2	6
Intake	0.026	0.000	0.004	3.47	24.60	4.02	3	2	6
FCR	0.000	0.000	0.003	15.05	12.17	4.17	3	2	6
Survival	0.199	0.870	0.559	1.63	0.14	0.82	3	2	6
Protein retention	0.189	0.000	0.002	1.68	23.44	4.41	3	2	6
Lipid retention	0.138	0.000	0.000	1.96	12.55	6.11	3	2	6
Energy retention	0.004	0.000	0.002	5.37	24.92	4.31	3	2	6

Table 4 Shrimp growth and feed utilisation parameters

Treatment	36P	42P	48P	54P	36P5M	42P5M	48P5M	54P5M	36P10M	42P10M	48P10M	54P10M	Pooled SEM
Initial weight (g/shrimp)	2.99	3.08	3.02	3.02	2.96	2.96	3.05	2.90	3.02	2.92	2.95	2.96	0.01
Final weight (g/shrimp)	5.26^{a}	5.91^{a}	7.04^{b}	7.05^{b}	6.94^{b}	7.50^{b}	7.61^{b}	8.61^{c}	8.71^{c}	8.91^{c}	9.17^{c}	9.41^{c}	0.18
Rate (g/wk)	0.38^{a}	0.47^{ab}	0.67^{b}	0.67^{b}	0.65^{b}	0.76^{b}	0.76^{b}	0.95^{c}	0.95^{c}	1.00^{c}	1.04^{c}	1.07^{c}	0.04
Intake (g/shrimp)	11.26^{b}	10.29^{a}	10.58^{ab}	9.54^{a}	11.24^{b}	12.75^{c}	10.74^{ab}	16.57^{f}	16.82	13.07^{c}	14.24^{d}	15.29^{e}	0.38
FCR (feed/gain)	5.02^{c}	3.69^{b}	2.64^{ab}	2.38^{a}	2.82^{ab}	3.03^{b}	2.40^{a}	2.92^{b}	2.99^{b}	2.21^{a}	2.29^{a}	2.44^{a}	0.11
Survival (%)	71.9^{a}	78.1^{ab}	90.6^{c}	87.5^{bc}	90.6^{a}	78.1^{ab}	81.3^{ab}	78.1^{ab}	65.6^{a}	87.5^{b}	84.4^{b}	81.3^{ab}	1.95

FCR : Feed conversion ratio. Different superscripts within rows indicate significant differences (P<0.05). An absence of superscripts implies that there were no significant differences (P>0.05).

Table 5 Nutrient and energy retention efficiency (%) parameters

Treatment	36P	42P	48P	54P	36P5M	42P5M	48P5M	54P5M	36P10M	42P10M	48P10M	54P10M	Pooled SEM
Protein	5.4 ^{a}	8.5 ^{b}	13.5 ^{c}	14.3 ^{c}	13.0 ^{c}	15.3 ^{cd}	16.2 ^{d}	11.1 ^{bc}	19.6 ^{e}	24.0 ^{f}	18.7 ^{de}	15.4 ^{cd}	0.8
Lipid	1.0 ^{a}	3.7 ^{b}	10.2 ^{d}	7.8 ^{c}	10.4 ^{d}	5.6 ^{b}	12.2 ^{e}	6.3 ^{bc}	14.7 ^{f}	13.2 ^{ef}	8.5 ^{c}	8.9 ^{c}	0.7
Energy	2.5 ^{a}	4.9 ^{b}	9.1 ^{c}	10.2 ^{c}	8.8 ^{c}	8.5 ^{c}	10.9 ^{c}	7.8 ^{bc}	10.9 ^{c}	14.5 ^{d}	12.2 ^{d}	11.3 ^{cd}	0.5

Figure 1. Effect of inclusion level of microbial biomass combined with varying protein inclusion level on the gain rate (g/wk) of shrimp. Diet protein content is percent as fed basis.

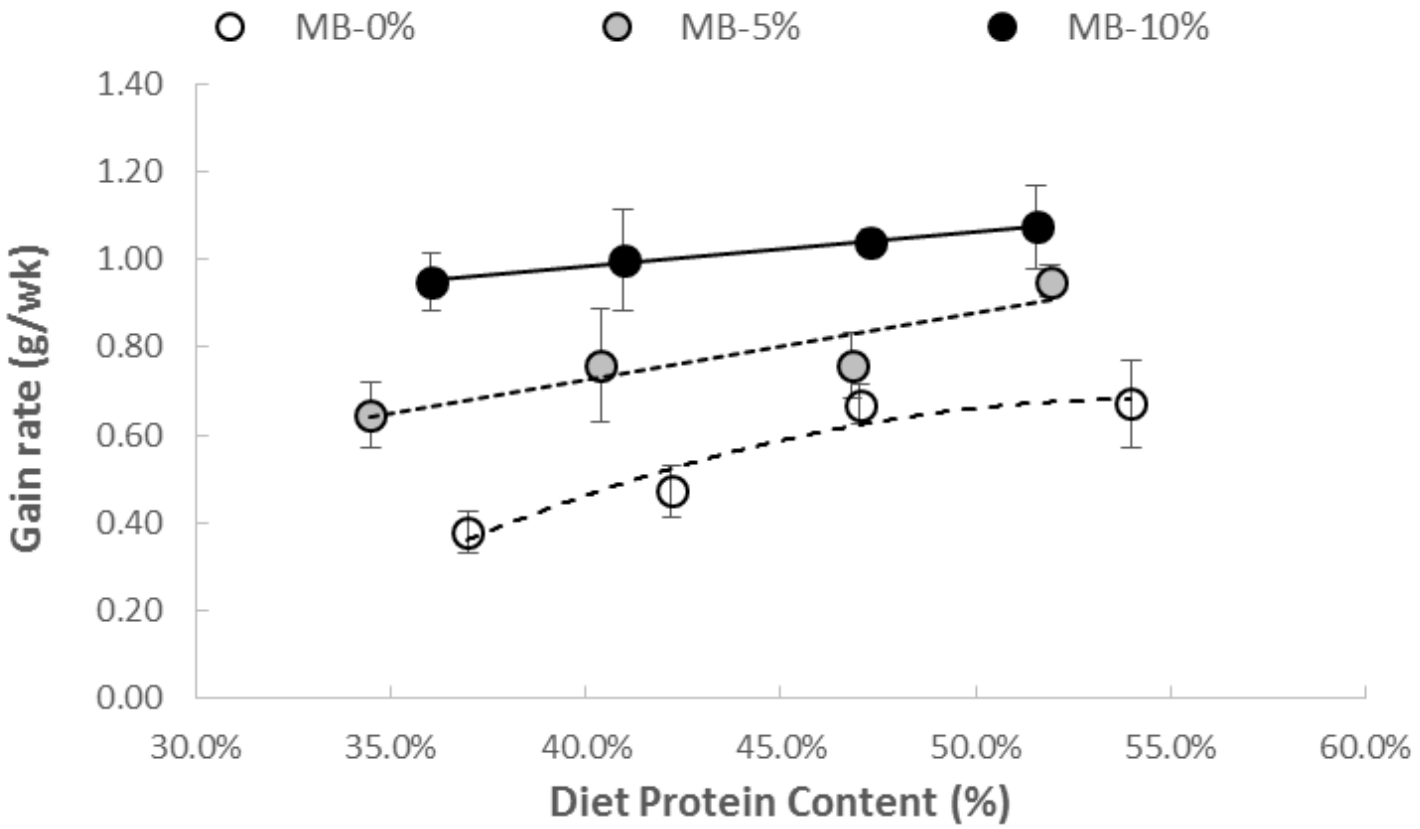

Figure 2. The relationship between feed intake and weight gain for each of the treatments (O : MB-0%; ● : MB-5%; ● : MB-10%). Shown is the regression line fitting for all treatments combined which was defined as; $y = 0.406x - 0.4643$, $R^2 = 0.5762$.

.

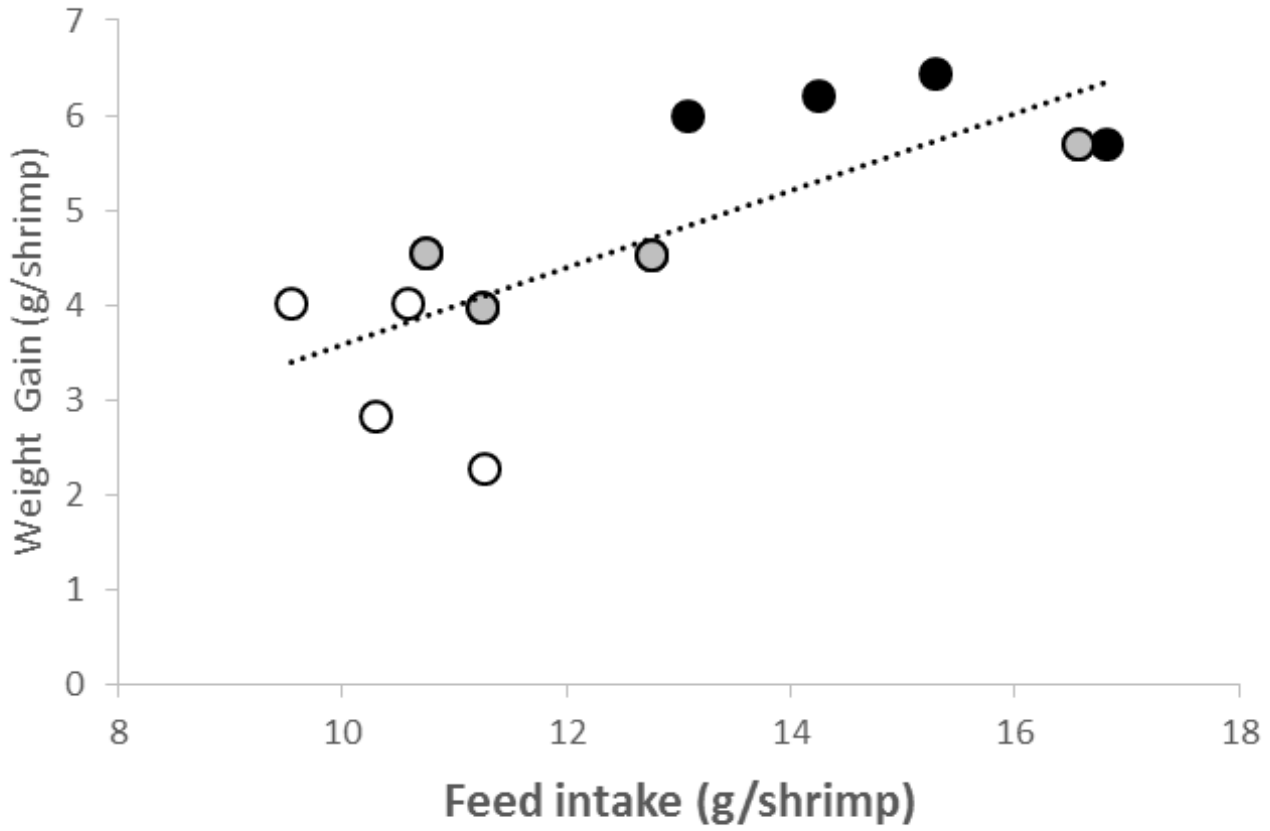

Figure 3. Effect of inclusion level of microbial biomasss combined with varying protein inclusion on the protein retention efficiency of shrimp. Diet protein content is percent as fed basis.