FINAL REPORT TO FRDC

Development of cost effective diets for prawn growout using local ingredients

Project No.: 90/67

DPI QUEENSLAND

DEPARTMENT OF PRIMARY INDUSTRIES

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2. PROJECT SUMMARY

Prawn aquaculture has been rapidly expanding in Australia, from an infant industry in 1986, to an annual production of 1100 tonnes. The industry uses approximately 2150 tonnes of pelleted feed annually.

Prawn feed is expensive (average $A1500/tonne) and represents up to 60% of the operating cost. The main reason for the high cost of feed is the need for expensive marine protein ingredients.

The black tiger prawn (*Penaeus monodon*) is the major prawn aquaculture species in Australia and the world. It's dietary protein requirement is around 40%. Dietary protein is utilised not only for protein requirements, but also as an energy source, since prawns digest proteins more efficiently than carbohydrates. The use of protein as an energy source is expensive, and the cost of diets may be reduced substantially if more of the prawn's energy requirements can be met from carbohydrates.

Australia is an agricultural country and produces large and regular quantities of terrestrial proteins, such as legume seeds, yeast and animal by-products. They have been very successfully used in cost effective diets for pigs and poultry.

Ineffective feeding practices also reduce efficient use of feed by prawns and increase the cost. Several factors, including feeding behaviour of prawns, stocking density, mortality, molting cycles and water quality determine which feeding methods are most appropriate. In South-east Asia where aquaculture is a traditional industry, efficient feeding methods are well established. However, in Australia methods are still being developed to match the needs of the prawn with the cost and availability of labour.

This project was established in 1990 to research least cost diet formulation for *P. monodon* using Australian ingredients and to develop effective feeding methods.

Initially the work concentrated on developing an effective diet for prawns. Approximately 45 feedstuffs were analysed for proximate composition, fatty acids, minerals and amino acids. This information was stored in a database (Paradox) and in the master file of the Least Cost Diet Programme (LCDP), designed by QDPI for pig and poultry nutrition. Later we modified LCDP for diet formulation for prawns.

Pipi (*Plebidonax* spp) was used as a standard diet in initial trials. However after evaluation of commercial prawn feeds, the most efficient one was selected as the standard diet. Five commercial diets were evaluated in terms of their effect on growth of prawns and food conversion ratio. The diets were analysed for chemical composition, and using this information four diets were formulated through LCDP. Trials showed that the diet coded B4-91 was the most efficient diet, and that it was as effective as the average commercial prawn feed.

B4-91 was used in ongoing studies to reduce the cost of the formulation. We used the information that we obtained from concurrent experiments on essential amino acid and protein requirements, enzyme application and dietary energy in prawn feeds to make B4-91 more cost effective.

Our initial work in developing B4-91 identified three key areas requiring nutritional research. These were:

(a) obtaining a measure of the energy value of feed ingredients for prawns;
(b) quantifying the requirements of prawns for certain key amino acids important for prawn growth; and
(c) investigating ways of improving energy availability in feeds, particularly those based on plant proteins.

The first of these needs was addressed by developing a net energy system for testing feed ingredients. Based on comparative slaughter procedures we have been able to quantify the net energy value to prawns of a number of feed ingredients. The second was addressed by initiating a series of experiments to measure the growth response of prawns to various levels of individual amino acids. The third need was investigated by looking at the effects of enzyme additions to feed on the digestion and growth of prawns. Enzyme application to canola based diets resulted in a 28% increase in weight gain, indicating that prawns grow better if the digestibility of the diet is improved. The higher digestibility provided more non-protein energy and spared protein for growth. A similar result was obtained by 10% inclusion of sucrose, a highly digestible carbohydrate, as a non-protein energy source.

Our findings indicate that, inclusion of an exogenous enzyme or sucrose (sugar) can:

- improve the efficiency of prawn diets; and
- reduce the dietary protein level required by prawn without reducing the efficiency of diets.

Both results suggest a substantial reduction in feed cost. More research is necessary to identify the precise enzyme requirements for the digestion of various feed ingredients.

We have assessed several feeding methods used throughout South-east Asia. The method which is commonly practised in Thailand seemed to be the most efficient method. This involves using feed trays to check food consumption. Thai farmers obtain around 1.3 to 1.7 food conversion ratios by using this method. An extension officer from Bribie Island Aquaculture Research Centre (BIARC) attended a workshop on feeding methods and pond management in Thailand. After the return of the officer details of the method were distributed to all prawn farmers in Australia. Today some farmers are successfully using the method.

Our investigations demonstrates the feasibility of formulating cost effective prawn diets from local ingredients. Our results have shown the importance of using carbohydrate sources to meet the energy needs of prawns, and the potential to enhance digestibility of some carbohydrates through the use of enzymes. It appears that there is an optimum mixture of carbohydrate types, sugar and starch, for prawns. A quantification of the energy value (net energy) of various feed ingredients for prawns is essential.

3. BACKGROUND

Prawn aquaculture is the fastest growing aquaculture industry in Australia. Between 1989 and 1992 the production increased 54% while the two biggest aquaculture groups, oyster and finfish have increased their production only 32% and 5% respectively. In 1985 the total prawn production from aquaculture was only 28 tonnes (Treadwell *et al.*, 1992). It is estimated that the annual production will reach 1100 tonnes in the 1992/93 season (Figure 1).

Although there has been a rapid increase in prawn aquaculture, annual production is currently less than 1300 tonnes which is below that predicted for the 1989/90 season (Hardman *et al.*, 1991). There are several factors that affect prawn production in Australia. One of the main factors is the high production cost which limits profitability, secondly the domestic market is the only market for the cultured black tiger prawn *P. monodon* which represents more than 95% of the cultured prawn species in Australia. There is no overseas market for this prawn produced in Australia due to the high production costs.

Hardman *et al.* (1991) estimated that up to 60% of the operating cost in prawn aquaculture was the cost of feed. Feed with 40% crude protein costs between $A1300 to $A1800/tonne. By comparison feed of the same crude protein level costs only $A800/tonne to farmers in the USA (Rosenberry, 1991).

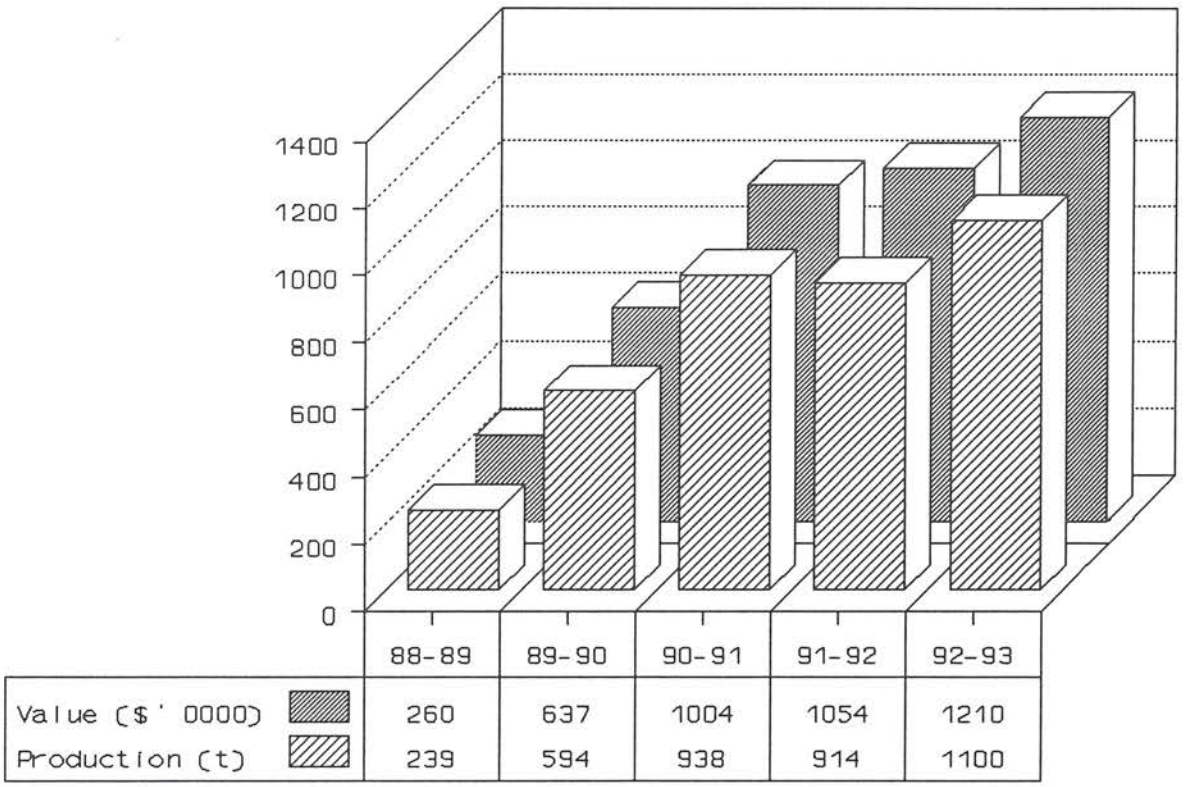

Figure 1. Prawn production and its market value in Australia (ABARE, 1991 and 1992; Lobegeiger and Barlow, 1992; Treadwell *et al.*, 1992).

The industry used about 2150 tonnes of feed in 1992/93. The majority of the feed (75%) was imported. The rest was manufactured in Australia using ingredients, 75% of which were imported.

Prawn feed heavily depends on high quality, but expensive, marine protein sources. Fish meal and squid meal are the main protein sources and they cost around $A1100/tonne and $A5000/tonne respectively. The black tiger prawn requires 40% crude protein (Alava and Lin, 1983; Shiau *et al.*, 1991). However all dietary protein is not utilised for protein requirement. Some is utilised as energy source, since prawns digest proteins more efficiently than carbohydrates (Alayama and Dominy, 1989). To reduce the cost of prawn diet carbohydrates should be used as energy sources rather than proteins.

Australia, produces large and reliable quantities of terrestrial protein and carbohydrate sources. They are cheaper than marine protein sources. The pig and poultry industries successfully use these terrestrial feedstuffs to formulate cost effective diets. However, direct use of these feedstuffs in prawn diets could be limited due to their low digestibility and negative effect on the palatability and chemo-attractiveness of the prawn diet. The nutritive value of terrestrial feedstuffs for poultry has been enhanced by the inclusion of enzymes in the diet (Marquardt, 1993). Similarly, it was reported that addition of chemo-attractants to pelletised feeds for crustacea may increase ingestion rate and improve growth, survival and food conversion (Heinen, 1980).

Protein sparing by supplying dietary energy through non-protein energy sources is not enough to promote optimum growth. Prawns do not have a requirement for protein but rather for essential amino acids. The balance of essential amino acid in diets is most important for maintenance and growth of prawns. Kanazawa (1993) reported that ten amino acids, arginine, methionine, valine, threonine, isoleucine, leucine, lysine, histidine, phenylalanine and tryptophan were essential for penaeid prawns. However quantitative requirements of these amino acids are not known. It is necessary to determine the quantitative requirements for individual essential amino acids to formulate more efficient diets and to spare proteins in prawn feeds.

Effective feeding practices are economically very important since it is very easy to waste significant amounts of feed in ponds. Prawn feeding habits, difficulties of estimating survival due to pond conditions, supplementary feed through natural productivity and environmental conditions such as water temperature, are factors that affect the feeding levels. Although some research was carried out on feeding habits of prawns (McTigue and Feller, 1989; Kuttyamma, 1974) and feeding methods (Villegas *et al.*, 1980; Murtaugh, 1984) there is no published information on estimation of feed levels in prawn ponds. However in South-east Asia where aquaculture is a traditional industry, efficient feeding methods are well developed through practical experience.

Development of least cost diet formulations and efficient feeding methods are directly for the benefit of the Australian prawn aquaculture industry. Annual feed consumption in 1992/93 season was valued around $A32 million. The average food conversion ratio (FCR) was 1.9. In South-east Asia FCR is as low as 1.4, mainly due to well established feeding practices. If FCR could be reduced by 0.1 in Australia, the present saving to farmers would be $A165 000. By reducing FCR to 1.4, as achieved in South-east Asia, would presently help farmers to save $A915 000. Similarly reducing the cost of feed by $A100 from $A1500/tonne to $A1400/tonne would create a saving of $A215 000 per year.

4. PROJECT OBJECTIVES

(i) To develop least cost diet formulations for prawn growout from local ingredients, with particular emphasis on protein sources.

(ii) To maximise the efficiency with which protein is used, with emphasis on amino acid requirements and energy balance in diet.

(iii) To develop practical feeding systems which ensure efficient use of feed.

5. TECHNICAL INTRODUCTION

Successful least cost diet formulations depend on knowledge of:

- Nutritional requirements of target species.
- Composition and availability of nutrients in feed ingredients.
- Availability of locally produced feed ingredients.
- Feed processing techniques.

Information on quantitative and qualitative requirements of nutrients for target species is the key point in establishing a master file for least cost diet formulations. Requirements of *P. monodon* for nutrients such as fatty acids, and some minerals and vitamins have been established (Kanazawa, 1981; Chuang, 1990; Kanazawa, 1993). However there is no information on dietary energy and quantitative essential amino acid requirements.

The main problem in studies of essential amino acid requirements is the difficulties of using crystalline amino acids. Dose-response method is the most recognised method for this kind of work and was commonly used in pig and poultry nutrition (Leveille and Fisher, 1958; Hurwitz *et al.*, 1978; Fuller *et al.*, 1989). However this method is not easy to apply to work with prawns due to:

1. high solubility of crystalline amino acids in water; and

2. occurrence of a diabetic-like effect in prawns at high inclusion levels due to rapid absorption of crystalline amino acids by prawns.

Various binding and coating methods were tested to reduce the leaching of crystalline amino acids in water (Teshima *et al.*, 1986). However none had been completely successful to date. A new method using minimum amounts of crystalline amino acids needs to be used to determine the quantitative essential amino acid requirements of prawns.

Biological measurements of feed ingredients must also be included in diet formulations for prawns (Everington *et al.*, 1990). Digestibility data (energy, protein etc) are the most common and the basic biological measurement. In fish and crustacean nutrition, the indirect digestibility method, using chromic oxide (Cr_2O_3) as an inert marker, is commonly used (Akiyama *et al.*, 1989; Law *et al.*, 1990; Catacutan, 1991). This method assumes that all the chromic oxide consumed will be excreted without being digested. Digestibility is calculated from the relative changes in chromic oxide percentage in the feed and faeces. Previous digestibility studies carried out using this method, however, did not consider the leaching in both faeces and feed and loss of feed due to the feeding behaviour of prawns. In contrast to finfish which ingest their food in tanks within seconds, it takes up to three hours for prawns to consume their feed. In water, prawn feed looses considerable amounts of dry matter due to leaching and breakdown. To obtain accurate digestibility values of feedstuffs for prawn feeds, a more reliable method has to be developed.

The loss of feed in water also affects feed intake measurements. An accurate estimate of feed intake enables better indices of feed quality to be measured including feed conversion and protein efficiency ratios. A method to determine water stability of pelleted feeds is a prerequisite for accurate measurement of feed intake. Water stability of aquaculture feeds was tested by simply leaving a certain amount of pellets in water (Lim and Destajo, 1979; Viola *et al.*, 1985; Ali, 1988; Dominy and Lim, 1991). However this method does not consider the effect of water movement due to the water current, aeration and prawn activity. A more accurate estimation of water stability depends on the development of a reliable method which can produce repeatable results.

Feed intake may also be affected by the palatability and attractiveness of the feed. There are several studies on the effect of various chemical compounds on the feeding behaviour of marine crustaceans (McLeese, 1970; Hindley, 1975; Carr, 1978; Harpaz *et al.*, 1987). However there is little published information on chemo-attractants for the black tiger prawn *P. monodon*. Akiyama and Dominy (1989) reported that some attractant components such as free amino acids and possible small peptides were available in marine protein sources such as fish and squid meals. However use of these feedstuffs are expensive and their substitution with cheaper protein sources such as those of plant origin reduces the palatability and attractiveness of feeds. Therefore a specially formulated cheap and effective attractant must be prepared.

Feed ingredients used in diets for prawns should be available all year round in sustainable amounts and at low prices. A constant change in quality and quantity of feed ingredients cause problems in diet formulation, manufacturing process and cost fluctuations. In this project we tried to select feed ingredients that were produced in large quantities in Australia.

Although feed processing techniques affect the quality of feeds, this project only involved feed formulations and feeding methods.

An accurate feeding method is an important part of the pond management during the growout period of prawn aquaculture. Over feeding in ponds deteriorates the pond environment and results in high mortalities as well as increase the cost of production. On the other hand underfeeding slows down the growth and in extreme conditions can increase mortality. An appropriate feeding level is determined by:

- number of prawns in pond;
- water temperature;
- size of the prawn; and
- molt stage.

There has been no scientific study on determining appropriate feeding rates for prawns in ponds. However in South-east Asia, prawn farmers successfully estimate feeding levels through their experience in traditional aquaculture practices. However aquaculture, in general, is very new in Australia and feeding rate must be established by either developing a new feeding method suitable to Australian conditions or adopting the methods used in South-east Asia to Australian situations.

6. EXPERIMENTAL STUDIES

The project was carried out in various stages.

- Construction of bioassay systems.
- Preliminary trials (for digestibility, establishing methods for feed intake calculations, estimating time period for the acclimatisation before trials etc).
- Feeding trials.
- Search for practical feeding systems in ponds.

6.1 Construction of Bioassay Systems

Four bioassay systems were constructed to carry out various feeding trials. Three of these consisted of 20 x 250 litre black polyethylene flat bottom tanks which were placed in temperature controlled rooms. These systems utilised pre-filtered (10 μ) fresh seawater which was heated to 28°C in a header tank and delivered by gravity to experimental tanks at 0.175 litres/minute, giving a daily exchange of 100%. Aeration was provided to individual tanks using air stones (Figure 2).

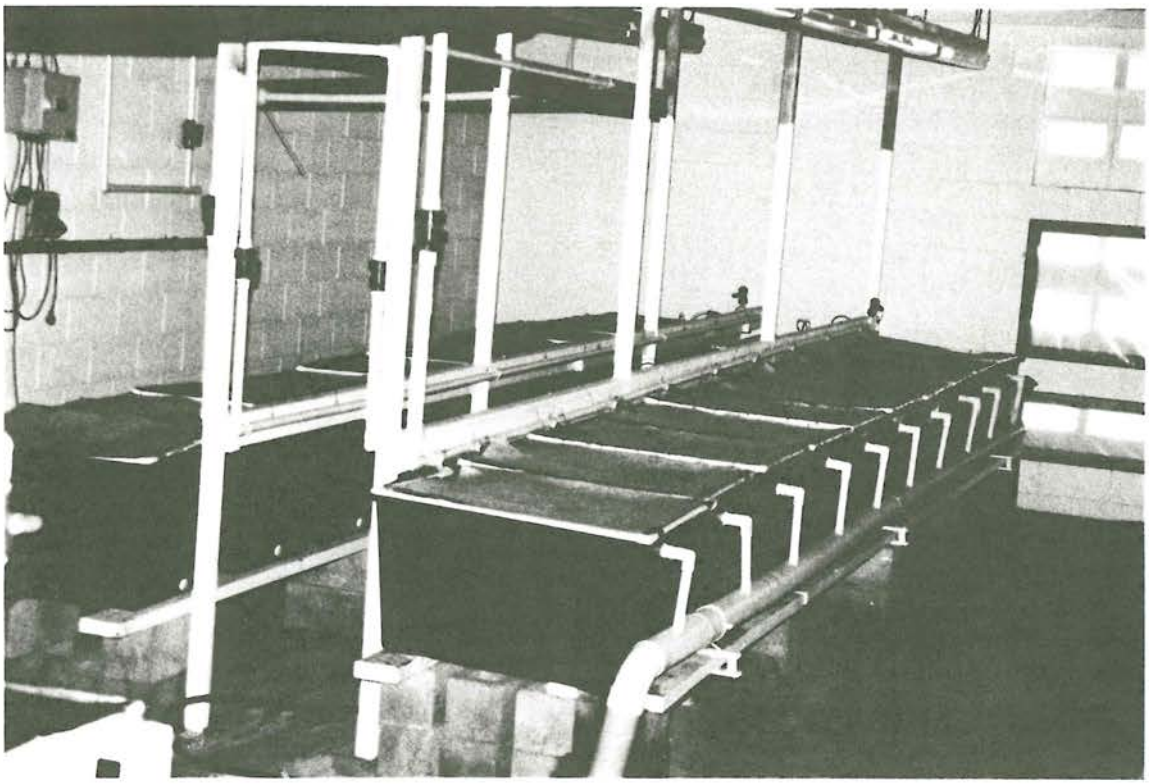

Figure 2. The bioassay system that consists of 250 litre black polyethylene tanks.

The rooms were equipped with air-conditioners for controlling the room temperature and computerised lighting systems for providing controlled day and night cycles.

The fourth bioassay system was established with 40 x 60 litre rectangular fibreglass tanks. Ten of these tanks were placed in a 300 cm x 50 cm x 40 cm fibreglass water bath to maintain a constant water temperature.

In this system, the seawater was also pre-aerated in a header tank and delivered to each experimental tank by gravity. The flow rate was 0.125 litres/minute, allowing 300% water exchange daily. The room was temperature and light controlled.

6.2 Preliminary Trials

Although prawn aquaculture has been practiced over the last 30 years, there is still only a rudimentary understanding of prawn nutrition. At the beginning of this project we identified some of these problems as being very important to the overall outcome of the project. These were:

- determination of the length of the acclimatisation period before a feeding trial;
- effect of chemo-attractants on prawns feeding behaviour;
- effects of non-digestible dietary compounds on feed intake;
- water salinity and dietary protein level and feed intake relationship;
- measuring feed residues and faeces output for prawns in aquaria; and
- development of a system for water stability test on prawn feeds.

6.2.1 Digestibility trials

During the first year of the project the main work on digestibility focussed on developing a bioassay system that allowed faeces to be collected without causing any stress to animals. A prototype of a digestibility system was developed using 5 litre (15 cm H x 20 cm ϕ) polyethylene cylinders (Figure 3).

These cylinders had a conical bottom with a valve at the end of it. Individual cages made of 6 mm x 6 mm mesh polyethylene were put into the cylinders to separate prawns from the conical compartment and to allow faeces to go through to the collection valve. Some faeces were caught on the side of the conical bottom, which required a gentle sweeping inside to collect all faeces. Prior to collection, prawns were removed to another cell by transferring the whole inner cage. The cells had mesh lids and from the top half way down the wall of the cages had holes to let fresh and aerated seawater through. Six cells were placed in a 250 litre water bath and were resting on a rigid 12 mm x 12 mm platform. The bottom end of the conical part was pushed through a hole (ϕ 30 mm) and sealed with a grommet. The seawater was delivered to the water bath at a rate of 0.175 litres/minute and an air stone was provided in each water bath for aeration.

Several digestibility trials carried out using this system failed, due to:

- stress of mesh bottom and daily handling on prawns;

- deterioration of faeces due to static water column in the conical bottom of the cells, and high water temperature (28°C). This deterioration occurred on the second day of the collection, even when faeces were collected twice daily; and
- problems in offering food to prawns on a mesh floor area.

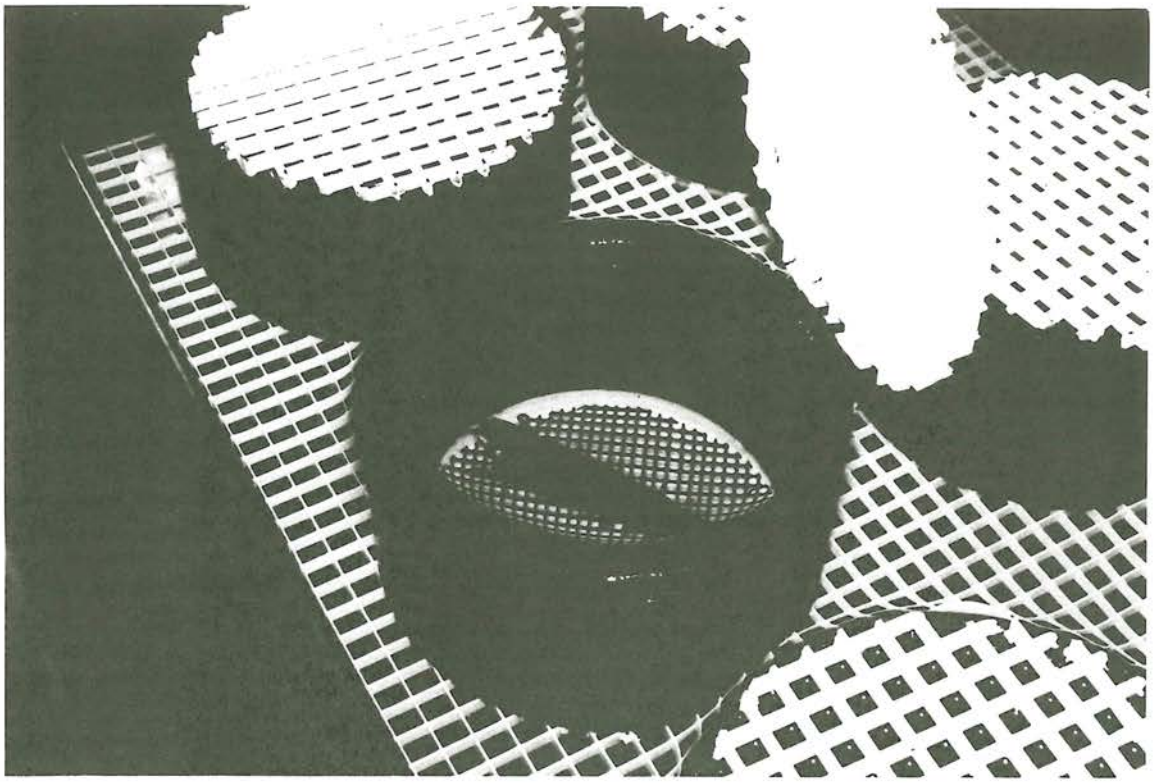

Figure 3. The bioassay system for digestibility studies.

This failure, in fact, helped us to decide on evaluating feed ingredients by estimating their net energy values rather than digestible energy. Use of net energy in feed formulations is more accurate as net energy, directly expresses the level of energy in a particular feed ingredient used for production. The net energy will be discussed in future chapters of this report.

6.2.2 Effect of feeding level and changing diet on food intake and digestibility in juvenile prawns *P. monodon* (estimation of the acclimatisation periods)

The measurement of food digestibility is essential in collecting data suitable for diet formulations and in comparing diets as sources of nutrients for growing prawns. In collecting these data it is necessary to know how quickly animals adapt to a new diet and what is the effect of level of food intake on digestion of nutrients. It is often recommended that at least five days be allowed for animals to adapt to the feed under test (De Silva, 1989), though much shorter periods are often used (Cliffor and Brick 1978; Smith *et al.*, 1985). The digestibility may also be affected by the level of food intake (Cho and Kaushik, 1985) and in comparing diets it may be necessary to have a common level of food intake.

This trial was carried out to determine the time required for *P. monodon* to adapt to a new food and the effect of food intake on diet digestibility.

Prawns were given fresh pipi muscle (*Donax deltoides*) at 5% of live weight for four weeks then fresh pipi was abruptly changed to the pelleted diet. This diet was given at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5% of live weight daily. Food was given in two approximately equal portions each day, at 8 am and 4 pm.

Prawns (*P. monodon*) of average live weight 4 g were maintained in the bioassay system with 250 litre tanks which was described earlier.

Ten prawns were allocated to each tank and there were two replicates. Beginning three days before the change to the pelleted diet faeces and food residues were collected by siphoning from the bottom of each tank twice daily, before feeding. The total sample was separated into feed residues and faeces by hand sorting. These samples were pooled for each two day period, dried and weighed. No adjustment was made for losses of soluble material from faeces and feed residues. The experiment was continued for 14 days (seven periods) after the introduction of pelleted feeds. The prawns were weighed before and after the 17 day measurement period.

Data were analysed by analysis of variance. Intakes are expressed as g DM/day for ten prawns.

Intake of pelleted feed was complete from day one at levels of feeding up to 3.0% live weight. Above this level of feeding animals took up to six days to consume most of the feed offered (Figure 4). Apparent dry matter digestibility was depressed in the first few days after the change of diet (Figure 5). At the low levels of feeding the depression was large, but variable, with apparent digestibility values for each two day period ranging from 20 to 70%. At higher feeding levels the depression in digestibility was less marked and dry matter digestibility stabilised after about eight days (Table 1).

After adaptation feed intake was closely correlated with level of feed offered (Table 1). Dry matter digestibility remained variable at the 0.5 and 1.0% level of intake, the standard deviation for these two treatments being 2.5 times that of other treatments. Above the 1.0% level of intake there was little difference in dry matter digestibility. Live weight gain was closely correlated with level of food intake ($r = 0.98$, $P < 0.01$). Survival was reduced at the low levels of feeding, with all deaths occurring between days two and seven after the change of diet. There was no difference in the frequency of molting between treatments (Table 1).

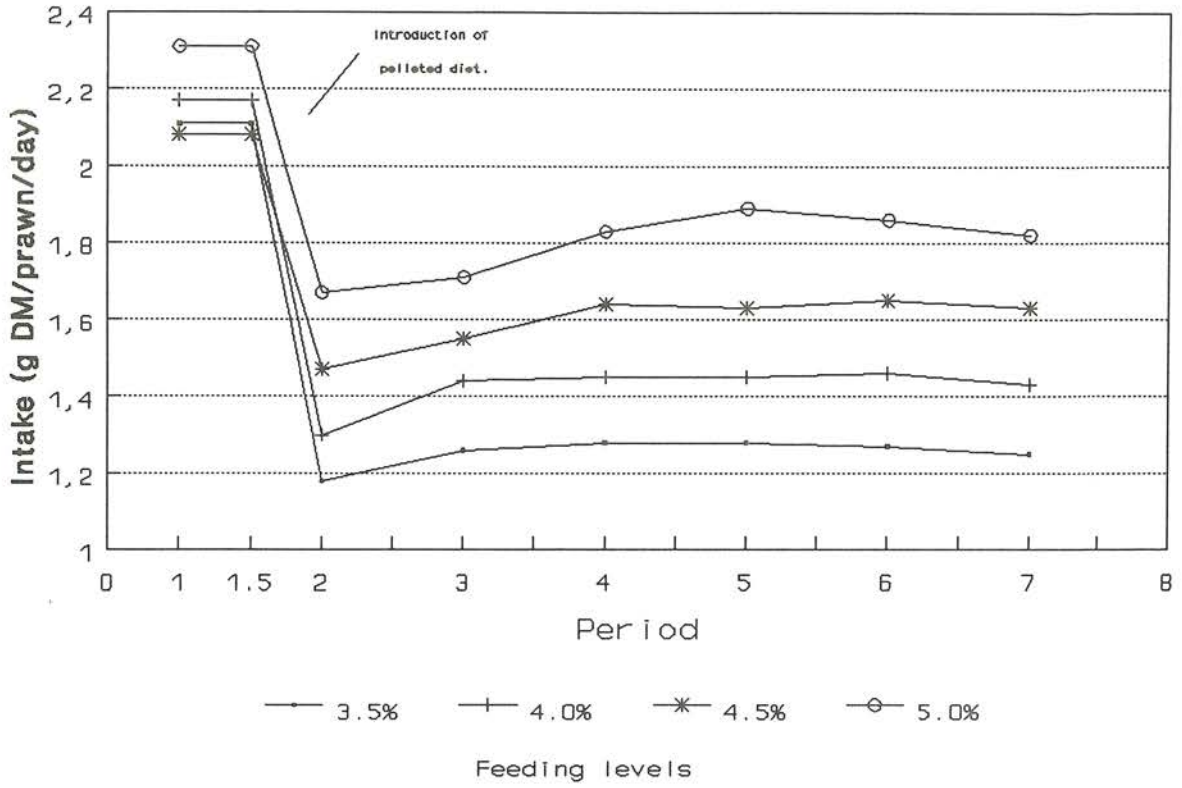

Figure 4. Adaptation in intake after the introduction of a pelleted diet.

Table 1. Effect of level of food intake on weight gain, digestibility, survival and molting of prawns. Intake and digestibility results are for the period 7 to 14 days after the diet change.

Measurement	Level of food offered (% live weight)										
	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5	5.0	LSD (5%)
Food intake (g DM/day)	0.16	0.35	0.51	0.67	0.90	1.06	1.27	1.44	1.64	1.85	0.05
Digestibility (% DM)	80.5	94.6	91.4	92.3	92.1	91.2	93.0	93.5	90.8	92.5	7.5
Live weight gain (g/prawn/week)	0.26	0.40	0.32	0.42	0.74	0.79	1.02	1.14	1.11	1.29	0.31
Survival (%)	80	85	100	95	95	100	100	100	100	100	-
Molting (No.)	2.5	1.0	4.0	4.0	4.0	2.5	4.5	4.0	7.5	5.5	6.5

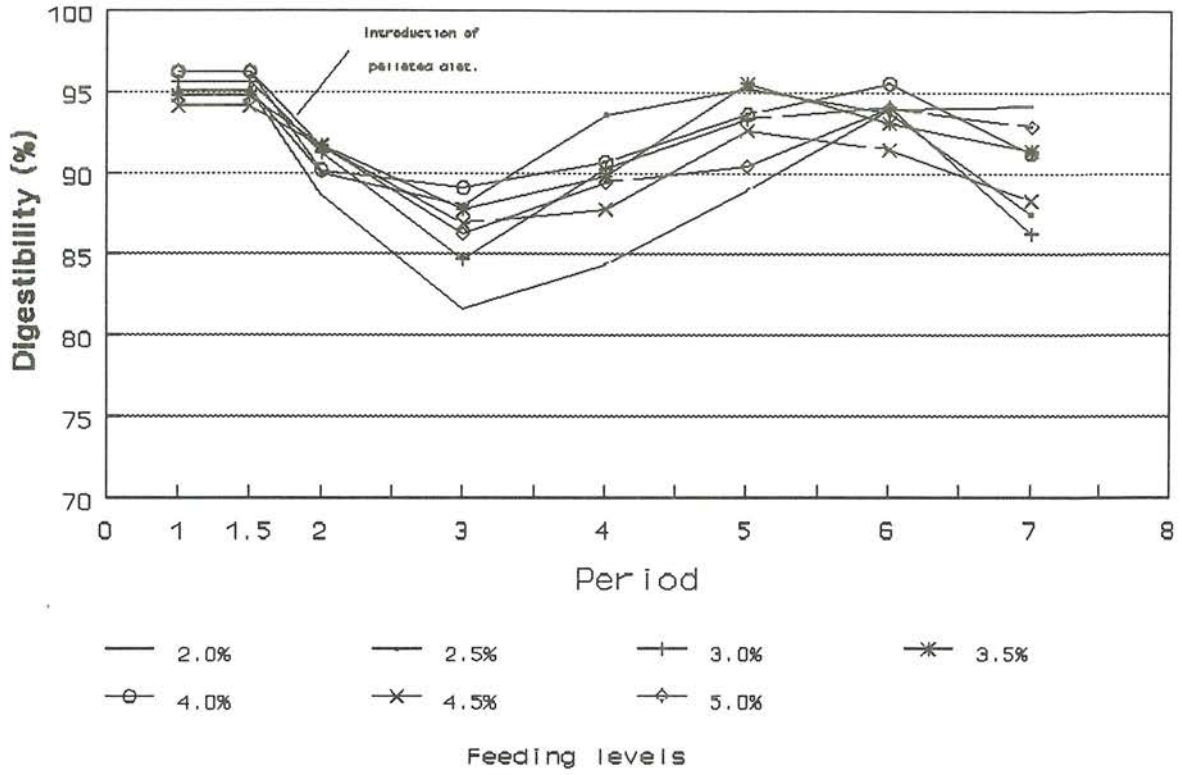

Figure 5. Adaptation of dry matter digestibility after the introduction of a pelleted diet.

6.2.3 Effect of chemo-attractant on prawn feeding behaviour

Optimal feeding regimes may result in reduced feed costs by minimising expenditure of metabolic energy of prawns. Attractive foods are located and consumed quickly, reducing losses of essential water soluble components from leaching (Meyers, 1987). Furthermore, the addition of chemo-attractants to pelletised feeds may increase ingestion rate and improve growth, survival and food conversion (Heinen, 1980).

The objective of this study was to investigate relative attractiveness of seven individual amino acids (alanine, arginine, glutamine, glycine, isoleucine, serine and taurine), betaine, adenosine 5'-monophosphate and an equimolar mixture of seven amino acids plus betaine to *P. monodon*. Laboratory observations of substrate probing by the chelate walking legs (chelipeds), antennular flicking rate and maxilliped activity of the prawn were used to measure the attractiveness of these components at seven different concentrations (10^{-1}M to 10^{-7}M). All individual amino acids and betaine resulted in higher rates of chilopod substrate probing (CSP) (Table 2), antennular flicking and maxilliped activity in *P. monodon* at high pipette concentrations ($> 10^{-2}$M). The poorest response was obtained in prawns which were exposed to adenosine 5'-monophosphate (AMP). Glutamine, betaine and taurine were the most effective single compounds tested and resulted in significantly higher activities ($P < 0.05$) in prawns at concentrations above 10^{-6}M (except for taurine at 10^{-2}M) than the control (seawater only).

Table 2. Average of the transformed ($\sqrt{\ + 0.5}$) CSP scores of *P. monodon* exposed to different chemicals at various concentrations.

Chemicals	Concentration (10^{-x} M)						
	1	2	3	4	5	6	7
Control (seawater)	0.68	0.68	0.68	0.68	0.68	0.68	0.68
Amino acid + betaine mixture	7.77	4.74	4.82	4.29	3.18	3.13	2.29
Alanine	3.90	3.59	2.45	2.88	1.13	-	-
Arginine	3.74	4.14	3.44	2.85	1.62	-	-
Betaine	6.59	5.60	3.08	3.89	3.07	1.64	-
Glutamine	8.07	4.14	4.71	2.76	2.81	2.71	1.77
Glycine	5.51	3.14	2.71	0.71	-	-	-
Isoleucine	4.80	4.90	2.83	3.36	1.64	-	-
Serine	3.61	3.19	2.87	1.81	-	-	-
Taurine	5.23	2.76	3.68	3.28	2.86	1.38	-
AMP	-	1.12	-	-	-	-	-
LSD (0.05)	2.56	2.32	2.31	1.96	2.06	1.91	1.62

The equimolar amino acid plus betaine mixture was also found to be an effective stimulant to *P. monodon* at concentrations above 10^{-6}M and continued to elicit search responses in prawns at a concentration lower than single compounds. The strong response of *P. monodon* to the mixture of amino acids and betaine is consistent with synergistic interactions of the mixtures.

The information obtained from this trial helped us to develop an attractant mixture. The attractant mixture was based on enriching mullet extract with crystalline amino acids and fish oil. This mixture is simply sprayed on to dry feed pellets.

Whole mullet was minced two to three times and later thoroughly mixed with water (1:1, v:v) in a blender. This process was repeated a second time using ethanol after extracting the water from the first mixture. This way water and fat soluble constituents of mullet were extracted. Two fractions were mixed with each other thoroughly and then crystalline amino acids and fish oil were added to it. The levels of individual amino acids and fish oil was 0.1 g of each per litre mixture. The mixture was kept frozen in plastic bottles until it was needed.

6.2.4 Effect of non-digestible dietary compounds on feed intake

The replacement of fishmeal, in growout diets for the black tiger prawn (*P. monodon*) with protein meals of plant origin will result in an increase in crude fibre levels in these diets. This is due to the fibre that is associated with the protein meal. Fibre can have both positive and negative effects on digestibility and food intake. The aim of this experiment was to determine the effect of fibre inclusion in a balanced diet on growth, digestibility and food intake, and to establish optimum and maximal levels for practical diet formulation. Fibre could affect food intake by diluting digestible nutrients so that the animal must consume more to fulfil its requirements or by adding bulk so that intake is depressed due to a gut fill effect.

A low fibre basal diet was diluted by substituting proportionally with indigestible material, α-cellulose (fibre) or ground pipi shell (ash). The diet was substituted at 2, 4, 8, 16, 32%. These treatments were replicated twice and allocated randomly. All diets were marked with Ytterbium acetate (Yb) at a rate of 40 mg/kg.

Eight prawns of 1.73 ± 0.35 g were placed in each tank of a flow-through aquaria system (20 x 250 litre tanks) as described previously. These animals were fed *ad libitum* four times daily at 0800, 1200, 1700 and 2200 hours. Residual food and faeces was removed from the tanks immediately prior to the 0800 and 1700 feedings. The experiment commenced following a seven day acclimation period.

The trial was conducted for six weeks and divided into six collection periods of five days, with two days between these periods. During these periods all residual food and faeces were collected and frozen after a subsample of faeces was collected for Yb analysis. During periods three and five, all residue was totally sorted into components of uneaten food and faeces before being frozen. At the completion of the trial all residues were filtered through Whatman No. 52 papers, dried and weighed before being analysed for Yb.

Upon completion of the trial all animals were weighed and slaughtered for chemical analysis.

Table 3. Growth, digestibility and food intake of prawns fed experimental diets.

Diluent	Substitution level (%)	DMD (%)	Growth (g/prawn)	Food intake (g/prawn/d)
Ash	2	84.47 ± 2.80	3.02	0.1725 ± 0.0447
	4	83.35 ± 1.62	2.55	0.1948 ± 0.0470
	8	76.06 ± 2.81	3.03	0.2155 ± 0.0673
	16	66.13 ± 2.92	2.78	0.1816 ± 0.0332
	32	51.12 ± 3.40	3.39	0.1992 ± 0.0500
Fibre	2	85.76 ± 2.19	2.72	0.1634 ± 0.0322
	4	84.01 ± 3.14	2.55	0.1676 ± 0.0285
	8	77.13 ± 2.39	3.08	0.1835 ± 0.0403
	16	66.84 ± 2.00	2.80	0.1735 ± 0.0359
	32	48.50 ± 4.22	2.36	0.1694 ± 0.0322

Growth over the trial period was not affected by the incorporation of either ash or fibre into the diets (Table 3). Dry matter digestibility (DMD) decreased as the level of ash and fibre increased. There does not appear to be any difference in response to fibre compared to ash. Intake was not affected by level of inclusion of ash or fibre. These results suggest that juvenile *P. monodon* are able to adapt to fibre levels of up to 32% of the diet.

6.2.5 The effect of salinity on protein requirement, food conversion ratio and growth of the juvenile prawn *P. monodon*

Knowledge of the protein requirement of the black tiger prawn in different environmental conditions is essential in developing a feed which provides good growth and survival in intensive aquaculture. Previous studies have aimed at determining the dietary requirements of penaeids under controlled conditions and have neglected the

interaction between environmental factors and dietary requirements. Salinity is one of the more important environmental factors that could affect nutritional requirements of prawns. It affects the osmo-regulatory mechanisms (Potts and Parry, 1964; Lockwood and Andrews, 1969; Baldwin and Krishner, 1976), oxygen consumption (Bishop *et al.*, 1980), and growth (Navas and Sebastian, 1989) in crustaceans and subsequently can affect feed utilisation.

Salinity in commercial prawn ponds fluctuates all year round in Northern Queensland where half of the Australian annual production is produced. The extreme climatic conditions such as extended periods of hot weather or continuous rain cause large fluctuations in salinity. This trial was carried out to determine the effect of salinity on dietary protein requirement, growth, survival, and food conversion ratio of prawns.

A factorial trial was conducted using juvenile *P. monodon* of an average weight of 4.51 g. The experimental system consisted of eight 250 litre tanks with recirculating water and stocking density was 10 prawns/tank. Overflow from each tank passed down a biological filter filled with polyethylene bio-balls (ϕ 50 mm).

A three hour immersion heater in the reservoir tanks raised the water temperature to 28 ± 1°C. Water was pumped to the experimental tanks by a power head aquarium pump, each tank received filtered water at the rate of 600 ml/min. Total water exchange occurred every four hours.

Experimental tanks were divided into two blocks and each block was supplied with seawater at one of two salinities (35 ppt and 40 ppt). Each block had an independent biofilter system.

Following a one week acclimatisation period, prawns were fed either of two diets (crude protein content of 35 or 55%) for 60 days. Dietary protein had a greater affect on growth, FCR and PER than water salinity. 35 ppt salinity resulted in a significantly better growth than 40 ppt. However no difference was obtained in survival or digestibility (Table 4).

Table 4. Response of juvenile *P. monodon*, fed diets with different crude protein levels and reared at two different water salinities*.

Treatment**	Weight gain (%)	Survival (%)	FCR	PER	Digestibility (%)
A	102.3^{a}	95.0^{a}	3.88^{a}	1.42^{a}	96.82^{a}
B	90.6^{b}	100.0^{a}	5.11^{ab}	2.81^{b}	96.80^{a}
C	96.3^{a}	100.0^{a}	4.76^{ab}	1.75^{a}	96.70^{a}
D	79.9^{c}	90.0^{a}	6.19^{b}	3.20^{b}	96.60^{a}

* Treatment means with the same superscript are not significantly different ($P > 0.05$).

** A = 35% crude protein at 35 ppt salinity, B = 55% crude protein at 35 ppt salinity, C = 35% crude protein at 40 ppt salinity and D = 55% crude protein at 40 ppt salinity.

The results showed an interaction between levels of protein and salinity. The combined effects of low levels of each resulted in the best growth, FCR and PER. As salinity level was elevated food intake increased but growth rate was maintained at a similar level. It was suggested that the greatest economic benefit the dietary protein level should be kept low in low salinities and feeding levels should be increased as the water salinity rises.

6.2.6 Measuring feed residues and faeces output for prawns in aquaria

It is important in nutritional experiments to have an accurate measure of uneaten feed (residues) and faeces output in order to calculate food intake and digestibility. In aquaria the food residues and faeces are mixed together on the bottom of the tank, and must be physically separated. An efficient system of subsampling would reduce the time needed for this sorting.

During a longer term experiment comparing four commercial diets for *P. monodon* the opportunity was taken to compare subsampling routines for their efficiency in measuring total faeces output and feed residues. Subsampling treatments were confounded with the four replicates in the original trial design. Replicates contained prawns of 5 g average weight.

Food residues and faeces on the bottom of aquaria were siphoned off into buckets twice daily. After solid material settled water was poured off to reduce total daily volume to 400 ml. The sample was then treated in four ways, namely (a) the entire sample sorted daily into feed residues and faeces, (b) a 100 ml subsample sorted daily, (c) the entire sample frozen until the end of the collection period, defrosted, and then treated as in treatment (a), and (d) as for (c) but with a 25% subsample being taken before sorting as in (b). The subsample was taken by swirling the solid material with the associated water in a beaker, then quickly pouring into a 100 ml flask. Prawns had been adapted to the experimental diets over three weeks with feed residue and faeces collections being made over a period of 11 days.

Separation was made using pipettes and the sorted samples dried at 80°C for 24 hours.

Data were analysed by analysis of variance.

Taking a 25% subsample daily for sorting (treatment d) gave the same result as sorting the entire sample daily (Table 5). Estimates of uneaten food, food intake, faeces output and digestibility were all similar. After freezing and rethawing, sorting the total sample underestimated the amounts of food residues and faeces output. This could be due to the fact that after freezing, thawing, mixing, decanting and sorting a greater percentage of dry matter is lost in the water fraction than when the sample was sorted daily. It could be expected that there would be considerable breakdown of food and faeces after this treatment.

Table 5. Effects of subsampling and storage on the estimates of food residues and faeces in aquaria.

	Treatment			
	Daily sorting		Sorting after 10 days collection	
	Total (a)	0.25 subsample (b)	Total (c)	0.25 subsample (d)
Food offered (g DM)	52.63	52.69	52.57	52.54
Food residues (g DM)	18.05^{ab}	15.69^{a}	16.18^{a}	19.59^{b}
Food intake (g DM)	34.58^{ab}	37.00^{a}	36.39^{a}	32.95^{b}
Faeces (g DM)	3.83^{b}	3.45^{ab}	2.88^{a}	3.95^{b}
Dry matter digestibility	0.889^{bc}	0.906^{ab}	0.918^{a}	0.881^{c}

By contrast, sorting of the 25% subsample after freezing and thawing gave a similar result to sorting the entire sample daily. There was a tendency for the subsampling procedure to give higher estimates of food residues and faeces output when done after freezing and thawing than when done daily. This may be due to a tendency to select solid material or large particles in subsampling or sorting.

It would appear that subsampling after freezing and thawing gives a useful estimate of daily total sorting, and saves times and effort during a feeding trial.

6.2.7 Development of water stability test system

Prawns are slow eaters and there is a certain time period for some pellets to stay submerged in the water. This time period may change depending on the amount of feed presented to prawns, and their appetite. However it is a fact that pellets get effected by the water during that time. Initially some nutrients leach to water and gradually pellets swell with water and breakdown, resulting in loss of some solid components as well as water soluble fraction. Determination of losses from pellets in water helps to estimate accurate food intake which is directly related to calculation of other feed quality indicators such as food conversion ratio and protein efficiency ratio.

In this work we tried to develop a water stability test system which would give us repeatable results under controlled situations and that simulates the conditions in experimental tanks.

A water bath (HAAKE SWB20) was used as the stability test system. A platform with 15 cells (ϕ 65 mm) was attached to the shaker of the bath. 150 cc cylindrical clear plastic jars were used as cells. 50 cc containers with 200 μ mesh on the bottom were used to hold feed pellets (Figure 6). These small containers were placed in cells which were previously filled with seawater preheated to 20°C. The agitator was adjusted to 30 strokes per minute. One stroke was the movement of the platform from one end of the bath to another (ca 8 cm). The water bath was filled with freshwater (28°C) to protect the bath from corrosion. Three cells were taken out at the end of the first and

the second half hour periods and every hour after that. Pellets were filtered, dried and dry matter loss was calculated.

Water stability results obtained from three replications are given for some commercial and laboratory diets in Table 6. This system was used for every diet used in feeding trials.

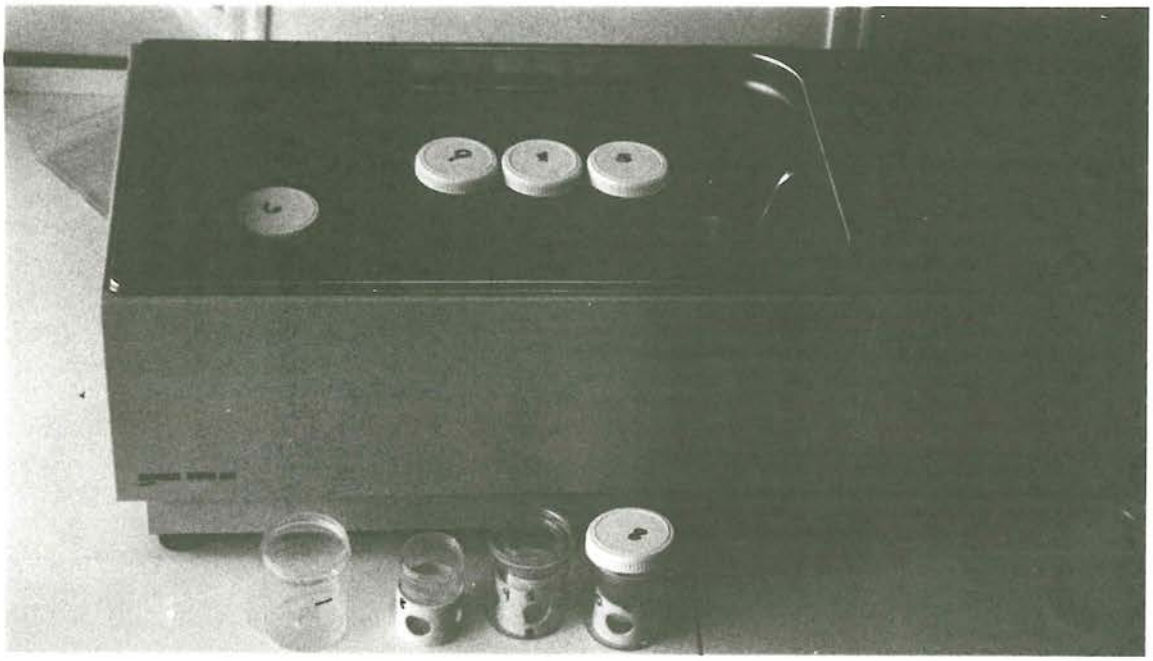

Figure 6. The water stability test system.

Table 6. Dry matter loss of some commercial and laboratory diets for prawns.

Time (min)	Commercial diets			Test diets	
	CP	Fong-Leng	Chin-da	B4-91	B4-91(1)
30	8.76 ± 0.84	11.34 ± 0.20	5.42 ± 0.57	4.22 ± 0.88	7.04 ± 0.97
60	11.06 ± 0.36	12.69 ± 0.33	6.78 ± 0.37	7.71 ± 0.92	8.16 ± 0.62
120	12.67 ± 0.25	13.93 ± 0.34	7.84 ± 0.65	11.64 ± 3.44	12.71 ± 0.62
180	13.03 ± 0.78	15.10 ± 0.71	9.94 ± 0.86	14.11 ± 0.71	15.91 ± 0.52
240	13.53 ± 0.21	15.50 ± 0.69	9.48 ± 0.75	15.16 ± 0.60	16.95 ± 0.26

6.3 Feeding Trials

During the three year period of the project various feeding trials were conducted. These feeding trials were designed to:

- evaluate commercial prawn feeds;
- evaluate Australian feed ingredients;
- develop our own feed formulations;
- investigate the protein/energy ratio; and
- determine the dietary essential amino acid requirement.

6.3.1 Evaluation of commercial prawn feeds

Five well known commercial prawn feeds were chemically analysed and their effects on growth and survival of prawns, food conversion ratios, body nutrient retention and feed intake were compared. This trial was carried out with the objective of developing a reference diet at BIARC by simulating the nutritional balance of the best commercial diet.

The two trials were conducted in the temperature controlled rooms using 250 litre polyethylene tanks. Prawn growout feeds were stored in a cool, dry environment and used within two months of purchase. These feeds represented the major sources of feed used for prawn growout in Australia during 1990.

In the first trial prawn (*P. monodon*) of average live weight 32 g were acclimated to the experimental tanks for two weeks, then weighed and grouped on the basis of live weight into four blocks, each containing five groups of six prawns. Within blocks groups were then randomly allocated to treatment. The treatments were four commercial diets (Aquafeed grower 1, AQ1; Aquafeed grower 2, AQ2; CP and President) and a natural diet pipi (*Plebidonani* sp.). The trial was carried out for eight weeks.

In the second trial prawns of average live weight 1.5 g were similarly acclimated to the same tanks, weighed, grouped into four blocks, each containing six groups of ten prawns, and allocated to treatments. The sixth group of prawns was frozen for later analysis of body composition. There were ten prawns in each tank and the trial was conducted for six weeks.

In each period prawns were individually weighed after towelling dry, at fortnightly intervals in Trial 1 and at ten day intervals in trial 2. Dead animals were replaced with animals of similar live weight throughout the trials. At the completion of trial 2 all animals were freeze-dried and ground for analysis of gross energy, crude protein, crude lipid and ash.

Diets were fed to approximately 110% of the previous days consumption at 0830 and 1630 hours. Before each feeding, food residues and faeces were siphoned from tanks. During the initial and final two weeks of each trial food residues and faeces were hand sorted daily, dried at 60°C for 24 hours and weighed.

Initial and final prawn samples, all diets and pipi were analysed for proximate chemical compositions. Amino acid and fatty acid analysis were also carried out on all diets and pipi.

Digestibility of dry matter (DMD) and crude protein (DCP) were calculated as the difference between feed intake and faeces output, expressed as a percentage of feed intake. Digestible dry matter intake (DDMI) was calculated as dry matter intake x DMD.

Animal performance data were analysed by analysis of variance using the mean value for each tanks as a single observation and treatment means compared using the least

significant difference at P = 0.05. Data for intake and digestibility were analysed as a split plot on time.

There was little variation in the crude protein content of the commercial diets, averaging 43.6% (Table 7). The calculated essential amino acid index was high for all diets (0.94 - 0.97). Substantial variation occurred in the crude lipid (4.94 - 9.02%), crude fibre (1.4 - 6.5%), ash (9.0 - 15.5%), nitrogen free extract (30 - 35%) as well as the calcium (1.3 - 3.3%) and phosphorus (1.2 - 2.0%) levels. Variation in n-3 fatty acid content was associated with different concentrations of 18:2n6, 20:5n-3 and 22:6n-3 (Table 8). The pipi was high in crude protein (75%), but low in crude fibre, NFE, calcium and phosphorus, and very low in the above indicated n-3 fatty acids.

Table 7. Proximate and amino acid composition (percent dry matter) of five commercial diets, pipi mussel (*Plebidonax* sp) and whole *P. monodon*.

Parameter	Diet						
Proximate composition	**1**	**2**	**3**	**4**	**5**	**Pipi**	**Whole *P. monodon***
Dry matter	92.4	91.6	89.6	90.4	89.6	15.4	26.6
Crude protein	43.3	43.8	42.6	45.5	43.0	74.5	70.0
Crude lipid	9.02	9.02	8.74	4.94	7.31	4.15	5.88
Crude fibre	6.48	6.38	2.56	1.46	1.84	--	--
Ash	9.0	9.9	15.5	13.3	13.4	12.6	16.35
NFE	32.2	30.9	30.6	34.8	34.5	8.75	--
Gross energy (MJ/kg)	20.54	20.57	19.14	18.91	19.20	19.33	19.17
Phosphorus	1.18	1.23	1.72	2.04	1.51	0.69	--
Calcium	1.36	1.83	3.28	2.95	3.33	0.50	--
Ca:P ratio	1.15	1.49	1.91	1.45	2.21	0.72	--
Amino acids							
Lysine	2.83	3.14	2.67	3.31	2.16	4.29	4.40
Histidine	1.19	1.18	1.02	1.16	1.00	1.26	1.18
Arginine	3.21	3.27	2.37	2.78	2.18	5.80	4.99
Threonine	1.56	1.64	1.53	1.77	1.56	3.23	2.69
Valine	1.97	2.06	1.86	2.08	1.87	3.14	3.04
Methionine	0.88	0.93	0.89	1.04	0.88	1.76	1.50
Isoleucine	1.76	1.87	1.86	1.95	1.63	3.08	2.65
Leucine	3.22	3.44	3.49	3.44	3.10	5.46	5.96
Phenylalanine	2.07	1.98	1.89	1.86	1.69	2.51	2.97
Tryptophan	0.47	0.38	0.37	0.50	0.34	0.46	0.66
Aspartic acid	4.72	4.72	4.17	4.84	3.21	7.18	6.63
Serine	1.89	1.90	1.75	1.99	1.73	3.03	2.67
Glutamic acid	7.53	7.60	7.70	7.96	7.10	10.77	9.55
Proline	1.88	2.28	2.54	2.17	2.64	2.64	4.64
Glycine	2.34	2.44	2.33	2.64	2.28	4.68	5.29
Alanine	2.16	2.27	2.28	2.46	2.12	3.96	3.59
Tyrosine	1.26	1.34	1.33	1.08	1.16	2.49	0.80

Table 8. Cholesterol and fatty acid content (percent dry matter) of five commercial diets and pipi mussel (*Plebidonax* sp).

Parameter	Diet					
	1	2	3	4	5	Pipi
Cholesterol	0.32	0.28	0.27	0.21	0.24	0.22
Fatty acid	0.13	0.16	0.26	0.21	0.27	-
14:0	0.13	0.16	0.26	0.21	0.27	-
16:0	1.08	1.06	1.31	1.00	1.27	0.16
16:1	0.13	0.17	0.28	0.23	0.28	0.02
18:0	0.24	0.24	0.37	0.19	0.23	0.12
18:1	0.87	0.84	0.93	0.79	1.10	0.04
18:2n-6	1.57	1.25	0.96	0.64	0.65	-
18:3n-3	0.13	0.10	0.11	0.07	0.08	-
20:1	0.12	0.13	0.08	0.09	0.17	-
20:4n-6	0.03	0.04	0.10	0.05	0.06	0.06
20:5n-3	0.23	0.28	0.40	0.48	0.63	0.09
22:6n-3	0.40	0.45	0.77	0.55	0.75	0.33
Total	5.46	5.30	6.51	4.86	6.27	1.11
n-3:n-6	0.48	0.64	1.21	1.59	2.06	7.00
Total n-3	0.76	0.83	1.28	1.10	1.46	0.42

Apparent digestibility was reduced ($P < 0.05$) in those diets containing high levels of crude fibre, and was very high for pipi (0.97) (Table 9). Food intake was not related to dry matter digestibility, but was lowest for the diet containing the lowest total quantity of n-3 fatty acid. Intake of pipi was high initially but declined to levels below that of the commercial diets as the experiment progressed ($P<0.01$). Growth rate of prawns fed the commercial diets was related ($r^2 = 0.92$; $P<0.01$) to digestible dry matter intake in small (Trial 2) but not in large prawns (Trial 1) (Figure 7). Differences amongst diets with respect to growth of prawns were generally consistent between small and large prawns, although small prawns appeared more sensitive to differences in dietary fatty acid content. We concluded that differences amongst diets in food intake and prawn growth were associated with variation in the non-protein fractions, and in particular the n-3 fatty acid, crude fibre and NFE content of the diets.

Table 9. Weight gain, feed conversion ratio, feed intake and digestibility for prawns feed various experimental diets.

Parameter	Diet						
	1	2	3	4	5	Pipi	Pooled sem
Large prawns (Expt. 1)							
Initial weight (g)	32.8	31.9	32.4	31.1	-	31.40	0.63
Weight gain (g)	1.66	3.17	4.44	4.14	-	3.63	0.63
FCR	21.87	11.54	8.83	8.18	-	12.25	1.30
Food intake (g DM/6 prawns/11 days)	42.8	43.1	45.0	39.9	-	52.4	1.0
Crude protein intake (g/6 prawns/11 days)	19.8	20.3	19.6	18.1	-	39.5	0.52
Dry matter digestibility	0.86	0.86	0.92	0.93	-	0.97	0.006
Crude protein digestibility	0.94	0.94	0.96	0.96	-	0.99	0.004
Digestible DM intake (g/6 prawns/11 days)	37.0	37.3	41.2	37.2	-	51.3	1.1
Small prawns (Expt. 2)							
Initial weight (g)	-	1.40	1.43	1.32	1.34	1.44	0.15
Weight gain (g)	-	3.77	4.57	2.21	3.34	1.58	0.11
FCR	-	1.87	1.69	2.34	2.10	2.98	0.60
Food intake (g DM/10 prawns/6 days)	-	10.0	11.1	7.4	10.1	6.7	0.2
Crude protein intake (g/10 prawns/6 days)	-	5.75	6.65	3.99	5.68	5.11	0.15
Dry matter digestibility	-	0.86	0.90	0.90	0.92	0.98	0.008
Crude protein digestibility	-	0.92	0.94	0.91	0.93	0.98	0.007
Digestible DM intake (g/10 prawns/6 days)	-	8.5	9.9	6.7	9.2	6.6	0.18

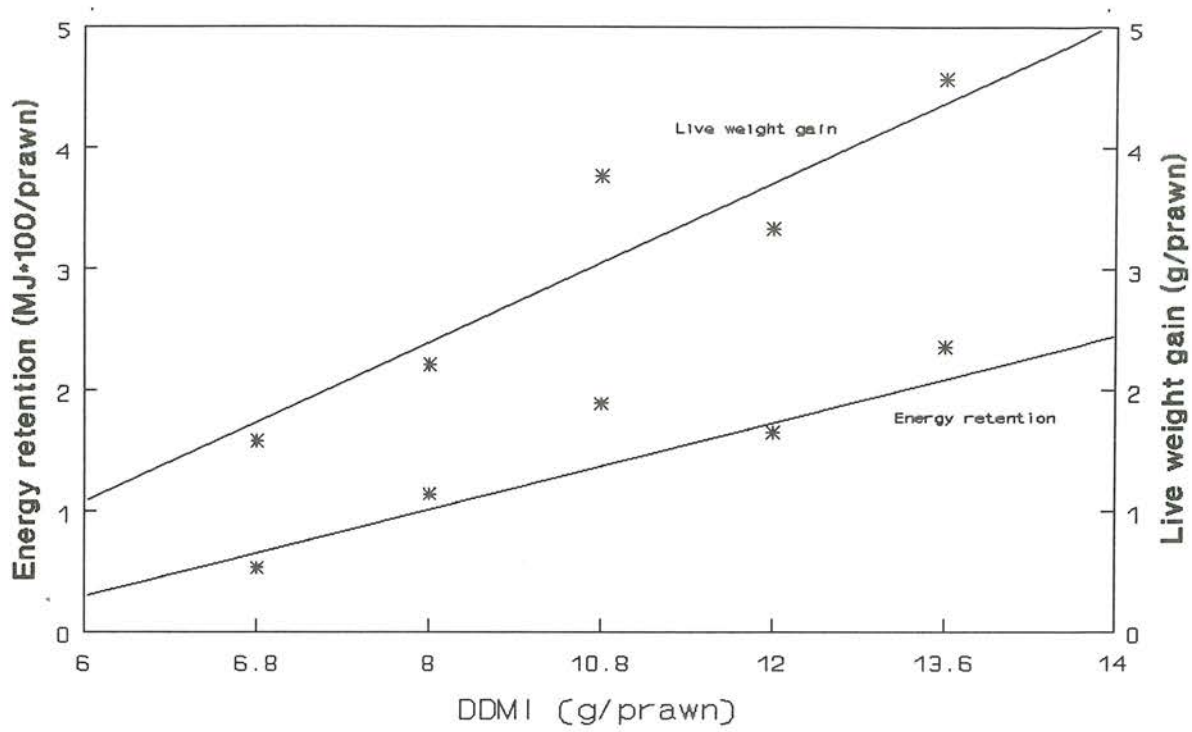

Figure 7. Relationships between digestible dry matter intake (DDMI) and live weight gain (L) and energy retention (E) in small prawns. The equations describing these relationships were:

$L = -1.038 + 0.408\ DDMI\ (R^2 = 0.92;\ RSD = 0.091)$
$E = -0.887 + 0.237\ DDMI\ (R^2 = 0.90;\ RSD = 0.038)$

6.3.2 Evaluation of Australian feed ingredients

6.3.2.1 Chemical analysis of feed ingredients

Approximately 70 samples representing 23 different Australian feed ingredients were collected, mainly in Queensland. They were chemically analysed for proximate composition, amino acids, fatty acids and minerals. Essential amino acid index (EAAI) of each ingredient was calculated to compare essential amino acid ratios of feed ingredients to that of prawn body.

Dy Penaflorida (1989) has rated feed ingredients as good quality protein source with EAAI 0.90 and above, useful with EAAI between 0.70 and 0.90 and inadequate with EAAI below 0.70. According to this rating none of the feed ingredients which were assessed in this study was found to be in the inadequate group (Table 10). Plant protein sources had EAAI between 0.83 (lupin meal and peanut meal) and 0.95 (canola meal). They were mainly low in lysine and methionine ratios. The ratios of histidine, valine, isoleucine, plenylanine and tryptophan were sufficient. Legume seeds were particularly low in methionine. Essential amino acids were present at sufficient levels in canola meal. Cottonseed meal was low in lysine (0.66), leucine (0.68) and

methionine (0.71), chickpea in methionine (0.70), wheat gluten in lysine (0.33) and arginine (0.76), sunflower meal in lysine (0.53) and peanut meal in lysine (0.61), threonine (0.76) and methionine (0.53).

Amongst terrestrial animal proteins, offal meal had the best EAAI (0.92), although it was low in methionine (0.76) and isoleucine (0.68). Blood meal and meat and bone meal gave the lowest EAAI, 0.76 and 0.71 respectively, not only amongst the terrestrial animal protein but also amongst all feedstuffs tested. Arginine, methionine and isoleucine ratios were low in both meals. Meat and bone meal was also low in tryptophan ratio (0.63).

All marine protein sources gave a good EAAI. Particularly fishmeals and squid meals had EAAI over 0.95. The lowest EAAI was obtained in prawn meal (0.91). That could be due to the source of prawn meal which was mainly prepared from head and shell with a minimum level of muscle in it. The ratios of individual amino acids were above 0.77 in all marine meals.

Cereals displayed EAAI between 0.88 (wheat flour) and 0.93 (oat meal). They were all low in lysine ratio. The ratio of other essential amino acids were above 0.85 except arginine ratio in cornflour (0.76) and wheat flour (0.73).

EAAIs of wheat bran and lucerne hay was 0.91 and 0.90 respectively. Wheat bran was particularly low in lysine (0.70) while lucerne hay was low in methionine (0.69).

Although all feed ingredients tested in this study showed either a good or a useful EAAI, their inclusion levels in prawn diets are determined by limiting essential amino acids and other nutritional and antinutritional factors which are present in them. The nutritional requirements of prawns also determines the inclusion levels of these ingredients.

The proximate analysis of feed ingredients is given in Table 11. Plant protein sources are high in crude protein but also in fibre. Apart from wheat gluten (0.04% CF) and full fat soybean meal (2.8% CF) all plant protein sources include over 10% crude fibre and in sunflower meal it reaches to 27%. Ether extract levels of plant protein sources were at below 6% apart from full fat soybean meal. This makes these ingredients easy to store without any problem with rancidity. The full fat soybean meal had 24% ether extract, which might be useful for prawns due to high 18:2w6 fatty acid level, which is, essential for the growth of penaeid prawns.

Animal protein sources have the highest crude protein level amongst all ingredients tested. Their fibre level is either zero or negligible. However most of them have high levels of ash. Ash levels are 58.8% in prawn meal, 35.2% in meat and bone meal and 29.9% in offal meal. This is because of the high bone or shell content in the feedstuffs. Terrestrial animal sources except blood meal have high fat content which is rich in saturated fatty acids. Marine protein sources have lesser amounts of ether extract but they are high in unsaturated fatty acids which are essential for prawn growth.

Table 10. EAA ratios* and EAAI of feed ingredients for prawn diets.

	LYS	HIS	ARG	THR	VAL	MET	ISO	LEU	PHE	TRY	EAAI
Plant protein sources											
Soybean meal	0.93	1.50	1.02	0.96	1.04	0.60	1.15	0.83	1.20	1.36	0.92
Full fat soybean meal	0.96	1.32	1.00	0.99	1.04	0.65	1.15	0.86	1.13	1.53	0.94
Canola meal	0.85	1.45	0.82	1.21	1.23	1.06	1.10	0.87	0.96	1.46	0.95
Cottonseed Meal	0.66	1.55	1.61	0.83	0.99	0.71	0.83	0.68	1.26	1.56	0.86
Lupin (whole)	0.78	1.43	1.63	0.90	0.91	0.35	1.09	0.81	0.92	0.93	0.83
Chickpea	0.98	1.36	1.34	0.86	0.93	0.70	1.00	0.82	1.24	-	(0.91)**
Wheat gluten	0.33	1.54	0.76	0.88	1.21	0.96	1.28	1.08	1.59	1.55	0.86
Sunflower meal	0.53	1.34	1.31	0.97	1.17	1.05	1.14	0.79	1.12	1.25	0.91
Peanut meal	0.61	1.32	1.80	0.76	1.01	0.53	0.93	0.80	1.21	-	(0.83)
Terrestrial animal protein meals											
Blood meal	1.08	2.72	0.50	0.98	1.40	0.49	0.28	1.10	1.28	1.41	0.76
Meat and bone meal	1.05	1.39	0.13	0.97	1.13	0.72	0.78	0.85	0.95	0.63	0.71
Poultry offal meal	0.69	0.79	1.02	1.17	1.48	0.61	1.19	0.93	1.06	0.86	0.88
Offal meal	1.05	1.43	1.07	1.02	1.23	0.76	0.68	0.92	0.97	0.93	0.92
Cereals											
Cornflour	0.56	1.74	0.76	0.97	1.07	1.05	0.90	1.29	1.12	1.47	0.91
Oat meal	0.62	1.39	1.01	0.92	1.22	0.88	1.09	0.93	1.28	1.53	0.93
Wheat flour	0.43	1.75	0.73	0.98	1.28	0.99	1.25	1.08	1.56	-	(0.88)
Wheat (whole)	0.50	1.72	0.85	0.96	1.26	1.02	1.18	1.04	1.44	-	(0.90)
Marine protein sources											
Fishmeal (orange ruffy)	1.08	1.11	1.06	1.10	1.06	1.39	1.02	0.82	0.91	-	(0.97)
Squid (fresh)	1.15	0.96	1.12	1.05	0.88	1.07	1.07	0.88	0.81	1.00	0.95
Cuttlefish (fresh)	1.11	1.08	1.03	1.13	0.92	1.16	1.10	0.88	0.86	0.70	0.93
Plant fibre meals											
Wheat bran	0.70	1.99	1.12	0.96	1.18	0.80	0.92	0.79	1.02	2.42	0.91
Lucerne hay	0.80	1.10	0.70	1.20	1.25	0.69	1.17	0.93	1.26	2.14	0.90

* $$\text{EAA} = \frac{\text{EAA / Total EAA in feed ingredient}}{\text{EAA / Total EAA in whole prawn body}}$$

** Calculated in the absence of tryptophan analysis

Table 11. Proximate analyses and gross energy content of potential feed ingredients. Descriptions of the feeds are given in the text. NFE has been estimated by difference.

Feed	Analysis							
	n	DM (%)	CP (% DM)	EE (% DM)	Ash (% DM)	CF (% DM)	NFE (% DM)	GE (MJ/kg DM)
Plant protein meals								
- soybean meal	5	88.1 ± 0.6*	51.8 ± 0.4	1.6 ± 0.1	7.6 ± 0.2	7.5 ± 0.5	31.5 ± 0.9	19.6 ± 0.2
- full fat soybean	1	92.8	39.0	24.0	5.6	2.8	28.6	23.5
- canola meal	1	89.4	43.3	2.8	7.1	16.2	30.6	20.6
- cottonseed meal	5	89.9 ± 0.6	41.6 ± 0.5	5.2 ± 0.8	6.9 ± 0.2	14.8 ± 0.6	31.5 ± 1.0	20.9 ± 0.7
- lupin (whole)	2	94.6	34.6	5.6	3.3	17.0	39.5	19.9
- peanut meal	3	92.3 ± 0.6	49.7 ± 0.8	1.4 ± 0.7	6.4 ± 1.0	11.2 ± 1.0	31.3 ± 0.2	19.3 ± 0.3
- chickpea (whole)	5	89.2 ± 0.6	24.7 ± 1.6	5.0 ± 0.3	3.1 ± 0.3	10.8 ± 0.6	56.4 ± 0.9	19.2 ± 0.02
- wheat gluten	5	91.8 ± 0.2	82.2 ± 0.3	1.4 ± 0.5	1.2 ± 0.2	0.4 ± 0.04	14.8 ± 0.3	23.2 ± 0.1
- sunflower meal	5	90.2 ± 0.4	34.1 ± 1.1	1.2 ± 0.1	7.2 ± 0.2	27.1 ± 1.7	30.4 ± 0.7	19.3 ± 0.2
Cereals								
- cornflour	1	89.7	9.8	6.5	1.9	3.6	78.2	18.9
- oat meal	1	89.6	11.4	8.4	1.0	0.5	78.7	19.5
- wheat flour	1	88.3	11.9	1.1	0.5	0.9	85.6	18.1
- wheat (whole)	5	88.3 ± 0.2	17.0 ± 0.3	1.9 ± 0.2	1.7 ± 0.1	-	79.5 ± 0.5	18.5 ± 0.08
Terrestrial Animal Protein Meals								
- blood meal	5	93.8 ± 0.7	95.6 ± 0.9	1.3 ± 0.8	2.5 ± 0.6	0.6 ± 0.2	0 ± 0.1	22.9 ± 1.1
- meal and bone meal	6	94.7 ± 0.9	53.6 ± 1.4	9.6 ± 0.4	35.2 ± 1.5	0	1.6 ± 0.5	16.0 ± 0.6
- poultry offal meal	3	95.1 ± 1.4	67.6 ± 2.3	19.2 ± 2.4	10.5 ± 1.1	0	2.7 ± 0.6	23.9 ± 0.1
- offal meal	1	93.8	58.6	9.7	29.9	0	1.8	17.7
Marine Protein Sources								
- fishmeal (orange ruffy)	1	91.6	63.9	9.4	26.0	0.7	0	18.8
- squid (fresh)	5	12.9 ± 1.4	85.4 ± 0.8	6.0 ± 0.5	7.0 ± 0.9	0	1.6 ± 1.3	22.5
- cuttlefish (fresh)	1	26.0	70.2	3.1	19.6	1.4	5.7	19.0
Plant Fibre Meals								
- rice bran	2	92.2 ± 0.8	14.2 ± 0.1	19.1 ± 1.3	7.0 ± 1.4	23.5 ± 2.1	36.2 ± 4.4	18.0
- wheat bran	1	95.4	17.9	4.7	5.9	11.8	59.7	19.0
- lucerne hay	5	89.5 ±0.5	20.5 ± 1.7	2.1 ± 0.3	10.5 ± 1.0	28.8 ± 1.5	38.1 ± 0.3	-

* Standard error of the mean.

Cereals have the lowest crude protein level of all feedstuffs. However they are also low in crude fibre and ash. Cornflour and oat meal show higher levels of ether extract than wheat flour and whole wheat. Cereal feedstuffs are mainly energy sources providing above 78% NFE.

Rice and wheat bran and lucerne hay are high in fibre while ether extract levels vary considerably.

Mineral levels of feed ingredients are given in Table 12. Calcium and phosphorus are the two most important minerals for prawns. Kanazawa (1983) recommended that prawn diets should have a minimum of 1% of both minerals and a dietary Ca:P ratio of 1:1. Most of the animal protein meals had more than the required levels of Ca, especially prawn meal (not Australian), meat and bone meal, fish meal (not Australian) and offal meal showed very high levels of Ca; 17.7%, 10.2%, 7.78% and 6.44% respectively. Amongst other feed ingredients only lucerne hay had a desirable Ca level (1.34%). Levels of phosphorus, on the other hand, were more close to the requirement level. In plant protein meals canola meal, cottonseed meal and sunflower meal had P levels around 1%. Although lucerne hay was short of P, rice bran and wheat bran had P levels 1.2% and 1.09% respectively. Animal protein meals, especially meat and bone meal (5.3%), offal meal (5.13%) and Australian fishmeal (4.26%) were high in P. None of the feed ingredients apart from imported fishmeal and squid meal, had Ca:P ratio of 1:1.

Recommended dietary levels of magnesium and potassium has been reported as 0.3%, and 0.9%, respectively (Kanazawa, 1983). Lupin, and wheat gluten in plant protein meals, all animal protein meals except imported squid meal and all plant energy meals were low in magnesium. Potassium levels were below the recommended dietary level in wheat gluten, all animal protein meals except imported fishmeal and fresh cuttlefish meal, and in all plant energy meals.

It was reported that manganese and iron inhibited prawn growth when dietary levels exceed 30 ppm and 60 ppm respectively. All feed ingredients apart from lupin, cornflour, oat meal and rice bran were high in iron. However all ingredients except lupin, imported prawn meal, fresh cuttlefish and wheat bran had low levels of manganese.

Requirements of prawns for cholesterol and unsaturated fatty acids were reported to be 0.5% and 1% (of C20:5w3 and C22:6w3) respectively (Kanazawa, 1993). The feed ingredients could be divided into two extreme groups in terms of their cholesterol levels (Table 13). The cholesterol level was above the requirement in animal protein sources while all feed ingredients of plant origin did not have any cholesterol. However unsaturated fatty acid levels were above the suggested requirement level in all feed ingredients except sunflower meal, blood meal and prawn meal.

Table 12. Mineral content of Australian feed ingredients (% dry matter). The number of samples refers to number analysed; standard errors are not shown where the number of samples analysed was less than three.

Feed	Analysis								
	n	Ca	Mg	Na	P	K	Zn	Mn	Fe
Plant protein meals									
Soybean meal	5	0.40 ± 0.03	0.32 ± 0.01	0	0.75 ± 0.01	2.86 ± 0.10	68	19	207
Full fat soybean meal	1	0.19	0.27	0	0.62	2.61	53	33	137
Canola meal	1	0.81	0.49	0	1.02	1.51	54	42	93
Cotton seed meal	5	0.22 ± 0.01	0.66	0.23	1.15 ± 0.04	1.70	62	14	100
Lupin (whole)	2	0.23	0.25	0.03	0.39	0.94	31	103	50
Chickpea (whole)	5	0.16 ± 0.05	-	-	0.45 ± 0.06	-	-	-	-
Wheat gluten	3	0.08 ± 0.01	0.05 ± 0.00	0.03 ± 0.01	0.21 ± 0.01	0.10 ± 0.01	37	27	78
Sunflower meal	5	0.37 ± 0.02	0.55	-	1.13 ± 0.05	1.96	86	29	156
Peanut meal	3	0.16 ± 0.02	-	-	0.66 ± 0.03	-	-	-	-
Terrestrial animal protein meals									
Blood meal	5	0.20 ± 0.13	0.02 ± 0.01	0.32 ± 0.03	0.23 ± 0.14	0.17 ± 0.02	25 ± 3	3.5 ± 0.5	2176 ± 33
Meat and bone meal	5	10.2 ± 1.1	0.23 ± 0.00	0.59 ± 0.00	5.30 ± 0.70	0.44 ± 0.03	94	9	190
Poultry offal meal	3	2.76 ± 0.65	0.10	0.35	1.27 ± 0.04	0.56	138	17	789
Offal meal	1	6.44	0.13	0.45	5.13	0.31	95	12	611
Cereals									
Cornflour	1	0.02	0.16	0	0.43	0.64	33	10	59
Oat meal	1	0.03	0.06	0	0.22	0.28	11	27	26
Wheat flour	1	0.03	0.03	0	0.11	-	-	-	-
Wheat (whole)	5	0.06 ± 0.01	-	-	0.33 ± 0.02	-	-	-	-
Marine protein sources									
Fishmeal (orange ruffy)	1	7.78	0.19	0.58	4.26	0.69	97	10	967
Squid (fresh)	5	0.11 ± 0.01	-	-	1.09 ± 0.05	-	-	-	-
Cuttlefish (fresh)	1	4.87	0.20	1.29	1.04	1.31	317	38	274
Plant fibre meals									
Rice bran	1	0.06	0.69	0.01	1.20	1.33	50	-	50
Wheat bran	1	0.11	0.48	0	1.09	1.60	145	121	133
Lucerne hay	4	1.34 ± 0.22	-	-	0.31 ± 0.02	-	-	-	-

Table 13. Fatty acid and cholesterol levels of Australian feed ingredients (% dry matter).

Feed ingredients	Analysis																		
	n	Cholesterol	Total fatty acids	14:0	16:0	16:1w9	18:0	18:1w9	18:1w7	18:2w6	18:3w3	20:1w11	20:1w9	20:1w7	20:4w6	20:5w3	22:1w9	22:1w7	22:6w3	24:1w9
Plant protein meals																			
Soybean meal	2	0	1.53	0	0.27	0	0.07	0.24	0.03	0.86	0.09	0	0	0	0	0	0	0	0	0
Full fat soybean meal	1	0	19.89	0	2.25	0	0.67	3.55	0.29	11.46	1.67	0	0	0	0	0	0	0	0	0
Canola meal	1	0	2.12	0	0.17	0.03	0.04	0.88	0.27	0.55	0.12	0	0.01	0	0	0	0	0	0	0
Cottonseed meal	1	0	4.40	0.03	1.06	0.03	0.14	0.77	0.04	2.30	0.02	0	0	0	0	0	0	0	0.02	0
Lupin (whole)	1	0	4.91	0	0.63	0	0.42	1.80	0.04	1.72	0.18	0	0.01	0	0	0	0.09	0	0.02	0
Chickpea meal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-			
Wheat gluten	1	0.25	2.92	0.01	0.58	0.01	0.10	0.48	0.03	1.57	0.06	0	0.02	0	0	0	0.01	0	0	0
Sunflower meal	1	0	0.79	0	0.09	0	0.04	0.16	0	0.49	0	0	0	0	0	0	0	0	0	0
Peanut meal	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Terrestrial animal protein meals																				
Blood meal	3	0.90	1.04	0.03	0.28	0.03	0.22	0.36	0.01	0.04	0	0	0	0	0	0	0	0	0	0
Meat and bone meal	2	0.62	7.63	0.27	2.12	0.20	1.63	3.00	0.09	0.13	0	0	0.01	0	0.005	0.005	0	0	0.005	0
Poultry offal meal	1	2.07	11.92	0.17	3.06	0.70	1.25	4.76	6.25	1.15	0.06	0	0.05	0	0.06	0	0	0	0	0
Offal meal	1	1.59	9.05	0.25	2.18	0.19	1.94	3.46	0.33	0.18	0.03	0	0.01	0	0.02	0	0	0	0	0
Cereals																				
Cornflour	1	0	11.29	0	1.31	0	0.19	3.03	0.08	6.47	0.13	0	0.02	0	0	0	0	0	0	0
Oat meal	1	0	6.03	0.01	0.92	0	0.12	2.60	0.05	2.19	0.07	0	0.05	0	0	0	0	0	0	0
Wheat flour	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wheat (whole)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Marine protein sources																				
Fish meal (orange ruffy)	1	3.89	4.47	0.09	0.08	0.41	0.38	2.30	0.19	0.24	0.02	0.04	0.38	0	0.06	0.05	0.03	0	0.20	0
Squid (fresh)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cuttlefish (fresh)	1	10.49	1.79	0.04	0.41	0.02	0.24	0.07	0.03	0	0	0.02	0.04	0	0.17	0.17	0	0	0.58	0
Plant fibre meals																				
Rice bran	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wheat bran	1	0	4.20	0	0.61	0	0.05	0.80	0.03	2.52	0.16	0	0.03	0	0	0	0	0	0	0
Lucerne hay	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

6.3.2.2 Determination of net energy in feed ingredients

Problems related to determining accurate digestibility values for feed ingredients encouraged us to evaluate the nutritional value of feed ingredients using a net energy method. There are four different types of energy to express the energy content of a feed or feed ingredient. They are gross energy, digestible energy, metabolisable energy and net energy. The net energy is the amount of energy of a particular feed or feed ingredient which has contributed to the growth of a farmed animal. It is widely used for feed ingredients of terrestrial animals (Moe *et al.*, 1972; Moe and Tyrrell, 1976; Suleiman and Mathison, 1979).

When using the net energy (NE) system for evaluating feed ingredients or diets it is necessary to record two values:

1. value of feed at intakes up to maintenance (NEm); and
2. value of feed at intakes above maintenance, that is, growth (NEg).

In a preliminary trial, to determine the maintenance level, prawns of two average body size (2.99 ± 0.50 g and 23.38 ± 1.38 g) were fed at one of seven feeding levels. Two prawns of an average size were held in a 20 litre bucket until the end of a one week acclimatisation period, during which prawns were fed *ad libitum*. Then all prawns were weighed and one prawn from each tank was killed and analysed for gross energy, crude protein, ether extract and ash.

The remaining prawns were fed for 28 days. The feeding levels were set at equal increments from 0 to *ad libitum* on a percent body weight per day basis. The daily food allowance for each prawn was divided into three feeds at 0700, 1400 and 2200 hours, or for the lower levels, two feeds at 0700 and 2200 hours. Water quality measurements were carried out daily and all exuvia were collected and recorded. At the end of the trial all prawns were killed and analysed for gross energy, crude protein, ether extract and ash. Prawns on zero feed were removed from the trial, weighed and analysed on day 12. These data were extrapolated to 28 days.

Regression analysis of total energy on wet weight was performed for each group (Table 14). The equation obtained from regression analysis was used to estimate the initial energy content of experimental prawns of the same size and average weight group. The values of slope and intercept for different sizes were compared and no significant difference was observed.

Table 14. Relationship between gross energy (kJ) and live body weight of prawns (g).

Size	Regression	r^2
Small	GE* = -4.9163 + 7.2472 WW**	0.91
Large	GE = -60.388 + 8.9219 WW	0.87

*GE = gross energy
**WW = live body weight

The energy retention by each prawn was determined as the difference between the energy content at the end of the trial and the estimated content at the start of the trial. Energy retention per gram average body weight was regressed against energy intake per gram average body weight to obtain the efficiency of utilisation of energy for each group of prawns (Table 15). There was no significant difference between the sizes in their maintenance requirements per gram body weight for energy.

The second trial was carried out to determine the net energy value of eight feed ingredients, namely: sorghum, wheat, rice, sago, soybean meal, fishmeal, prawn meal and wheat gluten. The comparative slaughter method was used for this study. Test ingredients were incorporated into a basal diet at a rate of 15% test ingredient to 85% basal diet. The net energy for growth (NEg) value of the basal diet was determined by the difference in energy retention (kJ) due to intake at maintenance and just under *ad libitum* divided by the difference in intake (g). The NEg value for each test ingredient was determined by comparison of the performance of the test diet with the basal diet fed at maintenance level. The test diets were fed just under *ad libitum*.

Two of the temperature controlled experimental systems was used for this study. Four replicates each of the basal diet at each of two intake levels and three replicates of each of the four test diets were allocated in a randomised block arrangement to both systems.

The basal diet consisted of a mixture of squid meal, wheat flour, vitamins, minerals and a binder. Crude protein content of the basal diet was 48%, and in test diets it varied between 40% and 55%. The test diets and one basal diet treatment were fed just below *ad libitum*. The other basal diet treatment was fed at a level approximating maintenance which was calculated from the results of the preliminary trial. Prawns were fed four times/day at 0800, 1200, 1700 and 2200 hours.

Table 15. Relationship between energy retention (RE, kJ/g body weight) and energy intake of prawns (I, kJ/g body weight).

Size	Regression	r^2
Small	$Re^{*} = -2.9669 + 0.3103\ I^{**}$	0.79
Large	$Re = -4.8472 + 0.53410\ I$	0.90

*Re = energy retention (kJ/g body weight)
**I = energy intake (kJ/g body weight)

The amount of energy available for growth after the energy required for maintenance was fulfilled was calculated for each diet by comparison with the basal diet fed at maintenance (Table 16 and 17). The two systems were treated as separate entities. Within each test diet total intake was divided into that due to the test ingredient (15%) and that due to the basal diet portion (85%). The test diet was then compared with the basal diet fed at maintenance and the differences in food intake due to the basal diet component and test ingredient and total energy retained calculated. Energy retention due to the basal diet can be calculated by use of the NEg value for this diet. The difference between total energy retained and that due to the basal diet is then

attributed to the test ingredient. If follows that from knowledge of the amount of test ingredient consumed the NEg (kJ/g) of the ingredient can be determined.

Table 16. Net energy for growth (NEg) of carbohydrate sources.

Item		Base diet (maintenance)	Sorghum	Wheat	Rice	Sago
Total feed intake:	Base (g)	3.254	8.0331	8.1164	8.0852	8.6136
	Ingredient (g)	0	1.4176	1.4323	1.4268	1.5201
Total energy gain (kJ)		-0.4149	15.5917	16.6478	17.0509	15.8766
Differences food	Base (g)	-	4.7791	4.8624	4.8312	5.3596
	Ingredient (g)	-	1.4176	1.4323	1.4268	1.5201
	Energy (kJ)	-	16.0066	17.0627	17.4658	16.2915
Energy from base (1.3825 kJ/g)		-	6.6066	6.7218	6.6787	7.4091
Energy from ingredient (kJ)		-	9.4	10.3409	10.7871	8.8824
∴ NEg of ingredient (kJ/g)		-	6.6309	7.2198	7.5603	5.8433

Table 17. Net energy for growth (NEg) of protein sources.

Item		Base diet (maintenance)	Soybean	Fish	Prawn	Gluten
Total feed intake:	Base (g)	3.197	8.5881	8.3110	8.6791	8.3011
	Ingredient (g)	0	1.5156	1.4667	1.5316	1.4649
Total energy gain (kJ)		-0.6052	24.7159	19.9265	17.8741	20.7075
Differences food	Base (g)	-	5.3911	5.114	5.4821	5.1041
	Ingredient (g)	-	1.5156	1.4667	1.5316	1.4649
	Energy (kJ)	-	25.3211	20.5317	18.4793	21.3127
Energy from base (2.9813 kJ/g)		-	16.0725	15.2464	16.3438	15.2169
Energy from ingredient (kJ)		-	9.2486	5.2853	2.1355	6.0958
∴ NEg of ingredient (kJ/g)		-	6.1023	3.6035	1.3943	4.1612

6.3.2.3 The assessment of some local protein and energy sources as dietary ingredients of P. monodon

Feed is a large component of cost in intensive prawn production, partly due to a heavy dependence on marine protein meals as dietary sources of protein and energy. Australia has large quantities of agricultural feedstuffs available at lower cost than marine protein meals, and the cost of feed would be reduced if these were used to partly replace marine protein meals in the diet.

Three experiments were conducted to measure the effects of inclusion of 10 local feedstuffs, each at two levels, on liveweight gain in juvenile *P. monodon* of 2 to 3 g initial liveweight. Each of the experiments contained a common control diet, and a

factorial combination of three to four feedstuffs and two levels of inclusion. The upper level of inclusion was assessed from the literature to be the maximum for the feedstuff, and the lower 0.5 of this. Diets contained a similar crude protein content (44%) and were offered *ad libitum* to groups of eight prawns in flow through, seawater aquaria of 250 litre capacity. There were two replications of the test diets and four replications of the control, and each experiment was continued for six weeks.

The feedstuffs tested and relative performance levels, calculated using the control diet common to each experiment, are shown in Table 18. There was considerable variation between feedstuffs in inclusion levels. Food intake was generally maintained with the use of protein feedstuffs, though values were reduced for canola meal and high levels of offal meal. Intakes were lower for the carbohydrate feedstuffs. Similarly liveweight gain was maintained by most protein feedstuffs, being reduced only by high levels of offal and lupins. Liveweight gain by prawns given soybean meal was higher than for the control diet ($P < 0.05$). Inclusion of 20% wheat was effective in maintaining liveweight gain, though values were reduced with maize and oatmeal. The higher levels of inclusion reduced liveweight gain for each of the carbohydrate feedstuffs. FCR was maintained at or below values for the control diet for most feedstuffs. The relatively low food intake by prawns given canola was reflected in a low FCR, and the low liveweight gain by prawns given the higher level of oatmeal resulted in a high FCR.

Table 18. Effect of feedstuff and level of inclusion on the relative food intake, liveweight gain and food conversion ratio (FCR) of juvenile *P. monodon*.

Feedstuff	Level of inclusion (% as fed)	Food intake	Liveweight gain	FCR
		(Relative to control = 100)		
Control (50% marine protein)	-	100	100	100
Protein feedstuffs				
Meat and bone meal	10	90	103	87
	20	96	100	93
Blood meal	7.5	90	109	80
	15	100	97	100
Soybean meal	20	100	130	73
	40	106	115	87
Offal (chickens)	10	96	94	100
	20	85	85	100
Cottonseed meal	5	110	103	107
	10	100	109	87
Lupin	20	83	97	87
	40	94	88	107
Canola	7.5	69	106	60
	15	75	106	69
Carbohydrate feedstuffs -				
Wheat	20	90	118	73
	40	88	88	100
Maize	20	80	73	107
	40	75	67	107
Oatmeal	15	85	88	93
	30	81	46	173

The results demonstrate a substantial opportunity to partly replace marine protein meals with local feedstuffs. Further information is needed on the efficiency with which prawn use carbohydrates, including the high starch meals and the non-protein fraction of protein meals.

6.3.2.4 Substitution of marine proteins with local protein sources in diets for prawns

A mixture of local protein sources was added to the diet formulated at BIARC (B4-91) (see Section 6.3.3) at various levels to reduce the dietary levels of marine feed ingredients. The mixture consisted of blood, canola, cottonseed and poultry meals at the ratio of 30:17:23:30 respectively. The mixture was added to the diet to replace 10%, 15%, 30%, 25% and 30% of the total marine proteins. All diets were isonitrogenous and their essential amino acid composition was similar.

Eight prawns averaging 8.93 ± 0.26 g ($\bar{x}$ ± SD) were placed in each experimental tank. The experimental system was the 20 x 250 litre polyethylene tank system as described earlier. Prawns were fed twice daily at 0900 and 1700 hours. The experimental period was six weeks.

The results showed a significant difference in weight gain and FCRs between treatments ($P<0.05$) (Table 19). Both weight gain and FCR were effected by substitution of marine proteins with terrestrial protein sources. A positive significant linear regression ($P<0.05$) was found between FCR and substitution levels (r^2 = 0.79). The regression between weight gain and substitution levels was also significant ($P<0.05$) but negative (r^2 = 0.64). It was speculated that the level of available energy might be lower in diets containing terrestrial protein sources. Pre-treatment of feed ingredients with enzymes or by cooking may be employed to increase the available energy.

6.3.2.5 Use of exogenous enzymes in canola based diets for prawns

Feed ingredients of plant origin have low digestibilities (Akiyama and Dominy, 1989) and this could be attributed to the high level of carbohydrates, especially non-starch polysaccharides (Choct and Annison, 1992; Choct et al., 1992). Exogenous enzymes are often used to increase the nutritive value of feed ingredients of plant origin in pig and poultry feeds (Annison, 1992; Wang et al., 1992). The use of exogenous enzymes in prawn feeds has also been shown to increase the digestibility of these feeds and to improve growth rate (Maugle et al., 1983). However, more information is needed on the effectiveness of exogenous enzymes in improving the nutritive value of prawn feeds.

This study investigates the effect of an exogenous enzyme mixture in a canola based prawn feed on the growth, survival and FCR of prawns and the net energy value of canola meal.

Table 19. Weight gain and FCR of prawns fed experimental diets*.

Parameters	Diets						
	B4-91	1	2	3	4	5	6
Substitution level (% of marine protein)	-	10	15	20	25	30	30 + amino acids
Initial weights (g/prawn)	9.22 ± 0.27	8.64 ± 0.24	8.95 ± 0.05	8.82 ± 0.17	8.92 ± 0.39	9.17 ± 0.18	8.92 ± 0.12
Weight gain (g/prawn)	6.12 ± 0.25^{a}	5.23 ± 0.28^{abc}	5.40 ± 0.64^{ab}	5.17 ± 0.39^{bc}	4.84 ± 0.70^{bc}	4.34 ± 0.37^{c}	4.46 ± 0.60^{c}
FCR	3.68 ± 0.25^{a}	4.25 ± 0.31^{ab}	4.35 ± 0.54^{ab}	4.19 ± 0.31^{ab}	4.59 ± 0.81^{ab}	5.38 ± 0.58^{c}	5.06 ± 0.53^{bc}

* Treatment means with the same superscript are not significantly different (P>0.05).

Table 20. Weight gain, food conversion ratio (FCR), feed intake, protein efficiency ratio (PER) and dry matter digestibility for prawns fed experimental diets.

Parameter	Diets					
	Basal	Low canola	Low canola + enzyme	High canola	High canola + enzyme	High canola + sucrose
Initial weight (g/prawn)	1.02 ± 0.10	1.00 ± 0.11	0.96 ± 0.08	0.94 ± 0.09	0.92 ± 0.10	1.02 ± 0.11
Weight gain (g/prawn)	2.33 ± 0.49^{ab}	2.29 ± 0.27^{ab}	2.40 ± 0.16^{b}	1.79 ± 0.42^{c}	2.29 ± 0.36^{ab}	2.01 ± 0.22^{ac}
FCR[2]	2.28 ± 0.23^{ab}	2.49 ± 0.19^{bc}	1.98 ± 0.29^{a}	3.06 ± 0.53^{d}	2.31 ± 0.34^{ab}	2.88 ± 0.13^{cd}
Feed intake (g/prawn/42 days)	5.23 ± 0.65	5.67 ± 0.32	5.42 ± 0.15	5.34 ± 0.41	5.20 ± 0.39	5.79 ± 0.55
PER[3]	0.87 ± 0.08^{ab}	0.84 ± 0.06^{a}	1.09 ± 0.16^{c}	0.80 ± 0.12^{a}	1.02 ± 0.16^{bc}	0.95 ± 0.04^{abc}
Dry matter digestibility (in proportion to basal diet)	100^{c}	89^{ab}	89^{ab}	93^{b}	101^{c}	86^{a}

1. Means with the same superscript in the same row are not significantly different (P >0.05).

A 42 day prawn feeding study was carried out to evaluate six diets. One of the diets was a basal diet which was squid meal based, four contained canola meal at percentage inclusions of either 20 or 64,% with and without enzyme addition. The last diet contained 54% canola and to that 10% sucrose was added to supply readily available energy to prawns. The concentrations of the binder and micro-nutrient premix were held constant for all diets and inclusions of canola, sucrose and enzyme were made at the expense of, and in proportion to, the other constituents of the basal diet. The enzyme mixture (porzyme) (0.25% dry weight) was added to low and high canola diets. Ytterbium acetate was added to each diet at a concentration of 40 mg/kg as an inert marker for digestibility determinations.

Ninety prawns (*P. monodon*) of average liveweight ($\bar{x}$ ± SD) of 0.96 ± 0.09 g were randomly distributed amongst the 30 x 40 litre experimental tanks and acclimated to the experimental conditions for a week. Prawns were fed to appetite in six treatments and at maintenance level in two treatments with basal and high canola diets. Feeding was three times daily at 0800, 1200 and 1700 hours. Before each feeding, tanks were cleaned by siphoning, food residues and faeces separated by hand sorting and the respective samples held at -20°C pending analysis. Food intake of the prawns was determined as the difference between the amount offered and the collected food residue, assessed with reference to its Ytterbium concentration. Prawns were individually weighed after towelling dry at the beginning and the end of the experiment.

High canola diet gave significantly lower growth rates (1.79 ± 0.42 g) ($P<0.05$), and enzymes increased the liveweight gain from this diet by 28%, to a level similar to the basal (2.33 ± 0.49 g) ($P>0.05$) (Table 20). The low canola + enzyme diet also resulted in higher liveweight gain (2.40 ± 0.16 g) than the basal and low canola diets (2.29 ± 0.27 g) but it was not significant. Addition of enzyme mixture to diets also gave a significant improvement in FCRs. The addition of sucrose to the canola based diet resulted in a significantly higher liveweight gain (high canola + sucrose 2.01 ± 0.22 g), but FCR was not affected.

The net energy value of canola meal was 6.07 kJ/g. This was increased to 9.17 kJ/g when exogenous enzymes were included in the diet.

These results indicate that dietary enzymes have considerable potential to improve the nutritional value of feeds and feedstuffs.

6.3.3 Developing feed formulations at BIARC

Initially we wanted to test if diets made locally at the Research Centre could maintain growth of prawns similar to that of commercial diets. In the first trial of a series, four diets based on fish meal, prawn meal, squid meal and soybean meal as main protein sources were formulated using a Least Cost Diet Formulation Program (Table 21). Formulations were based on the amino acid profile of a commercial prawn feed which was also used as a control in this experiment. Crude protein and gross energy levels of the diets were similar, between 45.0 - 48.0% and 18 - 20 MJ/kg respectively. The control diet had a lower protein level (43%) but the gross energy level was the same (19.0 MJ/kg).

Table 21. Composition of the experimental diets (% dry matter).

	Diet			
Ingredients	1	2	3	4
Squid meal	10.00	5.00	10.00	6.00
Fish meal	14.40	30.00	12.00	24.00
Prawn meal	5.50	5.00	25.00	-
Broken rice	11.00	5.00	5.00	-
Sago	4.00	-	2.25	-
Wheat gluten	10.00	10.00	10.00	10.00
Sorghum meal	1.50	5.00	-	-
Rice bran	2.00	-	3.00	14.00
Soybean meal	15.00	5.00	13.00	20.00
Wheat flour	5.00	18.50	3.00	14.40
Minced cuttlefish	10.00	5.00	5.00	-
Cod liver oil	3.00	3.00	3.00	3.00
Soybean oil	1.00	1.00	1.00	1.00
Lecithin	2.00	2.00	2.00	2.00
Cholesterol	0.30	0.30	0.30	0.30
Vitamin mix[1]	2.00	2.00	2.00	2.00
Mineral mix[2]	3.00	3.00	3.00	3.00
L. Histidine	0.03	0.02	0.08	-
L. Leucine	0.18	0.18	-	0.3
L. Lysine	0.12	-	0.29	-
L. Methionine	-	-	0.03	-
L. Isoleucine	-	-	0.05	-

(1) The vitamin mixture includes p-aminobenzoic acid 6.32 mg; biotin 0.252 mg; inositol 252.8 mg; Ca-pantothenate 37.92 mg; nicotinic acid 25.28 mg; pyridoxine-HCL 7.584 mg; riboflavin 5.056 mg; thiamine-HCL 2.528 mg; cyanocobalamin 0.052 mg; Na-ascorbate 1264 mg; folic acid 0.504 mg; choline-HCL 379.2 mg; menadione 2.536 mg; β-carotene 6.068 mg; α-tocopherol 12.64 mg and calciferol 0.76 mg.

(2) The mineral mixture contains K_2HPO_4 0.74 g; $MgSO_4$-$7H_2O$ 1.13 g; NaH_2PO_4-$2H_2O$ 0.29 g and $CaHPO_4$ 1.00 g

All test diets were produced in a laboratory at BIARC by mixing all dry ingredients thoroughly and adding cod liver oil, soybean oil and water to this mixture. The doughy mixture was extruded through a Hobart Mixer (2.0 mm) after steam cooking at 100°C for 15 minutes. The spaghetti shape diet was then dried in an oven at 60°C overnight, broken down to 2 - 7 mm lengths and refrigerated at 4°C.

Eight prawns, averaging 5.85 ± 0.11 g were placed in each of 20 x 250 litre black polyethylene flat bottom tanks, contained in a temperature controlled room. The animals were fed twice a day to appetite.

Three diets (1, 2 and 3) gave a promising result in terms of weight gain and FCR. The weight gain of prawns fed on diet 4 was significantly lower ($P<0.01$) than those of prawns fed on any of the other diets (Table 22). Diet 1 resulted in the best weight gain, which was greater than that of prawns fed on either the control, diet 2 ($P<0.05$) or diet 4 ($P<0.01$).

Table 22. Growth rates of the prawns fed on experimental diets and a popular commercial diet.

	Diets				
Measurements	**Control**	**1**	**2**	**3**	**4**
Initial weight (g/prawn)	5.94 ± 0.184	5.88 ± 0.199	5.68 ± 0.233	5.92 ± 0.257	5.83 ± 0.175
Final weight (g/prawn)	10.48 ± 0.270	11.13 ± 0.438	10.16 ± 0.770	10.82 ± 0.422	8.61 ± 0.346
Total weight gain (g/prawn)	4.54 ± 0.281	5.25 ± 0.626	4.48 ± 0.711	4.90 ± 0.491	2.78 ± 0.474
Daily weight gain (g/prawn/day)	0.14 ± 0.009	0.16 ± 0.020	0.14 ± 0.022	0.15 ± 0.015	0.09 ± 0.009
FCR	2.8	2.6	3.0	2.7	4.7
Protein efficiency ratio	0.90 ± 0.043	0.82 ± 0.134	0.74 ± 0.116	0.87 ± 0.084	0.48 ± 0.072

The results showed that diet 1, which was coded as B4-91, was the best in terms of growth of the prawns and FCR. In a separate experiment B4-91 was compared to four commercial prawn diets most commonly used in Australia. The same experimental system was used and water quality parameters were very similar to those of the first trial. Prawns (6.58 ± 0.299 g) were randomly allocated to each tank and fed as described previously. The weight gain and FCR ratios varied between 7.99 to 10.35 g and 2.19 to 2.66 respectively. The survival rate ranged from 93.8% to 100%. Statistical analysis showed no significant difference between B4-91 and the other diets in terms of weight gain, FCR and survival.

In the first trial, B4-91 (diet 1) was higher in plant protein and lower in marine protein than diets two and three (Table 21). This indicates that marine protein sources could be replaced with plant protein sources in prawn diets. However more information is needed on the availability of amino acids and energy in ingredients of plant origin.

6.3.4 Investigation of dietary protein to energy ratios for prawns

Well balanced diet formulations are important for successful farming of prawns. Protein and energy are two main nutritional components in prawn feeds. In studies with fish Garling and Wilson (1976) showed that the weight gain was reduced due to a diet low in both protein and energy. Optimum dietary ratio of protein to energy plays

an essential role in the growth of prawns. There are limited numbers of studies in this area. It is important for the Australian prawn aquaculture industry to establish relevant data for the black tiger prawn which represents the majority of the farmed prawn species.

Prawns averaging 5.83 ± 0.16 g were obtained from a local prawn farm. The diet which was formulated at BIARC (B4-91) was used as basal diet. Four other diets were formulated by modifying B4-91. They had different protein levels varying from 40% to 25% (Table 23). The ratio of protein to energy varied between 23.4 (B4-91) and 12.8 (25% protein diet). The sixth diet was a commercial control diet.

Table 23. Chemical composition of experimental diets (% dry matter basis).

Diets	Dry matter	Crude protein	Ash	Ether extract	Fibre	Gross energy (MJ/kg)	P/E (g protein/MJ)
B4-91	93.92	47.87	10.70	8.40	2.27	20.47	23.4
1	91.00	41.97	9.40	13.69	2.49	21.32	19.7
2	90.80	37.37	8.40	13.81	2.32	21.19	17.6
3	91.10	32.29	7.30	13.41	2.22	21.04	15.4
4	90.30	26.85	6.50	14.40	1.75	21.02	12.8
Commercial control	90.70	44.98	10.20	8.40	2.18	21.50	20.9

B4-91 and the commercial control had four tanks and the other four diets had three tanks each as replications. Each tank was stocked with eight prawns. The experimental system was a temperature controlled system with 20 x 250 litre tanks. Prawns were fed to satiation twice daily at 0900 and 1630 hours.

Results showed significant differences in weight gain and FCR ($P<0.05$) (Table 24). The diet with the lowest P/E ratio and the commercial diet resulted in the poorest weight gain and FCR which were significantly different ($P<0.05$) from those obtained from other diet groups. No significant difference was observed in weight gain and FCR of prawns fed diets B4-91, 1, 2 and 3.

Results indicate that optimum P/E ratio in diets for the black tiger prawn could be as low as 15.35 with a dietary protein level of 30%. However more research is needed to estimate accurate P/E ratio using digestible protein and energy values rather than gross P/E.

Table 24. Performance of prawns fed on experimental diets with various ratios of protein to energy ($\bar{x}$ ± SD)*.

Performance indicator	Diets					
	Commercial (control)	B4-91 (control)	1	2	3	4
Initial weight (g/prawn)	5.81 ± 0.24	6.06 ± 0.12	5.89 ± 0.10	5.83 ± 0.15	5.94 ± 0.11	5.74 ± 0.23
Weight gain (g/prawn)	1.25 ± 0.25[a]	2.07 ± 0.24[b]	1.79 ± 0.02[b]	2.00 ± 0.41[b]	1.77 ± 0.23[b]	1.01[a] ± 0.32[a]
FCR (relative to commercial control diet)	100	85.35[a]	96.10[a]	89.93[a]	97.28[a]	143.08[b]
P/E ratio (g protein/MJ)	20.92	23.39	19.68	17.64	15.35	12.77

* Treatment means with the same superscript are not significantly different (P>0.05).

Table 25. Essential amino acid and crude protein composition of test diets fed to juvenile prawns.

Component (% Dry matter)	Diets									
	1	2	3	4	5	6	7	8	9	10
Crude protein	46.27	46.13	45.31	45.48	43.71	43.09	39.55	37.88	31.55	36.72
Threonine	1.78	1.76	1.73	1.74	1.64	1.65	1.49	1.47	1.18	1.19
Cystine	0.46	0.47	0.45	0.46	0.43	0.44	0.39	0.39	0.30	0.31
Valine	2.62	2.31	2.15	2.25	2.29	2.24	2.04	1.97	1.61	1.65
Isoleucine	2.11	2.00	1.95	1.81	1.99	1.97	1.81	1.79	1.43	1.47
Leucine	3.85	3.80	3.73	3.69	3.58	3.60	3.26	3.22	2.56	2.61
Tyrosine	1.67	1.91	1.92	1.84	1.75	1.75	1.66	1.62	1.23	1.27
Phenylalanine	1.97	2.15	2.23	2.21	2.10	2.12	1.89	1.95	1.45	1.57
Lysine	2.93	2.52	2.50	2.50	2.42	2.49	2.18	2.25	1.75	1.84
Histidine	1.19	1.14	1.17	1.29	1.14	1.12	1.01	0.98	0.89	1.01
Arginine	1.84	1.81	1.78	1.74	1.75	1.71	1.55	1.35	1.12	1.22
Tryptophan	0.55	0.53	0.53	0.50	0.50	0.54	0.44	0.46	0.35	0.38
Methionine	1.04	1.01	1.02	1.00	0.98	0.94	0.87	0.86	0.68	0.73

6.3.5 Determining the dietary essential amino acid requirements for prawns

Tacon (1990) has described three methods for determining the optimum requirements of dietary essential amino acids for finfish and crustaceans. Amongst those three methods the most used method in practice for prawns is the carcass analysis method. For more accurate estimations dose response or carcass deposition (amino acid retention) methods should be used. The dose response procedure is not a reliable method in aquaculture application because of the problem of crystalline amino acids leaching out of the feed. However, the carcass deposition method does not have this disadvantage. Ogino (1980) has described the carcass deposition method for carps and rainbow trout.

In this project the carcass deposition method was used to determine dietary essential amino acid requirement for prawns *P. monodon*.

Juvenile prawns averaging ($\bar{x} \pm$ SD) 2.17 $\pm$ 0.09 g were used during the feeding trial. Ten diets varying in crude protein level (between 31% and 43%, Table 25) were prepared and fed to prawns four times a day at saturation level. Each diet had four replications. The experimental system was the same as described previously.

A group of prawns were subsampled at the beginning of the trial. Initial prawns and prawns after the trial were analysed for amino acid compositions. The essential amino acid retention was calculated from the difference between initial and final compositions of whole prawn body.

Results were calculated for an 80% digestible diet with 40% crude protein at a feeding level of 8% body weight (Table 26). The requirement level of EAA for *P. monodon* found in this study was lower than the recommended levels given by Tacon (1990) for any one prawn species. The high levels reported by Tacon (1990) could be to cover the requirement of carnivorous species which need higher dietary protein requirement. However there were similarities in ratios of individual EAA to total EAA.

In this study, the values found for requirements could change depending on feeding levels, digestibility of diets, dietary protein level and the moisture level of the diet. Therefore a constant adjustment of dietary EAA levels must be carried out in order to determine the actual requirement of EAA at any particular condition.

Although carcass deposition method is a well known method to estimate the requirements of EAA for prawns, these values only indicate the requirement for growth. Therefore more research has to be carried out to determine the EAA requirement for maintenance.

When essential amino acid ratios of the requirement levels found in this study were compared to the ratio of essential amino acids in the body of *P. monodon*, a strong relationship was found. This relationship was also observed in other aquatic species (Benitez, 1989).

Table 26. Essential amino acid (EAA) retention and requirements for *P. monodon*.

Amino acids	EAA retention (g/100 g prawn/day)	EAA requirement (g/100 g protein)	EAA requirement		Recommended dietary EAA requirements for prawns (Tacon, 1990)		EAA compositions in prawn body (*P. monodon*)	
			% dry diet	Ratio to total EAA (%)	% dry diet	Ratio to total EAA (%)	% dry weight	Ratio to total EAA (%)
Threonine	0.023	2.18	0.87	7.51	1.34	9.59	2.70	8.12
Met + cystine	0.019	1.89	0.76	6.56	1.14	8.16	2.30	6.92
Valine	0.026	2.54	1.02	8.81	1.19	8.51	3.04	9.15
Isoleucine	0.037	3.68	1.47	12.69	0.95	6.80	2.65	7.97
Leucine	0.040	3.77	1.51	13.04	1.96	14.02	5.96	17.93
Phenylalanine + Tyrosine	0.044	4.27	1.72	14.85	2.17	15.53	5.44	16.37
Lysine	0.043	4.20	1.68	14.51	2.06	14.74	4.39	13.21
Histidine	0.016	1.55	0.62	5.35	0.62	4.44	1.18	3.55
Arginine	0.043	4.19	1.68	14.51	2.17	15.52	4.99	15.01
Tryptophan	0.007	0.64	0.25	2.16	0.38	2.72	0.59	1.77

6.4 Search for Practical Feeding Systems in Ponds

Inappropriate feeding practices increase the feed costs of production and reduces the profitability of prawn aquaculture. Therefore, precise feeding strategies that provide accurate estimation of feeding levels are needed. Australian farmers do not have as much expertise in feeding management in ponds as do South-east Asian farmers. In South-east Asia, farmers have developed methods that estimate daily feeding levels through their experience. To bring these methods to Australia and to adapt them to Australian conditions, officers from BIARC visited Thailand in 1991. The methods of practical feeding of prawns in ponds as used in Thailand were compiled into a report and distributed to all prawn farmers in Australia.

The Thai feeding method is based on the average consumption of feed by all prawns present in a pond in a 24 hour period. This level is expressed as a percentage of mean body weight of prawn. Estimation of feeding levels is based on survival of prawn, total biomass of prawn in pond, water temperature and molt stage. Assumptions are made daily.

Six feeding trays per 1 hectare pond are used to estimate feeding levels. The size of trays varies depending on the stocking density; for stocking densities between 25 to 30 prawn/m^2 the recommended tray size is 50 cm x 50 cm. Feed trays should not be positioned at muddy areas, in front of aerators or too close to the pond edge. The correct position for feed trays is behind the aerators and at an adequate distance from the pond edge. The amount of feed which would be put in feeding trays is calculated in proportion to the total feeding area in ponds:

$$\% \text{ feed in one tray} = \left(\frac{\text{Area per feed tray} \times \text{number of feed trays}}{\text{feeding area in ponds}} \right) \div \text{number of feed trays}$$

The actual amount of feed to put in ponds is calculated from the biomass of prawns in the pond. Prawns are sampled at two to three different points in a pond using a cast net. Average body weight is calculated by weighing the total weight of prawns and dividing that weight by the number of prawns caught. Estimation of survival rate is the most difficult part. In Thailand it is expected that the survival rate is around 70% at the time of harvesting. In actual terms the survival rate is calculated using the following equation:

$$\text{Number of prawns in pond} = \frac{\text{feeding area in pond}}{\text{surface area of the cast net}} \times \text{number of prawns caught in a cast net}$$

Once the total biomass of prawns in the pond is calculated from sampling of the number and size of prawns, the daily amount of feed can initially be estimated as a percentage of the biomass (Table 27). Amount of feed per feeding tray is calculated from the total daily feeding level and placed to trays.

A fixed schedule for checking feeds in trays is necessary for different sizes of prawns. Small prawns take a longer time to feed while big prawns consume their feed relatively quickly. Therefore time duration for checking feeds in trays varies between 2.5 hours for prawn 2 - 3 g size to 1 hour for 30 - 40 g size prawns.

Table 27. Rate of feed consumption per day of prawns at each phase of development.

Live body weight (g)	Feed (% of biomass)
2 - 3	8.0 - 7.0
3 - 5	7.0 - 7.5
5 - 10	5.5 - 4.5
10 - 15	4.5 - 3.8
15 - 20	3.8 - 3.2
25 - 30	3.2 - 2.9
20 - 35	2.9 - 2.5
35 - 40	2.5 - 2.3
	2.3 - 2.1

Daily feed adjustments are made according to the amount of feed left in the trays. However water temperature and molting stage also affect the level of feeding. The feeding level should be decreased when the water temperature drops since prawns drop their feed intake at low water temperatures.

Above 10 g live weight, the molting stage should be taken into consideration in estimating feeding levels. The amount of feed should be decreased by about 20% when prawns molt. Molting can be synchronised by initiating large water exchange when there is a new and full moon.

Estimation of daily feeding levels with this method is labour intensive. However, when it is carried out strictly, it can save considerable amounts of money for farmers. Today there are some Australian prawn farmers that use this method successfully.

7. OVERSEAS TRIP ON PRAWN NUTRITION

An overseas trip was organised by BIARC to:

1. determine the level of usage in prawn feeds of novel protein sources available in Australia;

2. adapt new techniques for measuring the digestibility of feed ingredients;

3. determine the current level of knowledge of prawn nutritional requirements;

4. observe current feeding methods carried out in South-east Asia for adaptation to Australian conditions;

5. establish collaborative research projects with feed manufacturers in South-east Asia; and

6. establish BIARC as a member of the International Working Group on Crustacean Nutrition in Puerto Rico.

Provision for the trip was made in the first year programme of the FIRDC funded prawn nutrition project which commenced at the BIARC in the 1990-91 financial year.

Two members of the prawn nutrition group, Dr David Hewitt and Mr H Zafer Sarac embarked on a six week trip during which several government and university research centres and commercial operations in Thailand, Taiwan, the UK and the USA were visited. The World Aquaculture Society conference and the meeting of the International Working Group on Crustacean Nutrition in Puerto Rico were also attended.

Total production of farmed prawn in Thailand has been increasing rapidly and was approximately 120,000 tonnes in 1990. The Charoen Pokphand group of companies (CP) dominates the prawn feed industry (80% of market share), with the remaining 20% of the industry under the control of Aquastar. Both companies have large prawn farms in the Songkhla region of Thailand near the Malaysian border and are concerned about the long term effects of the discharge of nutrient enriched seawater from intensive prawn farming. This is one of the concerns of the scientists at the National Institute of Coastal Aquaculture (NICA) and those of the University of Prince of Songkhla in Songhkla province. While CP appears satisfied with the prawn feeds they have developed, Aquastar are working toward more efficient prawn feeds. Their feed development project has two main aims, the first is to replace the fish meal component of prawn feed with vegetable proteins such as soybean meal, cottonseed meal etc and the second is to produce prawn feeds which are more highly assimilated by prawns and produce lower levels of organic waste. Aquastar is willing to participate in collaborative research with the BIARC.

Taiwan lacks both the size and length of coast line of Thailand. Due to crowding of farms, intensive prawn aquaculture in Taiwan gave rise to water pollution that caused an outbreak of disease, virtually destroying the industry. The production of prawns

from aquaculture is now 20 to 30% of the 100 000 tonne peak of 1987. The Tungkang Marine Laboratory (TML) is one of the largest government research centres in southern Taiwan and research at this centre involves eight prawn species which are grown in culture in China and Taiwan. Studies examine the effects of environmental parameters on the growth of prawns, development of formulated feeds, assimilation and digestion of feed ingredients and improvement of pond management. Plans for collaborative work between TML and BIARC were discussed and were positively received.

The University of Stirling in Scotland, the fisheries laboratory of the Ministry of Agriculture, Fisheries and Food at Conwy and the University of Wales in northern Wales were the three research institutions visited in the UK. The Institute of Aquaculture, University of Stirling carries out nutritional studies on tilapia and freshwater and marine prawn species. Some of these studies have been done in conjunction with BP Nutrition in Thailand. At the fisheries laboratory of the Ministry of Agriculture, Fisheries and Food, the main research areas involve lobster (*Homarus gammarus*) cultivation and husbandry and the enhancement of natural fisheries with hatchery reared animals. Studies on the nutritional requirements of prawn larvae and the development of microencapsulated diets for prawn larvae are the primary research projects at the University of Wales. Commercial concerns involve the University in the testing of ingredients for juvenile prawn diets and this is a significant source of funding for this institution. In all institutions the experimental systems utilising recirculated seawater were innovative and effective, particularly the biological filters.

The World Aquaculture Society conference in Puerto Rico was held between the 16 and 20 June. Sessions on the nutritional requirements of prawns, feed ingredients and the technology of fish and crustacean farming were attended and useful contacts established with researchers from Thailand and the US. The nutritional problems in prawn aquaculture and the standardisation of nutritional systems and control diets for studies on crustacean nutrition were discussed during the meeting of the International Working Group on Crustacean Nutrition (IWGCN). The next meeting of IWGCN will be held in Singapore in October 1992. Significantly, the formulation of the standard reference diet for crustaceans was agreed upon and BIARC will possibly be involved in testing this diet for *P. monodon*.

Some of the world's best work in prawn nutrition has come from Texas A & M University Experiment Stations in Corpus Christi and Port Aransas. Their work involves the replacement of fish meal in prawn diets with soybean meal and other possible plant proteins such as lupin and cottonseed, in addition to the study of vitamin, mineral and amino acid requirements. The design of the unique cage system developed by A & M University scientists which allows them to carry out nutritional experiments in ponds is directly transferable to BIARC and will be developed for later dietary trials. Researchers at Texas A & M are also willing to be involved in collaborative work with the BIARC.

Bodega Marine Laboratory (BML) (University of California) is one of the major research centres working on lobster (*Homarus americanus*) nutrition. The nutrition research group at BML has been working on essential nutrient requirements and the determination of optimum levels of both essential and growth enhancing dietary

components. They are currently extending their nutritional studies to penaeid prawn species. Testing of vitamin additives to prawn feeds is also a source of funding at BML. Nutritional research similar to that under way at BML is being carried out at the University of California, Davis for several fish species such as sturgeon and striped bass.

The two main research areas of the Oceanic Institute in Hawaii are the development of prawn diets using cheap vegetable protein sources such as soybean meal etc and the determination of the nutritional contribution of pond water to prawn production. The OI specialises in the area of very high intensity (high stocking density) prawn grow out using plastic lined ponds with central drains. Their results from lined ponds are very important due to the similarities in design with ponds to be constructed at BIARC. Collaboration between OI and BIARC in both diet development and grow out areas was discussed. A new $US14 million project will increase the total area of the research centre and reorganise the OI research facilities. Similarly, reorganisation of research facilities has been under way at the research centre of the Hawaii Institute of Marine Biology.

The information obtained from this overseas trip was compiled in a report and copies were sent to FRDC in 1991.

8. IMPLICATIONS AND RECOMMENDATIONS

This project was mainly concerned with reducing feed related costs by investigating inclusion levels of local feed ingredients and adopting cost effective feeding managements. During the course of the project, a substantial amount of time was spent on developing new techniques and bioassay systems to obtain more reliable results under less labour intensive conditions. A considerable amount of information was obtained in determining feed intakes, net energy values of feed ingredients and feeding levels in ponds. Furthermore dietary requirements for essential amino acids, dietary protein to energy ratios and the effect of attractants on feeding behaviour of prawns were investigated. Nutritional value of various Australian feed ingredients was evaluated for diets of prawns.

The results of this project indicate that:

1. Australian feed ingredients contain nutritional components that fulfils the dietary requirements of prawns. However to formulate more efficient feeds net nutritional contribution of these ingredients to the prawns growth has to be investigated.
   Recommendation 1: Net energy value of all feed ingredients used in feed formulations must be determined.

   Present digestibility method used for feed ingredients and feeds for prawns was not found effective.
   Recommendation 2: A more efficient and reliable digestibility method (that is, use of radio-activity labelled inert markers) must be developed.

2. Attractants in feeds are important to increase feed intake of prawns. They become more in need when less marine proteins are used in prawns feeds.
   Recommendation 3: The optimum levels of dietary attractants must be determined with regard to quality and quantity of terrestrial feed ingredients in prawn feeds.

3. Dietary protein level in feeds could be reduced to 30% which is a 25% reduction from the level currently recommended. However this could be possible only if more digestible carbohydrate sources are used as the energy source to compensate for the energy loss associated with the reduced protein level. Secondly exogenous enzymes are used to increase the digestibility of carbohydrate sources to provide more digestible energy.
   Recommendation 4: A mixture of exogenous enzymes must be developed to increase the digestibility of a wide range of feed ingredients of plant origin.

5. Management of feeding levels in ponds could be more efficient when the amount of feed consumed by prawns is monitored by using feeding trays. However prawn biomass in ponds, molting stages of prawns and the quality of pond water (that is, temperature, salinity) should also be considered.

Savings on feed costs resulted from the findings of this project could be considerable. Reduction in dietary protein levels by 10% units could reduce the cost by up to $A150/tonne. The cost of fishmeal, prawn meal and squid meal are around $A1000/tonne, $A900/tonne and $A5000/tonne respectively. However Australian feed ingredients are relatively cheaper (wheat $A180/tonne; maize $A210/tonne; soybean meal $A460/tonne and mill run $A150/tonne). Feeds for the black tiger prawn contains 40% protein, and on average 60% is supplied by inclusion of marine protein sources. Contribution of marine protein to the cost of feed is about $A500/tonne when marine protein supplies 60% of the protein in a 40% protein feed. Table 28 shows the estimated savings in feed cost by reducing the dietary protein level by 10%.

Table 28. Estimated savings in feed cost by reducing the dietary protein level.

	Dietary inclusion level (%)	Contribution to dietary protein (%)	Cost ($A/tonne)	Savings ($A/tonne)
Today's price				
Marine proteins (25% fishmeal, 5% shrimp meal and 5% squid meal)	35.0	60.0	500.00	-
After 10% reduction in the protein level				
Marine proteins	19.4	68.0	317.57	182.43
Substitution of cereals such as sorghum, wheat	15.6	0	28.08	
			Actual savings 182.43 - 28.08 = 154.35	

Management of feeding in ponds using 'feed tray method' will also reduce feed cost. FCR in Thailand is around 1.4 to 1.7, whereas in Australia it is about 1.9. This means if we reduce FCR by minimum 0.2 by using 'feed tray method' the savings will be about $A330 000 a year for the Australian prawn farming industry.

9. TECHNICAL SUMMARY OF INFORMATION DEVELOPED

The aims of the project were to reduce the effect of feed on production cost in prawn aquaculture by using local ingredients in feeds and to develop effective feeding management strategies.

Results obtained from this project could be compiled in three major groups:

Nutritional requirements of prawns

Requirements for protein and protein/energy ratio for prawns were investigated. The current recommended dietary protein requirement of prawns is about 40%. However, studies in this project indicated that 40% protein is excessive, resulting in some being used for energy. The trial (Section 6.3.4) on dietary protein/energy ratios suggested that the dietary protein level could be reduced to 30%. However its success depends on improving the digestibility of non-protein energy sources (mainly carbohydrates) by addition of exogenous enzymes to diets (Section 6.3.2.5).

Essential amino acid requirements for prawn growth were investigated. It was found that the requirements for EAA were similar to the composition of EAA in the prawn body (Section 6.3.5).

Evaluation of local feed ingredients

Approximately 23 Australian feed ingredients were chemically analysed for proximate composition, amino acids, fatty acids and minerals. Their suitability for prawn diets was discussed with emphasis on chemical composition (Section 6.3.2.1).

Since the present digestibility methods are not reliable, a net energy estimation method was adapted to prawn nutrition to evaluate net energy levels of some feed ingredients for prawn growth. Net energy value of those feed ingredients was: sorghum 6.6 kJ/g; wheat 7.2 kJ/g; rice 7.6 kJ/g; sago 5.8 kJ/g; soybean meal 6.1 kJ/g; fishmeal 3.6 kJ/g; prawn meal 1.4 kJ/g and gluten 4.2 kJ/g (Section 6.3.2.2) and canola meal 6.07 kJ/g (9.17 kJ/g with enzyme treatment) (Section 6.3.2.5). However, net energy estimations must be carried out on more ingredients to formulate better least cost diet formulations.

Furthermore, inclusion levels of seven local protein sources and three carbohydrate meals were tested in prawn diets. Results showed that the growth of prawn could be maintained when local protein sources, wheat and bone meal, blood meal, soybean meal, cottonseed meal and canola were added to diets. However chicken offal and lupin resulted in depressed growth when high inclusion levels of these feed ingredients were used. Local carbohydrate meals, maize, oat and high inclusion levels of wheat depressed growth (Section 6.3.2.3). On the other hand, addition of exogenous enzymes to diets improved growth and digestibility of feed ingredients (Section 6.3.2.5). More research on utilisation of local feed ingredients and the use of exogenous enzymes in prawn feeds is warranted.

Feeding strategies

Australia farmers loose considerable amounts of money due to wrong feeding management in ponds. In South-east Asia, methods for economical feeding management are well established. During this project, a feeding method from Thailand was adapted to the Australian prawn farming industry. This method uses feed trays which are filled with a certain amount of feed based on the ratio of tray size to the pond feeding area. The total daily amount of feed was reduced or increased depending on the amount of feed left in the trays. However pond water temperature and salinity, and molting stage of prawns also determine the feeding levels (Section 6.4).

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