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Preliminary evaluation of meatmeal in aquaculture diets for prawns
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MEAT & LIVESTOCK
AUSTRALIA

PROJECT SUMMARY

OBJECTIVES

- Determine the apparent digestibility of the dry matter, energy, phosphorous, protein and essential amino acids in three meatmeals.
- Determine whether it is possible to measure assimilation and retention of protein from the meatmeal in a diet by examining the stable isotope ratios in the muscle of prawns fed that diet.
- Obtain preliminary data on the effect on prawn growth rate of replacing up to 50% of fishmeal with meatmeal.

In the agreement with the Meat Research Corporation, CSIRO undertook to recommend in the Final Report, appropriate research strategies to:

- Determine the maximum inclusion level of meatmeal in prawn diets.
- Evaluate the impact of the inclusion of meatmeal on prawns' flavour and nutritional value.
- Improve the meatmeal for use in prawn diets.
- Investigate the use of chemical attractants, amino acids and other supplements to increase the inclusion level of meatmeal in prawn diets.
- Investigate the impact of feed residues containing meatmeal on pond sediment conditions.

MATERIALS AND METHODS

Three meatmeals were tested: Provine (Aspen Technology Pty Ltd), and two meat and bone meals (Fletcher International and Beef City). The meatmeals were compared with Tasmanian fishmeal.

1. APPARENT DIGESTIBILITY

1.1 DIET PREPARATION

To test the digestibility of the meals, they must be bound into a water-stable pellet, and certain essential nutrients must be added. A base diet containing fishmeal, binders, vitamins, minerals and lipids was made. Each meatmeal and the fishmeal was mixed with an equal portion of the base diet. Thus, five diets were prepared: three meatmeal diets, a fishmeal diet and the base diet. To each was added the inert markers chromic oxide and ytterbium acetate. After the diets were extruded through a mincer to form the pellets, they were steamed and dried.

1.2 DIGESTIBILITY TRIAL

Power analysis of previously obtained data was used to determine the number of replicates required in the digestibility trial to detect a specified difference between two treatments with 80% probability. We decided obtain 10 replicates of dry matter and nitrogen digestibility data for each treatment which meant that we could detect a difference in digestibility of between 2% and 5% with 80% probability.

The trial was carried out in 100L tanks, each containing one 18-20g *Penaeus monodon*. The prawns were acclimatised to the tanks and their allocated diet for 4 days before the trial began. They were fed a known weight of food twice a day. Uneaten food was removed 40 min. after each feeding and its dry weight determined. Faeces was collected at 6-hourly intervals and all the collections over a 24 hour period were pooled. The faeces were dried, weighed and analysed to determine dry matter and nitrogen digestibilities. The trial ran over three successive days which provided us with three separate estimates of dry matter and nitrogen digestibility for each prawn

After the first phase of the experiment where faeces were collected for dry matter and nitrogen digestibility, faeces were collected from each prawn for a further 6 days. These collections were pooled to provide 4 replicates from each treatment for gross energy and amino acid analyses.

1.3 CHEMICAL ANALYSIS

Proximate composition of the major ingredients and diets was analysed using Association of Official Analytical Chemists (AOAC) procedures. Nitrogen and phosphorus content of the diets and faecal samples were determined colorimetrically in a continuous-flow analyser following Kjeldahl digestion. Chromium and ytterbium were analysed from the same digest solution by inductively-coupled plasma mass spectrometry (ICP-MS). Gross energy was determined by bomb calorimetry. Amino acids were analysed by HPLC. Water-soluble material in the meatmeals was determined by stirring a known weight of the meatmeal in water, then filtering and weighing the residue. Stable isotope analyses were carried out on a Europa Tracer-Mass mass spectrometer.

1.4 DIGESTIBILITY CALCULATIONS

Apparent digestibilities for dry matter, nitrogen, energy and amino acids in the diets were calculated both directly and indirectly. The indirect method used nutrient-to-marker ratios, and therefore eliminated errors due to non-quantitative collection of faeces.

As the test diets were made up of equal quantities of base diet and meatmeal or fishmeal, the digestibilities of the base diet and the test diets were used to calculate the apparent digestibility of the meatmeals and fishmeal.

The apparent digestible essential amino acid ratio (ADEAA) was calculated for each amino acid in each meatmeal and fishmeal. The ADEAA compares the availability of an amino acid in a particular protein source with the amino acid requirements of the target species. All of the ADEAAs for a meatmeal or fishmeal were combined to give an ADEAA index. This index is used to estimate and compare the suitability of proteins for the target species.

2. GROWTH

2.1 DIET PREPARATION

Seven diets containing identical levels of digestible protein were formulated. The control diet contained fishmeal, but no meatmeal. In the other six diets, 50% and 25% of the digestible protein from fishmeal was replaced with each of the three meatmeals. After extrusion through a mincer, the spaghetti-like strands of the diets were broken up into pellets then steamed and dried.

2.2 EXPERIMENTAL DESIGN

The growth trial was conducted in 42 90L tanks, each containing six 5g *Penaeus monodon*. Each diet was fed to 6 randomly selected tanks. The prawns were acclimatised to the tanks and the diets for 7 days before the trial began. During the trial, the animals were fed *ad libitum* twice daily. Before each feeding, residual food and faeces were siphoned out. The prawns were weighed individually at fortnightly intervals. The trial ran for 4 weeks.

3. NUTRIENT RETENTION USING STABLE ISOTOPE ANALYSIS

One of the objectives of the project was to determine whether it is possible to measure the assimilation and retention of protein amino acids from meatmeal in the diet. Over a period of time the muscle of a prawn will take on the stable isotope ratio of the nutrients it is assimilating and retaining. If the stable isotope ratio of the meatmeal is sufficiently different from that of the fishmeal it is replacing in the diet it is possible to calculate the assimilation rate of the meatmeal relative to fishmeal. Samples of fishmeal, the three meatmeals and the diets used in the digestibility trial were analysed on a mass spectrometer to obtain stable isotope ratios of nitrogen (N) and carbon (C). The $\delta^{15}N$ and $\delta^{13}C$ values were calculated using atmospheric air as a standard for ^{15}N and Peedee Belemnite carbonate as a standard for ^{13}C.

RESULTS

1. PROXIMATE COMPOSITION

The ash contents of fishmeal (14.3 %) and Aspen meatmeal (9.4 %) were lower than those of Beef City and Fletcher meatmeals, which were both about 38% (Table 1). This is probably due to a higher bone content in Beef City and Fletcher meatmeals. Correspondingly, the protein and lipid contents of fishmeal and Aspen meatmeal were higher than those of Beef City and Fletcher meatmeals. Aspen meatmeal and fishmeal contained more soluble material than the other meatmeals (Table 1).

2. APPARENT DIGESTIBILITY

2.1 DRY MATTER, PROTEIN AND PHOSPHORUS

Since protein content is directly proportional to nitrogen content, protein digestibility is approximately equal to nitrogen digestibility. All the meatmeals had lower dry matter and nitrogen digestibility than Tasmanian fishmeal. Aspen meatmeal was more digestible than either Fletcher or Beef City meatmeal (Table 1). This is probably because of its lower bone content. Another possible reason for Aspen's higher digestibility is its high content of soluble material, as a high rate of leaching of water soluble material would lead to an erroneously high digestibility estimate. Because of the presence of bone pieces in the Fletcher and Beef City meals, phosphorus digestibility figures were inconsistent, and have not been reported here.

Table 1. Summary of composition of the protein sources (% of Dry Matter), Gross Energy (Mj/kg) and apparent digestibilities of dry matter (DM) and nitrogen (N).

	Fishmeal	Aspen	Beef City	Fletcher
Ash	14.3	9.4	38.6	36.9
Crude Protein	77.2	83.5	46.7	51.0
Total Lipid	9.2	17.1	7.4	6.3
WSM*	24.9	23.1	8.6	6.1
Gross Energy	21.3	25.4	13.9	14.5
DM Digest.(%)	85.9	77.8	59.6	57.2
N Digest (%)	93.3	82.5	76.5	73.8
DE (%)	88.5	63.9	61.3	55.2

* Water soluble material (WSM), Apparent digestible energy (%) (DE)

2.2 ENERGY

Aspen meatmeal contained more gross energy than fishmeal, and Fletcher and Beef City meatmeals contained less (Table 1). However, the apparent digestible energy (DE) of all the meatmeals was lower than that of fishmeal, and similar to each other. This suggests the lipid in the meatmeals is not as well digested the protein.

2.3 ESSENTIAL A NO ACIDS

The differences in the digestibility of individual amino acids from the three meatmeals follow the same pattern as observed for their nitrogen digestibility, with the amino acids in fishmeal having a higher average digestibility than those in Aspen, Fletcher or Beef City meatmeals. In general, all amino acids in a particular meatmeal had similar digestibility, however, cystine digestibility was lower in all meatmeals, and arginine was lower in Beef City and Fletcher meatmeals. From the ADEAA ratios, it appears that arginine is deficient in all the meatmeals and fishmeal. Isoleucine, leucine and lysine are limiting amino acids in Beef City and Fletcher meatmeals.

Feed ingredients with an essential amino acid (EAA) index of 0.90 or greater are considered good ingredients, those with values between 0.80 and 0.90 are considered useful and those with a value less than 0.80 are considered inadequate. The ADEAA indices for fishmeal, Aspen, Beef City and Fletcher were 0.95, 0.96, 0.88 and 0.83 respectively. Therefore, with respect to amino acid composition, fishmeal and Aspen are good ingredients, and Beef City and Fletcher meatmeals are useful ingredients.

3. GROWTH

There was no significant difference between the growth rates of the prawns fed the meatmeal diets and the control diet (MRC Standard), though there were differences in the growth rates between the meatmeal diets. However, the growth rates were lower than expected, and the mortality rate was higher. The cause of this has now been demonstrated to be due to the quality of the prawns that we purchased. Meatmeal was included in this trial at rates of up to 29% of the diet without any significant reduction in performance, and this is a strong indication that meatmeal may be used to replace at least 50% of the fishmeal in the diets of juvenile *P. monodon*.

4. ASSIMILATION AND RETENTION USING STABLE ISOTOPE ANALYSIS

Aspen meatmeal has a markedly different stable isotope ratio from the other meatmeals and from fishmeal. It appears that it would be possible to determine the relative contribution of fishmeal protein and Aspen meatmeal protein to synthesis and retention in the prawn. With the development of techniques to determine the ratio of two stable isotopes of sulphur, the meatmeals and fishmeal may be more clearly differentiated.

CONCLUSIONS

The apparent digestibilities of dry matter, energy, protein and essential amino acids in the meatmeals are lower than that of fishmeal. Phosphorus digestibility could not be accurately determined because of the high bone content of Fletcher and Beef City meatmeals and the fishmeal. Of the three meatmeals, Aspen has the highest apparent digestibility. The apparent digestible essential amino acid index rates Aspen meatmeal as a good ingredient, and Beef City and Fletcher meatmeals as useful ingredients.

It appears that it would be possible to measure the relative assimilation and retention of proteins from Aspen meatmeal and fishmeal using stable isotope ratios as they are markedly different from each other. Beef City and Fletcher meatmeals have similar stable isotope ratios to fishmeal. It is therefore unlikely that relative assimilation could be measured for these meatmeals using only stable isotopes of carbon and nitrogen. However, the use of stable isotopes of sulphur may lead to clearer differentiation between fishmeal and these meatmeals.

The results obtained from this study indicate strongly that meatmeal could be used to replace at least half of the fishmeal in diets for juvenile prawns. Because the meatmeals have lower dry matter digestibility and nitrogen digestibility than fishmeal, there would be significantly more faecal waste produced with these diets which could have an adverse effect on water quality.

RECOMMENDATIONS

FURTHER RESEARCH AND DEVELOPMENT

- *Determine the maximum inclusion level of meatmeal in prawn diets.* The maximum inclusion level of meatmeal in the prawn diets could be established using summit diet dilution experimental design using small tanks as used in the growth experiments described in this report. Once the maximum practical inclusion level of meatmeal in the feed has been established, a feeding trial in small scale prawn ponds or large tanks (3 m diameter) would be advisable before testing the diets in a commercial prawn farm pond. This would minimise the risk of a very expensive loss in production, given a crop in a 1 hectare pond is worth about $100,000 at harvest. The trial in small scale ponds or large tanks should be carried out to confirm the cost-effectiveness of the meatmeal based diets and to determine any effects to water or pond bottom quality. The results in this type of experiment would be much closer to those obtained in a commercial pond.
- *Evaluate the impact of the inclusion of meatmeal on prawns' flavour and nutritional value.* The flavour of the prawn flesh of prawns fed a base diet and diets containing meatmeal should be compared from prawns grown in the small scale ponds or large tanks. The CSIRO Division of Food Science and Technology and the Queensland Department of Primary Industries have the facilities and expertise to conduct these trials. This work could be carried out on a fee for service basis.
- *Improve the meatmeal for use in prawn diets.* The Beef City and Fletcher meatmeals could be significantly improved by reducing the amount of bone in them. This would have the effect of increasing their dry matter digestibility and possibly their nitrogen digestibility. The addition of other tissues or organs that are richer in arginine, lysine, leucine and isoleucine would improve the amino acid profile of the meatmeals, particularly with Fletcher and Beef City.
- *Investigate the use of chemical attractants, amino acids and other supplements to increase the inclusion level of meatmeal in prawn diets.* At this point we have not seen any evidence that the flavour of the meatmeal is a counter-attractant for prawns at the inclusion levels we have used. There seems to be no immediate need to investigate the use of attractants. However, the inclusion of crystalline amino acids, either free or in microencapsulated form, to balance the amino acid profile of the feeds does require further investigation. Supplementary amino acids are likely to enhance the use of meatmeals particularly if the arginine and lysine levels can be effectively increased in the feed.

- *Investigate the impact of feed residues containing meatmeal on pond sediment conditions.*
  A comparison of the amount of waste matter produced from a control diet and the meatmeal based diets can easily be made in the course of a feeding trial. It would be necessary to study the breakdown of the waste material in a simulated pond situation to determine the impact on the water quality. Parameters such as ammonia and nitrite, organic N and C, chlorophyll a, BOD, and bacterial numbers in the water column and in the sediment would need to be monitored.

PRACTICAL USE

The results of this study are a preliminary evaluation of the use of meatmeal in aquaculture diets. They have indicated that meatmeal could be used to replace at least half of the fishmeal in prawn feeds. It has also identified areas where the meatmeals could be improved and areas or further research.

COMMERCIAL EXPLOITATION

At this stage a recommendation for the commercial exploitation of the results would be premature.

BACKGROUND

Meatmeal may have the potential to be used as a major component in aquaculture diets for prawns.

Currently the major protein source in aquaculture diets is fishmeal and generally comprises between 25 and 30% of prawn diets. The demand for formulated prawn feeds is increasing with the transition from extensive to intensive prawn farming in many parts of the world. There is consequently a worldwide increase in the demand for fishmeal and fisheries biologists believe that stocks of fish being used for fishmeal are being over exploited (Barlow, 1989, New, 1991). Australia has very limited stocks of fish that can be used for fishmeal production and so relies heavily on imported fishmeal. Prawn feeds generally use the best quality, low temperature fishmeals which cost up to $1100/tonne.

The need to replace fishmeal in aquaculture diets is recognised as a major international research priority and there are considerable national and international marketing opportunities for successful alternative protein sources. Global and Asian aquaculture feed production in 1990 was estimated at about 3 million tonnes and 1.6 million tonnes respectively and the forecast for Asian production in 2000 is 2.6 million tonnes (Akiyama, 1991). The aquaculture feed market could offer an outlet for tens or even hundreds of thousands of tonnes of Australian products if these are shown to be well utilised by prawns and are competitively priced.

The aim of this project was to evaluate the digestibility and assimilation of meatmeal in the diet of the giant tiger prawn *Penaeus monodon*, and at the same time to obtain preliminary data on the effect on growth rate of replacing up to 50% of the fishmeal in the diet with meatmeal. *P. monodon* is the most commonly farmed prawn species in South East Asia and Australia and provides the most appropriate test species for evaluating the meatmeal.

PROJECT OBJECTIVES

- Determine the apparent digestibility of the dry matter, energy, phosphorous, protein and essential amino acids in three meatmeals selected in collaboration with the MRC.
- Analyse the stable isotope ratios of nitrogen and carbon and determine whether the level of assimilation and retention of protein from the meatmeal in a formulated diet can be measured from the stable isotope ratios in the muscle of prawns fed that diet.
- Obtain preliminary data on the effect on prawn growth rate of replacing up to 50% of fishmeal with the meatmeals as considered appropriate.

In the agreement with the Meat Research Corportation, CSIRO undertook to recommend in the Final Report, appropriate research strategies to:

- Determine the maximum inclusion level of meatmeal for a practical aquaculture diet for prawns.
- Evaluate the impact of including meatmeal on prawn flavour and nutritional value.
- Improve the meatmeal with respect to its use in prawn diets.
- Increase the maximum practical inclusion level of meatmeal in prawn diets by including chemical attractants, amino acid and other supplements.
- Investigate whether the feed residues containing meatmeal would have an adverse impact on pond sediment conditions.

MATERIALS AND METHODS

The Meat Research Corporation arranged for three meatmeal producers to supply material for us to evaluate in this study. Aspen Technology Pty Ltd of Preston, Victoria provided us with their 'Provine' formulation, and Beef City of Toowoomba, Qld. and Fletcher International of Dubbo, NSW both provided a standard meat and bone meal. Throughout this report the three meatmeals have been referred to by the abbreviated name of the company that manufactured them. The performance of the meatmeals was compared against that of Tasmanian fishmeal purchased from Aqua-feed Products Australia of Narangba, Qld. All the meals were sieved and milled to a particle size of less than 0.71 mm.

Prior to starting the digestibility experiments and the growth trials, previously obtained data was examined using power analysis to determine the level of variability in the data and the number of replicates that would be required to detect a 2% and a 5% difference between two treatments with 80% probability.

1. APPARENT DIGESTIBILITY

1.1 DIET FORMULATION

The diets used in the digestibility experiments were formulated so that 50% of the control diet was replaced with the ingredient being tested, these being; Tasmanian fishmeal, Aspen, Beef City and Fletcher meatmeals (Table 1). The physiologically inert markers, chromic oxide and ytterbium acetate were included in the diets, at 0.05 and 0.02% of dry matter (DM) respectively, to provide an indigestible reference in the calculation of apparent digestibility.

1.2 DIET PREPARATION.

The dry ingredients were mixed together with chromic oxide using a Hobart mixer. Ytterbium acetate was dissolved in 10mls of distilled water and sprayed through an atomiser onto the dry ingredients which were mixed regularly to ensure homogeneity of application. The fresh squid mince and oils were then added to the dry ingredients and mixed thoroughly. Water was added (if required) until a crumbly dough was formed. This dough was extruded twice through a Hobart mincer attachment with a 3mm die. The resulting spaghetti-like strands were cut into 6mm lengths and steamed.for 10 minutes. Pellets were then dried at 60°C until their water content was reduced to 10-12%. Diets were stored at 1°C until used.

Table 1. Composition of diets used in digestibility experiments. (% Diet DM).

Ingredient	Control	Fishmeal	Aspen	Beef City	Fletcher
Flour	45.95	22.995	22.995	22.995	22.995
Cholesterol	1	0.5	0.5	0.5	0.5
Lecithin	3	1.5	1.5	1.5	1.5
Squid Oil	4.01	2	2	2	2
Fishmeal	20.02	60	10	10	10
Squid †	10	5	5	5	5
Gluten	12.01	6	6	6	6
Vitamins	1.8	0.9	0.9	0.9	0.9
Minerals	2.2	1.1	1.1	1.1	1.1
Meatmeal	-	-	50	50	50
Coated Vit.C	0.01	0.005	0.005	0.005	0.005

† Whole fresh bait squid minced twice through a 3mm die, it was included on a dry matter (DM) basis, squid mince used was 17% DM.

1.3 DIGESTIBILITY TRIAL

Digestibility experiments were carried out in 25 specially modified 100L round, white, low density polyethylene tanks in a flow-through seawater system. The incoming seawater was heated to 30°C to maintain tank temperature at 28°C, and filtered through a multimedia filter and two cartridge filters (IBC) to 1μm. Flow into the tanks was set at 500 mL/min, an exchange rate of 720% per day. Tanks were all individually aerated.

One *Penaeus monodon* juvenile of 18-20g, supplied by Gold Coast Marine Hatcheries, was randomly assigned to each tank. The moult stages of the animals were determined and each was marked with a numbered eyestalk tag. Only intermoult animals were selected for the experiments. The prawns were weighed to 0.01 g and placed in a tank where they were acclimatised to the laboratory conditions and treatment diets for 4 days prior to the start of the trial. During the first 3 days acclimatisation, food was offered *ad libitum* twice a day but on the fourth day uneaten food and fragments were removed 40 minutes after the food had been placed in the tank. On the night before the digestibility experiments were to start, faeces were siphoned from the tanks about 6 hours after feeding to minimise the incidence of coprophagy.

During the first phase of the digestibility experiment, which lasted 3 days, the prawns were provided with a known weight of food, about 0.3g, twice a day, at 05:00 h and 17:00 h. After 40 minutes all uneaten food and residues were siphoned from the tank and filtered onto a 5.5 cm Whatman GF/C filter, dried at 105°C for 4 hours and weighed. The faecal material produced by individual prawns from each feed were collected by siphoning 6 h after feeding and again at 12 h, immediately before the next feed. The recovered material was filtered onto a 3cm diameter Whatman No.1 filter paper, lightly rinsed with distilled water and dried at 105°C for 4 hours before weighing. After the 6 h filtration a drop of 3% formalin was placed on each filter and they were stored undisturbed on the sealed filtration manifold at 1°C until they were used to filter the 12 h samples.

Faeces produced from the 05:00 h and 17:00 h feedings were pooled to provide the day's faecal production sample. Faeces were collected in this manner from each prawn for three days to provide triplicate samples from an individual animal for the estimation of dry matter (DM) and nitrogen digestibility. Over the following 6 days, faeces were collected for amino acid analysis and gross energy analysis. The faecal material recovered during this second phase was filtered onto a screen of 90μm nylon mesh, dried at 105°C for 4 hours and ground to a fine powder. This material was pooled to provide sufficient material for 2 replicate samples from each treatment.

As we had 25 tanks available for the digestibility tanks we were able to set up 5 treatments (diets) with 5 replicates (prawns) for each treatment. In order to obtain 10 replicates for each treatment the entire experiment was carried out a second time after the prawns had been randomly re-assigned to a different treatment. From the second experiment we obtained a further 2 replicates for the amino acid analyses and gross energy determinations.

1.4 CHEMICAL ANALYSIS.

Proximate composition of the major ingredients and diets were determined using standard Association of Official Analytical Chemists (AOAC) procedures as outlined in the Proceedings of the Analytical Techniques Workshop held by Fisheries Research and Development Corporation's Fishmeal Replacement Sub-Program in April 1994. (Ed. Allan and Frances)

Nitrogen and phosphorous content of samples were determined colorimetrically in a continuous flow analyser, following Kjeldahl digestion. The indophenol blue complex was used for the nitrogen analysis and the phosphomolybdenum blue complex was used for phosphorus determination. Chromium and ytterbium were analysed from the same digest solution by inductively coupled plasma mass spectrometry (ICP-MS).

Gross energy was determined by isothermal bomb calorimetry using a Leco AC200 Bomb Calorimeter.

Amino acids were analysed by HPLC after hydrolysis in 6N HCl. Methionine and cystine were determined as methionine sulphone and cysteic acid respectively following oxidation with performic acid and then hydrolysis with 6N HCl. Tryptophan is destroyed during acid hydrolysis and was not determined in this study.

Water soluble material in the meatmeals was determined by stirring a weighed portion of meatmeal (ca 0.5g) with 50 ml distilled water before quantitatively transferring and filtering through a pre-dried and weighed glass-fibre filter (Whatman GFC. 7 cm diam.). The filter and residue were dried at 105°C for 4 h before being reweighed.

1.5 CALCULATION OF APPARENT DIGESTIBILITY COEFFICIENTS.

Apparent digestibility for dry matter (DMD), nitrogen (ND), energy (DE) and amino acids were calculated both directly, and indirectly using the markers. The following formulae were used for these calculations:

Direct;

$$\text{Nutrient Digestibility} = \frac{\text{Nutrient ingested (g)} - \text{Nutrient in faeces (g)}}{\text{Nutrient ingested (g)}} \times 100$$

Indirect;

$$\text{Nutrient Digestibility} = \frac{\text{Marker Ratio in food} - \text{Marker Ratio in faeces}}{\text{Marker Ratio in food}} \times 100$$

$$\text{Marker Ratio} = \frac{\text{Concentration of nutrient in sample (mg / g)}}{\text{Concentration of marker in sample (mg / g)}}$$

Where 'Marker' is either elemental Chromium (Cr) or Ytterbium (Yb).

As the meatmeal diets were made up of 50% meatmeal and 50% control diet, the apparent digestibility coefficients of the diets can be used to calculate the apparent digestibility coefficient of the meatmeals using the following equation.

$$\text{Digest. of Meatmeal} = 2 \times \text{Digest. of Meatmeal diet} - \text{Digest. of Control diet}$$

1.6 CALCULATION OF APPARENT DIGESTIBLE ESSENTIAL AMINO ACIDS

The apparent digestible essential amino acid ratio (ADEAA) of a protein and the ADEEA index, which is calculated from it provides a very useful parameter to evaluate the suitability of a protein. They are derived from the amino acid profile of the ingredient, the apparent digestibility of the amino acids in it and the amino acid requirements of the target species.

$$\text{ADEAA Ratio} = \frac{\text{Apparent Digestibility of } AA_1 \times \%\ AA_1 \text{ in Protein}}{\%\ AA_1 \text{ in Prawn Muscle Protein}}$$

where AA_1 is any essential amino acid.

The ADEAA index is derived from the ADEAA ratios of all the essential amino acids but when ADEAA ratio is greater than 1.0 it is given the value of 1.0 (Penaflorida, 1989). The concentration of one essential amino acid, tryptophan, was not determined in this study and was not included in the calculation of the ADEEA index.

$$\text{ADEAA Index} = \sqrt[n]{ADEAA_1 \times ADEAA_2 \times \ldots ADEAA_n}$$

2. GROWTH TRIAL 1

2.1 EXPERIMENTAL DESIGN

This trial was conducted in a flow through seawater aquaria system of 42 x 90L black fibreglass tanks. These tanks were individually aerated, and supplied with preheated seawater exchanging 680% per day. Tank temperatures were maintained at 28±1°C. Subdued lighting was provided in a 12 h light /12 h dark regime.

Six *P. monodon* juveniles (4.86 ± 0.06g), supplied by a North Queensland prawn farm, were placed into each tank so that total weight and size range was similar across all tanks. The 7 treatments, a control and the 3 meatmeals at 2 inclusion levels, were replicated 6 times and assigned in a completely randomised design. The prawns were acclimatised to the laboratory conditions and diets for 7 days prior to the start of the trial. The animals were fed *ad libitum* twice a day at 08:30 h and 16:30 h, feeding level was monitored and adjusted daily. Residual food and faeces were siphoned from the tanks before each feeding. Moults, mortalities and tank temperatures were recorded daily. The trial ran for 4 weeks, and the prawns were weighed fortnightly.

2.2 DIET FORMULATION

The diets in the growth experiments were formulated to contain identical levels of digestible protein using apparent digestibility data obtained in the previous experiment. The diets were derived from a standard diet (MRC Standard) which was comprised of 57% base ingredients and 30% fishmeal, the balance being cellulose. Diets were prepared to contain each of the meatmeals at two inclusion levels, replacing one half and one quarter of the digestible protein from fishmeal in the standard diet (Table 2). The cellulose was used to balance the formulation. The small amount of protein from ingredients, other than fishmeal and meatmeal, in the diet was kept constant to maintain the protein profile of the base diet. The crude protein and digestible crude protein (fish and meat meals) levels of the diets were similar (Table 3).

2.3 DIET PREPARATION

The dry ingredients were mixed together using a Hobart mixer. The fresh squid mince and oils were added and mixed thoroughly. Water was then added to this mixture, if required, until a crumbly dough was formed. The dough was extruded twice through a mincer attachment with a 3mm die. The resulting strands were then cut into 6mm lengths and steamed for 10 minutes. The pellets were dried at 60°C until they contained about 10-12% moisture and were stored at 1°C until used.

Table 2. Composition of diets used in growth trials (% Diet DM).

Ingredient	MRC Standard	Aspen 1	Aspen 0.5	Beef City 1	Beef City 0.5	Fletcher 1	Fletcher 0.5
Flour	26.5	25.5	25.5	25.5	25.5	25.5	25.5
Gluten	6	6	6	6	6	6	6
Cholesterol	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Lecithin	3	3	3	3	3	3	3
Squid Oil	4	4	4	4	4	4	4
Fish Meal	30	15	22.5	15	22.5	15	22.5
Squid †	5	5	5	5	5	5	5
Minerals	1.1	1.1	1.1	1.1	1.1	1.1	1.1
Cellulose	13	10.65	12.34	0.2	7.12	3.3	8.67
Vitamins ‡	0.9	0.9	0.9	0.9	0.9	0.9	0.9
Soy Meal	10	10	10	10	10	10	10
Meatmeal	-	18.35	9.16	28.8	14.38	25.7	12.83

† Whole fresh bait squid minced twice through a 3mm die, it was included on a DM basis, squid mince used contained 17% DM.
‡ Coated Vitamin C was included in all diets at 500 mg/kg.

Table 3. Nutrient composition of growth trial diets (%DM).

	MRC Standard	Aspen 1	Aspen 0.5	Beef City 1	Beef City 0.5	Fletcher 1	Fletcher 0.5
Crude Protein	40.38	41.62	40.88	42.56	41.38	42.22	41.20
Dig. CP†	22.04	22.00	22.00	22.00	22.00	21.99	22.00
Lipid	11.63	13.30	12.44	12.41	12.00	11.86	11.73
Crude Fibre	14.60	12.37	14.03	0.95	8.31	4.23	9.94
Ash	7.00	6.51	6.75	16.35	11.66	14.67	10.82

† Apparent digestible crude protein from fishmeal and meatmeals but excluding soy meal, flour, gluten and squid. Total digestible protein estimated at 38%

3. GROWTH TRIAL 2

In a second growth trial, the experimental design was identical to the first experiment except that the prawns were obtained from another source. A commercial prawn feed for *Penaeus monodon*, made by the CP Group in Thailand was used as the control and the growth and survival of prawns compared with that on the MRC Standard diet. As in the first trial, 6 replicate tanks each containing 6 prawns (mean 5.24g, s.d. 0.45g) were assigned to each treatment. The prawns were acclimatised to the laboratory conditions and diets for 7 days prior to the start of the trial. They were fed *ad libitum* twice a day at 08:30 h and 16:30 h, feeding level was monitored and adjusted daily. Residual food and faeces were siphoned from the tanks before each feeding. Moults, mortalities and tank temperatures were recorded daily. The trial ran for 4 weeks, and the prawns were weighed fortnightly.

4. NUTRIENT RETENTION USING STABLE ISOTOPE ANALYSIS

One of the objectives of this project was to determine if it is possible to measure the assimilation and retention of protein amino acids from meatmeal in the diet. Over a period of time, the muscle of a prawn will take on the stable isotope ratio of the nutrients it is assimilating and retaining. If the stable isotope ratio of the meatmeal is sufficiently different from that of the fishmeal it is replacing in the diet, it is possible to calculate the assimilation rate of the meatmeal relative to fishmeal. This calculation requires data on the stable isotope ratios of the meatmeal, fishmeal, the diet that is used and the muscle of prawns that have grown substantially while being fed the test diet.

Samples of fishmeal, the three meatmeals and the diets used in the digestibility experiment were analysed on a Europa Scientific Tracer-Mass, mass spectrometer at Griffith University, Nathan, Qld. The percentage N and C and the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios were determined.

The ratio between the stable isotopes is frequently expressed as the $\delta^{15}N$ value for nitrogen and $\delta^{13}C$ for carbon. This expression is a refinement of the basic ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$ and includes the ratios from a particular standard compound for each element (Sholto-Douglas *et. al.* 1991). Our results have been expressed in this form.

$$\delta X(\text{‰}) = \frac{(R_{sample} . R_{standard})}{R_{standard}} \times 1000$$

where $X = {}^{15}N$ or ${}^{13}C$
$R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$
standard = atmospheric (AIR) nitrogen for ${}^{15}N$
standard = Peedee Belemnite carbonate for ${}^{13}C$

5. STATISTICAL ANALYSIS

The digestibility data was modelled using the Mixed Procedure in SAS (Proc Mixed, SAS Technical Report P229). Estimates of the digestibility of each meatmeal and the associated standard error were obtained for each method of measuring digestibility (ie. the gravimetric method and the inert markers, chromic oxide and ytterbium acetate). Nitrogen and DM digestibility data from each individual prawn, which were collected over three days, were treated as repeated measurements and a time series model (first order auto regressive model) was fitted to the data.

ACHIEVEMENT OF OBJECTIVES

All of the objectives that were planned for this project have been achieved. The estimates of the digestibility of energy, protein and essential amino acids have been obtained, the stable isotope ratios of the meatmeals have been measured and a growth trial was carried out to evaluate the three meatmeals at inclusion levels where they replaced 25% and 50% of the protein from fishmeal.

The digestibility data has been combined with the essential amino acid data to produce an index of nutritional value of the meatmeals. The results for the digestibility of phosphorous in meatmeals appeared erratic and have not been presented as they are considered inaccurate. This was probably due to relatively high levels of phosphorous in the bone which is poorly digested and was not homogeneously distributed through the feed pellets.

The stable isotope ratios of nitrogen and carbon in the meatmeals were obtained and it appears that the relative utilisation of protein from Aspen meatmeal in a prawn feed could be determined. However, with Beef City and Fletcher meatmeals the ratios appear too similar to the ratios in fishmeal to be useful on their own and may require the addition of stable isotope ratio data such as that from sulphur.

From the growth trials, replacement of 25% and 50% of the fishmeal gave results similar to the fishmeal based control diet, suggesting that an inclusion level of 25% meatmeal in a prawn diet may be feasible. However, the growth rates obtained with the control diet in the main growth trial were lower than expected, so another trial will be needed to confirm these findings.

RESULTS AND DISCUSSION

DIGESTIBILITY

DRY MATTER AND NITROGEN

Since protein content is directly proportional to nitrogen content, protein digestibility is approximately equal to nitrogen digestibility. The apparent digestibility of DM, energy and nitrogen in the meatmeals were all significantly lower than that in Tasmanian fishmeal. Aspen had significantly higher apparent digestibilities than Beef City or Fletcher which had similar digestibilities (Tables 4 and 5). Because of the presence of bone pieces in the Fletcher and Beef City meatmeals, phosphorus digestibility figures were inconsistent, and have not been reported here.

Table 4. Apparent digestibility (%) of Dry Matter (DM) and Nitrogen obtained using the gravimetric method (Grav) and using the inert markers, chromic oxide (Cr) and ytterbium acetate (Yb). Standard errors are in parentheses.

	DM (Grav)	DM (Cr)	DM (Yb)
Fishmeal	80.4 (0.82)	85.9 (1.79)	77.5 (1.11)
Aspen	79.8 (0.89)	77.8 (1.66)	64.0 (1.21)
Beef City	38.7 (1.26)	59.6 (2.02)	28.2 (1.49)
Fletcher	43.2 (1.29)	57.2 (2.04)	19.2 (1.58)

	Nitrogen (Grav)	Nitrogen (Cr)	Nitrogen (Yb)
Fishmeal	90.6 (0.41)	93.3 (0.54)	89.9 (0.31)
Aspen	85.4 (0.38)	82.5 (0.62)	75.2 (0.64)
Beef City	66.2 (0.68)	76.5 (0.80)	60.6 (0.67)
Fletcher	67.1 (0.73)	73.8 (0.86)	53.8 (0.82)

total fat are so consistent that we believe they are a true representation of the composition of the samples. This suggests that either, 6.25 is an inappropriate multiplier for the type of the protein in the meatmeal or that there is a significant amount of non-protein nitrogen present. Analyses of the meatmeals showed low levels of urea. In the Aspen meatmeal it was less than 0.5% of DM which would have a minimal effect on the total nitrogen content.

ENERGY

The digestible energy in the meatmeals comes mainly from the energy obtained from protein and fat. The Aspen meatmeal contained the highest level of gross energy which is a reflection of its low ash content and consequently its higher crude protein and fat levels. (Tables 5 and 6). The apparent digestible energy (DE) of the meatmeals were lower than the fishmeal (Table 5) but were not markedly different from each other with Aspen having the higher value. The relative DE (DE of meatmeal/DE of fishmeal) of all the meatmeals is lower than the relative nitrogen digestibilities suggesting that the fat in the meatmeals is not being digested as well as the protein. This suggestion is supported by the fact that Aspen has more than twice the total lipid content of Beef City and nearly twice the protein content, but the difference between the relative DE of Aspen and Beef City is significantly smaller than the difference between the relative nitrogen digestibilities of the two meatmeals. As with DM digestibility, the apparent DE of the Aspen meatmeal would appear higher than the true digestibility if the water soluble material in it was lost from the feed pellets and was of organic origin.

ESSENTIAL AMINO ACIDS

Using the amino acid profiles of the meals (Table 7) and their apparent digestibilities (Table 8), ADEAA's were calculated (Table 9). The differences in the digestibility of individual amino acids from the three meatmeals follow the same pattern as observed for their nitrogen digestibility (Table 8). In a particular meatmeal there was little variation in the digestibility of most amino acids. There appeared to be a closer relationship in the variation of the individual amino acid digestibility between Beef City and Fletcher meatmeals than with Aspen. Cystine consistently appeared to have a lower digestibility than the other amino acids while in Beef City and Fletcher arginine was also lower. In contrast, the digestibility of lysine was higher in two of the meatmeals but markedly lower in Fletcher. This suggests that the processing conditions of the Fletcher meatmeal may have reduced the lysine digestibility and if this is correct, it would probably have reduced the bioavailability of the digestible lysine. From the ADEAA ratios it appears that arginine is deficient in all the meatmeals and particularly in fishmeal (Table 9). Isoleucine appears to be the limiting amino acid in both Beef City and Fletcher, followed by leucine then lysine.

Feed ingredients with an essential amino acid (EAA) index value of 0.90 or greater are considered good feed ingredients, those with values between 0.80 and 0.90 are considered useful and those with a value less tan 0.80 are considered inadequate (Oser, 1959). We are applying a more rigorous criterion in that we are using the

Table 5. Gross Energy (GE) and apparent digestible energy (DE) of meatmeals and fishmeal as determined using chromic oxide and ytterbium acetate.

Ingredient	GE Mj/kg DM†	DE (%) Chromic oxide	DE (%) Ytterbium acetate
Fishmeal	21.3	88.5	87.3
Aspen	25.4	63.9	67.0
Beef City	13.9	61.3	63.7
Fletcher	14.5	55.2	57.4

† Values calculated from DE of diets rather than directly from ingredients.

Proximate analysis of the meatmeals showed that Aspen has a higher crude protein and lower ash content than Beef City or Fletcher (Table 6). The higher apparent digestibility of the DM in the Aspen meatmeal is partially due to it's lower bone content. However, it could also be partially due to the high level of water soluble material in this meatmeal (Table 6). The loss of water soluble material was measured from the free meatmeal and is likely to be lower when the meatmeal is incorporated into a feed pellet. Significant levels of water soluble material in the meatmeals will result in erroneously high estimates of DM digestibility as a proportion of this material would be lost from the feed to the surrounding water before and during feeding. Interestingly, the Tasmanian fishmeal has a high level of water soluble material, close to that of the Aspen meatmeal.

Table 6. Proximate composition of meatmeals and fishmeal expressed as a percentage of dry matter.

Ingredient	Ash	Crude Protein	Total Lipid	Water-soluble material
Fishmeal	14.3	77.2	9.2	24.9
Aspen	9.4	83.5	17.1	23.1
Beef City	38.6	46.7	7.4	8.6
Fletcher	36.9	51.0	6.3	6.1

The apparent digestibility of nitrogen in the Aspen meatmeal was greater than the other two meatmeals but the difference was less than the difference in the DM digestibilities (Table 4). As with the DM and energy, this value could be an over-estimate of the digestibility if significant amounts of nitrogen are being lost from the meatmeal once the food is placed in water. At this stage we have not measured the amount of nitrogen lost in this way from meatmeals either on their own or when incorporated into a prawn feed. The crude protein in Aspen as calculated by N x 6.25 gives a very high value for crude protein such that the sum of ash, crude protein and total fat exceed 100%. The results of the duplicate analyses for ash, nitrogen and apparent digestible EAA. With this criterion Aspen appears to be a good feed ingredient while Beef City and Fletcher are rated as useful.

The bioavailability of the amino acids is not determined with digestibility measurements. Processing conditions can have a significant effect on the bioavailability of amino acids, particularly of lysine and arginine, while their apparent digestibilities could remain unchanged. To determine apparent bioavailablity of amino acids in protein fed to prawns, the incorporation and retention of amino acids in the muscle of the prawns would have to be measured. This would require the use of stable isotope ratios to identify the proportion of the amino acids in prawn muscle that had come from a particular protein source in the feed. This methods for this work have been established except for development of the method to separate and purify the amino acids for stable isotope analysis in sufficient quantity in a time and cost efficient manner.

Table 7. Amino acid profiles of prawn muscle, fishmeal and three meatmeals expressed as the percentage of sum of the amino acids.

Amino Acid	Prawn	Fishmeal	Aspen	Beef City	Fletcher
Aspartic acid	9.61	8.73	9.11	9.47	8.90
Threonine	3.51	5.03	4.86	3.89	3.36
Serine	2.54	4.43	4.55	4.36	4.40
Glutamic acid	15.69	12.14	11.72	12.69	12.59
Proline	7.91	5.26	7.25	9.98	11.43
Glycine	7.84	6.63	7.81	12.50	14.24
Alanine	5.17	7.14	6.65	8.29	8.88
Cystine	0.52	1.21	1.24	1.12	0.88
Valine	4.35	5.81	5.83	4.47	4.32
Methionine	3.02	3.17	2.22	1.68	1.36
Isoleucine	4.35	4.90	4.71	3.02	2.77
Leucine	7.39	7.59	7.57	6.32	5.94
Tyrosine	3.65	3.75	3.57	2.03	1.87
Phenylalanine	3.85	4.84	5.27	3.91	3.63
Lysine	7.89	8.56	6.65	5.99	5.32
Histidine	2.08	3.86	2.35	1.93	1.65
Arginine	10.63	6.94	8.64	8.34	8.47

Table 8. Apparent digestibility (%) of amino acids in three meatmeals and fishmeal as determined using chromic oxide as an inert marker.

Amino Acid	Fishmeal	Aspen	Beef City	Fletcher
Threonine	91.0	58.0	51.7	40.4
Valine	90.5	56.5	53.3	42.1
Cystine	85.1	49.9	35.0	27.0
Methionine	92.7	59.5	64.3	57.7
Isoleucine	90.2	55.0	56.4	47.6
Leucine	90.5	54.1	55.1	43.4
Tyrosine	101.1	58.6	74.3	55.9
Phenylalanine	90.1	57.3	55.9	46.1
Lysine	94.8	62.4	61.6	46.5
Histidine	92.8	61.1	59.3	55.5
Arginine	93.1	65.4	44.9	29.6
Average digest.	92.0	58.0	55.6	44.7

Table 9. Apparent digestible essential amino acid (ADEAA) of three meatmeals and fishmeal expressed as a ratio to the amino acid profile of prawn muscle.

Amino Acid	Fishmeal	Aspen	Beef City	Fletcher
Threonine	1.43	1.39	1.11	0.96
Valine	1.34	1.34	1.03	0.99
Cystine[1]	2.34	2.39	2.16	1.70
Methionine	1.05	1.34	1.03	0.99
Isoleucine	1.13	1.08	0.70	0.64
Leucine	1.03	1.02	0.85	0.80
Tyrosine[1]	1.03	0.98	0.56	0.51
Phenylalanine	1.26	1.37	1.02	0.94
Lysine	1.08	0.84	0.76	0.67
Histidine	1.86	1.13	0.93	0.79
Arginine	0.65	0.81	0.78	0.80
ADEAA Index	0.95	0.96	0.88	0.83

1 These non-essential amino acids were not included in the AEAA Index.

STABLE ISOTOPE RATIOS

The stable isotope ratios of the fishmeals, the meatmeals and the diets made up with them were measured. The Aspen meatmeal has markedly different stable isotope ratios from the other meatmeals, being the most separate from fishmeal in the $\delta^{15}N$ value and $\delta^{13}C$ value (Table 10, Figure 1).

Table 10. The $\delta^{15}N$ value for nitrogen and $\delta^{13}C$ for carbon obtained from fishmeal and three meatmeals and the values for diets containing those ingredients at a 50% inclusion level and 50% Control diet.

Sample	$\delta^{15}N$	$\delta^{13}C$
Tasmanian Fishmeal	12.88	-19.65
Aspen meatmeal	8.72	-24.32
Beef City meatmeal	10.62	-19.65
Fletcher meatmeal	11.77	-20.64
Control diet	10.73	-22.33
Fishmeal diet	12.93	-20.74
Aspen diet	9.66	-23.02
Beef City diet	11.29	-21.15
Fletcher diet	11.14	-21.72

The ratios of carbon and nitrogen may be used as a combination to provide a more powerful method of differentiating between fishmeal and meatmeal protein (Figure 1). From these results it appears that we would be able to determine the relative contribution of fishmeal protein and Aspen meatmeal protein to nitrogen retention (new protein) in the prawn. It is unlikely that using only $\delta^{15}N$ and $\delta^{13}C$ we would be able to determine these proportions with the Beef City and Fletcher meatmeals with a useful degree of accuracy. This is partly because the meatmeal is only 50% of the diet and that there is some protein in the other 50%, and partly because of the similarity between the stable isotope ratios of the two meatmeals. However, our colleagues at Griffith University are developing the techniques to determine the ratio of the two stable isotopes of sulphur, ^{34}S and ^{32}S. Sulphur is contained in three of the protein amino acids and would provide a third dimension to the protein signatures of the meatmeals which could lead to the clearer differentiation between the three meatmeals and fishmeal.

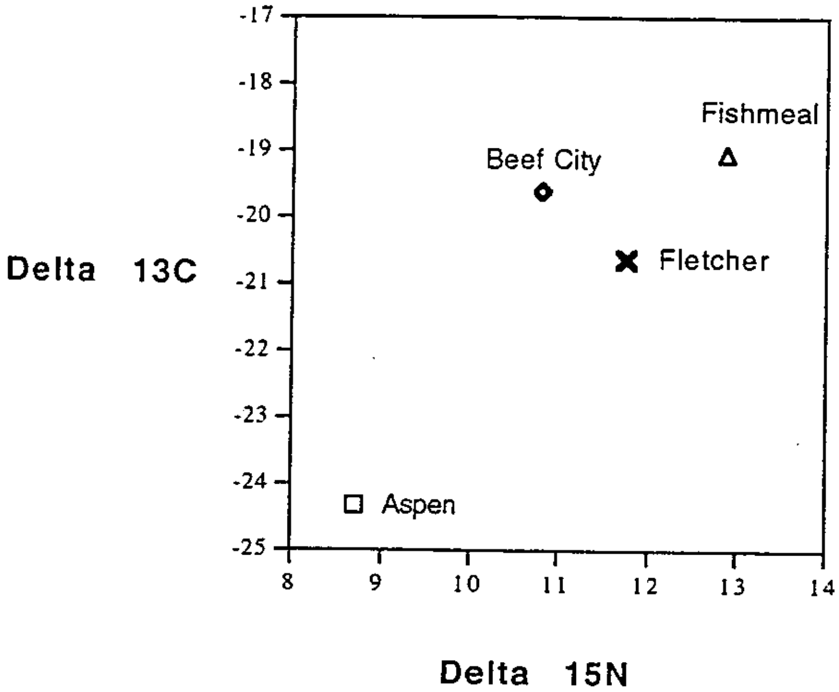

Figure 1. Delta ^{15}N and delta ^{13}C ratios of Tasmanian fishmeal and three meatmeals.

GROWTH

The preliminary results from this growth trial give a promising indication that meatmeals may be used successfully as a partial replacement for fishmeal in *P. monodon* diets. There is no significant difference between the growth rates of prawns fed any of the meatmeals, at both inclusion levels, and those fed the control diet, MRC Standard. However the performance of the diet containing the higher inclusion level of Beef City meatmeal was significantly lower than with the lower inclusion level. The opposite situation was observed with Fletcher meatmeal; it performed better at the higher replacement level. This result may be an artefact caused by a higher mortality rate in the smaller prawns biasing the apparent growth rate and producing erroneously high growth.

The growth rates in the prawns in this study were lower than expected and the mortality rate higher than expected (Table 11). In the second growth experiment we obtained growth rates with a commercial feed and with our MRC Standard diet of about 60% weight gain in 4 weeks (Table 12), which is equivalent to a gain of about 86 mg/day. This result is comparable to the best reported for *Penaeus monodon* of the same size range in aquaria systems (Cruz-Suarez *et. al.*, 1992). We established that the depressed growth rate in the first trial was not due to any procedural or dietary failings but was due to a health condition in the prawns we had purchased .

Meatmeal has been included in this trial up to 29% of the diet without any significant reduction in performance. Previously Sarac *et al.* (1994) have included meatmeal in diets for *P. monodon* juveniles at 10 and 20%, and reported no differences in live weight gain compared to a control diet based on fishmeal. These trials have given a strong indication that meatmeal may be used to replace at least 50% of the fishmeal in the diets of juvenile *Penaeus monodon* juveniles.

Table 11. Growth of *P. monodon* juveniles during the first four-week trial, expressed as a percentage of initial weight and average survival

Treatment	Growth (%)	Survival (%)
MRC Standard	38.71 ± 5.247 [abc]	75.00 ± 7.136 [ab]
Aspen (25%)	36.89 ± 2.982 [ab]	72.22 ± 8.240 [ab]
Aspen (50%)	36.15 ± 4.085 [abc]	86.11 ± 5.122 [a]
Beef City (25%)	39.15 ± 4.443 [a]	86.11 ± 5.122 [a]
Beef City (50%)	29.40 ± 2.911 [bc]	80.56 ± 5.122 [ab]
Fletcher (25%)	28.68 ± 2.228 [c]	83.33 ± 6.086 [ab]
Fletcher (50%)	34.73 ± 5.855 [abc]	69.44 ± 6.690 [b]

Values with the same superscript within a column are not significantly different ($p<0.05$).

Table 12. Growth of *P. monodon* juveniles during the second four-week trial, expressed as a percentage of initial weight and average survival

Treatment	Growth (%)	Survival (%)
Commercial Feed CP)	60.30 ± 4.57	100.0
MRC Standard	58.09 ± 3.81	100.0

INTELLECTUAL PROPERTY

At this stage the know-how associated with the use of meatmeal in prawn diets is at such a preliminary stage that I do not believe it constitutes Intellectual Property.

RECOMMENDATIONS

These preliminary results indicate that meatmeals may be incorporated into *P. monodon* growout diets to about 29%. Further tank trials are required to investigate the upper limit of meatmeal inclusion and reinforce the growth results before carrying out larger scale feeding trials in prawn ponds. Manufacturers of aquaculture feeds must be convinced that supplies of meatmeal are of consistent composition and quality before they start using meatmeals routinely. The use of meatmeals in their diets must not compromise the pellet stability or present problems in processing (eg. extra milling to reduce bone fragment size). Once we have established the maximum effective inclusion level of meatmeal a batch of feed should be prepared under commercial processing conditions and then tested in small scale prawn ponds or large tanks (ca 3 m diameter) before the diets are evaluated in commercial ponds. This would minimise the risk of a very expensive loss in production, given a crop in a 1 hectare pond is worth about $100,000 at harvest. The trial in small scale ponds or large tanks should be carried out to confirm the cost-effectiveness of the meatmeal based diets and to determine any effects on water or pond bottom quality. Previous research by the Oceanic Insitute of Hawaii (E. Duerr pers. comm) and by NSW Fisheries (Allan & Maguire, 1993) has demonstrated that growth rates and food conversion ratios in properly conducted experiments in large tanks are closely comparable to the results obtained in commercial ponds.

- *Determine the maximum inclusion level of meatmeal in prawn diets.*
  The maximum inclusion level of meatmeal in the prawn diets could be established using summit diet dilution experimental design using small tanks as used in the growth experiments described in this report.

- *Evaluate the impact of the inclusion of meatmeal on prawns' flavour and nutritional value.*
  The flavour of the prawn flesh of prawns fed a base diet and diets containing meatmeal should be compared from prawns grown in the small scale ponds or large tanks. The CSIRO Division of Food Science and Technology and the Queensland Department of Primary Industries have the facilities and expertise to conduct these trials. This work could be carried out on a fee for service basis.

- *Improve the meatmeal for use in prawn diets.*
  The Beef City and Fletcher meatmeals could be significantly improved by reducing the amount of bone in them. This would have the effect of increasing their dry matter digestibility and possibly their nitrogen digestibility. Since the lipid in the meatmeals appear to be poorly digested and it contains mainly saturated fatty acids, which feed formulators are not seeking to add to the feed, a reduction of the fat levels would be advantageous if it was not achieved at too greater cost. The addition of other tissues or organs that are richer in arginine, lysine, leucine and isoleucine would improve the amino acid profile of the meatmeals, particularly with Fletcher and Beef City.

- *Investigate the use of chemical attractants, amino acids and other supplements to increase the inclusion level of meatmeal in prawn diets.*
  At this point we have not seen any evidence that the flavour of the meatmeal is a counter-attractant for prawns at the inclusion levels we have used. There seems to be no immediate need to investigate the use of attractants. However, the inclusion of crystalline amino acids, either free or in microencapsulated form, to balance the amino acid profile of the feeds does require further investigation. Supplementary amino acids are likely to enhance the use of meatmeals particularly if the arginine and lysine levels can be effectively increased in the feed.

- *Investigate the impact of feed residues containing meatmeal on pond sediment conditions.*
  A comparison of the amount of waste matter produced from a control diet and the meatmeal based diets can easily be made in the course of a feeding trial. It would be necessary to study the breakdown of the waste material in a simulated pond situation to determine the impact on the water quality. Parameters such as ammonia and nitrite, organic N and C, chlorophyll a, BOD, and bacterial numbers in the water column and in the sediment would need to be monitored.

MRC CONTRIBUTION

a. Supply of meatmeals for evaluation.
b. Provision of $29, 964 towards the research costs.

IMPACT OF RESULTS AND CONCLUSIONS

During the 1992/93 financial year a total of approximately 2223 tonnes of prawn feed was used in Australia. Australian manufacturers supplied 24% of this total. If the meatmeals were included at the levels used in the growth trials, in the Australian feed production, based on current prices this would produce for the renderers;

Aspen (18.5%) @ $775/t	$76 725 per year
Beef City (29%) @ $445/t	$68 975 per year
Fletcher (26%) @ $445/t	$61 855 per year

If Australian manufactured feeds capture only 75% of the market in Australia and are using meatmeals to replace 50% of the protein currently being supplied by fishmeal, in 1995/96 with an estimated local market of 4,000 tonnes, manufacturers would require about 750 tonnes of meat and bone meal.

The aim of a number of research projects in Australia is to produce a feed that has wide market acceptance in Australia and overseas. We anticipate that in the next five years a much larger percentage of the prawn feed used in Australia will be manufactured locally and that the local market for prawn feed will be approaching 12,000 tonnes. Further opportunities will arise for exporting the Australian feed into the South East Asian market twhichis predicted to require about 1,000,000 tonnes of prawn feed in the year 2000.

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