Fishmeal replacement research for shrimp feed in Australia[1]

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ABSTRACT: The global expansion and intensification of aquaculture has brought with it an increasing requirement for formulated feeds. Feeds for carnivorous fish and shrimp contain a large proportion ingredient of marine origin, particularly fishmeal. However, despite the continuing increase in demand for fishmeal, world fishmeal production is unlikely to increase further. The identification and development of alternative feed ingredients that can replace fishmeal is recognised as an international research priority. A nationally coordinated research program in Australia has evaluated a wide range of ingredients produced by Australia's agricultural industries. Information has been obtained on the chemical composition, apparent digestibility (AD) and subsequent assimilation and utilisation of each of the ingredients evaluated. The research focused on three key warmwater species endemic to Australia: the omnivorous marine crustacean, black tiger shrimp *Penaeus monodon*; the euryhaline carnivorous fish, barramundi or Asian seabass *Lates calcarifer* and the freshwater omnivorous fish, silver perch *Bidyanus bidyanus*. Across the three key species, the AD for crude protein was highest for fishmeal though the more refined, high-protein ingredients of plant origin were equally well digested. The AD of energy was relatively low in ingredients with a high carbohydrate or fibre content. Dehulled lupins *(L. angustifolius)* and high-protein, low-ash meat meals were found to have the greatest potential to replace fishmeal in shrimp diets. Meat meal can replace about half of the digestible crude protein, and dehulled lupins can replace a quarter of the digestible crude protein, in a shrimp diet without significantly affecting biological performance, provided that the balance of the crude protein in the diet comes predominantly from high quality ingredients of marine origin. When the diets contain these ingredients of marine origin at inclusions of at least 50 $g.kg^{-1}$, specific feeding effectors do not appear to necessary to maintain the palatability of the diets.

KEYWORKDS: Feeding and nutrition – crustaceans, diet composition

INTRODUCTION

Aquaculture is the fastest expanding food producing sector in the world, growing at a rate of almost 12% p.a. since 1984, with production almost trebling from 13 to 36 million tonnes in the last 10 years (Tacon, 1999). This expansion will have to continue well into the next century if production is to keep pace with the increase in demand solely due to the increase in population. However, much of the increase in aquaculture production has been brought about through the adoption of intensive farming practices using formulated feeds. Fishmeal is one of the most important ingredients in formulated

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aquafeeds, and particularly in shrimp feeds where it is typically included at between 200 and 300 $g.kg^{-1}$ (Tacon and Akiyama, 1997). Its inclusion is primarily for its high quality protein but has the additional benefit of its oil content and associated highly unsaturated fatty acids.

Tacon (1999) estimated that almost one third of aquaculture production came from ‘feeding species’ which consumed almost 12 million tonnes of aquafeeds containing 2.3 and 0.7 million tonnes of fishmeal and fish oil respectively. World fishmeal production has remained relatively static at 6.2 million tonnes (1997) and it is unlikely to increase. However, it is subject to sharp, periodic declines such as that of 1998 when only 4.75 million tonnes were produced (Tacon, 1999).

It is evident from these statistics that continued expansion of aquaculture will not be possible if fishmeal is relied upon as the main source of protein in aquafeeds. Moreover, demand for fishery product from other high profit sectors, such as the pet food industry, will force fishmeal prices up until its usage in aquafeed will become uneconomical. In any event, if aquaculture is to become a net and increasing contributor to human food supplies, it is critical that aquafeeds become less reliant on fishery products, which will mean finding suitable and cost-effective terrestrial alternatives.

Australia’s agricultural industries produce an abundant supply of protein-rich feed ingredients of animal and plant origin that have potential as replacements of fishmeal in aquaculture diets. In 1993, the Australian Fisheries’ Research and Development Corporation (FRDC) established a nationally coordinated research program to assess and develop Australian-produced ingredients that had the potential to be used as cost-effective replacements to fishmeal in aquafeeds (Allan, 1997). Additional funding was provided by other Research and Development Corporations and considerable in-kind support was provided by aquaculturists and feed manufacturers.

A collaborative team was established that involved 11 aquaculture nutrition research groups in Australia. The research focused on three key warmwater species endemic to Australia: the omnivorous marine crustacean, black tiger shrimp *Penaeus monodon*; the euryhaline carnivorous fish, barramundi or Asian seabass *Lates calcarifer* and the freshwater omnivorous fish, silver perch *Bidyanus bidyanus*. Additional collaborative feeds research was carried out on the coldwater Atlantic salmon *Salmo salar*. Selection of ingredients for evaluation was based on their perceived potential as fishmeal replacements and also on the amount of the ingredient being produced (or its potential to be produced) in Australia. The ingredients (Table 1) were collected centrally and analysed to determine their proximate and amino acid composition before samples were distributed to participating research institutions. The nutritive value of the ingredients was assessed for each of the three key species by determining apparent digestibility (AD) and the biological response to diets containing serial increments of the ingredients. Further studies were carried out to determine the effect the ingredients had on palatability of the diets and the response to the addition of attractants or other feeding effectors. This paper summarises this work with a particular emphasis given to the research carried out with the black tiger shrimp, *P. monodon.*

Table 1. The average ash, crude protein (CP), total lipid (TL) and gross energy (GE) composition (dry matter) of feed ingredients evaluated in the fishmeal replacement research in Australia

Feed ingredient	Composition (dry matter basis)			
	Ash (%)	CP (%)	TL (%)	GE kJ/g
Fishmeals				
Fishmeal (Australian)	14.2	73.2	9.9	21.3
Fishmeal (Danish)	13.0	72.9	11.4	21.5
Fishmeal (Peruvian)	17.6	70.2	11.3	20.9
Terrestrial animal meals				
Blood meal	3.1	94.9	<1	23.9
Feather meal	3.0	84.3	11.2	24.9
Meat meal (low ash)	9.4	80.9	13.0	25.0
Meat meal (beef)	36.0	49.2	9.2	16.1
Meat meal (sheep)	34.5	54.3	7.2	16.2
Meat meal (mixed)	12.1	60.6	14.5	23.5
Poultry offal meal	15.0	60.3	18.2	22.7
Oilseed meals				
Canola (expeller)	6.3	31.8	12.5	21.8
Canola (solvent)	8.0	36.6	2.6	19.9
Cottonseed	8.3	48.1	4.6	19.9
Peanut	5.2	41.2	1.3	19.7
Soybean (full fat)	5.5	35.8	19.5	23.3
Soybean (expeller)	6.3	47.5	6.4	20.9
Soybean (solvent)	8.0	47.8	3.7	17.0
Grain legumes				
Chick pea	3.4	20.8	4.7	19.4
Field pea (Dunn)	3.4	25.5	1.1	17.0
Lupin (*L. albus*)	3.7	37.6	6.2	20.9
Lupin (*L. angustifolius*)	2.8	34.1	5.7	17.9
Lupin (*L. angustifolius*)[1]	3.5	44.8	7.1	20.6
Cereals & byproducts				
Gluten (corn)	2.0	62.0	<1	24.1
Gluten (wheat)	2.5	76.9	<1	23.1
Sorghum grain	2.3	14.5	1.5	18.8
Wheat grain (ASW)	1.8	12.2	1.9	18.3
Wheat grain	1.7	15.2	1.8	18.5
Wheat offal meal	4.9	22.3	5.0	19.6

Apparent digestibility determination

Method development

The most appropriate method for the measurement of digestibility was established for each of the key species. The methods varied primarily because of differences in the feeding behaviour of the species and the physical structure of their faeces. For silver perch, AD could be determined relatively quickly and reproducibly by collecting faeces using settling tanks based on the Guelph design (Allan *et al.*, 1999). Manual stripping of faeces from of the lightly anaesthetised fish was the preferred method with barramundi as the faeces were very loosely bound (McMeniman, 1998).

In establishing the protocol for measuring digestibility with shrimp (Smith and Tabrett, 1998), the suitability of chromic oxide as a digestibility marker was studied. This involved using radio-labelled chromic oxide (^{51}Cr) to determine the digestibility of the marker, rate of passage studies to examine marker excretion patterns and techniques to ensure the homogeneous distribution of the marker through the diet. Radio-labelled chromic oxide studies showed that less than 1% of the total radioactivity was absorbed into the tissues of the shrimp and 96% was recovered from the digestive tract and faeces. The remaining radioactivity was detected in the shrimp's exoskeleton, suggesting it came from surface contamination during feeding (Smith and Tabrett, 1998).

Concern about the partitioning of chromic oxide from other faecal material in the midgut and hind gut of shrimp led to a study of the rates of passage of marker and nutrients through the digestive tract. After feeding a diet containing chromic oxide, faeces were collected and pooled over 3-hourly intervals for 24-hours. The ratio of dry matter to marker, and nitrogen to marker were very consistent over the first 6 hours when >95% of the chromic oxide and 90% of the dry matter and nitrogen was excreted. Thereafter the faeces contained very little material and the peritrophic membrane contributed a significant proportion of the dry matter and nitrogen. Hence, if faeces were collected over a 3-hour or longer period, any inaccuracies associated with short-term partitioning of the marker would not seriously affect the accuracy of the AD determination (Smith and Tabrett, 1998).

The homogeneity of the distribution of the digestibility marker in the diet was found to be of critical importance for accurate determination of AD. As shrimp usually eat relatively small amounts of food in any one meal, uneven distribution of the marker in the diet can result in significant errors in the estimate of digestibility. By regrinding and re-extruding a diet that had previously been prepared using established procedures (Preston *et al.*, 1996), the coefficient of variation of the chromium concentration found in 10 replicate 0.2 g samples was reduced from 12.2% to 6.6%.

In determining the AD of an individual ingredient using the substitution approach (where the test ingredient substitutes for a proportion of the basal diet ingredient mixture), the level of substitution was found to have a very large effect on the precision of the measurement. This is due to a multiplication of errors that are inherent in the calculation of ingredient AD. At a 20% substitution, the variance of the AD estimate the ingredient is about 40 times larger than for the AD estimate of the whole diet. At a substitution of 50%, the variance of the test ingredient AD is only 5 times larger than the variance for the whole diet AD. Too large a substitution is also undesirable since this may sufficiently distort the nutritional balance of the diet being fed, to invalidate the inherent assumption of additivity of the digestibility of the constituent ingredients. Unless these aspects are carefully considered and the veracity of the underlying assumptions validated, serious inaccuracies are likely to occur in the measurement of AD.

The study confirmed the near zero absorption of chromic oxide and hence its suitability as a digestibility marker for shrimp. The data on gut passage rate of chromic oxide and undigested matter provided the basis for the design of faecal collection protocol. In preparing the diets used to determine the AD of ingredients, ingredients were substituted at the highest practical level (between 30 and 50%) to minimise amplification of errors in the calculations.

Apparent digestibility of ingredients

The AD of the feed ingredients was remarkably similar for all three species, despite their widely different feeding habits (Table 2). Across the species, AD was highest for the fishmeals, although other ingredients including some animal meals and gluten were equally well digested. Animal and plant proteins were generally well digested except for some meat meals, which were only moderately digestible (64 to 74%). Energy digestibility was high for protein-rich ingredients whether of plant (eg 100% for gluten) or animal origin, but was low for ingredients with a high carbohydrate content and especially those containing high amounts of fibre such as canola, cottonseed and most of the grain legumes.

Table 2. Apparent dry matter, protein and energy digestibility coefficients of various protein-rich ingredients determined with shrimp (S), silver perch (SP) and barramundi (B)[1]

Ingredient	Apparent digestibility coefficient (%)								
	Dry matter			Protein			Energy		
	S	SP	B	S	SP	B	S	SP	B
Fishmeals									
Fishmeal (Australian)	86	77	-	93	96	-	89	93	-
Fishmeal (Danish)	-	91	90	-	99	89	-	100	99
Fishmeal (Peruvian)	-	74	-	-	89	-	-	90	-
Terrestrial animal meals									
Meat meal (low ash)	78	89	-	83	84	-	64	95	-
Meat meal (beef)	60	43	-	77	66	-	61	73	-
Meat meal (lamb)	57	55	-	74	69	-	55	82	-
Meat meal (mixed)	-	76	-	-	83	-	-	85	-
Oilseed meals									
Canola (solvent)	49	50	49	79	83	81	53	57	56
Soybean (solvent)	67	73	56	92	95	86	71	82	69
Grain legumes									
Lupin[2] (whole)	39	-	-	88	-	-	45	-	-
Lupin[2] (dehulled)	67	68	61	94	100	98	68	74	62
Field pea (Dunn)	72	-	-	89	88	-	83	51	-
Cereals & byproducts									
Gluten (wheat)	96	97	100	100	100	100	100	100	99

[1] Most of the digestibility data for barramundi were produced by Dr N. McMeniman (University of Queensland).
[2] *L. angustifolius*

Ingredient utilisation growth assays

As has been amply demonstrated in pigs (Batterham *et al.* 1990), a considerable proportion of amino acids from heat-damaged proteins can be absorbed in a form that is not utilised, leading to their poor assimilation (utilisation) by the animal. Lysine, because of the reactivity of its epsilon amino group (malilard reactivity; Erbersdobler 1986), is most vulnerable to this type of damage with meat meals and high temperature processed vegetable protein meals such as cottonseed often suffering from this fate. Hence, digestibility alone may not be a reliable guide to the nutritive value of the ingredient.

To address this issue, experiments were carried out with shrimps to examine the effect of serial replacement, on a digestible protein basis, of the fishmeal in a basal diet. Experiments were also carried out with barramundi and silver perch to determine nutrient retention following the feeding of diets containing serial increments of selected ingredients. With all species, the experiments involved clear-water aquarium studies and subsequent on-farm studies with selected ingredients using various types of pond or cage systems.

In clear water aquarium experiments with shrimp, meat meal could be used to replace about two thirds of the dietary protein, which was of mostly marine origin, without having an adverse effect on shrimp growth rate or survival. Experiments with whole lupins, dehulled lupins and canola indicated that these ingredients could be used to replace about one fifth of the dietary protein, or used at an inclusion level of 200 $g.kg^{-1}$, without significantly affecting growth rate relative to that of the basal diet.

From the results of the aquarium experiments, a high specification meat meal and dehulled lupins were considered to have the greatest potential as fishmeal replacements, and were further evaluated in green water, pond experiments. These experiments involved the use of an array of cages (1 x 1 x 1 m) deployed in an outdoor raceway pond, supplied with water circulated from an adjacent commercial shrimp pond. Shrimp (3 to 4 g) were stocked at 15 per cage and fed the allocated diets five times daily using automatic feeders. This facility and the management protocol used, provided an environment similar to that of a commercial production pond, while providing adequate replication (5 cages per diet) to ensure statistically valid results.

In the first raceway pond experiment, we investigated a 56% protein, low ash, low fat meat meal when was used at inclusion levels of 150 $g.kg^{-1}$ and 300 $g.kg^{-1}$ to partially replace fishmeal in the basal diet. Inclusion of this meat meal at 300 $g.kg^{-1}$ represented a replacement of 43% of the digestible crude protein (41% of the total crude protein) in the diet. However, the balance of the protein in these diets was of marine origin, which was provided through the inclusion of fishmeal, 100$g.kg^{-1}$ squid meal and 100 $g.kg^{-1}$ shrimp shell meal. Growth rates of shrimps fed the meat meal diets (1.14 to 1.16 g/wk) were not significantly different from those obtained with the basal diet (1.14 g/wk) or with a commercial *P. monodon* diet (1.17 g/wk) that was used as a benchmark (Fig. 1). Average survival was >92% and not significantly different between treatments. There were also no significant differences in food intake or food conversion ratios. A trained taste panel evaluation of the flavour of shrimp grown at the end of the experiment indicated that the inclusion of high levels of meat meal in the diet did not impart an adverse or a distinguishable flavour to the shrimp

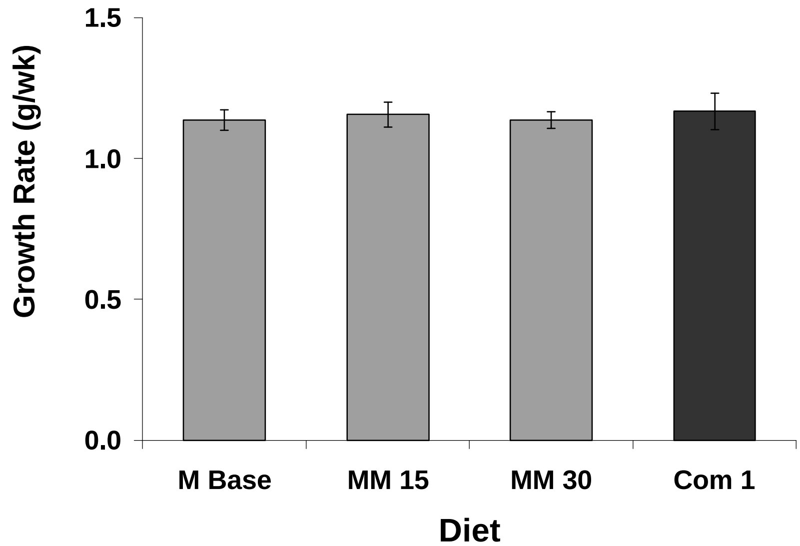

Figure 1. Weekly weight gain of caged shrimp from experiments where meat meal replaced part of the fishmeal in a basal diet (M Base) and compared with a commercial *Penaeus monodon* shrimp feed (Com 1).

The efficacy of dehulled lupin as a fishmeal replacement was examined under simulated pond conditions using cages situated in a raceway pond. Two varieties of *Lupinus angustifolius* were studied: the conventional variety, Warrah and a high-methionine, transgenic variety. The dehulled lupins were each used at an inclusion rate of 250 $g.kg^{-1}$ to partially replace an equivalent amount of fishmeal protein (on digestible protein basis) in a basal diet. The basal diet comprised 370 $g.kg^{-1}$ fishmeal, 100 $g.kg^{-1}$ crustacean meal, 50 $g.kg^{-1}$ squid meal and flour, binders and micronutrients. These diets were evaluated against a commercial diet formulated for *Penaeus japonicus (=Marsupopenaeus japonicus)* which had been shown to produce particularly high growth rates when fed to *P. monodon*. There were no significant differences ($P< 0.05$) between treatments in survival (>90%) but the growth rate of shrimp fed the commercial diet (1.6 g/week) was significantly higher than for the other three diets (1.4-1.5 g/week) which were not significantly different from each other (Fig. 2). These results demonstrated that dehulled lupin meals could be included at 250 $g.kg^{-1}$ to partially replace fishmeal in diets for *P. monodon* without adversely affecting growth or survival rates.

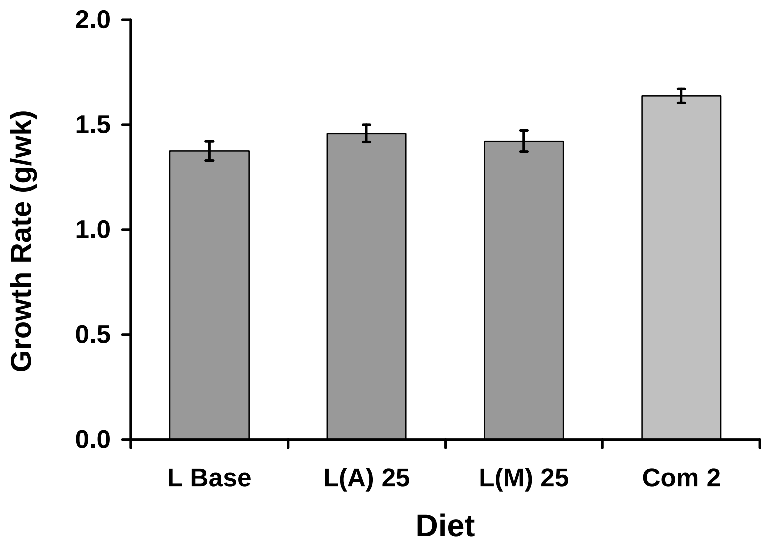

Figure 2. Weekly weight gain of caged shrimp from experiments where dehulled lupin meal (*L. angustifolius*) of the conventional variety L(A) and a high-methionine variety L(M), were used to partially replace fishmeal in the basal diet (L Base), and compared with a commercial *Penaeus japonicus* shrimp feed (Com 2).

Further work with dehulled lupins has shown that shrimp growth rate progressively declines at inclusion levels greater than 250 g.kg^{-1} in diets containing 400 g.kg^{-1} crude protein. The growth rate of shrimp fed the diet containing 600 g.kg^{-1} of dehulled lupins was two thirds of that of the basal diet. There was also a decrease in pellet water stability as the lupin meal content increased: dry matter loss during 4 hours of water immersion increasing from 6% for the basal diet to 16% for the diet containing 500 g.kg^{-1} of dehulled lupins. In a separate study, the nutritive value of a different species of lupins, *L. albus*, was found to be less than that of soybean meal, with a progressive decrease in performance as more lupin protein replaced soybean protein (Sudaryono *et al.* 1999).

Use of feed attractants

Because crustaceans are primarily chemosensory feeders, attractants and other feeding effectors are important components of their prey or feed. Proteins of marine origin such as fishmeal are likely to contain substances that are feeding effectors for shrimp, whereas proteins of terrestrial origin may not naturally contain these particular compounds. Therefore, the addition of feeding effectors to shrimp diets appeared likely to be important if a significant proportion of the fishmeal is replaced with terrestrial proteins.

Diet preference studies were carried out to determine the effect of a range of feeding effectors on feeding behaviour of the shrimp, *P. monodon*. Effectors compared included mono sodium glutamate, a number of products derived from shrimp and fish processing waste and a proprietary product rich in betaine. The product based on shrimp processing waste was the most effective feeding effector and little benefit was seen from using the other feeding effectors (Saraç and Smith, 1998). In a more recent study other feeding effectors, including fish and krill meals and hydrolysates, have been assessed. The most effective ones were the high quality marine products such as shrimp meal and squid meal used at inclusions of up to 5% of the diet.

CONCLUSIONS

The strength of the fishmeal replacement research in Australia has been the coordinated and cohesive way in which the research has been carried out across the three key species. In broad terms, the results have been remarkably consistent across species. Thus if an ingredient is an effective fishmeal replacement for one species, it is also likely to be effective for other species, particularly if of similar feeding habit. These studies have shown conclusively that terrestrial protein meals, if used on a digestible nutrient contribution basis, can completely replace fishmeal in grow-out diets for silver perch and barramundi without loss of fish productivity or impairment of eating quality of the fish. In shrimp diets, these ingredients can be used to partially replace the fishmeal, provided that the diet formulation includes at least 100 $g.kg^{-1}$ of a high quality ingredient of marine origin.

Meat meal has the potential to be used as a major component in aquaculture diets for fish and shrimps to replace or partially replace fishmeal (Williams *et al.*, 1997). The major constraint on its use is the market concern, particularly in Europe, of the potential for bovine spongiform encephalopathy (BSE) transmission through the meat meal. Australia is free of BSE and government officials are seeking international recognition of our BSE-free status. A lesser, though significant concern, is the relatively low digestibility of meat meals and the effect this would have on increasing nitrogen and phosphorous loads in ponds and discharge waters.

Dehulled lupin meal has been used effectively to replace a quarter of the marine protein in the diet without reducing productivity. It has been identified in Australia as the seed meal with the greatest potential to be used to replace fishmeal in shrimp diets. However, at inclusion levels greater than 250 $g.kg^{-1}$ there is a progressive decline in shrimp performance. Research is continuing to identify the limitations of its use, to identify alternative varieties of lupins more suitable for use in aquafeed and to develop strategies or processes that will improve its usefulness.

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