FISH MEAL REPLACEMENT IN AQUACULTURE FEEDS: SUB-PROGRAM ADMINISTRATION PROJECT NO. 93/120

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1 PROJECT DETAILS

Project Title: Fish meal Replacement in Aquaculture Diets Sub-Program

Project No: 93/120

The Sub-program included six separate core projects and two satellite projects. Separate final reports are available for all projects. Please see attached order form for details (Appendix 1).

Project Number	Title	Principal Investigator	Research Organisation
93/120-02	Fish meal replacement in aquaculture feeds for prawns	Mr David Smith	CSIRO Marine Research
93/120-03	Fish meal replacement in aquaculture feeds for silver perch	Dr Geoff Allan Dr Stuart Rowland	NSW Fisheries, PSRC and Grafton Research Centre
93/120-04	Fish meal replacement in aquaculture feeds for barramundi	Dr Kevin Williams	CSIRO Marine Research
93/120-05	Fish meal replacement in aquaculture feeds for salmon	Dr Chris Carter	UTAS, School of Aquaculture
93/120-06	Replacement of fish meal in aquaculture diets - feed processing	Dr Tony Evans	CSIRO Food Science
93/120-07	Amino acid supplementation of aquaculture feeds - a technology audit	Dr Paul Grieve	QDPI, International Food Institute of Queensland
93/120	Fish meal replacement in aquaculture feeds: *in vitro* studies on feed ingredients for aquaculture species	Dr Alex Anderson	QUT, School of Life Science
92/63	Dietary requirements and optimal feeding practices for barramundi	Dr Kevin Williams Dr Chris Barlow	CSIRO Marine Research QDPI, WFFAC
93/126	Development of more cost effective salmon feeds for the Tasmanian Atlantic salmon industry	Dr S Percival Mr P Lee	SALTAS
95/069	Replacement of fish meal in diets for barramundi: improving feedstuffs using synthetic amino acids	Dr Kevin Williams Dr Chris Barlow	CSIRO Marine Research QDPI, WFFAC

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2 NON-TECHNICAL SUMMARY

93/120	**Fish meal Replacement in Aquaculture Feeds: Sub-Program Administration**

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Objectives:

1 To develop coordinated and collaborative research and development to replace fish meal in feeds with cost-effective alternatives, and
2 obtain an early commitment from feed manufacturers (preferably Australian) to produce and market feeds.

Non-Technical Summary

If aquaculture is to continue to expand in Australia cost-effective diets based on Australian agricultural ingredients urgently need to be developed. The replacement of fish meal as the protein source of choice is a global research priority driven by static or declining supply of fish meal and rapidly expanding aquaculture and aquaculture feed industries. Australia has very poor supplies of fish meal and other aquatic meals but fortunately has abundant supplies of agricultural ingredients with potential for use in aquaculture diets.

In recognition of the need to develop diets for Australian aquaculture species, with reduced contents of fish meal, a number of institutions independently commenced this type of research in the early 1990's. The Fisheries Research and Development Corporation (FRDC) was approached by a number of institutions to financially support this research. In response, FRDC created their first 'Sub-Program' with the aim of coordinating research to develop Australian aquaculture diets. The two primary objectives were to replace fish meal and obtain an early commitment from commercial aquaculture feed manufacturers to adopt results.

Six separate projects were formed; four on species considered to represent most 'types' of species being farmed in Australia, one on feed processing and one on a technology audit for amino acids. The four 'model' species were prawns *Penaeus monodon*, silver perch *Bidyanus bidyanus*, barramundi *Lates calcarifer* and Atlantic salmon *Salmo salar*. Each project involved a number of collaborating scientists from different institutions and all projects were coordinated through a Sub-Program Steering Committee. Regular meetings with investigators from all projects as well as feed manufacturers, ingredient suppliers and R&D corporations were held twice each year.

This report describes the Sub-Program administration, lists the reports produced and provides a brief summary of conclusions. Final reports for all six projects are available (see Appendix

1). In addition, collaborative research on *in vitro* digestibility conducted on each of the four species within each of the "species" projects was reported separately. Two additional nutrition projects, which were funded by FRDC before the Sub-Program commenced, were brought under the Sub-Program umbrella. These projects were on the "development of more cost effective salmon feeds for the Tasmanian Atlantic salmon industry" and the "dietary requirements and optimal feeding practices for barramundi." Final reports for these are also available. Results with barramundi nutrition research prompted an extension to this research to study the efficacy of crystalline amino acids and to specifically evaluate meat meal as a replacement for fish meal. Results obtained during this extension are also reported separately. Finally, a series of reports from activities undertaken during the Sub-Program, including technical workshops and separate experiments funded by other R & D Corporations to investigate particular aspects of fish meal replacement, were produced and these are also available. (Please see Appendix 1 for information on how to obtain all reports).

Summary of Conclusions

* Precise and reproducible methods for determining apparent *in vivo* digestibility for diets and ingredients were developed (or evaluated) for prawns, silver perch, barramundi and salmon. The influence on digestibility of collection time and period, indicator type, fish size and stage, season and feeding pattern was investigated for one or more of the target species. Due to intrinsic differences between species, different methods of determining digestibility were developed. For prawns, silver perch and small salmon, faeces were collected by settlement while for barramundi and large salmon, faeces were collected by stripping.

* Digestibility coefficients for over 60 ingredients were determined for silver perch (the only omnivorous fish species targeted) and digestibility coefficients for the most promising ingredients were determined for other species.

* Rapid methods for *in vitro* digestibility determination were developed or evaluated and results were compared using *in vivo* methods. *In vitro* methods were very useful as qualitative rather than quantitative indications of digestibility.

* Digestive capacities of aquaculture species were determined. Barramundi and Atlantic salmon were adapted to carnivorous diets, silver perch and tiger prawns were omnivorous and redclaw crayfish and tilapia were adapted to more herbivorous diets.

* Carnivorous species had a limited capacity to digest starch while omnivorous and herbivorous species could digest starch very well. A possible pathological accumulation of glycogen in barramundi livers was identified. Not even the omnivorous silver perch could digest non-starch polysaccharides in lupins.

* Maximum practical inclusion contents for a large number of ingredients were determined using growth assays and comparative slaughter techniques to assess ingredient utilisation. Utilisation of meat meal was high for all species examined (prawns, silver perch and barramundi) and this ingredient could be used to replace 70-100% of fish meal. Other terrestrial animal proteins such as blood meal, poultry offal meal and feather meal were also well utilised in the species where they were tested. In general, plant protein sources were less well utilised. Silver perch and prawns were better able to utilise plant protein sources than barramundi and salmon. Reducing the carbohydrate content through dehulling and further protein concentration improved the

potential value of these ingredients. Cooking starch-rich plant protein ingredients improved their value.

* Studies on nutrient requirements concentrated on protein and protein:energy requirements:

  - The maintenance requirements of *P. monodon* for protein and energy were 0.007 g digestible crude protein, 0.3 KJ digestible energy/g body weight/day respectively.

  - For silver perch fed diets with 14-15 MJ/kg digestibility energy, increasing digestible protein and digestible lysine above 25.3% and 1.5% respectively did not increase growth.

  - For optimal growth rate and food conversion, barramundi held at high water temperature (26 to 29°C) require diets high in protein and energy (>45% protein and protein:DE of 30 mg/kj) and not less than 1.5% EPA+DHA.

  - High DE diets for barramundi (>16 kJ/g) were advocated as a management strategy to minimise the slow growth rate of fish held at low water temperatures during winter.

* The deterioration in amino acid profile which occurs when terrestrial protein ingredients are used to replace fish meal was considered unlikely to adversely affect barramundi performance if the dietary protein was >50% and fish were fed liberally.

* Crystalline amino acids were less effective than intact protein sources but were found to be more effective supplements for low protein diets than high protein diets.

* Exogenous enzyme supplements including proteases, carbohydrases and phytase were evaluated with Atlantic salmon. Only phytase elicited a growth response.

* For Atlantic salmon, adaptation to changes in the ingredient composition were identified and shown to be of great importance when assessing the performance of diets containing new or unusual ingredients.

* A survey of commercial diet pellet characteristics was completed.

* Optimal extrusion conditions for salmon and silver perch diets were described.

* An audit concluded that in the short-term, crystalline amino acids will be the major form of amino acid supplementation but development of peptic supplements has great long-term potential.

* Technologies which were identified as having possible long-term potential for the production of peptides include: chemical modification of proteins, chemical synthesis of peptides, enzymatic modification of proteins, enzymatic synthesis of peptides and generation of defined peptides by recombinant DNA technology.

* Practical diets for silver perch based on meat meal and grain legumes, with only 5 or 10% fish meal, out-performed earlier reference diets based on fish meal and soybean meal in large-scale experiments with fish grown to market size in earthen ponds.

* Barramundi diets containing high meat meal contents, with or without any fish meal, but with additional fish oil, produced equivalent production and fish with similar sensory characteristics to fish reared on fish meal-based diets.

* These results have already been commercialised. Silver perch diets with 5% fish meal, based on formulations developed during this Sub-Program, are available commercially at lower prices than earlier diets. Results for barramundi have also been adopted by industry as the basis for new commercial diets for this species. On-going evaluation of results with prawns and salmon is also attracting great commercial interest.

3 BACKGROUND

A large project to replace fish meal in aquaculture diets was submitted to FRDC for funding in January 1993. Total funds requested for the 3 year project ranged from $797 520 in Year I to $104 382 in Year III. The project involved collaboration between nine separate sub-projects with participation from some sixteen research institutions or companies. The number 93/120 was allocated to this project.

At the meeting in April 1993 the Board recognised the importance of the research proposal for Project 93/120 but decided that, as presented, the application was not sufficiently task-orientated and that the research proposed in the separate sub-projects was not adequately coordinated.

A decision was made to:

1 Create a FRDC aquaculture feeds 'Sub-Program'.
2 Appoint a Sub-Program Manager to develop a Sub-Program 'Concept and Action Plan' and to present this to the Board at the May 1993 meeting.
3 Allocate $500 000 for research toward Sub-Program objectives for 1993/94.
4 Encourage funding support from non-aquaculture sources.

Six separate but coordinated collaborative projects were funded. Four of these focussed on the four "key species", one was on feed processing and the final project was a technology audit of amino acid supplementation in aquaculture diets. These projects were numbered 93/120-02 to 93/120-07. Research on *in vitro* digestibility was coordinated by Dr Alex Anderson at QUT within each of the four "species" projects. Results were reported in a separate report.

Two additional nutrition research projects which had been previously funded by FRDC were brought under the Fish meal Replacement Sub-Program umbrella: 92/63 "Dietary requirements and optimal feeding practices for barramundi" and 93/126 "Development of more cost-effective feeds for the Tasmanian Atlantic salmon industry". Finally, the research with barramundi was extended to include investigations into the efficiency of crystalline amino acids and the potential of meat meal to replace fish meal. This was given the number 95/069 and was described in a separate final report. The need for the research is outlined below then specific Sub-Program objectives and strategies explained.

4 NEED

One of the major factors limiting the expansion of aquaculture is the development of nutritionally adequate, cost-effective diets. Feeds and feeding can contribute up to 70% of the

total operating costs for fish and shrimp farms (Wee, 1992). The most expensive component of pelleted feeds is protein, of which 25-55% is required, depending upon whether the species is herbivorous, omnivorous or carnivorous (NRC, 1983; Lovell, 1989). The major protein source for most aquaculture diets is fish meal (Lovell, 1989) and formulated diets can contain up to 70% fish meal and fish oil (New, 1991; Wee, 1992; Tacon, 1996).

There are however, some major problems with fish meal. Fish meal and fish oil production is declining (Barlow, 1989). The aquaculture feed industry currently uses 16.3 and 28.5% of the total world production of fish meal and fish oil (equal to 27.8 and 49.3% of the total amount available for export (Tacon, 1996)) excluding 'trash fish' fed directly to aquaculture species. As aquaculture production increases, demand for fish meal will also increase, inevitably forcing prices to rise. As higher quality fish meal is generally required for aquaculture feeds, species of fish currently used for human consumption will increasingly be targeted by fish meal manufacturers. In Malaysia much of the cheap fish used to produce salted fish for human consumption is now used for aquaculture instead (New, 1991). **While aquaculture remains dependant to this extent upon capture fisheries it will not be a net contributor to human food supplies.**

Apart from a relatively small quantity of fish meal produced in Tasmania during a limited period each year, very little fish meal is produced in Australia (Foster, 1992) and most required for aquaculture feeds is imported (ABARE, 1997). Imported fish meal varies in quality and prices in Australia have risen to over AUS $1 500/tonne for high quality fish meal. Improved growth and food conversion efficiency have been recorded for salmonids when low-temperature fish meals have been used. These special 'aquaculture grade' fish meals are more expensive than ordinary fish meal, some by as much as 35% (Foster, 1992).

Apart from problems with availability, quality and price of fish meal in Australia, changes in quarantine legislation are having a serious, detrimental effect upon the fledgling Australian aquaculture and aquaculture feed industries. The Australian Quarantine Inspection Services (AQIS) currently requires that all imported fish feeds have an import permit, and, from 1 August 1992, require that all fish meal also has a import permit. This will increase the price. In addition, following recent research which has shown that some pathogenic fish viruses and bacteria are not destroyed by high temperatures, the AQIS has issued draft conditions which, if imposed, will require that all imported fish meal and fishfeeds are treated at temperatures in excess of 100^{o}C for 30 minutes (or shorter periods for higher temperatures). This will reduce the nutritional value of fish meal.

If Australian aquaculture is to develop, suitable alternatives to fish meal must be found.

The need to replace fish meal in aquaculture diets is recognised as a major international research priority (Manzi, 1989; New, 1991; Tacon, 1996) and was recognised as one of the major challenges facing aquaculture nutrition researchers at the Aquaculture Nutrition Workshop held in 1991 (Allan and Dall, 1992).

Australian agriculture has much to gain from developing new products for use in aquaculture feeds and from selling existing products in this market. Forecasts of the worlds aquaculture feed production for the year 2 000 range from a projected 4.5 million tonnes (New and Csavas, 1993) to 7.5 million tonnes (Tacon, 1996). By far the largest consumer is the Asian region with a market estimated at 2.5 million tonnes in 1994 (Smith and Gueria, 1995). New's (1991) estimates are more conservative, predicting an Asian market of 1.9 million tonnes by 2000. For several reasons this market is expanding, and will continue to expand rapidly.

In many areas aquaculture is progressing from extensive production, which relies more upon natural food within large scale ponds, to semi-intensive and even intensive production which requires major feed inputs. As systems become more intensive, more of the culture animals nutritional requirements must be met by the added feed. The push throughout Asia to increase aquaculture production is leading to a much greater demand for formulated feeds. This is evident by the much greater increase in the production of aquaculture diets than in production of fish and crustaceans from aquaculture. Between 1986 and 1990 production of aquaculture feeds increased more than four fold (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 fish and crustaceans and are competitively priced.

Australian feed manufacturers also have the opportunity to enter the rapidly expanding aquaculture feeds market. Although Asian fish and crustacean feed manufacturing technology is currently at the forefront of international feed development, Australian companies could access this market if low cost ingredients could be produced from Australian agricultural products. This would require appropriate formulations and rigorous evaluation of diets. The development of new technology to improve the digestibility of Australian agricultural products to fish and the manufacture of new protein or amino acid supplements could give Australian feed manufacturing companies significant advantages over rival overseas companies. Value adding to our agricultural products by combining them into high value exportable aquaculture diets could greatly increase export earnings.

A large number of studies using different species and ingredients have already been conducted. The majority have investigated the potential of soybean meals or soybean products to replace fish meal (eg Dabrowski et al., 1989; Shiau et al., 1989; Smith et al., 1988; Mohsen and Lovell, 1990; Balogun and Ologhobo, 1989; Lim and Dominy, 1990) because of the excellent amino acid profile of soybeans.

Other studies have investigated a range of different products including rapeseed meal (Davies et al., 1990; Smith et al., 1988), cottonseed meal (El-Sayed, 1990; Robinson and Brent, 1989), mustard oil cake, linseed and sesame meals (Hosain and Jauncey, 1989a, 1989b) and other less common vegetable proteins (Martinez-Palacios et al., 1988; Olvera-Novoa et al., 1990). Unfortunately many of these studies have been conducted on an *ad-hoc* basis and, with the exception of channel catfish very little systematic research has been conducted for warmwater species. Although, the first task in evaluating the potential use of a feed ingredient is to assess its digestibility (Cho et al., 1982), digestibility of alternative protein sources to fish meal has not been determined for many warmwater species apart from catfish (NRC, 1983; Halver, 1989). The measurement of digestibility involves measuring the amount of energy, or a specific nutrient such as protein or fat, which is ingested, and subtracting the amount remaining in the faeces. For highly digestible ingredients like fish meal, very little energy or specific nutrients remain in the faeces. In terms of digestibility to fish, fish meal is generally superior to terrestrial protein sources which are in turn superior to vegetable protein sources (Lovell, 1989). Fish do not have well developed mechanisms to digest the large amounts of carbohydrate or fibre often present in vegetable protein ingredients (New, 1987) although omnivorous or herbivorous species are more capable of utilising carbohydrates than carnivorous species.

If digestibility of ingredients is not considered when diets to compare different ingredients are formulated, the different diets may vary considerably in the digestible energy levels and in the amounts of specific nutrients (eg protein) actually available to the fish. This completely

invalidates these types of comparisons. At the Third Asian Fisheries Forum held in Singapore in October 1992 approximately 25 research papers were presented which dealt with replacement of fish meal in aquaculture diets. Only two or three of these papers considered digestibility of the alternative protein sources to fish meal which were investigated. It is imperative that there is a change in approach to fish meal replacement research. Digestibility information must be a first priority and there is an urgent need for systematic, rigorous science to address this problem.

Central to the evaluation of fish meal replacements is an understanding of their assimilation by target animals. Assimilation is assessed suing growth studies, preferably involving carcass composition where the contribution of ingredients to nutrients retained within the fish flesh can be measured. The measurement of stable isotopes concentrations in feed ingredients and fish flesh has been proposed as a method of measuring the contribution to fish tissue deposition of individual ingredients.

The determination of optimum protein and energy requirements is also crucial when attempting to replace fish meal (Murai, 1992). Protein which is supplied in excess is used to provide energy and may be replaced by well digested carbohydrates or fats (El-Sayed and Teshima, 1991; Murai, 1992). For fish, the amino acid balance of fish meal is superior to alternative protein sources. Synthetic amino acids are widely used in fish diets although problems with leaching and poor assimilation have been observed (Lovell, 1989; Cowey, 1992; Murai, 1992). Methods of overcoming these problems, possibly by coating synthetic amino acids (Cowey, 1992; Murai, 1992) or developing, using recombinant protein technology, specific dipeptides or oligopeptides, need investigation in order to determine the true potential to supplement practical fish diets with synthetic amino acids.

In Australia there are a number of protein sources which are produced in abundance. These include both animal meals such as meat meal, bloodmeal and poultry by-product meals, and vegetable protein sources such as the oilseed meals (eg soybean meal, canola meal, cotton seed meal and peanut meal) and grain legumes (eg lupins, field peas, cow peas and chick peas).

Apart from alternative protein sources to fish meal, Australia has internationally renowned terrestrial animal nutrition research laboratories and a large and successful stock feed manufacturing industry. The problems which faced the pig and poultry industries in Australia 10-20 years ago were similar to those facing some aquaculture industries today. Feed costs had to be dramatically reduced. Fish meal was a major ingredient in early pig and poultry diets but as a result of sustained nutritional research, has now been almost completely replaced. Treatment used to increase digestibility and availability of alternative feed ingredients to pigs and poultry, such as extrusion, microwave processing or addition of exogenous enzymes (Batterham, 1992; Farrell, 1992), may all have application for fish. Australian agronomy has also successfully developed a number of plant cultivars which have reduced concentrations of anti-nutritional factors. The most obvious example is the Australian varieties of rapeseed or canola which now have glucosinolate concentrations as low as 14 m/g compared with cultivars grown in 1966 which had concentrations of 100 m glucosinolate/g (Bob Coulton, NSW Agriculture, Principal Agronomist, personal communication). New varieties of oilseeds and grain legumes are continuously being developed and tested by agriculture departments throughout Australia.

In 1991, NSW Fisheries, with funding from the Grains Research and Development Corporation, commenced preliminary research to investigate the digestibility of Australian

oilseeds and grain legumes in diets for silver perch and to investigate the potential to use these products to replace fish meal. This research has produced some encouraging initial results and indicates some of these products have considerable potential (Allan and Rowland, in press).

The development of cost-effective diets, with reduced contents of fish- and other aquatic-meals is an urgent priority for most fish and crustacean aquaculture industries. The problem is particularly important in Australia which has very poor supplies of aquatic meals. Consequently a number of research institutions and private companies are committed to research of this nature. Although there are a number of specific issues that must be addressed for each species (digestibility of different ingredients for example varies between species as do specific nutritional requirements) much of the research necessary to replace fish meal and develop cost-effective aquaculture diets is generic and will have general application to a number of species. A collaborative project involving a number of separate research institutions will foster the interchange of ideas and standardisation of methods and will greatly improve the quality of the research and usefulness of the results.

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5 OBJECTIVES

			Methods to develop coordinated, collaborative research and development by:
1	To replace fish meal in feeds with cost-effective alternatives	1.1	Review existing knowledge
		1.2	Identify relevant experts - research and commercial
		1.3	Identify target species
		1.4	Identify potential ingredients and their suppliers
		1.5	Evaluate promising ingredients and determine restrictions to their use
		1.6	Develop and evaluate methods of improving the usefulness of ingredients through processing and the use of enzymes and supplements
		1.7	Identify limiting nutritional requirements
		1.8	Optimise feed presentation - physical characteristics, feeding frequency and rate
		1.9	Optimise environmental compatibility of feeds
		1.10	Attract funding from alternative sources to FRDC
2	To obtain an early commitment from feed manufacturers (preferably Australian) to produce and market feeds	2.1	Involve manufacturers in all stages of this project
		2.2	To disseminate, identify and develop markets (domestic and export) for fish and other species
		2.3	Identify and obtain commitment from suppliers of raw products (in consultation GRDC, MRC, CMRC)
		2.4	Produce trial batches of feed for evaluation

6 METHODS

6.1 Structure of the Sub-Program

In April 1991 FRDC supported an Aquaculture Nutrition Workshop and funded a number of keynote speakers from overseas as well as within Australia. This three-day workshop attracted over 100 international and national delegates and a number of pressing research and development needs were discussed. One of the major research priorities identified was the need to find suitable replacements for fish meal in aquaculture diets. Following the workshop two industry-orientated seminars were organised, one at Salamander Bay and the other at Hobart. The seminar at Salamander Bay was designed to stimulate discussion between feed manufacturers, fish farmers and research workers. The common theme amongst all groups was the need to improve Australian made diets, reduce the cost of diets and to find viable alternatives to fish meal.

NSW Fisheries as well as many of the other research institutions associated with this project have developed strong linkages with commercial feed manufacturing companies. These companies have all supported the research proposed in this application. To facilitate industry involvement in this Sub-Program, one commercial feed company executive and one aquaculture industry executive were included on the Sub-Program Steering Committee. Several representatives from feed manufacturing companies and aquaculture companies were also included on the Scientific Committee.

The Sub-Program involved interactive, collaborative research at twelve institutions. Six separate projects were included which together address the Sub-Program objectives. Most projects involved several institutions and the research was coordinated both within and between Projects.

The Sub-Program was managed by Dr Geoff Allan who facilitated coordination between Projects, assessed performance indicators and reviewed milestones for all Projects, organised meetings and workshops and chaired the Sub-Program Steering and Scientific Committees. He also coordinated the collection of the protein sources and their distribution to other Project leaders for evaluation. CSIRO Division of Food Science and Technology, North Ryde provided protein concentrates from its own research and from contacts in the various Grain Pools. Other R & D corporations, including the Grains Research and Development Corporation, the Meat Research Corporation and the Wheat Board were contacted and separate funding proposals to support fish meal replacement research submitted (which were subsequently approved). Apart from financial support, these R & D Corporations facilitated access to protein sources, some of which were developed specifically for this Sub-Program.

The Australian Centre for International Agricultural Research funded research to investigate fish meal replacement in aquaculture diets involving NSW Fisheries (for research with silver perch) and Thailand Department of Fisheries (for research with hybrid walking catfish). The research with silver perch had complementary objectives and was led by Dr Geoff Allan.

The Steering Committee will determined overall research direction and reviewed progress. In addition to the Sub-Program Manager, it included Mr Peter Dundas-Smith, the Executive Director of FRDC, Dr Ted Batterham (Principal Research Scientist with NSW Agriculture) (after Dr Batterham's death in 1994 he was not replaced), Dr W Dall (CSIRO), Mr Roger Harrison (Morton Bay Prawn Farms) and Mr Ray Johnson (formerly of Rhone-Poulenc now with Greens Foods).

Coordination between Projects, including prioritising ingredients to be evaluated, standardising methodology and synchronising experiments was facilitated by a Scientific Committee comprising representatives of all institutions, FRDC and the feed manufacturing industry. On occasions, independent experts were invited to participate in Scientific Committee meetings to ensure the research targeted industry needs and embraced the most recent scientific advances.

To help standardise methodology used within the Sub-Program, and to ensure the analytical techniques used and the experimental approach taken were equal to the best in the world, a technical workshop was organised to review biochemical analyses. Replicate analyses of fish faeces, fish tissue, plant and animal protein sources and diets were done at each laboratory prior to this workshop and then results were compared and methods discussed. A separate study to compare lipid and fatty acid analyses at difference laboratories was also conducted. In conjunction with the 6th International Symposium on Fish Nutrition and Feeding being held in Hobart 4-7 October 1993 a meeting with the international experts participating at the Symposium and scientists involved in the Sub-Program was organised. At this meeting experimental approaches proposed by scientists in the Sub-Program to replace fish meal were discussed with international nutrition experts.

6.2 Species

The Sub-Program conducted research on 'key-representative' species. These were selected using the following criteria:

1 Formed the basis of a large industry or had outstanding potential to do so
2 Cost of feed was a major factor limiting the development of the industry
3 Fish meal was the primary protein source
4 There was a perceived potential to replace fish meal in diets for the species
5 Scientists within the Sub-Program had the capacity to conduct research with the species
6 Feed manufacturing company or companies manufactured diets for the species and were interested in replacing fish meal in these diets

The following species were chosen:

- Jumbo tiger prawn (*Penaeus monodon*) - warmwater, marine, carnivore, crustacean
- Silver perch (*Bidyanus bidyanus*) - warmwater, freshwater, omnivore, finfish
- Atlantic salmon (*Salmo salar*) - coldwater, marine, carnivore, finfish
- Barramundi (*Lates calcarifer*) - warmwater, catradromous, carnivore finfish

7 RESULTS

7.1 Coordination and Communication

Too often research projects achieve their objectives but fail to transfer the results to industry. Technology transfer was deemed to be a high priority by the Steering Committee and during the course of the Sub-Program regular Scientific Committee meetings were held, workshops and conferences convened and results published to rapidly transfer technology developed over the three years:

YEAR I

Sub-Program Scientific Committee Meetings

- 30-31 March 1994 at IFIQ, Brisbane
- 30 October to 1 November 1995 at IFIQ, Brisbane

Workshops, Meetings and Conferences

- Meeting on experimental procedures, Hobart 5 October 1993.

To take advantage of the presence in Australia of international nutrition experts, an evening meeting to discuss fish meal replacement and experimental procedures for nutritional research was held during the Sixth International Symposium on Fish Nutrition and Feeding. The agenda was as follows:

Welcome and Introduction - Dr Geoff Allan
Alternatives to fish meal in aquaculture diets - Professor C Cowey (Canada)
Measuring feed input in large scale facilities - Dr S Helland (Norway)
Experimental procedures at Guelph University - Professor C Y Cho (Canada)
Experimental procedures at University of Mississippi - Prof R Wilson (USA)
Experimental procedures at INRA, France - Dr S Kaushik (France)

The meeting was held over dinner and Sub-Program scientific committee members had ample opportunity to meet international experts and discuss fish meal replacement research in Australia.

- Workshop on Analytical Techniques, 13 April 1994

The accuracy and reliability of biochemical analyses is crucially important to nutritional research. In a large coordinated program such as ours, it is important that results between research workers are comparable. To investigate this a series of matched samples were sent to different laboratories for proximate and amino analyses. For amino acid analyses, laboratories involved in our Sub-Program took part in a large study comparing amino acid analyses throughout Australia and New Zealand. This larger study was funded by the Pig Research Council. Fatty acid analyses were not conducted because although they are of critical importance in aquaculture nutrition research, only one laboratory was prepared to carry out the analysis. The results from the amino acid and proximate analyses are somewhat sobering. Although there is reasonable agreement for some analysis, large differences occur in results for others. These differences were the focal point for discussions during the workshop. The aims of the workshop were to:

1 Improve the reliability of inter-laboratory comparison of results.
2 Minimise between-laboratory variation for analyses of identical samples.
3 Help standardise methodology where similar equipment is used.
4 Allow technicians conducting proximate and amino acid analyses to discuss methodology in an open workshop forum to foster exchange of ideas.
5 Help ensure research scientists are familiar with techniques used and with the limitations of these techniques.
6 Compile commonly used techniques for proximate and amino acid analyses.
7 Provide useful information about different methods of analyses and advise on the most appropriate analyses for fish nutrition research.

The Proceedings of the Analytical Techniques Workshop has since been published (ISBN 0 7310 36905). Extra copies are available from NSW Fisheries, Port Stephens Research Centre. Please see Appendix 1.

- Lipid Analysis Comparison Trial 1994-95

Aquaculture nutrition research laboratories are evaluating many alternate protein sources for aquaculture diets; analysing them to determine nutrient composition. Although amino acid and protein content are of particular concern, lipid content is also relevant.

Following the success of the Analytical Techniques Workshop, it was decided that a follow-up trial focussing on lipid analysis techniques would also be beneficial to laboratories in the group. The aims were similar to those of the previous trial.

- Allow participating laboratories to compare their results with others and review any perceived problems.
- Help standardise methodology where similar equipment is used.
- Ensure the limitations of any particular technique are understood.
- Provide useful information about commonly used techniques of lipid analysis and advise on those most appropriate for aquaculture nutrition research.

YEAR II

Sub-Program Scientific Committee Meeting

- 5-6 December 1995 at NSW Fisheries, Port Stephens Research Centre.

Workshops/Meetings and Conferences

- 20 December 1994. Meeting with Dr Roger Campbell (General Manager, Technical Sciences Bunge Meat Industries Inc.) and Dr Ray Johnson (General Manager, Rhone-Poulence Animal Nutrition) at PSRC with Sub-Program scientists.

- 5-7 December 1994. Fish meal Substitution Workshop, Iowa, USA. David Smith presented a paper co-authored with Geoff Allan describing the Sub-Program, the objectives, individual projects and collaborative arrangements.

- 22-24 April, 1995. Fifth International Working Group on Crustacean Nutrition Symposium, Kogashima, Japan. This Conference was organised by Dr Akio Kanazawa, one of the foremost experts in prawn nutrition. Geoff Allan presented a joint review paper with David Smith on crustacean nutrition research in Australia, David Smith spoke on digestibility research with *P. monodon* and Zafer Sarac spoke on nutrient utilisation efficiency with *P. monodon.* (See Appendices 2, 3 and 4).

- 29-30 May, 1995. GRDC Workshop, SARDI, Adelaide. This workshop was organised by SARDI and the GRDC to examine grain use in aquaculture feeds and the potential for increasing their use. A number of invited papers were presented to overview grain use, processing, nutritional requirements for fish and crustaceans and current aquafeeds practices. (See Appendices 5 and 6).

- Two Day Strategic Research Planning Meeting. On 12 September 1995 Sub-Program project scientists and Steering Committee members met in Brisbane at IFIQ to discuss priorities for new research. During this session, research results were very briefly outlined and priorities and future directions discussed. The following day the outcomes of this meeting were presented to, and debated with, representatives from FRDC, GRDC, MRC and Ridley Agriproducts. The aim of this two day meeting was to formulate a Strategic Research Plan which would assist with new nutrition project applications to FRDC and other R & D corporations for 1996/97 and beyond. The group considered it was important to continue the collaborative approach to research exemplified by the Fish meal Replacement Sub-Program and a decision was taken to apply for a new Sub-Program to be called the Aquaculture Diet Development Sub-Program.

Completed Reports

Allan, G.L., 1994. Preliminary evaluation of meat meal in aquaculture diets for silver perch (*Bidyanus bidyanus*). Final Report to Meat Research Corporation. NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW, 2316 Australia (ISBN 0 7310 4893 8).

Allan, G.L., 1994. Growth of juvenile silver perch (*Bidyanus bidyanus*) on diets based on modified wheat gluten. Final Report to Academy of Grain Technology and Fisheries Research and Development Corporation. NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW 2316 Australia (ISBN 0 7310 6400 3).

Allan, G.L., Rowland, S.J., 1996. Potential of meat meal to replace fish meal in commercial diets for silver perch (*Bidyanus bidyanus*). Final Report to Meat Research Corporation. (ISBN 0 7 310 9695 9).

Smith, D.L., 1995. Preliminary evaluation of meat meal in aquaculture diets for prawns (*Penaeus monodon*). Final Report (Part 2) to Meat Research Corporation. CSIRO Division of Fisheries, Cleveland Qld Australia.

Smith, D.L., Barclay, M.C., 1995. Lipid Analysis Comparison Trial. Report for FRDC Fish meal Replacement Sub-Program. CSIRO Division of Fisheries, Cleveland Qld Australia.

Smith, D., 1996. Evaluation of meat meal in aquaculture diets for the giant tiger prawn (*Penaeus monodon*). Final Report for Meat Research Corporation.

Williams, K.C., Barlow, C., 1996. Potential of meat meal to replace fish meal in commercial diets for barramundi (*Lates calcarifer*). Final Report to Meat Research Corporation.

Copies of these reports are available from NSW Fisheries, Port Stephens Research Centre. Please see Appendix 1.

YEAR III

Sub-Program Scientific Committee Meetings

This was held on 5-6 December 1995 at PSRC.

Sub-Program Scientific Committee Meeting

- 22-23 May 1996 at CSIRO Marine Laboratories, Cleveland, Queensland.
- 14-15 April 1997 also at CSIRO, Cleveland.

Conferences

- WAS 1996 meeting was held in Bangkok on 29 January to 2 February, 1996. The meeting attracted approximately 4000 delegates. Papers were presented by Kevin Williams, Alex Anderson and Geoff Allan about Sub-Program research. (See Appendices 7, 8 and 9).
- The Second International Conference on the culture of penaeid prawns and shrimps was held in Iloilo City, Philippines on 14-17 May 1996. Zafer Sarac presented a paper. (See Appendix 10).
- The World Fisheries Congress was held in Brisbane 29 July to 3 August 1996. More than 600 abstracts were submitted across six major themes, one of which was "How can aquaculture help sustain world fisheries?"
- Victam-Asia, "Feed Production on the Threshold of the Next Age." Bangkok, Thailand, November 1996. An invited paper on "Potential for replacement of marine ingredients in Asian aquafeeds" was presented. (See Appendix 11).

7.2 Non-Technical Summary of Individual Projects

7.2.1

93/120-02	**Fish meal Replacement in Aquaculture Feeds for Prawns**

Principal Investigator: D. M. Smith

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Objectives:

1 To determine, for prawns, the digestibility of the alternative protein sources and the assimilation of the nutrients in them.
2 To investigate methods of enhancing the digestibility of feeds and feed ingredients.
3 To develop methods to enhance the nutrient balance, attractiveness and palatability of diets formulated using alternative protein sources.
4 To determine the prawn's protein requirements in relation to different amounts of digestible energy available in the feed.
5 To use this information in the continued testing of potentially commercial diets using selected alternative protein sources to replace or partially replace fish meal.

Non-Technical Summary

The objectives of this project were to review the available feed ingredients that could be used to replace fish meal in aquaculture diets for prawns, and to select a number of them for detailed evaluation. We were then to determine how well the prawns could digest those ingredients in either their 'as used' form or any alternatively processed forms that were economically feasible. We would also determine how well the prawns could utilise those ingredients using various types of growth experiments. We proposed to determine the prawns' requirements for protein. This would assist in the cost-effective formulation of diets and ensure that excessive amounts of protein were not included in the diet. We planned to study the efficacy of supplementing diets with amino acids and attractants. These supplements could to enhance or extend the use of some of the alternative protein sources.

A number of methods used to assess the nutritional value of the alternative protein sources were evaluated or developed. A precise and reproducible method was developed for measuring the apparent digestibility (AD) of ingredients and the key nutrients contained in them. The procedure involved the use of inert markers (chromic oxide and ytterbium) in the diet and the recovery of those markers in the faeces. Using this method, we determined the AD of wheat gluten, lupin meal, canola meal, soybean meal, Dunn field peas and three types of meat meal. Generally the AD of the plant protein was about 90% or higher with the exception of canola meal (78%). The removal of the hulls from lupins resulted in a significant increase in the AD going from 88% to 94%. The AD of protein in the meat meals varied according to the type of meat meal and ranged between 74% and 83%. Investigation into the opportunities of improving digestibility, using techniques such as sprouting or malting indicated that these processes are unlikely to be cost-effective

The summit dilution method for assessing ingredients was tested and adopted to provide information on the potential inclusion levels of the ingredients in commercial diets. From these studies it appeared that the maximum practical inclusion level of wheat gluten was 30%, canola meal and lupins (whole, de-hulled and lupin protein concentrate) was 20%, and cotton seed meal was 10%. The need for the removal of anti-nutritional factors (such as gossypol) from cotton seed meal was highlighted in this study. The use of meat meal was investigated using supplementary funds from the Meat Research Corporation. This work clearly demonstrated that meat meal could be used to replace two thirds of the fish meal protein in the diet without adversely effecting the growth performance or flavour of the prawns

The maintenance requirement of *Penaeus monodon* for energy was found to be 0.3 kJ digestible energy/g body weight/day and 0.007 g digestible crude protein/g body weight/day. Larger prawns were significantly more efficient than smaller prawns in utilising both ingested crude protein and energy. In a second study to determine the prawns' response to different levels of dietary protein and energy the prawns' growth rate increase linearly with increasing digestible crude protein intake. The diets used contained digestible crude protein between 30% and 60% of the diets. There did not appear to be any response to the dietary energy though it was provided at levels between 14 and 19 MJ/kg in the diets. The objective of this study was to determine the optimum level of protein and energy to use in commercial feeds for *Penaeus monodon*. Though these experiments were carried out successfully, further work will need to be carried out to obtain a clearer picture of the optimum requirements.

In the study on the use of commercial attractants, the most effective attractant was a cooked shrimp powder. However, when included in a diet containing low levels of ingredients of marine origin (which on its own would appear to be low in attractiveness), the shrimp powder

did not elicit a substantial improvement in feed preference or feed intake. The cost-effectiveness of using attractants as compared to using a low inclusion level of a high quality marine protein meal needs to be looked at closely.

The use of stable isotopes (non-radioactive) either at their natural occurrence levels or enriched in specially grown ingredients was studied. It showed promise as a research tool in nutrient utilisation studies. However, it provided no advantage over the digestibility and the summit dilution methods for evaluating the ingredients for use in commercial diets. The stable isotopes were also used in pond studies to look at the utilisation of feeds in ponds by prawns, and to what extent other pond biota feed on the prawn feed and then become a 'live' food for the prawns. The information coming from this study is giving an insight into the impact of feeds on the water quality of the ponds, which has a major impact on production costs.

Though we have carried out successful experiments studying the protein and energy requirements of *Penaeus monodon*, we have not yet defined the optimal inclusion levels that will give maximum growth rate with minimum protein content. It does appear that prawns use protein preferentially as an energy source, which complicates this task. However, we have identified the maximum practical inclusion levels of the potentially most useful ingredients to be used to partially replace fish meal in prawn diets, and have determined their apparent digestibility. We have the information that will allow prawn feed formulators to reduce the inclusion levels of fish meal and other marine products by over 50%.

7.2.2

93/120-03 Fish meal Replacement in Aquaculture Feeds for Silver Perch

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Objectives:

1. To identify potential feed ingredients to replace fish meal in aquaculture diets for silver perch.
2. To evaluate promising ingredients in terms of their *in vitro* and *in vivo* digestibility and assimilation.
3. To develop and evaluate methods of improving the usefulness of ingredients through processing (eg extrusion or cooking) and the use of enzymes and supplements.
4. Identify areas where inadequate knowledge of nutritional requirements may restrict fish meal substitution and determine these requirements for silver perch.
5. To formulate and evaluate diets with reduced contents of fish meal for silver perch.

Non-Technical Summary

If aquaculture is to continue to expand in Australia cost-effective diets based on Australian agricultural ingredients urgently need to be developed. The replacement of fish meal as the protein source of choice is a global research priority driven by static or declining supply of fish meal and rapidly expanding aquaculture and aquaculture feed industries. Australia has very poor supplies of fish meal and other aquatic meals but fortunately has abundant supplies of agricultural ingredients with potential for use in aquaculture diets.

In recognition of the need to develop diets for Australian aquaculture species, with reduced contents of fish meal, a number of institutions independently commenced this type of research in the early 1990's. The Fisheries Research and Development Corporation (FRDC) was approached by a number of institutions to financially support this research. In response, FRDC created their first 'Sub-Program' with the aim of coordinating research to develop Australian aquaculture diets. The two primary objectives were to replace fish meal and obtain an early commitment from commercial aquaculture feed manufacturers to adopt results.

Six separate projects were formed; four on species considered to represent most 'types' of species being farmed in Australia, one on feed processing and one on a technology audit for amino acids. The four 'model' species were prawns *Penaeus monodon*, silver perch *Bidyanus bidyanus*, barramundi *Lates calcarifer* and Atlantic salmon *Salmo salar*. Each project involved a number of collaborating scientists from different institutions and all projects were coordinated through a Sub-Program Steering Committee. Regular meetings with investigators from all projects as well as feed manufacturers, ingredient suppliers and R&D corporations were held twice each year.

This report describes the progress achieved with the silver perch project: Replacement of Fish meal on Aquaculture Diets for Silver Perch. Silver perch are an omnivorous, freshwater species endemic to eastern Australia. They have shown outstanding potential for culture in static earthen ponds and are one of the few species being cultured, or considered for aquaculture, in Australia which might replace some of the more than 55 000 t/yr of imports of white-fleshed fish. Growth and production potential of silver perch are similar to channel catfish in the USA and carp and tilapia in south-east Asia.

Objective 1: To identify potential feed ingredients to replace fish meal in aquaculture diets for silver perch

Literature and data base searches were conducted and a comprehensive list of ingredients currently available for use in animal feeds in Australia was compiled. Available data on biochemical composition, price and availability of these ingredients was obtained. This was used to select ingredients for further evaluation.

Additional ingredients were identified following discussions with the Grains Research and Development Corporation, the Grain Pool, the Australian Wheat Board and the Meat Research Corporation. The specific features making ingredients worth considering for aquaculture were discussed and the various agencies recommended ingredients their stakeholders produced which might be suitable. Descriptions of ingredients and data on composition can be found in Sections 6.2, 6.3, 6.4, 6.7, 6.8, 6.10 and 6.12.

Objective 2: To evaluate promising ingredients in terms of their *in vitro* and *in vivo* digestibility and assimilation

Initially, a series of three experiments were done to determine the most appropriate techniques for *in vivo* digestibility determination. Results from these experiments demonstrated that collection of faeces by settlement over 18 h was a suitable method for determining digestibility in juvenile silver perch (see Section 6.1 of this report). As the sum of digestibility coefficients calculated separately for individual ingredients was similar to that calculated for a diet comprised of those ingredients, the assumption that digestibility coefficients are additive was validated (see Section 6.1 of this report).

Methods for *in vivo* digestibility were developed or evaluated by Dr Alex Anderson at QUT. Results from these experiments are reported separately (see Fish meal Replacement in Aquaculture Feeds: *in vitro* studies on feed ingredients for aquaculture species. Final Report to FRDC Sub-Program 93/120). In summary, *in vitro* methods were shown to be useful for ranking but not for accurately determining digestibility coefficients for use in diet formulation.

In vivo digestibility determination involves measuring the amount of dry matter, energy or a specific nutrient which is ingested and then subtracting what is excreted. This involves collecting and analysing faeces. It is the first critical stage in evaluating the potential of an ingredient for use in a formulated diet. Following method development and verification (see Section 6.1 of this report), digestibility coefficients for over 60 ingredients, including some processed in different ways, were calculated. Digestibility coefficients for dry matter, energy, protein and, in most cases, individual amino acids (except tryptophan) are presented (see Sections 6.2, 6.3, 6.7, 6.8, 6.10 of this report).

Once digestibility coefficients are available, the next step is to determine the maximum amount of an ingredient which can be used in formulated diets. Many ingredients, especially those derived from plants, have anti-nutrients, some of which affect utilisation of the ingredient but not digestibility. In addition, excessive amounts of some ingredients may reduce the attractiveness of the diet or suppress palatability. Information on how well ingredients are utilised is also critical for effective diet formulation using new ingredients.

Growth experiments were conducted to provide this information for meat meals, poultry offal meal, feather meal and dehulled lupins. This added to earlier research results to estimate maximum contents of soybean meal, canola meal, peanut meal and lupins. One experiment was also conducted where all fish meal was replaced with specially modified wheat gluten meal. The most promising ingredients evaluated are meat meal, especially low ash meat meal, poultry offal meal and dehulled lupins. The specially-modified wheat gluten meal also deserves further evaluation. These results are reported in Sections 6.4, 6.6, 6.9 and 6.12.

Objective 3: To develop and evaluate methods of improving the usefulness of ingredients through processing (eg extrusion or cooking) and the use of enzymes and supplements

Most ingredients with potential for use in aquaculture diets are inferior to fish meal in terms of their nutritional composition (especially total protein content and amino acid profile), carbohydrate content or presence of anti-nutrients. Some of these deficiencies can be overcome. Digestibility and utilisation of an ingredient can be improved by processes such as grinding, cooking, and removal of less digestible components such as carbohydrate (eg through dehulling and removal of starch and non-starch polysaccharides) and ash (eg through removal of bone).

We found grinding diets below a particle size of between 710 and 1 000 μm was unnecessary but that steam conditioning or extruding practical diets containing starch improved gelatinisation of starch, digestibility, gustatory characteristics and fish growth (see Sections 6.8 and 6.10). Removal of hulls, by dehulling, improved dry matter and energy digestibility of two species of lupins, field peas, chick peas and vetch but not faba beans (vetch was poorly accepted by silver perch). Further protein concentration, through the removal of starch and/or non-starch polysaccharides further improved energy and dry matter digestibility of lupins, field peas and faba beans (other protein concentrates were not available). Protein digestibility of most pulses was high (see Sections 6.3 and 6.7 of this report).

Removal of part of the ash fraction from meat meals increased total protein content and improved the value of meat meals for use in diets for silver perch (see Section 6.4 of this report).

Some of the most common supplements used to overcome nutritional deficiencies in ingredients and diets are crystalline amino acids. During this study, crystalline lysine, methionine and/or threonine were added during several experiments but there is no conclusive evidence that silver perch responded to these supplements at any time. This may be due to problems with utilisation of crystalline amino acids, and such problems have been widely reported with some species of fish, or indicate that the diets supplemented were not deficient in those amino acids. This area requires further evaluation.

Objective 4: Identify areas where inadequate knowledge of nutritional requirements may restrict fish meal substitution and determine these requirements for silver perch

When we formulated the first reference diets for silver perch we set nutritional specifications using published requirements for other species as a guide. In particular, species such as channel catfish and tilapia, which are omnivorous, were used.

Given the high cost of protein, the major initial task was to estimate requirements of this nutrient for silver perch. As energy might be able to spare requirements for protein, the interaction between energy and protein was also important to quantify.

Preliminary research indicated that growth increased with both protein and energy but that as early diets were formulated before we had accurate information on protein and energy digestibility, results were confusing and difficult to interpret. We were able to conclude that energy could spare requirements for protein but high lipid content diets led to high lipid content of fish tissue, a negative from a marketing perspective.

Recent research with pigs and poultry has introduced the concept of a protein dependant phase and an energy dependant phase for maximum protein deposition. With this concept, at a certain energy content protein deposition increases during the protein dependant phase and then plateaus out. Further increases in protein deposition require additional energy.

We applied this approach to determine optimum protein and lysine requirements for silver perch. We used a single digestible energy content, one used successfully in practical diets gaining wide commercial acceptance and which we knew did not produce excessive lipid deposition in the fish carcass. During this experiment we determined that for silver perch fed a diet with approximately 14-15 MJ/kg digestible energy the minimum digestible protein

content which produced maximum growth was only 25.2% (much lower than most estimates of optimum protein for cultured fish). We also estimated that 14-15 MJ/kg digestible energy diets do not need to contain more than 1.5% digestible lysine for optimum growth (see Section 6.11 of this report).

Objective 5: To formulate and evaluate diets with reduced contents of fish meal for silver perch

Most nutritional research is done using small juvenile fish and small tanks. The applicability of results generated using these methods is often questioned by farmers who wish to grow fish through to a market size in large ponds or tanks. Nutritional research needs to be validated in commercially relevant facilities.

In this study we utilised the results for ingredient digestibility and utilisation efficiency and the effects of diet processing to formulate two 'least-cost' diets for a large-scale farming experiment. Cost of ingredients was estimated as ingredient cost only, not including transport costs as this component is clearly dependant upon where the feed mill is located.

Our least-cost diets contained only 5 or 10% fish meal with the remainder replaced with meat meal, dehulled lupins or dehulled field peas. The least-cost diets out-performed the earlier fish meal/soybean meal reference diet and the cost of producing fish, based on ingredient costs and food conversion ratios, was lower (see Section 6.5).

Keywords: Fish meal Replacement; Silver Perch; Nutrition; Ingredient Evaluation; Nutrient Requirements; Aquaculture; Meat meals; Lupins; Pulses; Least-cost

7.2.3

93/120-04 Fish meal Replacement in Aquaculture Feeds for Barramundi

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Objectives:

Overall objective:

- Assess the nutritive value and suitability of identified locally produced feedstuffs as alternatives to fish meal in diets for grow-out barramundi

Specific objectives:

1. Determine the digestibility of alternative protein (and energy) sources to fish meal for barramundi using *in vitro* and *in vivo* (faecal and ileal) procedures.
2. Assess the animal's assimilation of nutrients from identified feedstuffs using comparative slaughter procedures.

3 Determine the effects on diet acceptability (physical characteristics and palatability to barramundi) and growth performance of barramundi when alternative feedstuffs are used as substitutes for fish meal.

4 Compare and validate information gathered on the nutritive value of alternative feedstuffs using growth assay procedures.

5 Improve feed formulations and strategies to reduce wastage of feeds and to increase the utilisation of nutrients.

Non-Technical Summary

One of the major factors limiting the expansion of barramundi farming in Australia is the development of nutritionally adequate, cost-effective diets. Feed and feeding can contribute up to 50% of the total operating costs. The most expensive component of pelleted feed is protein, of which 40-45% is required by barramundi and this has typically been provided by using fish meal at inclusion rates of up to 50%. Australia is particularly vulnerable to any world shortage of fish meal because of our reliance on imported fish meal. However, Australia has an abundant supply of terrestrial animal and vegetable protein feeds which have the potential to at least partly if not fully replace the fish meal presently used in compounded aquaculture diets. Successful and cost-effective replacement of fish meal by terrestrial proteins in aquaculture diets may provide export opportunities for Australian feed manufacturers to supply the large Asian aquafeed market.

The primary objective of this project was to evaluate the suitability of locally available and less expensive protein feed ingredients as alternatives to fish meal in diets for barramundi. This Project was one of six elements which together comprised the FRDC Sub-program on "Replacement of Fish meal in Aquaculture Feeds" (93/120). Details of the other constituent Projects are provided as separate Reports. The barramundi work involved collaboration between research staff of Queensland Department of Primary Industries at Bribie Island and Walkamin (and subsequently, at CSIRO Division of Marine Research, Cleveland), University of Queensland and Queensland University of Technology.

Feedstuffs identified to have potential as fish meal replacements were evaluated with emphasis given initially to assessment of digestibility, nutrient utilisation and growth assays to evaluate dietary formulations. The *in vitro* digestibility work carried out by Dr Anderson in this Project has been addressed as a separate report since companion studies were made for all four species (barramundi, prawns, silver perch and Atlantic salmon) comprising the Sub-program (93/120).

The major work carried out and findings were:

- Alternative methods of faecal recovery and digestibility markers (chromium, titanium or ytterbium) were evaluated for apparent digestibility determination. Total collection and sedimentation methods were unacceptable because of the rapid and quantitatively large amount of nitrogenous compounds lost from the faeces into the surrounding water, resulting in overestimates of protein digestibility; intestinal dissection gave reliable digestibility estimates but the small sample size and high cost were serious disadvantages; manual stripping of large barramundi produced more reliable digestibility estimates than with anal suction collection and the former is advocated as the procedure of choice for faecal recovery in barramundi. All three markers produced reliable digestibility estimates but Yb was preferred because of its solubility (facilitated even dispersion in the diet), precise analytical measurement and absence of toxicity.

- The apparent digestibility of two fish meals (Danish and tuna), three terrestrial abattoir meals (poultry offal meal and two meat meals) and six plant protein meals (solvent- and full-fat soybean, peanut, canola, dehulled-lupin and wheat gluten) was determined. Protein and energy apparent digestibilities were high for all meals although the animal feeds were slightly better digested than the plant feeds other than for wheat gluten which was completely digestible. The digestibility of meat meal was variable and lower than for fish meal and this was attributed to meat meal's high ash content. These results demonstrate the similarity of barramundi to other carnivorous fish in being able to digest protein and energy from a wide variety of different terrestrial feeds.

- The biological value of the protein feeds examined in the digestibility studies were compared relative to a high fish meal reference protein using comparative slaughter and rationed feeding procedures. Replacement of each of the plant protein meals for the fish meal in the reference diet caused barramundi to refuse the diet and prevent the determination of biological value. Diets based on meat meal or poultry offal meal were well accepted by barramundi and the protein of these diets was utilised as efficiently as for diets based on Tasmanian fish meal.

- The efficiency with which protein and energy was utilised by juvenile barramundi when provided from six different protein meals (meat, Peruvian fish, casein, solvent- and full fat-soybean and dehulled lupin) was characterised in this project using comparative slaughter and summit dilution procedures. Each of the test protein meals was substituted for a high fish meal summit diet at inclusions incremented up to 70%. In terms of fish weight gain and nutrient retention, the three animal protein meals – Peruvian fish meal, casein and meat meal – were clearly superior as substitutes for the summit diet than the dehulled lupin or soybean meals which were of similar nutritive value. The lower nutritive value of the soybean and lupin meals was thought to be due to their lower digestible energy content and an apparent higher metabolic energy requirement for their utilisation. The present work provides presumptive evidence that the inferior amino acid balance of plant protein meals was not the reason for their lower nutritive value relative to fish meal.

- The nutritive value and palatability of ring-dried blood meal for juvenile barramundi was examined by growth assay. Supplementation with blood meal at rates of up to 22.5% had no adverse effect on the apparent palatability of the diet but did cause a slight worsening of food conversion. The results show that barramundi will readily accept diets containing high inclusions of blood meal and that blood meal may be a useful attractant to improve the unpalatability of other dietary constituents such as casein and plant protein meals.

- This project has shown that terrestrial animal protein sources such as meat meal have considerable potential as dietary feed ingredients for barramundi and could be used to replace most, if not all of the fish meal. Plant protein feeds, although not well accepted by barramundi, were capable of being digested and utilised *albeit* at an energetic efficiency apparently lower than that for animal protein feeds.

Keywords: Barramundi, Digestibility, Protein, Energy, Utilisation, Palatability, Comparative slaughter, Growth assay

93/120-05 Fish meal Replacement in Aquaculture Feeds for Atlantic Salmon

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Objectives:

1. To obtain a range of enzyme and feed supplements for use in Atlantic salmon feeds
2. To establish an effective experimental protocol for measuring the food consumption and growth and calculating growth efficiency of Atlantic salmon
3. To establish an effective *in vivo* digestibility method
4. To determine the effectiveness of enzyme supplements and feed components in improving the growth and growth efficiency of Atlantic salmon
5. To determine the effectiveness of feed supplements and feed components in improving the growth and growth efficiency of Atlantic salmon
6. To establish whether improvements in digestibility due to enzyme and feed supplements are translated into improvements in growth efficiency
7. To establish whether *in vitro* digestibility data can be used to screen and select feed components and suitable combinations of enzyme and feed supplements for inclusion in Atlantic salmon feeds

If aquaculture is to continue to expand in Australia cost-effective diets based on Australian agricultural ingredients urgently need to be developed. The replacement of fish meal as the protein source of choice is a global research priority driven by static or declining supply of fish meal and rapidly expanding aquaculture and aquaculture feed industries. Australia is very poorly supplied with fish meals but fortunately has abundant supplies of agricultural ingredients with potential for use in aquaculture feeds.

In recognition of the need to develop diets, with reduced contents of fish meal, for Australian aquaculture species a number of institutions independently commenced this type of research in the early 1990's. The Fisheries Research and Development Corporation (FRDC) was approached by a number of institutions to financially support this research. In response, FRDC created their first 'Sub-Program' with the aim of coordinating research to develop Australian aquaculture diets. The two primary objectives were to replace fish meal and obtain early commitment from commercial aquaculture feed manufacturers to adopt results.

Six separate projects were formed, four on species considered to represent most 'types' of species being farmed in Australia, one on feed processing and one on a technology audit for amino acids. The four 'model' species were Atlantic salmon *Salmo salar,* barramundi *Lates calcarifer,* silver perch *Bidyanus bidyanus* and prawns *Penaeus monodon.* Each project involved a number of collaborating scientists form different institutions and all projects were coordinated through a Sub-Program Steering Committee. Regular meetings with investigators form all projects as well as feed manufacturers, ingredient suppliers and R&D corporations were held twice each year.

This report describes the progress achieved with the Atlantic salmon project: Replacement of Fish meal in Aquaculture Diets for Atlantic salmon. Atlantic salmon is a carnivorous species, originally from the Northern hemisphere, that spawns in freshwater and can undergo growth and maturation in fresh and sea water. They are the most important species of intensively cultured fin fish in Australia as well as globally. The majority of Atlantic salmon production is in Tasmania where the industry had an annual production of 7647 t worth $ 58.5 M in 95/96. These figures are expected to treble in the next five years.

Objective 1: To obtain a range of enzyme and feed supplements for use in Atlantic salmon feeds

Literature searches and consultation with ingredient suppliers and aquaculture feed manufacturers were used to compile a comprehensive list of feed supplements, principally feed enzymes, that were commercially available for use in animal feeds or had been trialed with fish. Effective feed supplements generally target specific dietary components or physiological processes of the animal. Consequently, objective 1 was expanded after consultation with Gibson's Ltd to include feed ingredients that had potential for use in salmon feeds. Very few feed enzymes had been shown to have potential in salmon or fish feeds and even fewer were commercially available. Two commercially available enzymes and two mixes made from commercially available enzymes were judged worthy of testing and initial screening is described under Objective 4 (Section 6.1). The description of other feed supplements judged to have potential for salmon is described under Objective 5 (Section 6.6).

High quality feeds manufactured in Australia (by Gibson's Ltd) were and are available for Atlantic salmon. This meant that the choice of feed supplements with ingredients had to be very specific in order to have an impact on their potential use in future commercial feeds. Modern extruded salmon diets require ingredients that have very high protein contents (identified as at least 50%) and this meant that only processed plant meals could be considered. Meals and protein concentrates made from soybean, lupin and pea were selected. Phytase was selected as a feed enzyme with the most potential for use with plant proteins. It was predicted to improve phosphorous and nitrogen utilisation, it had a proven record with other farmed animals including fish and a commercial product was readily available. The assessment of the performance of these plant meals and of phytase in terms of growth and growth efficiency is discussed under Objective 4 (Sections 6.2, 6.3, 6.4,6.5).

Objective 2: To establish an effective experimental protocol for measuring the food consumption and growth and calculating growth efficiency of Atlantic salmon

Atlantic salmon can be difficult to maintain and grow quickly in experimental systems, experimental systems and procedures were successfully established and used in this project and are available for future research. The salmon grew at relatively high rates (when rations

permitted) and juvenile salmon typically doubled their weight in 6 to 7 weeks and sizes of between 1 and 500 g were successfully used in experiments. Feed intake was accurately measured using settlement traps and provided vital information on the efficiency of dry matter and nutrient gain. Feed intake was measured in all experiments.

The effect of switching feed ingredients on feed consumption was specifically investigated and consideration of how salmon can adapt to a new dietary ingredient made, the results are discussed under Objective 4 (section 6.9). Daily monitoring of feed intake highlighted differences in appetite that were related to differences in dietary composition and previous nutritional history.

Objective 3: To establish an effective in vivo digestibility method

Feed and ingredient digestibility provides important data necessary for the assessment of performance of fish meal replacements. The traditional settling-column collector developed at Guelph University was not of an optimum design. Sample collection was time consuming (~5-10 minutes/sample) due to the tendency of faeces to become lodged in the settlement area. In addition, turbulent mixing of faeces with water as they were withdrawn from the settling area occurred. This promoted a loss of faecal material since the quantity of water required to remove faeces was such that freeze drying all the water to retain nutrients was impracticable and some had to be discarded. A simple conical fibre-glass collector with a screw-top lid was attached to the bottom of the settlement column. The faeces was collected in plastic bottles that could be removed quickly and easily and dried *in situ*. This improvement was shown to be effective because digestibility values were lower indicating that leaching had been reduced.

A standard sampling regime was developed. *Fish were* fed on the experimental feeds containing a marker (chromic oxide) for at least 7 days and faeces collected from replicated groups. Faecal samples were collected between the evening and morning feeds. A trial in which samples were taken every four hours showed that there was no difference between these samples and a sample collected over the entire period or collected between feeds. The procedure is discussed in Section 6.7 and was used successfully during the project.

Objective 4: To determine the effectiveness of enzyme supplements and feed components in improving the growth and growth efficiency of Atlantic salmon:1

Originally, two components that concerned the use of feed enzymes (proteases, carbohydrases and phytase) with soybean and with cereals were identified. After the first experiment and in consultation with Gibson's Ltd the focus was changed to concentrate on 1. the use of two further plant protein sources from pea and lupin and 2. the effect of using phytase as a feed enzyme.

In the first experiment (Section 6.1) none of the feed enzymes resulted in significant improvements in growth performance when added to diets in which 40 % of the protein was supplied by soybean meal. However, there was an indication that the feeding response of the salmon fed on a diet containing phytase *was greater* than on the other diets. When this diet was fed to satiation the addition of phytase was shown to result in significantly higher growth rates (Section 6.2).

Further experiments were then conducted to investigate the potential of soybean, pea protein concentrate and lupin protein concentrate to replace fish meal protein and on the effect of adding phytase to diets containing only fish meal or a plant protein replacement. The plant

protein meals added to replace 40% of the protein from fish meal resulted in weight gains that were within 10% of the growth achieved by salmon fed fish meal only diets. This showed the potential of each of these meals to be used in salmon feeds. The performance of the three plant meals was further tested by replacing 25 and 33% of the fish meal protein in extruded feeds. Collaboration with C. Foster (Gibson's Ltd) and with T. Evans and V. Gleeson (CSIRO North Ryde) meant that the parameters used to extrude the feeds were controlled in order to ensure the experimental feeds matched the commercial salmon feeds produced in Australia. The growth on each of the plant proteins and at both inclusion levels was the same as the control, fish meal only, diet (Section 6.4). In a final trial, in which salmon were held under commercial conditions of high stocking density and high feed rates, the performance of pea meal and soybean meal in extruded feeds was equal to that of a fish meal only control diet as well as a commercial feed formulation. Pea meal, as a protein concentrate, was shown to have the most potential as a protein replacement for fish meal. Soybean and lupin were also shown to have considerable potential.

Nutritional adaptation was shown to be of great importance in assessing the performance of novel feeds. A diet in which 50% of the protein was replaced with pea meal resulted in decreased feed intake. This was most dramatic on the second day of feeding when it fell to 50% of the intake on the control feed. However, feed intake increased and after 12 days had returned to the previous level. Salmon were shown to adapt to the pea meal based diet and ate more and grew faster than the fish on the control diet. Furthermore, when feed intake was matched between two treatments the salmon that had adapted to a pea meal diet grew faster and more efficiently than salmon switched to the diet for the first time. These results have great significance in the organisation of trials for assessing novel feed ingredients and are discussed in Section 6.9.

Phytase had significant effects on digestibility of major nutrients and on growth (Section 6.3) and when added to an extruded diet containing soybean (33 %) the growth response was greater than that on the control diet and equal to that of a diet containing only fish meal as the protein source (Section 6.4). These experiments demonstrated that phytase improves the performance feeds when fed to salmon. The most important functions of phytase appeared to relate to improvements in phosphorous utilisation and stimulation of appetite so that feed intake increased.

Objective 5: To determine the effectiveness of feed supplements and feed components in improving the growth and growth efficiency of Atlantic salmon:2

The original objectives were to investigate the use of commercially available supplements to improve fat utilisation in high fat feeds (identified as specialist yeast products and biosurfactants) and to investigate the use of ferrous sulphate with cotton seed meal. Cotton seed meal was identified as an unimportant ingredient and was not investigated and the objectives were changed to allow more detailed and more relevant trials described under Objective 4. The potential of readily available additives, lecithin products (Aquagran; Nutrpur S; Lecisoy N-2; Emulbesto 100A; Emulbesto 100E) and products containing betaine (Finnstim; Betafin BCR), which have been linked to improved fat utilisation by fish was investigated.

Growth performance of fast growing salmon of two different sizes was not influenced by the inclusion of Finnstim, at three different concentrations (0.3, 1.0 and 1.5 %). However, there was a suggestion than a low inclusion of Finnstim had an effect since it improved the growth performance of the larger salmon by 10-15%. It should also be noted that the salmon were

maintained in freshwater and the experiment did not aim to investigate smoltification. Lecithin had no significant effect on final body weight or wet weight gain of the smaller salmon.

Objective 6: To establish whether improvements in digestibility due to enzyme and feed supplements are translated into improvements in growth efficiency

Nutrient digestibility was measured as part of experiments in which feed enzymes were tested. Increases in digestibility were clearly demonstrated when phytase was added to diets containing high inclusion levels of plant meals (Sections 6.2, 6.3, 6.4). No differences in digestibility were found when four different enzyme supplements were included in diets containing soybean (Section 6.1) but there were no differences in growth either.

Phytase inclusion was shown to result in an increase in the digestibility of nutrients when used in conjunction with plant meals: phosphorous when soybean and pea meals were included (Section 6.2, 6.3, 6.4); calcium when soybean and pea meals were included (Section 6.3, 6.4); zinc when soybean and pea meals were included (Section 6.3, 6.4). Significant differences in nitrogen digestibility were measured with soybean meal in some experiments (Section 6.4) but not others (Sections 6.2, 6.3).

Differences in digestibility resulted in increased growth and growth efficiency in some experiments (Section 6.2, 6.4) but this was not always the case (Section 6.3). In the latter cases it is proposed that feeding regimes need to be such that increases in the rate of digestion and gastric emptying and consequently the return of appetite are matched by feed availability.

Objective 7: To establish whether in vitro digestibility data can be used to screen and select feed components and suitable combinations of enzyme and feed supplements for inclusion in Atlantic salmon feeds

In vitro digestibility of ingredients, used in making the experimental diets fed during the project, was investigated for dry matter and nitrogen. A multi-enzyme procedure, based on established methods, that used digestive enzymes to digest samples of ingredients and based digestibility on measurements of the insoluble nitrogen and dry matter remaining after 12 hours of digestion or on the change in pH over the first ten minutes of the digestion was followed. Enzyme preparations were either made from commercially available porcine enzymes or from extracts of the pylorus of salmon (Section 6.8).

The ingredient digestibility showed a range of values and values were lower for dry material than for crude protein. There were differences between the different enzyme systems and the wider range of values from the salmon enzyme system suggested greater sensitivity compared to the porcine enzyme system. The pH change assay also provided a range of crude protein digestibility values but these did not correlate with the digestibility values from the multi-enzyme digestion assays. There were significant correlations between *in vivo* and *in vitro* digestibility values for the ingredients. The porcine enzyme system produced stronger correlations with both *in vivo* protein and dry material digestibility.

Correlations between growth efficiency and digestibility were also investigated and showed few significant correlations. Nitrogen digestibility was significantly and negatively correlated with change in wet weight and phosphorus digestibility was significantly and positively correlated with feed conversion efficiency (data from Section 6.3) but no other

significant correlations were found. It was concluded that it is difficult to predict differences in the performance of diets when there are small differences between the digestibility of nutrients in the diets.

Keywords: Atlantic salmon; *Salmo salar*; Digestibility; Feed additives; Feed evaluation; Fish meal replacement; Nutrition; Supplementary enzymes; Voluntary feed intake.

7.2.5

93/120-06	**Replacement of Fish meal in Aquaculture Diets - Feed Processing**

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Objectives:

1. To provide protein enriched fractions from grain legumes for nutritional evaluation.
2. To evaluate plant polysaccharide materials for their nutritional utilisation and role in aquaculture diets.
3. To produce by extrusion processing a range of commercially acceptable feed pellets with nutritional and physical attributes optimised for the selected target species.
4. To evaluate and review the opportunities for the encapsulation of additives into aquaculture diets.

This project related to the Sub-Program by providing the support to the species-based projects (ie for salmon, silver perch, barramundi and monodon prawns) in the following areas:

- the provision of protein-rich fractions from grain legumes,
- the utilisation of plant polysaccharide materials in the diet
- the production of pelleted aquaculture feeds by extrusion processing.

The division of time between these areas was determined by the requirements and interests of the other projects. Ultimately extrusion processing became the major focus of the activities.

Protein-rich fractions from grain legumes

Grain legumes represent broadacre crops of high protein content. Their availability, together with a demonstrated technology for the separation of these grains into protein and starch rich fractions, indicated a potential opportunity for their evaluation as a protein source for replacement of fish meal in aquaculture diets. The separation technology involves a fine milling stage followed by separation of the major fractions with the use of air classification. A milling plant (at Dulwich Hill NSW) with this capability was made available by Goodman Fielder Milling and Baking Group.

Grain legumes were sourced and supplied through this project. Initially a wide range of milled whole and dehulled grains were provided to other projects for preliminary growth trials. From this range four (narrow-leafed lupins, field peas, faba beans and vetch) were used in the preliminary fractionation milling trials. Some of this material was evaluated in further growth trials. However because of the extensive range of other protein materials already being evaluated, further work on grain legumes became restricted to narrow-leafed lupins and Dun-type field peas.

These grains were subjected to further fractionation trials and evaluated in growth and digestibility trials with silver perch and salmon.

It has been demonstrated that protein-rich fractions, with a protein content close to 50% can be readily obtained from many grain legumes, but certainly from lupins and field peas. These protein sources have also been successfully incorporated into aquaculture feeds.

Utilisation of plant polysaccharide materials in the diet

Three of the species in this sub-program, salmon, barramundi and monodon prawns are carnivorous, while the other, silver perch is considered omnivorous. In general carnivorous species have a limited ability to utilise starch and no capacity to digest non-starch polysaccharides. Plant materials which are used as alternative sources of protein to fish meal, will inevitably contain significant amounts of carbohydrate, predominantly in the form of non-starch polysaccharides and starch. Feeding plant materials to aquaculture species will therefore be most efficient when the carbohydrate material is utilised (ie by an omnivore or herbivore). Consequently a study was undertaken in collaboration with the silver perch program to determine the potential of this species to utilise plant carbohydrate. In this study two varieties of lupin, both whole and dehulled were fed incorporated into complete diets. Methods were developed for the measurement of carbohydrates in small amount of carbohydrate material. The results obtained indicated that non-starch polysaccharide of either lupin or cereal origin was not digested, but starch by contrast was well digested

Extrusion processing of ingredients and feeds

Aquaculture feeds are produced with the use of a pellet press or an extruder, either single or twin screw. The choice of equipment and process is determined by a variety of considerations, such as diet composition, sinking rate and stability. For example, slow sinking or floating feeds are produced by extruders as the product is required in an expanded form. For each target species the important characteristics of the feed pellets may be different, as determined by a particular feeding behaviour. To assess the importance of pellet characteristics for the four species in this project, opinion was sought from a survey of feed manufacturers and aquaculture producers throughout Australia. This provided ranked information for pellet characteristics for the four species. Feeds were also obtained from the feed manufacturers for a benchmarking study of the four species and information was requested regarding the methods used for measuring feed pellet quality. Some feeds were also obtained from visits to overseas feed companies. These included Trouw, Ewes and Biomass in the UC and Delta Western in USA (Mississippi). The first three companies are major producers of salmon feed and the latter produces catfish feed. It has been suggested previously that the catfish industry in the USA might serve as a model for the silver perch industry in Australia. During the course of these visits, there were opportunities to visit full scale, pilot and R&D plant, and discuss

issues concerned with production and pellet quality. Since there is little prawn feed produced in Australia samples of overseas product were obtained from some prawn farmers.

Keywords: Fish meal replacement; Aquaculture; Extrusion; Lupins; Diet processing; Nutrition; Protein concentration.

7.2.6

93/120-07	**Fish meal Replacement in Aquaculture Feeds: Amino Acid Supplementation of Aquaculture Feeds - A Technology Audit**

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Objectives:

1 Carry out a technology audit of current and potential amino acid products for use as nutritional additives.

2 Prepare a review article on the results of the technology audit for publication.

3 Report the results of the technology audit, including recommendations on how to proceed, to the FRDC.

Non-Technical Summary

A technology audit of current and potential amino acid products for use as nutritional additives in animal feeds has been completed. Information was obtained on:

- amino acid forms (eg crystalline, encapsulated, etc) used in nutritional studies of land-based intensive livestock and aquaculture industries;
- amino acid products commercially available as nutritional supplements and the cost and manufacturers/suppliers/users of such products; and
- present technologies used for the production of commercial amino acid products and non-commercialised technologies which may have potential in the manufacture of peptidic supplements.

The main producers of amino acid products world-wide are Degussa, Novus International, Rhone-Poulenc, Nippon Soda, Sumitomo Chemical, Nippon Kayaku, Ajinomoto, Kyowa Hakko Kogyo, Asahi Kasai and Miwon. These manufacturers distribute their products in Australia through subsidiary companies or affiliates. Information obtained confirmed that crystalline amino acids and amino acid analogues are the major amino acid forms used in the supplementation of animal diets and are produced by fermentation and enzymatic and

chemical synthesis technologies. The audit did not reveal any commercially available amino acid products that are coated or encapsulated for use in aquaculture diets.

The production and use of peptides as nutritional supplements is the focus of several research institutes and companies and is thought by many animal nutritionists to be the next major area of nutrition research (personal communications; Jerry Weigel, Vice-President Nutrition, ADM). Technologies identified with possible long-term potential for the commercial production of peptidic supplements include: chemical modification of proteins (by incorporation of lysine and or methionine into the protein); chemical synthesis of peptides (eg synthesis of multioligo(L-methionyl) poly-L-lysine); enzymatic modification of proteins (eg transglutaminase or the plastein reaction); enzymatic synthesis of peptides (eg use of peptidases and proteinases in low water environments) and generation of defined peptides by recombinant DNA technology. However, there are presently no peptidic supplements commercially available for nutritional fortification of animal feeds.

In the short term, the results of the audit indicate that crystalline amino acids will be the major form used for the supplementation of aquaculture diets based on cheap protein sources. However, this observation does not diminish the long-term potential of peptidic supplements. The considerable world-wide research focus on the non-chemical synthesis of functional peptides which have immense potential in food and feed applications, will ensure the development of commercial peptide synthesis technologies.

7.2.7

93/120	**Fish meal Replacement in Aquaculture Feeds: *In Vitro* Studies on Feed Ingredients for Aquaculture Species**

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Objectives:

1 To evaluate the differences among the target species in their abilities to digest dietary protein and starch.
2 To evaluate existing and new methods for *in vitro* determination of protein digestibility in potential feed ingredients in the target species, and the effects of processing.
3 To evaluate the *in vitro* digestibilities of various starches in the target species.

Non-Technical Summary

The justification for *in vitro* studies of digestion and digestibility in the four target species was originally included in the applications for the four separate projects on the individual species. Here we have dissected out the rationale and objectives for carrying out *in vitro* laboratory studies as part of these projects.

A comparative study of digestion in the target species

One major aim of the sub-program was to develop knowledge from study of the target species which could be extrapolated to other, as yet uninvestigated, species as they became potential candidates for aquaculture in the future.

This was to be accomplished by a comparative study of the target species, to identify those traits which might be associated with different feeding habits and ways of life. These differences became one of the criteria in the selection of the four target species. The details of those species are listed in the table below.

Species	Characteristics
Lates calcarifer	warmwater, carnivorous, euryhaline finfish
Bidyanus bidyanus	warmwater, omnivorous, freshwater finfish
Salmo salar	coldwater, carnivorous, anadromous finfish
Penaeus monodon	warmwater, omnivorous, marine crustacean

In addition to the four target species, we included the following only for comparative purposes.

Cherax quadricarinatus	warmwater, herbivorous, freshwater crustacean
Oreochromis mossambicus	warmwater, herbivorous, euryhaline finfish

The selected species thus covered carnivorous, omnivorous and herbivorous, warmwater and coldwater, marine and freshwater, and finfish and crustacea. Comparative studies of digestion in these species can show the traits in digestion consistent with different ways of life, allowing extrapolation of existing knowledge to new species.

In vitro assays for nutrient digestibilities

While the assay of nutrient digestibilities is most reliably carried out using *in vivo* tests with live animals, there are a number of drawbacks to such tests. They are very time-consuming, they require expensive facilities and many replicate analyses, and require large numbers of experimental animals. For the screening of digestibilities of many potential feed ingredients, it would be very beneficial to have a rapid laboratory based procedure which could then be used to select the most promising ingredients for further investigation. The nutrients of particular interest are protein and starch.

Quite a few procedures have been described for the *in vitro* assay of protein digestibilities. Some of these have yielded figures which correlate very closely with those measured *in vivo*. The aim of this part of the study was to investigate the applicability of these techniques to the problem of finding replacement ingredients for fish meal in aquaculture feeds, in order to reduce the cost associated with measuring digestibilities in a large number of ingredients in several different species.

The digestibility of starch is known to depend on a number of factors, and in particular the method of processing the feed can cause major changes to the digestibility. It is very beneficial to have a rapid laboratory screening test to identify promising ingredients and the effects of processing. The results from these studies are summarised below:

1. A survey of the digestive capacity of aquaculture species was carried out. This showed that the barramundi and Atlantic salmon were adapted to carnivorous diets, while the silver perch and black tiger prawn were omnivorous feeders. The redclaw crayfish and tilapia, included for comparison, seemed adapted to more herbivorous diets.

2. A search of the literature revealed that many *in vitro* methods for determining digestibility of protein in feed ingredients have been reported.

3. Two published methods were investigated for use in this project. One method had shown very good correlation with *in vivo* digestibilities determined in rats. We found that, although the method was relatively simple to use, our results did not correlate with reported digestibility values in fish. Also, some feed ingredients were difficult to handle in the apparatus. The other method, developed for use in prawns, was selected because of its technical simplicity. However, we were totally unable to duplicate the results reported by the authors.

4. A new method of *in vitro* measurement of protein digestibility was developed, but proved to have a descriptive value in ingredient assessment rather than a quantitative value. This method was capable of showing the course of digestive process, and could detect alterations to feed ingredients such as damage from rendering and changes resulting from feed processing. The method could be quantified by image analysis, but the results were variable and did not agree with tests carried out *in vivo* in fish.

5. The method was shown to be able to detect heat damage in field pea samples, which is the first time that such damage has been demonstrated in a laboratory test.

6. The digestibility of starch was investigated *in vitro*. The ratio of the components in the starch (amylose and amylopectin) had some effect on digestion in most species. Carnivorous species had a limited capacity to digest starch while the omnivorous and herbivorous species could digest it very well. Those species also showed some ability to utilise non-starch polysaccharides from their diet.

7. A possible pathological accumulation of glycogen and fat in the livers of barramundi was identified. The cause or causes of this condition, and its effect on fish health and production, remain to be identified.

Keywords: Fish meal Replacement; Aquaculture; *In Vitro* digestibility; Digestive enzymes; Starch.

7.2.8

93/63	**Dietary requirements and optimal feeding practices for barramundi (*Lates calcarifer*)**

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Objectives:

1. To develop feeding strategies and diets for periods of fast growth (summer) and slow growth (winter) which optimise food conversion and growth rate

2 To investigate the potential for sparing of fish meal in barramundi grow-out diets using synthetic amino acids and cheaper sources of supplementary protein

Non-Technical Summary

Feed is by far the single largest cost component of barramundi farming and accounts for up to 50% of on-farm operating costs. Reducing feeding costs by better tailoring dietary specifications to the nutrient requirements of the fish, by providing these nutrients at least cost and by adopting feeding practices that optimise productivity will greatly assist farm profitability and aid the continued expansion of the barramundi industry. In Australia, barramundi are pond-reared over latitudes from 5°S to 22°S and this results in large seasonal variations in water temperature and consequently, in growth rate of the fish. The research conducted in this project sought primarily to characterise the effect of water temperature on fish productivity and how modification of dietary nutrient specifications and/or feeding practices could assist in improving farming profitability. This entailed research to define the fish's response to changes in dietary supply of critical nutrients over a range of water temperatures. Research examining the efficacy of synthetic amino acids in diets for barramundi was carried out in a supplementary project (FRDC 95/69).

Effect of water temperature on food intake and growth of barramundi

The effects of water temperature, feeding frequency and fish size (weight) on food intake and fish growth were examined in order to define optimal feeding practices for juvenile (_30 to 300 g) barramundi. For each fish size, intake of an extruded dry pellet of acclimatised fish increased essentially linearly as water temperature rose from 20 to 29°C. The 'as fed' chemical composition of the extruded food pellet was: dry matter (DM), 95%; crude protein, 44%; and estimated digestible energy (DE), 15 kJ/g. As a percent of biomass, intake of a 50 g fish ranged from 2.15 to 4.4% at 20 and 29°C respectively whereas the respective values for a 300 g fish were 2.15 and 0.67%. Absolute growth rate also increased linearly with water temperature and size while food conversion (FCR) improved slightly with water temperature and deteriorated slightly with size. Growth rates of 50 g fish ranged from 0.7 to 2.0 g/d at 20

and 29°C, respectively and increased to 2.05 and 4.5 g/d for 300 g fish respectively. Varying the feeding frequency from 1 to 3 times daily increased food intake of small fish (<100 g), but the extra food did not result in significantly better growth rate. It is recommended that fingerlings up to about 100 g should be fed twice daily but thereafter once daily feeding is adequate. For fish above 300 g, skipping a day's feeding at the weekend (a common industry practice) resulted in a commensurate decrease in fish growth.

Dietary protein and energy requirements of juvenile barramundi

The response of barramundi to diets varying in protein concentration over the range of 29 to 57% was examined. The protein used was a high quality mixture of fish meal and casein (with an amino acid profile similar to that of the protein in barramundi) and the DE content of the diet was held constant at 15 kJ/g. Fish were held in recirculated fresh water at 28°C and fed twice daily to satiety. Increasing the amount of protein in the diet significantly improved FCR but food intake showed a pronounced reduction such that the growth of the fish was improved only slightly at dietary protein contents above about 45% (48.5% DM). These results suggest that with a 15 kJ/g DE diet, growth rate and FCR were optimised at a protein:DE ratio of 30 mg/kJ. Reducing the dietary protein:DE ratio from 29 to 25 and 21 mg/kJ caused growth to decline and FCR to worsen with these effects being more pronounced at high (26 or 29°C) than at low (20 or 23°C) water temperature. Increasing the DE content of the diet by 30% (from 14 to 18 kJ/g) resulted in a commensurate improvement in fish growth rate at 29°C and a doubling of growth rate at 20°C, ie, dietary energy was used more efficiently at low than at high water temperature. Moreover, energy utilisation efficiency progressively improved as the DE content of the diet increased from 14 to 16 and 18 kJ/g. The efficiency of protein utilisation also improved with increasing dietary protein concentration up to a maximum response at about 40%. However, in contrast to energy, protein utilisation efficiency was better at high than at low water temperature.

Essential fatty acid requirements

The amount of the critical eicosapentaenoic (EPA) and docosahexaenoic (DHA) n-3 fatty acids required in the diet of barramundi was investigated with fish held at water temperatures of either 20 or 29°C. Fish were fed twice daily to satiety on diets where the EPA+DHA content was varied serially from 0.5 to 2.1%. At low water temperature, the amount of dietary EPA + DHA had no effect on fish response but at high water temperature, fish responded to increasing dietary EPA+DHA much in the same way as for increasing protein. Namely, food intake declined, FCR improved and growth increased slightly, reaching a plateau when the dietary EPA+DHA content exceeded 1.8%. It is recommended that diets for barramundi contain not less than 1.5% of EPA+DHA.

The research has shown that growth rate of barramundi increases linearly with increasing water temperature over the range of 20 to 29°C. For optimal growth rate and food conversion, barramundi held at high water temperature (26 to 29°C) require diets high in protein and energy (>45% protein and protein:DE of 30 mg/kJ) and not less than 1.5% EPA+DHA. At lower water temperatures, barramundi growth is more dependent on the energy than on either the protein or essential fatty acid contents of the diet. High DE diets (>16 kJ/g) are advocated as a management strategy to minimise the slow growth rate of fish held at low water temperatures during winter.

Keywords: Barramundi, Water temperature, Feeding, Nutrient requirements, Protein, Fatty acids, Energy

7.2.9

93/126	**Replacement of Fish meal in Aquaculture Diets: Development of more cost-effective salmon feeds for the Tasmanian Atlantic salmon industry**

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Objectives:

1 To validate a technique for determining apparent digestibility in large Atlantic salmon in seacages.
2 To compare apparent digestibility in seawater Atlantic salmon at four different stages of the production cycle.
3 To determine the effect of feeding regime on apparent digestibility results.
4 To investigate digestibility for currently used commercial feeds.

Non-Technical Summary

This project comprised five separate studies.

(i) *Validation of a faecal collection technique for determining apparent digestibility in large (up to 5 kg) Atlantic salmon in sea cages.*

This study was undertaken to assess whether collection of faeces by a stripping method was suitable for measuring digestibility in large Atlantic salmon in sea cages under commercial conditions. Experiments were conducted to determine the significance of a number of factors associated with variable apparent digestibility coefficients (ADCs) when faeces is collected by stripping. Results of these experiments showed that the stripping method was suitable for collecting faeces from these fish for the purposed of calculating apparent digestibility and a robust and practical procedure which takes account of the factors which can cause major variation in data is suggested.

(ii) *Investigation of within day and between day variation in apparent digestibility, with observations on crude fibre as an internal marker.*

This study was undertaken to investigate whether apparent digestibility results determined using the stripping method validated in Study 1 vary within each day and/or between days so that this variation (if present) may be taken into account in digestibility studies. In addition, efficient mechanisms for overcoming such variation were tested during this study so that modifications could be made to the overall technique as appropriate. Results showed that there is variation in apparent digestibility between days and suggest that variation may also

occur within each day. Modifications based on results from this study are suggested for the technique developed in Study 1 to overcome this variability.

(iii) *Effect of feeding regime on apparent digestibility results*

The effect of four different feeding regimes on apparent digestibility was investigated to determine whether apparent digestibility results derived using one feeding regime can be extrapolated to other feeding regimes and whether the digestibility of the diet may be improved in a commercial situation through manipulation of feeding regime. Short term changes in feeding regime 36 hours prior to faecal collection were also investigated to assess the potential impact that such changes may have on apparent digestibility results. Within the limits of the feeding regimes tested in this study, it appears that apparent digestibility derived using one feeding regime can be extrapolated to other feeding regimes and there appears to be little potential to improve the digestibility of the diet through manipulation of the feeding regime. However, sudden changes in feeding pattern during digestibility experiments in Atlantic salmon in sea cages may affect apparent digestibility results and these factors must be kept minimum during such studies wherever possible.

(iv) *Comparison of apparent digestibility at different stages of the production cycle with further observations on between day variability in apparent digestibility*

The aim of this study was to compare apparent digestibility at 4 different stages of the production cycle in seawater over a 15 month period with each experiment being conducted over 5 weeks. This was done to investigate whether apparent digestibility determined at one stage can be extrapolated to other stages. Within each stage of the production cycle, apparent digestibility was compared on each of 6 different days over a 12 day period to further evaluate between day variation in results. Significant differences in apparent digestibility were evident between days at all stages of the production cycle tested. Results in large salmon (4.2 kg) in summer were highly variable because many fish became anorexic during the experiment, probably due to a combination of the onset of maturation, high water temperatures and increased handling stress associated with larger fish. Comparison of results between different stages of the production cycle in seawater suggest that apparent digestibility data can be extrapolated across stages, although there may be a slight increase in digestibility during winter.

(v) Effect of dietary composition on growth rate; apparent digestibility; relative weights of abdominal components; and histology and electron microscopy of the gastrointestinal tract

The aim of this study was to compare the growth performance, abdominal composition and apparent digestibility of 3 commercially available Atlantic salmon feeds. Histology and electron microscopy of the gastrointestinal tract was performed to determine physiological factors that may have contributed to differences in performance. Growth was highest in fish fed the extruded diet with 30% fat, however, the steam pelleted diet with 17% fat performed better than the extruded diet with the same fat content. This is contrary to expectation and may have been associated with reduced intake of the extruded diet. Apparent digestibility was higher for the extruded diets than for the steam pelleted diet and higher in diets with higher fat content. The weight (expressed as a percentage of body weight) of the proximal intestine (includes pyloric caecae and pyloric fat) was higher in fish fed the extruded diet with 30% fat than in fish fed the steam-pelleted diet with 17% fat, however, there were no other differences in abdominal composition between diets. There were no differences found in the histology or electron microscopy of the gastrointestinal tract between different diets.

7.2.10

95/069	**Replacement of Fish meal in Diets for Barramundi:- Improving Feedstuffs using Synthetic Amino Acids**

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Objectives:

Crystalline amino acid investigations

Primary

1. Determine the efficacy of crystalline amino acids as dietary supplements for barramundi
2. Improve the nutritional quality of fish meal alternatives using crystalline amino acids

Secondary (contingent on crystalline amino acids shown **not** to have high efficacy)

3. Improve the nutritional quality of fish meal alternatives using complementary intact protein sources

Potential of meat meal to replace fish meal investigations

1. Demonstrate on commercial barramundi farms the suitability of meat meal based diets for rearing fingerling fish to market size (400 to 500 g).

2. Using trained taste panels, compare the sensory characteristics of barramundi when reared on diets based on either meat meal or fish meal.

Non-Technical Summary

Australia has an abundant supply of terrestrial animal and vegetable protein feeds which has the potential to at least partly if not fully replace the fish meal presently used in compounded aquaculture diets. A major difference between marine and terrestrial protein sources is the marked difference in the amino acid make up of the protein. Compared to fish meal with an amino acid index of 100, terrestrial plant protein sources are very low in methionine (20 to 80), lysine (20 to 85) and threonine (55 to 85). Terrestrial animal protein sources score higher but the same three essential amino acids are often deficient. An imbalanced essential amino acid profile of the protein markedly reduces the nutritive value of the diet for terrestrial monogastric animals such as pigs and poultry. In these species, crystalline amino acids are a proven and cost-effective way of restoring the dietary amino acid balance. However, the efficacy of crystalline amino acids in aquaculture diets is quite equivocal. A clear understanding of the essential amino acid requirements of barramundi and the extent to which crystalline amino acids can improve the nutritive value of terrestrial protein feed ingredients is pivotal to further diet development for this species.

Crystalline amino acid investigations

The primary objective of this one-year study was to assess the efficacy of crystalline amino acids as supplements in diets for barramundi. Three growth experiments were carried out. The first (E1) examined the effectiveness of crystalline amino acids for restoring the amino acid balance of a low protein, high meat meal containing diet when food intake was controlled to ensure equivalent energy intake. The second (E2) and third (E3) experiments directly compared the efficacy of crystalline amino acids (C-AA) and protein-bound amino acids (Protein-AA) as amino acid supplements under conditions of either high (54%, DM) or low (39%, DM) dietary protein respectively, and when fish were fed once daily to satiety.

In Expt E1, reducing the fish meal content of the high protein (50% DM) control diet by increasing the inclusion content of meat meal until it supplied 60% of the total protein resulted in no significant change in growth rate or food conversion (FCR) of the fish. Reducing the DM protein content of the diet from 50 to 39%, primarily by reducing the meat meal content, caused a 30% reduction in growth rate and a 38% worsening of FCR. Addition of crystalline lysine and methionine to the lowest protein diet, but not lysine alone nor further additions of threonine and arginine, brought about a significant ($P<0.05$), but marginal improvement in growth rate and FCR.

In Expts E2 and E3, a high gluten basal-diet markedly deficient in lysine was incrementally supplemented with either a mixture of C-AA (including lysine) or Protein-AA (as casein) to restore the amino acid profile of the protein to a balance similar to that recommended for channel catfish diets. In Expts E2 and E3, the lysine content of the basal diet was 3.5 and 3.1% of protein, respectively. Thus, the amino acid balance of the dietary protein was similar in each experiment but the absolute concentrations of the amino acids were much higher in E2 compared to E3. In both expts, the amino acid balance of the basal diet was serially improved without altering the dietary protein or energy contents by the addition of either a mixture of C-AA or the isonitrogenous substitution of gluten by casein (Protein-AA). Increasing the amino acid content of the diet in Expt E2 (eg for lysine, from 1.87% to 3.2%), resulted in a significant ($P<0.05$) quadratic improvement in average and specific growth rates and FCR for both the C-AA and protein-AA diet series. The maximum response to amino acid enrichment occurred at a dietary lysine content of about 2.8% (5.2% of protein) for both types of amino acid supplements but the response was slightly better with the protein-AA compared to the C-

AA supplement. However, a statistical difference between the two types of amino acid supplements was observed only for FCR where C-AA was only about 50% as effective as protein-AA in eliciting the response. In Expt E3, growth rate and FCR improved quadratically with increasing amino acid content of the diet (eg for lysine, from 1.19% to 1.8%) as for Expt E2 except that a clear plateau response was not achieved at the highest rate of supplementation. Moreover, no significant difference in efficacy was observed between C-AA and Protein-AA supplements although the former tended to be slightly more effective than the latter, ie an opposite trend to that seen with the high protein diets (E2).

This research supports the following conclusions:

- The response to amino acid enrichment was relatively more marked at low compared to high dietary protein. And at low dietary protein, C-AA were equally as effective as protein-bound amino acids. However, efficacy of crystalline amino acids may be inferior to that of protein-bound amino acids when fish are provided with high protein diets and absolute amino acid intake is high. This effect may be due as much to altered efficiency of energy metabolism as to amino acid supply *per se*.
- The importance of the essential amino acid balance of the dietary protein as a factor influencing fish productivity increases inversely with absolute dietary protein (amino acid) intake. Where there is a critical shortage of an essential amino acid, barramundi will conserve the limiting amino acid, irrespective of whether supplied as free or protein-bound form, and will show the greatest benefit to amino acid enrichment. However, where the absolute deficiency of the essential amino acid is not so critical as was the case with the high protein diets, the response to improved amino acid balance of the dietary protein was small and in this situation, protein-AA was a more effective supplement than C-AA. Further, when the amino acid quality of the dietary protein was the same, the absolute productivity of the fish was much better for high compared to low protein diets which illustrates the extent to which dietary protein is used for meeting the animal's energy requirements.
- Substitution of fish meal by terrestrial protein feeds and the attendant deterioration in the essential amino acid profile of the dietary protein is unlikely to adversely affect fish productivity provided the protein content of the diet is maintained above about 50% (DM) and fish are fed liberally.
- To achieve at least 95% of maximum fish productivity, the essential amino acid requirement of barramundi was calculated to be 4.33, 3.92, 4.87, 2.46, 3.49 and 1.04 % of dietary protein for arginine, isoleucine, lysine, methionine plus cystine, threonine and tryptophan, respectively.

Potential of meat meal to replace fish meal

Two on-farm experiments (Expts F1 and F2) were carried out to compare the growth performance and taste characteristics of juvenile barramundi fed one of four diets, a high fish meal (control) diet, two experimental diets where most or all of the fish meal was replaced by meat meal and a commercial barramundi diet. Both experiments were carried out using caged fish (400 per $2m^2$ cage) in an aerated freshwater pond. The experimental fish were managed as for other fish on the farm, being fed to satiety once daily except on the weekend when fish were fed only on one of the days. A third growth assay in the Walkamin laboratory (Expt L1) was carried out using the same diets as fed in Expt F2 to validate the results of that on-farm experiment. In each of the 10-week on-farm studies, a 4x4 randomised block design was employed. For the 6-week laboratory experiment, the number of replicates was increased to 6 and fish were stocked at 8 fish/tank (180 l). At the conclusion of the two on-farm experiments, fish from each cage were sampled for sensory evaluation.

In Expt F1, diets comprising high inclusions of meat meal (contributing approximately 55% of the dietary protein) enabled barramundi to be farm-reared as successfully as those fed on either the high fish meal control diet or the commercial diet. However, the meat meal diets used in Expt F1 did contain a small amount of fish meal (contributing 15% of the dietary protein) which was included to ensure good palatability of the diets and their ready acceptance by the barramundi. Exclusion of fish meal from the experimental meat meal based diets fed in Expt F2 had no deleterious effect on barramundi productivity with growth rate of the fish being equal, or superior, to both the high fish meal control diet and the commercial barramundi diet. Rank order between diet treatments for all productivity traits was similar for Expts F2 and L1 although there was a heightened differentiation between the diets for food conversion in the laboratory experiment. The food conversion of the farmed fish in Expt F2 was nevertheless very good and only about 15% worse than that achieved by fish in Expt L1 (average across all diets of 1.34 vs 1.13, respectively). Use of a low-ash, 60% protein meat meal conferred no nutritional advantage over that of a conventional 52% protein meat meal when each was included to provide similar protein contributions in diets formulated to be isoenergetic and isonitrogenous. In all of the on-farm and laboratory experiments, the ingredient cost of diets formulated with the 52% protein meat meal was appreciably less than that for all other diets. Moreover, the productivity cost of the diet (expressed as food cost per unit weight increase of fish) for diets containing 52% protein meat meal was from 73 to 84% lower than for diets containing predominantly fish meal. In Expts F2 and L1, increasing the estimated digestible energy content of the diet from 15.0 to 16.2 kJ/g (with a concomitant increase in protein content to maintain a constant protein to energy ratio) caused an almost 10% increase in its ingredient cost. However, this increased ingredient cost was offset by improved fish performance such that the productivity cost of the two meat meal diets was either similar (Expt F2) or less (Expt L1) for the higher energy diet.

Trained taste panels were used to assess the flesh of farm-reared fish from each of the diets fed in Expts F1 and F2. Differences in sensory scores between the diets were few and confined to Expt F1 where fish fed the meat meal based diets had higher scores for "fishy" and "sweet" flavours and "firm" texture than those fed the high fish meal control diet. Importantly, strong and undesirable taints such as "muddy", "weedy" or "metallic" which might otherwise mask more subtle differences in taste of the flesh were only occasionally detected. The strong liking by taste panellists for fish fed the high meat meal content diets indicates that fish meal can be completely replaced in the diet of barramundi without reducing consumer acceptance. However, particular attention was taken in the present work to ensure that all experimental diets were supplemented with sufficient fish oil to satisfy the fish's dietary requirement for highly unsaturated fatty acids.

Conclusions from the research were:

- Diets based on meat meal and containing no fish meal were as palatable to barramundi and supported equivalent or superior fish productivity as those where fish meal was the predominant protein source.
- Fish reared on diets containing high inclusions of meat meal, with or without some fish meal but supplemented with fish oil, was found by trained taste panel assessment to be liked as well or better than fish reared on a diet formulated with a high fish meal content.
- The meat meal based experimental diets were equal to or better than a commercial barramundi diet in supporting fish growth and in producing fish with flesh of high sensory value.
- Using conventional high-ash meat meal as a partial or full replacement of fish meal in nutritionally complete diets resulted in an appreciable reduction in the ingredient cost of

the diet and a 16 to 27% lowering of the ingredient cost of the food per unit fish weight increase.

- Other than for potential environmental benefits, there was no advantage in using low-ash meat meal over that of conventional high-ash product.
- These results demonstrate unequivocally the suitability of meat meal to be used for the partial or complete replacement of fish meal protein in grow-out diets for barramundi.

Keywords: Barramundi, Amino acids, Efficacy, Meat meal, Sensory, Fish meal replacement

8 BENEFITS

Four major benefits were identified: 1) Firstly, the research has lead to better, cheaper diets for aquaculture. This has improved the economic viability of aquaculture, and contributed to containing prices for aquaculture products and improved the chance of replacing some of the 68 000 t of fish and fish products imported annually. 2) Secondly, marketing opportunities for Australian agriculture products have substantially increased, both from an increase in production of aquaculture feeds for the growing Australian industry and as ingredients for aquaculture feeds produced in Asia. The global market for aquaculture feeds is enormous. In Asia, the region where aquaculture is growing most rapidly, the feeds market was estimated at around 2.6 million tonnes in 1990 and this market grew more than four fold between 1986 and 1990 (Akiyama, 1991). There is a great potential to market Australian agriculture products, including oilseed, grain legumes, other cereal crops and animal protein sources like blood meal and meat meal as ingredients in aquaculture diets. Research into cost-effective methods of increasing the value of agricultural products in aquaculture diets, through processing or the addition of enzymes or amino acids is further expected to improve the marketing potential of these products. 3) Thirdly, Australian feed manufacturers have benefited from this research as they are using the results to manufacture better diets. The possibility of selling diets in Asia offers major marketing opportunities for dynamic Australian feed manufacturers. 4) Finally, Australian aquaculture research workers have benefited from close interaction with scientists from other disciplines who have contributed to this research topic.

9 RISK ANALYSIS

In addition to the high cost of fish meal, one of the major reasons aquaculture diets are currently so expensive in Australia is the low demand for these products. Many of the larger feed manufacturers are simply not interested in the currently small Australian aquaculture feed market. Based on production figures for 1990/91 available when the Sub-Program commenced, (Treadwell et al., 1992) and assuming a food conversion efficiency of 2:1 (dry feed: wet weight aquaculture product) the current total feed market for cultured fish and crustaceans in Australia was under 12 500 tonnes per year.

The project considered the risk of feed manufacturers not utilising the research was low because:

1. Reducing feed costs will increase the commercial viability of aquaculture in Australia, stimulate production and hence increase demand for feeds;
2. attempting to solve international problems with aquaculture feeds, such as identifying new ingredients, developing technology to improving the digestibility of ingredients to fish and developing novel protein supplements (to combat deficiencies in essential

amino acids), could give Australian feed manufacturers a competitive advantage in the international, especially Asian, aquaculture feed market and

3. a better understanding of the nutritional requirements of major aquaculture species will improve the ability of Australian feed manufacturing companies to produce superior formulations.

Another identified threat to the project results being adopted was that the technology could be tied up by one feed manufacturing company. To reduce this risk most results were public domain and widely disseminated. Intellectual property agreements and commercialisation strategies will be designed to prevent this occurring.

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10 COLLABORATING INSTITUTIONS

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PROJECTS

93/120-02
Fish meal replacement in aquaculture feeds for prawns (BIARC)

Collaborators: CSIRO Fisheries, Queensland Department of Primary Industries BIARC), Queensland University of Technology, Curtin University of Technology, CSIRO Food Science, CSIRO Meat Research

Total budget 1993/94: $204 030

93/120-03
Fish meal replacement in aquaculture feeds for silver perch

Collaborators: NSW Fisheries, NSW Agriculture, CSIRO Fisheries Queensland, University of Technology, CSIRO Food Science, Queensland Department of Primary Industries (IFIQ)

Total budget 1993/94:(includes Sub-Program administration costs) $150 526

93/120-05
Fish meal replacement in aquaculture feeds for salmon

Collaborators: University of Tasmania at Launceston, Queensland University of Technology, SALTAS, CSIRO Food Science.

Total budget 1993/93: $54 724

93/120-04
Fish meal replacement in aquaculture feeds for barramundi

Collaborators: QDPI (DFAM, BIARC), Queensland University of Technology, University of Queensland, CSIRO Fisheries.

Total budget 1993/94: $57 550

93/120-06
Replacement of fish meal in aquaculture diets - feed processing

Collaborators: CSIRO Food Science, NSW Fisheries, CSIRO Fisheries, Queensland Department of Primary Industries (BIARC), University of Tasmania at Launceston.

Total budget 1993/94: $32 465

93/120-07
Amino acid supplementation of aquaculture feeds - a technology audit

Collaborators: Queensland Department of Primary Industry (IFIQ)

Total budget 1993/94: $6 000

11 APPENDICES

May 1998 *Special Edition* Issue no. 3

Aquaculture Diet Development Sub-Program Newsletter

Welcome to our third issue of the Newsletter. This is a *Special Edition* to inform you of the pending publication of our set of final reports in the *Fishmeal Replacement in Aquaculture Diets Sub-Program*. The three year interactive and collaborative Sub-Program involving twelve institutions, embracing six core projects and three auxiliary projects has produced some excellent results. One of the final reports - *Amino Acid Supplementation of Aquaculture Feeds: A Technology Audit* by Dr Paul Grieve from QDPI, International Food Institute of Queensland, was published and distributed in 1994. As well as the final reports to Fisheries Research and Development Corporation for individual projects, we would also like to inform you of the availability of final reports to the Meat Research Corporation, the Academy of Grain Technology, the *Proceedings of the Analytical Workshop* and the results of a *Lipid Analysis Comparison Trial* which were published during the course of the Sub-Program. Many of you may already have these reports but if you require extra copies, please refer to the order form overleaf.

Printing Costs

In line with a recent change of policy and in common with many government departments and organisations, a nominal fee will be charged for all reports to help cover the ever-increasing cost of photocopying, collating, binding, postage and handling.

Monies received will be paid into the Aquaculture Diet Development SubProgram administration account. Project collaborators and Steering Committee members will of course receive all reports free of charge plus an additional four copies of their own report.

Sub-Program Administration Final Report

One additional Final Report in the *Fishmeal Replacement in Aquaculture Diets Sub-Program* series will be mailed out free of charge - the Sub-Program Administration Final Report which will encompass a general overview of the sub-program and will contain non-technical summaries of individual projects. This will enable you to browse through and identify your particular areas and species of interest before placing an order. It will also allow us to gauge the level of interest in the reports and reduce the risk of surplus numbers being copied and bound.

3rd Scientific Committee Meeting

The 3rd Scientific Committee Meeting was held on 18-19 May at the CSIRO Marine Laboratories, Cleveland, Qld. It was very well attended with good representation from the private sector. Everyone was eager to hear the latest on the three core projects of the sub-program as well as being brought up to date on tuna, abalone and snapper nutrition research. The 18 Month Progress/Milestone Report accompanies this special edition Newsletter and Issue 4 of our Newsletter will follow shortly.

ORDER FORM

Fishmeal Replacement in Aquaculture Diets Sub-Program 93/120 - Final Reports

Project Number	Title	Principal Investigator	Research Organisation	Cost*	No. Required
96/120-01	Fishmeal replacement in aquaculture diets: sub-program administration	Dr Geoff Allan	NSW Fisheries PSRC	Gratis	
93/120-02	Fishmeal replacement in aquaculture feeds for prawns. 167 pp.	Mr David Smith	CSIRO Marine Research	$50	
93/120-03	Fishmeal replacement in aquaculture feeds for silver perch. 305 pp.	Dr Geoff Allan Dr Stuart Rowland	NSW Fisheries PSRC and GRC	$50	
93/120-04	Fishmeal replacement in aquaculture feeds for barramundi. 134 pp.	Dr Kevin Williams	CSIRO Marine Research	$50	
93/120-05	Fishmeal replacement in aquaculture feeds for Atlantic salmon.	Dr Chris Carter	School of Aquaculture, UTAS	$50	
93/120-06	Replacement of fishmeal in aquaculture diets - feed processing. 117 pp.	Dr Tony Evans	CSIRO Food Science	$50	
93/120-07	Amino acid supplementation of aquaculture feeds - a technology audit. 60 pp.	Dr Paul Grieve	QDPI, International Food Institute of Queensland	$50	
93/120	Fishmeal replacement in aquaculture feeds: *in vitro* studies on feed ingredients for aquaculture species. 58 pp.	Dr Alex Anderson	QUT, School of Life Science	$50	
93/126	Development of more cost effective salmon feeds for the Tasmanian Atlantic salmon industry. 55 pp.	Dr S Percival Mr P Lee	SALTAS	$50	
92/63	Dietary requirements and optimal feeding practices for barramundi	Dr Kevin Williams Dr Chris Barlow	CSIRO Marine Research QDPI	$50	
95-069	Replacement of fishmeal in diets for barramundi: improving feedstuffs using synthetic amino acids	Dr Kevin Williams Dr Chris Barlow	CSIRO Marine Research QDPI	$50	
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NSW FISHERIES

Other Reports and Proceedings

Title	Cost*	No. Required
Allan, G.L., 1994. Preliminary evaluation of meatmeal in aquaculture diets for silver perch (*Bidyanus bidyanus*). Final Report to Meat Research Corporation. NSW Fisheries, Port Stephens Research Centre, Taylors Beach, NSW 2316, Australia. (ISBN 0 7310 4893 8).	$20	
Allan, G.L., Frances, J. (Eds.), 1994. Proceedings of the Analytical Techniques Workshop Brisbane 13 April 1994. NSW Fisheries. ISBN 0 7310 3690 5. 102 pp.	$20	
Allan, G.L., Rowland, S.J., 1996. Potential of meatmeal to replace fishmeal in commercial diets for silver perch (*Bidyanus bidyanus*). Final Report to Meat Research Corporation. 90 pp.	$20	
Allan, G.L., 1995. Growth of juvenile silver perch (*Bidyanus bidyanus*) on diets based on modified wheat gluten. Final Report to Academy of Grain Technology and FRDC. ISBN 0 7310 64003. 61 pp.	$20	
Smith, D.M., Barclay, M.C. (Eds.), 1995. Lipid Analysis Comparison Trial. CSIRO, Division of Fisheries. ISBN 0 643 05786 2. 31 pp.	$20	
Smith, D.L., 1995. Preliminary evaluation of meatmeal in aquaculture diets for prawns (*Penaeus monodon*). Final Report Part 1 to Meat Research Corporation. CSIRO Division of Fisheries, Cleveland Qld, Australia.	} $20	
Smith, D., 1996. Evaluation of meatmeal in aquaculture diets for the Giant Tiger prawn (*Penaeus monodon*). Final Report Part 2 to Meat Research Corporation. CSIRO Division of Fisheries, Cleveland Qld, Australia.	Bound together	
Williams, K.C., Barlow, C.G., 1996. Potential of meat meal to replace fishmeal in commercial diets for barramundi (*Lates calcarifer*). Final Report to Meat Research Corporation. 25 pp.	$20	
	Total:	

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To: Ms Helena Heasman, NSW Fisheries, Port Stephens Research Centre, Taylors Beach Road, Taylors Beach NSW 2316. Tel: 02 4982 1232 Fax: 02 4982 1107
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Meat Research Corporation

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AUSTRALIAN CENTRE FOR INTERNATIONAL AGRICULTURAL RESEARCH

MEASUREMENT OF DIGESTIBILITY IN PRAWNS*

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Abstract

A detailed knowledge of the processes involved in ingestion and digestion of food by prawns are required in order to design the appropriate procedures to measure digestibility of nutrients in a diet. The apparent digestibility of a diet or ingredients in a diet can be determined either gravimetrically or by using inert markers in the diet. With both methods a number of assumptions are made which need to be tested to establish the validity of the method. The gravimetric method relies on the ability to measure precisely the mass of food consumed by the prawn and the mass of all faeces produced from that food. The inert marker technique relies on: (a) the marker not being absorbed into the test animal or lost from the faeces, (b) the ratio of nutrient to marker ingested is the same as in the food and (c) the marker and food pass through the digestive system at the same rate.

In this study the apparent digestibility of dry matter and of total nitrogen were determined using the gravimetric method and with two inert markers: chromic oxide and ytterbium acetate. The leaching loss of dry matter, total nitrogen and digestibility markers from diets left undisturbed in seawater for 40 min was determined and used to assess the validity of both methods. Using ^{51}Cr-chromic oxide, it was established that in excess of 96% of ingested chromic oxide was excreted in the faeces. A significant proportion of the remaining radioactivity was likely to have come from contamination of external surfaces of the prawn rather than absorption from the digestive system. The clearance of chromic oxide, ytterbium, dry matter and nitrogen from the gut, and its appearance in the faeces, occurred at the same rate, indicating no partitioning of the tracers over the 3 hourly intervals of collection. When prawns were fed at 6 hourly intervals, the apparent digestibility of nitrogen was about 1% higher that when they were fed at 12 hourly intervals.

In carrying out a digestibility experiment, the homogeneity of distribution of the marker in the diet mixture is of critical importance. Where the apparent digestibility of an ingredient is being measured, the ingredient should be included in the test diet at its maximum practical inclusion level in order to obtain the most accurate estimate of its apparent digestibility. A protocol for carrying out digestibility experiments with prawns is described in the paper.

* Paper presented at the 5th International Working Group on Crustacean Nutrition Symposium. Kagoshima 22-24 April, 1995.

Abstracts of the Fifth International Working Group on Crustacean Nutrition Symposium, 1995
Kagoshima University. Kagoshima, Japan

Nutrition Research with Australian Penaeids

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Penaeid prawn culture in Australia is a relatively new industry and occurs mainly in theeastern states of Queensland and New South Wales. Total annual production has risen slowly over the past 6-8 years to about 1,230 t of *Penaeus monodon*, 50 t of *Penaeus japonicus* and 1 t of *Metapenaeus macleayi*. Other penaeids including *Penaeus esculentus* and *Penaeus plebejus* have been cultured in small quantities.

Research on the composition of natural foods provides a rational basis for formulation of artificial diets. Bivalves, gastropods, ophiuroids, crustaceans and polychaetes, together with algae and detritus are the most abundant food items. Common prey items for *P. esculentus* contained 67.83% protein, 10-21% lipid and 6.22% carbohydrate. Amino acid profiles were similar to prawn muscle, except for isoleucine, which was lower, and there was a high proportion of polyunsaturated fatty acids in the lipid fraction. Broodstock diets for *P. monodon* and *P. semisulcatus* have been formulated based on the biochemical profile of the natural diet during periods of maximum reproduction performance.

Natural foods in ponds can make a significant contribution to prawn nutrition. In ponds, optimum protein contents for *M. macleayi* were 27%, in contrast to studies with *P. plebejus* and *P. esculentus* in tanks where optimum protein contents were between 40 and 47%. Faster growth of *P. esculentus* on a 40% protein diet, compared with 30% and 50% protein diets, was attributed to reduced rates of protein degradation rather than increased protein synthesis.

Research in ponds indicated that optimum feeding rates for *M. macleyi* (average 5% prawn biomass/day) were considerably lower than those estimated for prawns in tanks, presumably because of the contribution from natural food. Further research with *P. monodon* on the interaction of pond preparation and feeding rates to quantify the contribution of natural food will be discussed.

As the foregut of penaeids occupies only about 2-3% of the total body volume, almost constant feeding activity is required for maximum growth. *P. esculentus* filled its foregut about six times a night when food was available and digestion is rapid; abaout 75% of the contents of the foregut can be cleared within 60 minutes. This research and other studies on feeding behavior will be reviewed and the implications for diet development and determining feeding frequencies discussed.

Replacement of expensive, imported aquatic meals is a high priority for aquaculture nutrition research in Australia. A national collaborative research program is underway to evaluate and improve alternative protein sources to fish meal. *P. monodon* is one of the target species for this research. One of the limitations to the use of different feed ingredients has been lack of information on bioenergetics for crustaceans. Recently, improved techniques to determine digestibility in *P. monodon* have been developed. These will be described and results for a range of ingredients presented. Net energy utilization studies have also been used to estimate assimilation efficiencies for different feed ingredients.

Abstracts of the Fifth International Working Group on Crustacean Nutrition Symposium, 1995
Kagoshima University, Kagoshima, Japan

Maintenance Requirement and Nutrient Utilization Efficiency in Juvenile Black Tiger Prawn, *Penaeus monodon*

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The comparative slaughter technique was used to determine the maintenance requirements and the efficiency of utilization of energy and crude protein in two sizes of the juvenile prawn *Penaeus monodon* (average weight 2.99 g and 22.38 g).

Twenty-eight prawns (14 male and 14 female) from each of the two size groups were taken from two separate populations and kept individually in 20 litre tanks in a flow-through aquarium system for 28 days. The prawns were fed a commercial diet at one of seven feeding levels ranging from zero to *ad libitum* based on a percent body weight. The initial gross energy (GE) and crude protein (CP) levels of the whole prawn body for each size group were analyzed in prawns subsampled from the initial populations. At the end of the trial, all prawns were euthanazed and analyzed for GE and CP. Prawns on zero feed were removed from the trial and analyzed on day 12 and this data was extrapolated to 28 days. A regression analysis was carried out between energy retention (ER) and energy intake (EI) and CP reterntion (CPR) and CP intake (CPI).

Regression relationships between ER and EI were ER = -2.9669+0.3103 EI (r^2: 0.79) for small prawns and ER = -4.8472+0.5341 EI (r^2: 0.90). Maintenance requirements (MER) for small and large prawns were estimated as 340 J/gW/d (0.08 kJ/kg$W^{0.75}$/d) and 279 J/gW/d (0.11 kJ/kg$W^{0.75}$/d) respectively. There was no significant difference between MER. Efficiency of use of absorbed energy of small (0.34) and large (0.59) prawns were significantly different ($P<0.001$).

Regression relationships between CPR and CPI were CPR = -0.08618 + 0.48355 CPI (r^2: 0.78) and CPR = -0.13971 + 0.71517 CPI (r^2: 0.85) for small and large prawns, respectively. Estimated maintenance requirements (MR) were 0.007 gCP/gW/d for small and 0.006 gCP/gW/d for large prawns. The efficiency of use of absorbed CP was 0.52 and 0.77 for small and large prawns. MR for small and large prawns was not significantly different. Lower efficiency of use in small prawns could be due to possible limitation in essential amino acids and/or preference of using dietary protein as an energy source.

The MR of both GE and CP do not appear to change with the size of prawn. The estimated ad libitum intake level for the large prawns only satisfied MR. The intake range for the large prawns was more limited than the small prawns. Direct comparison of efficiency of utilization values is therefore not possible.

Fishmeal replacement using grains: Present and future

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Abstract

Aquaculture production throughout the world is increasing rapidly and industries are increasingly reliant on formulated diets which currently contain large amounts of fishmeal. However, global fishmeal production is declining and very little is produced in Australia. Australia is fortunate in having large quantities of agricultural protein sources, including grains, with potential for use in domestic and international aquaculture feed manufacturing industries.

To evaluate the potential to use more grains in aquaculture diets, the biochemical composition and the digestibility of grains, and the effects of feeds containing these grains on the growth of target aquaculture species need to be determined. Methods to achieve this are described. Increasing the use of grains in aquaculture feeds can be limited by the high carbohydrate (including fibre) content of grains, low protein quality, presence of anti-nutritional factors and contamination by mycotoxins. Methods of improving the nutritive value of grains include selective breeding programs, removal of carbohydrate through dehulling and protein concentrating, and the use of synthetic amino acids and enzymes.

A national research program to coordinate research on fishmeal replacement, aimed at developing cost-effective diets for aquaculture species, is described.

Introduction

Although total aquaculture production of crustaceans and finfish in Australia is low by international standards, culture of a number of species is increasing. In 1993/94, production of penaeid prawns reached 1 549 t, salmon (*Salmo salar*) 4 200 t (5 200 t estimated for 1994/95), barramundi 370 t, tuna 1 275 t and freshwater crayfish 357 t. In addition, several new industries are emerging, including for silver perch culture, and several new species are being investigated including greenback flounder, stripey trumpeter, golden snapper, mangrove jack, snapper and mulloway. Total production of crustaceans and finfish is around 10 406 t per year (ABARE, 1994; pers. comm. grower association representatives and State Fisheries Department officials).

Aquaculture feed usage in Australia may be estimated from production. All the species mentioned above require feeding with the exception of freshwater crayfish which are sometimes cultured extensively. Tuna are currently fed frozen whole fish

Processing Technologies for the production of grain-based meals suitable for fishmeal substitution.

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CSIRO Division of Food Science and Technology[a]
Goodman Fielder Milling[b]

ABSTRACT

The opportunities and limitations for the use of grains in aquaculture feeds is considered. Components of grains which are likely to be least utilised by most farmed species in Australia are the major carbohydrate materials, starch and non-starch polysaccharides (NSP). Carnivorous fish are very limited in their ability to utilise carbohydrate and hydrolyse NSP. Alternatively omnivorous, and particularly herbivorous species, have a greater potential to utilise and digest carbohydrate materials. In herbivorous fish NSP is fermented to short chain fatty acids in a similar manner to that occurring in terrestrial hindgut fermenting herbivores. The two principal strategies for the use of grain for aquaculture species is either to target herbivorous/omnivorous species or to reduce the level of polysaccharide in the grain by a low cost fractionation process. Incorporation of a high level of grain material into aquaculture feeds, without the adoption of either approach could have adverse consequences for environmental and water quality.

INTRODUCTION

Grains do not unlikely to occur in the natural diets of aquaculture species being farmed in Australia. The concern with incorporating these in the diet of such species is therefore that they may lack necessary nutrients, contain antinutrients or may be poorly digested. To some extent, the first problem, where it is identified, can be addressed by nutrient supplementation. With regard to antinutrients, the best solution is to select a raw material which is free of antinutrients, but where this unavoidable, a processing treatment may be used to reduce antinutrient activity. The problems associated with reduced digestibility are less likely to be so readily solved. However previous aquaculture research, and also the experience of the pet food industry indicates that some aquatic and terrestrial carnivores can adapt to diets containing a significant content of grain material. The major and specific problem for the aquaculture industry, is that where feed components are poorly digested this leads to the increased release of waste materials into the environment, with adverse effects on water quality.

The bulk of poorly digestible material in grains is likely to be polysaccharide in origin, ie starch and cell wall materials. The polysaccharides content of grains can therefore be a major drawback to their greater use in aquaculture diets. This paper addresses the nature of this problem, and considers alternative solutions to an increased utilisation of this commodity in aquaculture feeds.

THE NEED FOR THE PROCESSING OF GRAINS TO REMOVE POLYSACCHARIDES OR INCREASE THEIR UTILISATION

OOCYTE GROWTH AND VITELLOGENIN LEVELS IN GROUPER (*Cephalopholia pachycentron*)

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A study was conducted to investigate the relationship between oocyte development and plasma vitellogenin levels in brown-coral cod (*Cephalopholia pachycentron*). Six to twelve wild brown-coral cod were caught monthly for one year from the sea near Sri-Chang Island, Thailand. A total of 107 fish were studied for gonadosomatic index (GSI) and two peaks were found. One peak appeared during April to May and another peak during August to November with a GSI of about 1. In 70 plasma samples vitellogenin levels measured by ELISA technique were undetectable up to 4 µg/ml and 4 - 32 µg/ml when oocytes were at stages 1 - 3 and 4 - 6, respectively. The results suggest that plasma vitellogenin might be useful as a tool for determining the developmental stage of growing oocytes.

KEYWORDS: Marine fish culture; ELISA; Grouper culture; *Cephalopholia pachycentro*

EFFECT OF FAECAL COLLECTION METHOD ON THE APPARENT DIGESTIBILITY OF DIETS FOR ASIAN SEA BASS, *Lates calcarifer* (BLOCH)

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Information on the apparent digestibility of alternative feedstuffs is critical for the development of cost-effective aquaculture diets. However, loss of faecal nutrients to the water is a possibility which could result in erroneous estimates of digestibility. An experiment was conducted to determine the apparent digestibility (AD) of five diets using three methods of faecal collection. The diets were a basal (B) and four test diets in which either Danish fishmeal (DF), tuna fishmeal (TF), microwaved full-fat soybean meal (FS) or poultry offal meal (PO) was substituted for 30% of B. The chemical composition of the diets is given in Table 1. The three faecal recovery methods were: sedimentation and siphon collection after contact with water for periods not exceeding either three hours (S-3); or 16 hours (S-16); and by intestinal resection with contents collected from the terminal 50 - 80 mm of the small intestine (IR). AD was determined using titanium dioxide as an indigestible marker.

Fish were equally distributed to 20 tanks (250 L) arranged as a flow-through system in which filtered (≈25 µm) and heated (27 ± 0.5°C) seawater (33 ppt) was exchanged at a daily rate of 700%. The experiment was repeated in time using 80 fish (mean weight ± SD) of 627 ± 54.3 g and 40 fish of 699 ± 76.4 g, respectively.

Fish were acclimatised to the conditions for 14 days after which S-3 and S-16 collections were taken daily for a consecutive 7 d period. At the conclusion of these collections, fish were anaesthetised and IR samples collected. Data were analysed as a 5 x 3 randomised block ANOVA. The effects of collection method and diet composition on AD are shown in Table 2.

Table 1 Dry matter chemical composition of the diets

Analysis	Diet				
	B	B+DF	B+TF	B+FS	B+PO
Moisture %	8.7	8.7	8.1	8.8	8.4
Protein %	51.1	53.5	52.8	48.2	52.5
Fat %	14.8	9.9	9.9	12.8	11.0
Energy (kJ/g)	22.56	22.42	20.95	22.87	22.95

Table 2 Effect of collection method and diet composition on apparent digestibility (AD)

AD (%)	Collection method				Diet					
	S-3	S-16	IR	±sem	B	B+DF	B+TF	B+FS	B+PO	±sem
Protein	84.0^{B}	88.5^{A}	77.2^{C}	0.96	87.6^{X}	87.9^{X}	89.5^{X}	79.4^{Y}	71.8^{Z}	1.24
Fat	80.9^{C}	85.8^{B}	93.7^{A}	0.92	87.8^{Y}	89.8^{XY}	80.9^{Z}	92.7^{X}	82.7^{Z}	1.20
Energy	75.5^{A}	76.6^{A}	73.3^{A}	1.20	73.8^{YZ}	83.3^{X}	72.1^{YZ}	76.2^{Y}	70.4^{Z}	1.55

$^{A,B,C;\ X,Y,Z}$ - Within rows and main effects, means without a common letter differ ($P<0.05$).

Increasing the faecal water contact time from 3 to 16 h caused digestibility estimates to be elevated ($P<0.05$) for protein and fat but energy was not affected ($P > 0.05$). IR collection resulted in the lowest and highest estimates for protein and fat respectively ($P < 0.05$). These results imply that soluble nitrogenous nutrients readily leach from the faeces, leading to protein and fat digestibilities being over- and under-estimated respectively. The study showed FS and PO to have low protein digestibility but that the digestibility of FS lipid was high and equal to, or better than all other meals examined.

KEYWORDS: Marine fish culture; Artificial diets; Seabass; *Lates calicifer*

EVALUATION OF MEATMEAL AS A REPLACEMENT FOR FISHMEAL IN DIETS FOR THE OMNIVOROUS, FRESHWATER SILVER PERCH, *Bidyanus bidyanus*

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More than 470,000 tonnes of meat meal are produced in Australia each year and are relatively inexpensive sources of protein. In this study, we determined the digestibility of four meatmeals fed to juvenile silver perch at two inclusion levels. We then measured growth and performance of fish fed diets where a mixture of two of the meatmeals were used to replace different amounts of fishmeal. Diets for digestibility determinations contained one of four meals; lamb meal (LM), beef meal (BM), a mixed species, low ash meal (MM) and Provine® (P), a high protein, low ash meal. Nine diets were formulated: the reference diet of Allan and Rowland (1992; Austasia Aquaculture, 6: 39-40) and eight others each of which contained one of the meatmeals at either 15 or 30% with the remainder of the diet being the reference diet. Chromic oxide at 1% inclusion was used as an inert marker. Eight 6 g fish were stocked into each of 27 tanks, three replicate tanks for each diet. Faeces were collected over 16 hours each day for 15 days from 160 l cylindroconical tanks fitted with a collection chamber which tapered into a 150 mm length of a 12 mm diameter silicon tubing. Faeces were chilled to < 5°C during collection. For each tank faeces were pooled over time. Methods of Cho and Kaushik (1990; World Rev. Nutr. Diet, 61: 132-172) were used to determine coefficients.

Digestibility coefficients for ingredients were analysed in a two-factor ANOVA with ingredient type and inclusion both considered fixed. Inclusion level and the interaction were not significant ($P > 0.05$). Apparent digestibility coefficients (ADC's) ($n = 6$) for BM, LM, MM and P were: dry matter - 42.6^a, 54.9^b, 75.7^c and 88.9^d; energy - 72.9^a, 81.5^b, 85.1^b and 95.2^c; protein - 66.5^a, 69.4^a, 82.7^b and 83.6^b respectively (different letters in superscripts indicate means are significantly different $P > 0.05$; Student Newman-Keuls test). Results indicated digestibility of meatmeals was high and increased when protein content was elevated through the removal of ash (bone).

Five diets were formulated for the growth experiment; the reference diet with 27% fishmeal, three others with 13%, 6% or 0% fishmeal with the rest replaced by a mixture of Provine® and lamb meal (for these L-lysine, DL-methione and L-threonine were added if necessary to adjust these amino acids to levels in the reference diet) and a fifth diet with no fishmeal and no added synthetic amino acids (a.a). Eighty-five 12 g fish were stocked into each of fifteen, 10,000 l tanks, three replicates for each diet. Fish were grown at 24 - 27°C for 65 days.

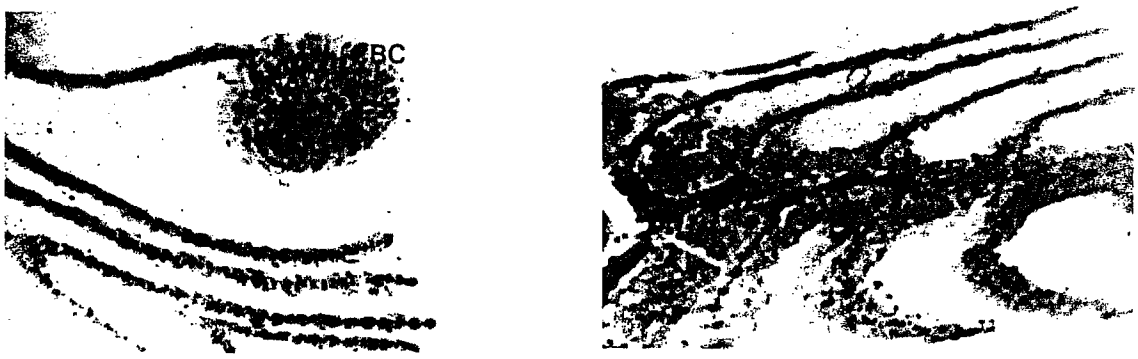
Diet (% fishmeal)	Weight gain[1] (g/fish)	FCR[1]
Ref (27)	60.4 ± 2.3^a	1.48 ± 0.01^a
2 (13)	60.2 ± 1.0^a	1.44 ± 0.02^a
3 (6)	53.9 ± 2.3^{ab}	1.46 ± 0.02^a
4 (0)	52.3 ± 1.8^b	1.50 ± 0.02^a
5 (0 - no a.a)	50.0 ± 1.5^b	1.47 ± 0.02^a

[1] Values are means ± se. For each column, means with a common letter in the superscript are not significantly different (P>0.05; Student Newman-Keuls test)

Weight gain and Food Conversion Ratio (FCR) (for diets with 92% dry matter) were analysed using single-factor ANOVA. Performance data (see below) support results from the digestibility experiment that meat products are excellent protein sources for silver perch.

It was reported that mucus secretions, clavate lamellae and blood vessel congestion are some of the defensive responses of fish to irritants (Mallat, 1985) which are manifestations of the direct effect of toxicants (Temnik et al., 1983; Abel, 1976).Our results have shown that the milkfish in the study area are exposed and affected by toxicants.

Figure 1: Photomicrograph of milkfish gills exhibiting aberrant structures.



The Office of Research Coordination (ORC), U.P. Diliman and the Natural Sciences Research Institute (NSRI) funded and housed the project.

KEYWORDS: Gill histology; Environmental contaminants

NUTRITIONAL IMPLICATIONS OF DIGESTIVE ENZYME ACTIVITY IN SILVER PERCH (*Bidyanus bidyanus*)

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The silver perch (*Bidyanus bidyanus* (Mitchell) (Teraponidae) is a native freshwater Australian fish with considerable potential for aquaculture. We have carried out a study of the digestive enzyme activity in this species, an omnivore, compared with that in two carnivores (barramundi ,*Lates calcarifer,* and Atlantic salmon,*Salmo salar*) and a herbivore (a tilapia, *Oreochromis mossambicus*). Previous work has suggested that the digestive enzymes present in fish vary according to their different feeding habits as carnivores, omnivores and herbivores.

As shown in Table 1, silver perch possesses higher levels of gastric protease than the herbivorous tilapia, but considerably lower than that in the carnivorous barramundi. Salmon and barramundi differ as to the relative activities of gastric and intestinal protease, while protein digestion in tilapia appears to be relatively

more important in the intestine than in the stomach. Using specific substrates, the enzymes trypsin, chymotrypsin and elastase were identified in the intestinal extract of silver perch. Amylase activity in silver perch was intermediate between the very low levels in the carnivores and the high level in the herbivore *O. mossambicus* (Table 1). Chitinase and lysozyme were not detected in any of these species. Of the disaccharides maltose, sucrose, lactose and cellobiose, the only one digested was maltose.

Table 1. Protease and amylase activities in fish gut extracts.

Species	Stomach protease	Intestinal protease	Intestinal Amylase
B. bidyanus	3.9	1.9	6.5
L. calcarifer	7.1	0.7	0.9
S. salar	2.5	8.6	0.4
O. mossambicus	0.6	2.4	16.6

Our results agree with the postulate that digestive enzymes reflect feeding habits in fish. They also indicate that silver perch have a higher capacity to utilise dietary starch than do fish with more carnivorous habits, therefore practical diets for this species may include higher levels of starch.

KEYWORDS: Fish nutrition; Silver perch; *Bidyanus bidyanus*

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Inclusion of Whole and Dehulled Lupin Diets for the Black Tiger Prawn (*Penaeus monodon*)

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Fish meal is a major component of the feed formulated for most farmed aquatic animals. Demand for fish as a food source for human and farmed animals has been increasing continuously. This situation forces the aquafeed industry to look for more readily available substitutes which will provide similar or better economic benefit to the aquaculture industry. One of the alternative groups of feed ingredients is legumes which consists of high protein seeds such as soybeans, faba beans and lupins. The present experiment examined the growth rates of prawns with diets based on substitution of fish meal with various inclusion levels of whole or dehulled lupin.

The experiment consisted of three groups of diets. In the first group, a fish meal based basal diet was formulated and later diluted with cellulose at 10%, 30% and 40% levels. The second and the third groups had 7 diets each with inclusion of either whole or dehulled lupin (instead of cellulose) at a level of 10%, 20%, 30%, 40%, 50%, 60% and 70%. The experimental design was 19 x 2 randomised block. Six prawns (average body weight 2.5 g) were placed in each 250 litre tank. Prawns were acclimatised to experimental conditions for a week during which the maximum feed intake was established. During the trial, prawns were fed at a restricted feeding level (5% body weight). Feeding level was adjusted every week based on the weekly growth of prawns.

The best growth (0.1 g/prawn/day) and FCR (1.7) were obtained with prawns fed the basal diet. The difference in daily growth was not significant ($P>0.05$) between the basal diet and diets with inclusion of 10% cellulose and 10% and 20% of either whole or hulled lupin. No significant differences were found in FCR between the basal diet and inclusion of cellulose (up to 30%), whole lupin (up to 40%) and hulled lupin (up to 50%). As the basal diet was diluted with cellulose, a linear reduction in daily growth was observed ($P<0.05$; $r^2=0.99$). A similar result was obtained with whole lupin ($P<0.05$; $r^2=0.96$). However, the slope of the basal diet was significantly different from the slopes of both lupin diets. Results indicate a possible deficiency of some essential amino acids such as methionine and lysine in lupin seeds. Without the essential amino acid supplementation up to 20% of whole or dehulled lupins could be included in prawn diets.

Abstract

The use of marine ingredients in aquafeeds has increased rapidly over the last decade as aquaculture production and the use of formulated feeds has increased. Unfortunately, the availability of marine ingredients such as fishmeal, trash fish and fish oil, is declining. A large range of alternative ingredients are available although in general these are of lower nutritional value than marine ingredients. Potential substitutes or partial substitutes include grains, such as oilseeds, grain legumes and cereals (or cereal by-products), and animal by-products such as blood meal, poultry meals and meat and bone meals. The composition and price will have a major influence of the potential use of all ingredients in aquafeeds. Other factors influencing their use include consistency of composition and availability. Systematic evaluation is critical if maximum use is to be made of these ingredients. Digestibility, needs to be determined for target species and then restrictions on the use of ingredients determined. Constraints to replacing marine ingredients with alternatives include the presence of anti-nutritional factors, deficiencies in essential nutrients and excessive levels of less digestible components such as carbohydrates, fibre and ash. Poor processing and contamination can also reduce the nutritional value of some ingredients. Plant breeding programs have successfully improved the value of some ingredients. New or improved processing techniques, eg dehulling, concentration of protein by removing carbohydrate fractions and removal of bone, also increase the value of some agricultural ingredients. Data on the effects of some of these techniques on digestibility of some grains and meat and bone meals fed to an omnivorous species, silver perch, are presented. A coordinated research program on fishmeal replacement in aquafeeds in Australia and Thailand is discussed.

Introduction

Demand for seafood is escalating as global population rises and the popularity of seafood increases (Anon, 1994; Liao, 1996). Production from capture fisheries is declining and aquaculture offers the only chance to meet this demand. Aquaculture production has risen from 15% of total fisheries production in 1989 to 22% in 1993 (Liao, 1996).

A shift to more intensive culture practices, made possible by the availability of better, formulated diets, has been partly responsible for the increase in aquaculture production. From 1986 to 1990, Akiyama (1991) estimated that demand for aquafeeds in Asia increased more than four-fold and New and Csavas (1993) predicted the Asian aquafeed market would reach 2.6 million tonnes by the year 2000. The number of feed mills in Asia increased from 5 in 1985 (producing
27 000 t) to 53 in 1988 (producing 264 000 t) (Boonyaratpalin and Akiyama, 1989). Most species, however, are still cultured extensively using farm-made aquafeeds and, although declining, this is still expected to be the predominant method of culture in the year 2000 (New et al., 1993).

Regardless of whether feeds are commercially manufactured or farm-made, marine based ingredients, especially fishmeal and trash-fish, are highly sought after as they provide high levels of essential amino and fatty acids, are low in carbohydrates and are well digested and utilised. However, production of fishmeal already uses approximately 30% of the total global fish catch and the proportion of fishmeal used for aquaculture is predicted to rise to between 25-30% within the next decade (Anon, 1996). In the same period, the proportion of fish oil used for aquaculture is expected to reach 30-50% of total production (Anon, 1996). As about 4 kg wet fish is needed to produce 1 kg fishmeal, if diets contain more than 17% fishmeal and/or the food conversion ratio exceeds 1.5:1, the aquaculture operation entails a net loss of fish protein.

In this paper, ingredients with the potential to replace fishmeal and other marine ingredients will be reviewed and constraints to their use discussed. Methods for evaluating these ingredients and increasing their use will be examined.

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