Strategic planning, project development and facilitation of nutrition research and extension across all Australian aquaculture sectors

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Australian Government

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2001/251: Strategic planning, project development and facilitation of nutrition research and extension across all Australian aquaculture sectors

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2001/251: Strategic planning, project development and facilitation of nutrition research and extension across all Australian aquaculture sectors

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OBJECTIVES:

- 1 Establish what the fundamental non species specific nutritional constraints are to aquaculture production (eg. diet form, nutritional requirements, feeding strategies, feed evaluation or a combination of several factors) and then establish and manage a collaborative core research program to address this.
- 2 Develop a risk management strategy for all aquaculture industries in relation to nutrition to assist the research priority setting process.
- 3 Forge an agreement between the FRDC and other relevant RDC's and research providers for the management of research relating to aquaculture nutrition under the auspices of an aquaculture nutrition subprogram accounting for variations in priorities between agencies.
- 4 Define and facilitate a mechanism for lead agencies for the commercialisation of all relevant nutrition research arising from FRDC funded projects.
- 5 Provide a single point of contact for aquaculture nutrition research in Australia and improve the aquaculture nutrition skills base in Australia.
- 6 Facilitate the functions of an aquaculture nutrition "Expert Working Group" to ensure ongoing research programs have a high degree of industry relevance and focus.
- 7 Improve communication of nutrition research outcomes and current nutrition research between scientists, aquaculturists, ingredient suppliers-and feed manufacturers.

NON TECHNICAL SUMMARY:

The Aquaculture Nutrition Subprogram was established to maintain communication and momentum in aquaculture nutrition research in Australia, and to ensure focussed investment in this research discipline and optimum return on research investment. The Subprogram was successful in establishing an Expert Working Group used for the evaluation of research projects and strategic research priorities, a strategic plan for 2002-2007, a number of short courses, workshops and conference sessions, and the basis for a more standardised and relevant approach to aquaculture nutrition research through a methodology manual.

OUTCOMES ACHIEVED

- The Aquaculture Nutrition Subprogram utilised an Expert Working Group and a variety of industry stakeholders to develop Strategic Directions for 2002-2007. This established fundamental non species specific nutritional constraints are to aquaculture production in Australia.
- Communication between aquaculture nutrition researchers and industry was maintained through the delivery of annual workshops and short courses.
- Additional investment was attracted from the Grains Research and Development Corporation to facilitate a more coordinated approach to aquaculture nutrition research across research agencies.
- The Expert Working Group reviewed methodology associated with aquaculture nutrition research and developed the basis for a "methodology manual" which will be further developed, published and distributed.
- The Aquaculture Nutrition Subprogram is now recognised as a single point of contact for aquaculture nutrition research and advice in Australia.

KEYWORDS: aquaculture, nutrition

BACKGROUND

Why is aquaculture nutrition research important?

Aquaculture nutrition research is a fundamental component in the establishment of new aquaculture industries and in the further development of existing aquaculture industries for the following reasons:

- 1. Feed costs represent a significant proportion (30-50%) of the production costs in most aquaculture systems. The most effective way to reduce feed costs is to improve the feed conversion efficiency of the target species which can only be accomplished by improving definition of feed ingredients, defining the nutritional requirements for different production phases, improving diet form and feed stability and increasing the range of feed ingredients available for use all of which require significant research inputs.
- 2. Development of cost-effective manufactured feeds for aquaculture species is a difficult process because the research has to be conducted underwater. This makes collection of information on fundamental parameters such as feed intake difficult. In addition, most aquaculture diets needs to be extruded which is a research discipline in itself and requires highly specialised equipment.
- 3. Many aquaculture diets have a heavy reliance on fresh fish, fish meals and fish oils. To provide long term stability to new and established aquaculture industries and to reduce the reliance on these ingredients, there is a need to identify alternative nutrient sources that facilitate maintenance of similar production levels and product quality.
- 4. Improved nutrition of aquaculture species is one of the most effective ways to reduce any environmental impacts from aquaculture by reducing nutrient loads in effluent.
- 5. Some aquaculture sectors, such as prawns, still rely heavily on imported feeds due to a lack of alternatives in Australia.
- 6. Given the large number of unknowns often associated with the nutrition of new aquaculture species and the difficulties associated with conducting the research, significant improvements in the efficiency of conducting this research can be achieved by developing a coordinated approach to the definition of research priorities and research methods, such as that achieved within a managed subprogram.

Protecting investment in aquaculture nutrition research

There is a significant investment in aquaculture nutrition research in Australia. Since 1990, FRDC has funded more than 50 projects that focus on nutrition or have components of research that focus on nutrition. In addition, a range of species specific FRDC Subprograms have, or have had, nutrition research as a high priority within their research portfolio including the Abalone, Rock Lobster Enhancement and Aquaculture and Southern Bluefin Tuna Aquaculture subprograms. Current investment in nutrition research projects by the FIRDC, the AQUAFIN CRC and research providers will exceed \$AUD5 million over the next 3 years.

To date FRDC has invested in two nutrition specific subprograms - the Fishmeal Replacement (FMR) and Aquaculture Diet Development (ADD) Subprograms. The FMR and ADD Subprograms were successful in meeting the objectives of managed subprograms on the basis that they:

Promoted a high level of collaboration between scientists working within a common discipline; Successfully delivered nutrition research expertise to infant aquaculture industries that otherwise would have not had access to this level of nutritional skill; Reduced the level of duplication of research effort towards a common goal; Applied outcomes were delivered to industry improving the profitability and viability of these industries; Facilitated a coordinated delivery of research funding submissions and research reports to the FRDC. Advanced the overall international knowledge base for aquaculture nutrition.

Why maintain a subprogram approach to generic nutrition research?

The smaller aquaculture industries benefiting from research conducted within the FMR and ADD Subprograms (ie. barramundi, salmon, prawns, silver perch) have suggested they would value the continuation of further coordinated research in the area of aquaculture nutrition. Industries such as the barramundi industry rate nutrition as the highest research priority above all other disciplines, and believe that nutrition research on behalf of their industry would be severely compromised in the absence of a nutrition specific subprogram. Research providers and researchers operating within the FMR and ADD Subprograms valued their involvement as the Subprograms, through the Subprogram Leader, managed to breakdown many institutional boundaries that previously existed. The Subprogram workshops also represented a valuable form of peer review for research results. Research providers and researchers conducting nutrition research projects within species-based subprograms found the FIVIR and ADD to be a valuable resource. Workshops conducted as part of these subprograms provided an outlet for related research results and a valuable forum for critical review of the research. Many researchers operating outside the FIVIR and ADD used these subprograms as their reporting vehicles to FRDC.

All individuals involved with, or benefiting from, the FMR and ADD Subprograms were highly complimentary of the efforts of the Subprogram Leader, Dr Geoff Allan. His efforts were largely responsible for the initiation of the subprogram, initiation of collaborations between research groups, involvement of other funding agencies and promotion of the results. While all parties, without exception, would be keen to see Dr Allan continue as the leader of any future subprogram, he has come to a mutual agreement with FRDC to no longer continue in this role. He has, however, indicated that he will lend his full support to the new Subprogram Leader, and is keen to maintain an active involvement in any future Subprogram.

The future role of an aquaculture nutrition subprogram

The infrastructure (ie collaborations, industry contacts, mailing lists etc) developed as part of the FIVIR and ADD Subprograms represents a valuable resource to the Australian aquaculture industries, and at a minimum, this resource should be maintained.

In 1999 the FRDC commissioned the BECAN Consulting Group to undertake a review of both the FMR and ADD Subprograms. The primary objective of this review was:

"To provide a recommendation to the Fisheries Research and Development Corporation Board on a potential basis and structure of an Aquaculture Diet Development (or similar) Subprogram considering research completed to date (within the Fishmeal Replacement Subprogram and the Aquaculture Diet development Subprogram) and nutrition research being completed as part of other FRDC research programs".

Despite a strong recommendation to maintain an aquaculture nutrition subprogram in some form, a number of areas were identified that could be improved in the future, including:

- While the Subprograms were successful in capturing funding from a variety of sources, there is scope for a future subprogram to take a lead role in the coordination of aquaculture nutrition research on behalf of other research investors. Other research investors have indicated that if FRDC and an aquaculture nutrition subprogram initiated liaisons, they would be keen to capitalise on the existing infrastructure and in many cases would request that FRDC manage research funds for aquaculture nutrition projects on their behalf.
- Submission of aquaculture nutrition projects to other funding agencies after they have been rejected by the Subprogram and/or the FRDC Board is a cause for concern by other research and development corporations. Agencies other than FRDC are not as well equipped to scrutinise aquaculture nutrition priorities and are keen to establish mechanism that ensures a coordinated approach to priority setting and informed decision making.
- End-user industries felt the FIVIR Subprogram had a highly defined research goal that was best addressed through a collaborative research program. This common goal was diluted during the establishment of the ADD Subprogram, and the funding and Subprogram momentum was accordingly

diminished. The establishment of the ADD Subprogram suffered because of a lack of well defined research priorities. In contrast, researchers felt the ADD Subprogram was a substantial improvement over the FIVIR Subprogram in terms of research direction.

- A major limitation identified by end-users of technology developed as part of the Subprograms was the process for commercial adoption of the research.
- While Scientific Committee meetings were valuable for the review of research completed to date, they were not used successfully to critically evaluate research prior to commencement, and they were not an effective means of delivering results to the primary beneficiaries of the research (ie the aquaculture industries). In addition, progress reports to research investors cannot be distributed to a wider audience. Outcomes from scientific meetings need to summarised and distributed to participants exclusive of progress reports.
- Despite some review during Scientific Committee meetings, some research providers were not sufficiently responsive to critical comments of their research approach. Over time, this diminished the value of some research results. Despite a significant planning process for many of the experiments, further changes could have improved the standard of the work.
- The process for the establishment of core species within the FMR Subprogram was adequate at the time of establishment of the subprogram, but did attract criticism from other groups.
- Many issues remain unresolved in relation to the most appropriate methods for the distribution of intellectual property arising from research conducted within the Subprograms, or the protection of intellectual property during the conduct of the research. A number of end-users expressed concern over the handling of IP and this should represent a key area for attention in any future subprogram.

The objective of this proposal is to address many of the issues described above by developing an aquaculture nutrition subprogram that can service not only emerging aquaculture industries, but research programs within existing species-based subprograms.

The Australian aquaculture industries have a heavy reliance on imported nutrition technologies, feeds and ingredients for the supply of nutrients to target species. This includes feed manufacturing technologies, ingredients such as bait fish, fish meals, crustacean meals and fish oils, and complete feeds such as those utilised by the prawn industry. Not only does this create issues in relation to imported disease risks, continuity of supply and cost, but it means that many local products are being under utilised. Given the broad range of issues associated with the provision of aquaculture diets, the real cost of aquaculture diets in Australia will only be reduced through a coordinated research approach such as that proposed within the Aquaculture Nutrition Subprogram. The infancy of the Australian aquaculture industries also means that a coordinated research approach is required to maximise the outcomes from research investment in the area

Many aquaculture nutrition research projects within the FRDC portfolio are currently managed within species based subprograms. Obviously the focus of these subprograms is the target aquaculture species rather than nutrition, and hence the nutrition research is undertaken in a variety of ways, often with no consideration for nutrition research being undertaken elsewhere. To ensure all aquaculture nutrition research is being conducted to the highest standard and to ensure the most efficient use of limited resources, a subprogram dedicated to nutrition is required to maintain linkages across species and to provide additional resources that will ensure existing projects have the best chance of delivering their defined outcomes.

Before it has even been commissioned, the Australasian Experimental Stockfeed Extrusion Centre has been approached by more than 15 groups consisting of aquaculturists, feed manufacturers and researchers seeking assistance with the development and manufacture of aquatic diets. This emphasises the strong need for inputs into aquaculture diet development for both new and existing aquaculture industries.

Emerging aquaculture industries in Australia generally have a requirement for small tonnages of feed. The specifications for the feed are often poorly defined, and research and development is required to establish an appropriate form for the diet. In many cases, it is not cost effective for feed manufacturers to develop new diets for emerging industries, and hence assistance is required from elsewhere if these aquaculture systems are to progress to a point where commercial feed manufacture is possible. In the past, the FIVIR and ADD Subprograms have proved to be an invaluable resource to the smaller aquaculture industries for the above reasons, and an Aquaculture Nutrition Subprogram is required to ensure this resource is available in the future.

- 1 Establish what the fundamental non species specific nutritional constraints are to aquaculture production (eg. diet form, nutritional requirements, feeding strategies, feed evaluation or a combination of several factors) and then establish and manage a collaborative core research program to address this.
- 2 Develop a risk management strategy for all aquaculture industries in relation to nutrition to assist the research priority setting process.
- 3 Forge an agreement between the FRDC and other relevant RDC's and research providers for the management of research relating to aquaculture nutrition under the auspices of an aquaculture nutrition subprogram accounting for variations in priorities between agencies.
- 4 Define and facilitate a mechanism for lead agencies for the commercialisation of all relevant nutrition research arising from FRDC funded projects.
- 5 Provide a single point of contact for aquaculture nutrition research in Australia and improve the aquaculture nutrition skills base in Australia.
- 6 Facilitate the functions of an aquaculture nutrition "Expert Working Group" to ensure ongoing research programs have a high degree of industry relevance and focus.
- 7 Improve communication of nutrition research outcomes and current nutrition research between scientists, aquaculturists, ingredient suppliers-and feed manufacturers.

GENERAL METHODS

Establishment of a recognised "expert working group"

A core function of the Aquaculture Nutrition Subprogram was the facilitation of an Expert Working Group that undertook the following:

- 1. Developed a 5 year research and development plan for generic Australian aquaculture nutrition research priorities.
- 2. When requested by FRDC, scrutinised nutrition research planned within the species based subprograms.
- 3. Provided advice to the FRDC Board and other Australian research and development investors on aquaculture nutrition research priorities.
- 4. Acted as an identifiable point of contact as an expert working group on nutrition for all Australian stakeholders in aquaculture.
- 5. Provided industry feedback and views.
- 6. Reviewed existing nutrition research based on contractual obligations when requested by FRDC.
- 7. Ensured nutrition research outcomes are commercially focused.
- 8. Identified those research providers and researchers best equipped to address defined research priorities.
- 9. Commissioned tendered research to address defined priorities.
- 10. Defined an appropriate basis for the distribution of intellectual property on a case by case basis.
- 11. Facilitated extension and technology transfer of nutrition research outcomes.

The Expert Working Group met face to face once per year in conjunction with the annual workshop. Teleconferences were convened as required during the year. Membership of the Expert Working Group was skills based, and included scientists, feed manufacturers, aquaculturists, and others as appropriate. This project supported a membership of 8-10.

Strategic planning and establishment of a core project base

Based on the inputs of the Expert Working Group, consultation with the species-based subprograms and consultation with industry, a strategic plan was developed for the Subprogram to address aquaculture nutrition research issues that are common to all species.

Based on the strategic plan, the Subprogram commissioned a number of core research projects as appropriate and manages these projects on behalf of FRDC in a similar way to species-based subprograms. If a research project existed that is consistent with the strategic plan for the Subprogram, a recommendation was made to FRDC to make it a core subprogram project. New research proposals were developed through the use of facilitated strategic planning meetings. Using priorities published in the Aquaculture Nutrition Subprogram Strategic Plan, the Subprogram Leader convened meetings with relevant researchers and research institutions to:

- 1. Define the planned outcomes of the new proposal;
- 2. Manage an indicative budget for the research;
- 3. Identify which researchers/institutions are best placed to undertake the research;
- 4. Promote collaboration between researchers and institutions where appropriate;

- 5. Seek external expertise and inputs as required.
- 6. Ensure the new proposal meets the objectives of the subprogram and that the research remains relevant and focused.

The Subprogram Leader ensured new research proposals were distributed to FRABS and the Expert Working Group for comment and ratification before submitting the proposals to FRDC on behalf of the lead agencies, or facilitating adjustments to the proposals prior to submission.

In the first instance, core projects incorporated a funding component to facilitate travel to Subprogram events, and eventually to support a Subprogram Steering Committee.

Development of formal relationships between FRDC and other investors

The Subprogram forged an agreement between the FRDC and other relevant RDC's (such as ACIAR and GRDC) and research providers for the management of research relating to aquaculture nutrition under the auspices of the Aquaculture Nutrition Subprogram. This process accounted for variation in priorities between agencies. This mechanism was to provide information to other RDC's rather than making decisions on their behalf.

Delivery of an annual aquaculture nutrition workshop

A core service of the Aquaculture Nutrition Subprogram was the organisation and delivery of an annual aquaculture nutrition workshop. This workshop provided a forum for the delivery of research results from all FRDC funded aquaculture nutrition projects. Where possible, invited speakers were utilised to address issues relevant to nutrition research. The workshops also provided an opportunity for feed manufacturers and end users to present their views in relation to aquaculture nutrition research priorities.

Presentations at the workshop were based on research outcomes rather than as a review progress within individual projects.

Industry consultation and communication

The Subprogram Leader promoted the activities of the Aquaculture Nutrition Subprogram through a website, industry newsletters, and direct communication with industry organisations and representatives.

Communication with FRABs

Communication with FRAB's was via distribution of an annual operating plan for the Aquaculture Nutrition Subprogram in December of each year combined with direct communications. The Subprogram Leader also attended the annual FRDC FRAB workshop to promote the activities and objectives of the aquaculture nutrition subprogram.

RESULTS/DISCUSSION

Establish what the fundamental non species specific nutritional constraints are to aquaculture production (eg. diet form, nutritional requirements, feeding strategies, feed evaluation or a combination of several factors) and then establish and manage a collaborative core research program to address this.

Through an extensive consultation process and a series of annual workshops, the Aquaculture Nutrition Subprogram developed a strategic directions document for use in the development of research programs aimed at addressing fundamental non species specific nutritional constraints to aquaculture production. Key elements of the strategic directions document for 2002-2007 are as follows:

Vision

Efficient, innovative and sustainable delivery of nutrients to aquaculture production systems to optimise aquatic animal health and production and aquaculture product quality while minimising impacts on the aquatic environment.

Introduction

Aquaculture nutrition research is a fundamental component in the establishment of new aquaculture industries and in the further development of existing aquaculture industries for the following reasons:

- 1. Feed costs represent a significant proportion (30-50%) of the production costs in most aquaculture systems. The most effective way to reduce feed costs is to improve the feed conversion efficiency of the target species which can only be accomplished by improving definition of feed ingredients, defining the nutritional requirements for different production phases, improving diet form and feed stability and increasing the range of feed ingredients available for use all of which require significant research inputs.
- 2. Development of cost-effective manufactured feeds for aquaculture species is a difficult process because the research has to be conducted underwater. This makes collection of information on fundamental parameters such as feed intake difficult. In addition, most aquaculture diets need to be extruded which is a research discipline in itself and requires highly specialised equipment.
- 3. Many aquaculture diets have a heavy reliance on fresh fish, fish meals and fish oils. To provide long term stability to new and established aquaculture industries and to reduce the reliance on these ingredients, there is a need to identify alternative nutrient sources that facilitate maintenance of similar production levels and product quality.
- 4. Improved nutrition of aquaculture species is one of the most effective ways to reduce any environmental impacts from aquaculture by reducing nutrient loads in effluent.
- 5. Some aquaculture sectors, such as prawns, still rely heavily on imported feeds due to a lack of alternatives in Australia.

This summary of strategic directions outlines the key nutrition-related issues facing the Australian aquaculture industries, identifies the common nutritional challenges facing these industries and forms the basis of an innovative core aquaculture nutrition research and training program.

Strategic directions for aquaculture nutrition research in Australia is a source of information for:

- Australian aquaculture industries;
- Feed manufacturers and suppliers, suppliers of feed ingredients used in aquatic feeds and suppliers of feed manufacturing equipment;
- Investors in research and development either directly involved with aquaculture, aquatic feeds or components of aquatic feeds;

- Research providers and research organisations that contribute, or can contribute to a broad aquaculture nutrition research and development program.
- Local, State and Federal governments and their agencies associated with the management of resources used by the aquaculture sector;
- Private and public organisations interested in becoming involved in the Australian aquaculture sectors.

Aquaculture Nutrition Subprogram

The Fisheries Research and Development Corporation established the Aquaculture Nutrition Subprogram (ANS) in September, 2001. Given that a wide scope of nutrition research already exists within the FRDC portfolio, as well as a range of other research investors, the ANS aims to take nutrition research into a higher risk area that is not limited by industry development restrictions and is beyond individual species based projects.

Subprogram Mission

To enhance the Australian aquaculture nutrition expertise base and to utilise combined knowledge to identify and solve common aquaculture nutrition challenges and limitations to development of the Australian aquaculture industries.

Role of the Aquaculture Nutrition Subprogram

• Develop and implement a core research program based on fundamental, non species-specific nutritional constraints to aquaculture production. In developing this core research program, the ANS will develop a risk management strategy for all aquaculture industries in relation to nutrition to assist the research priority setting process.

In developing a core research program, the ANS applies five criteria against potential areas of core research and innovation including:

- 1. Must be of national significance/benefit.
- 2. Must have multi-species significance.
- 3. Must require multiple knowledge inputs to achieve an outcome.
- 4. Must be limiting the development and/or viability of aquaculture industries.
- 5. Must not duplicate existing research efforts.
- Establish basic research standards and preferred methodologies for nutrition research.
- Facilitate technology transfer in the field of aquaculture nutrition.
- Develop and provide training for research investors, research providers, and end-users associated with aquaculture nutrition.
- Act as a point of contact for aquaculture nutrition in Australia.
- Develop links between research investors, research providers and end-users associated with aquaculture nutrition.

The Aquaculture Nutrition Subprogram will utilise the following to fulfil its roles:

- 1. A Subprogram Leader with the combined skills and knowledge to identify research priorities, implement effective research programs, develop effective communication and deliver outcomes to the Australian aquaculture sectors.
- 2. Expert working groups established as and when required to address specific aquaculture nutrition related research and development priorities. Expert working groups will capture inputs from research investors, research providers, aquaculture industries, and feed manufacture and supply industries, but will have a strong skill base.
- 3. Peer review and independent scientific advisers to ensure the objectives of the Subprogram are being met and to ensure that research implemented through the ANS is to the highest possible standard.

4. An annual workshop aimed at all those interested in aquaculture nutrition in Australia and overseas to collect information relating to research and development priorities and to disseminate outcomes from the conduct of any nutrition research and development undertaken within Australia.

Core Research and Development

A range of aquaculture nutrition research programs are underway within the following aquaculture sectors (some with established Subprograms):

- Southern Bluefin Tuna
- Abalone
- Prawns
- Rock Lobster
- Snapper
- Salmon
- Barramundi
- Other (Silver Perch, Yellowtail Kingfish, Sea Urchin, King George Whiting, Crabs).

A collation of aquaculture nutrition research and development issues faced by these aquaculture sectors together with inputs from related sectors (facilitated through the first annual ANS workshop held in February, 2002) is summarised as follows (in no specific order):

FEED EVALUATION

- Poor capacity to accurately measure feed intake (*All*).
- Capacity to manipulate dietary amino acid supply without utilising crystalline amino acids (*Prawns, Abalone*).
- Influence of manufactured aquatic feeds on aquaculture product quality and food safety hazards (All).
- Influence of nutrition on aquatic animal health (Abalone, Salmon, Tuna, Snapper, Rock Lobster).
- Basal larval, broodstock and production diets for existing and emerging species (*All*).

NUTRITIONAL REQUIREMENTS

- Application of inappropriate research methodologies in some cases resulting in inaccurate or misleading results (particularly in the definition of nutritional requirements) (*Prawns, Tuna, Abalone*).
- Poor capacity to accurately measure feed intake (*All*).
- Influence of feed form, feeding strategies and feeding regime on the surrounding aquatic environment (*All*).
- Alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils (*All*).
- Suitable larval, broodstock and production diets for existing and emerging species (*All*).

FEED PROCESSING

- Basal larval, broodstock and production diets for existing and emerging species (All).
- Fat stability in extruded pellets (*Salmon*).
- Capacity to manufacture extruded pellets with a diameter less than 1.5 mm (*All*).
- Effects of mechanical energy inputs during diet manufacture on water stability (All).
- Influence of feed production processes on micro-ingredient losses and stability (All).
- Starch and protein losses through degradation during feed processing (All).
- Interaction between ingredient source and type and extrusion processing parameters (*All*).
- Production of shelf stable semi-moist pellets (*Tuna, Sea urchins*).

An Expert Working Group was convened by the Aquaculture Nutrition Subprogram to identify additional nutritional constraints to aquaculture production in Australia. Aquaculture nutrition constraints and/or requirements in addition to those listed above include:

- Routine evaluation of feed ingredients suitable for use in aquatic diets.
- Availability/reliability of energetic and other models for use in managing feeding systems and aquatic animal responses.
- Assessment of feed attractants.
- Strategies for feed delivery to aquaculture systems.
- Techniques to facilitate rapid weaning of aquaculture species onto manufactured diets.
- Feed formulation approaches to meet both the nutritional needs of the target species and the manufacturing requirements.
- Nutriceuticals (eg. probiotics, enzymes) suitable for use in aquaculture feeds.
- Availability of nutrition research infrastructure and the nutrition research capacity of research providers in and out of Australia.

Based on the five criteria defined for core research and development projects suitable for inclusion within the auspices of the Aquaculture Nutrition Subprogram (ie national significance/benefit, multi-species significance, multiple knowledge inputs required, limiting development and/or viability of aquaculture industries, no duplication of existing research efforts) the collation of research and development issues can be reduced and grouped into the following categories:

NUTRITION RESEARCH TOOLS

- Capacity to accurately measure feed intake in aquaculture species.
- Development of energetic and production models for simulating the responses of aquaculture species to nutritional inputs.
- Availability of nutrition research infrastructure and the nutrition research capacity of research providers in and out of Australia.

AQUATIC FEED PROCESSING

- Basal larval, broodstock and production diets for existing and emerging species.
- Interaction between ingredient source and type and extrusion processing parameters.

FEED EVALUATION

• Alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils.

NUTRITION AND AQUATIC ANIMAL HEALTH

- Interaction between nutrition and aquatic animal health.
- Nutritional prevention of disease and maintenance of animal health.

NUTRITION AND THE ENVIRONMENT

• Influence of feed form, feeding strategies and feeding regime on the surrounding aquatic environment.

NUTRITION AND PRODUCT QUALITY

• Influence of manufactured aquatic feeds on aquaculture product quality and food safety hazards.

Short and Long Term Research Needs

From the above list of ten priority research areas, three can be declared as having an urgent research requirement:

1. Alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils.

A clear need has been identified for the development of a vegetable protein isolate available for use in Australian aquafeeds as an alternative to fishmeal. Critical to achieving this goal is the involvement of industry with the technical expertise to produce such an isolate, end-users of both mid-and high protein components of the isolate and any additional co-products, other research investors, and research providers.

Lupins, particularly new cultivars such as *L. luteus*, hold the greatest potential for the production of a high protein isolate in Australia. Research and development is required to identify the most appropriate process for the production of such an isolate, the efficiency of such a process, the need to identify multiple end-users for the graded isolates, and the suitability of such an isolate for aquaculture. This process holds potential to produce a range of additional co-products such as non-starch polysaccharide isolates, isoflavones and oils that would require further investigation by multiple end-users.

The Grains Research and Development Corporation have also expressed an interest in this research area and could work together with the Aquaculture Nutrition Subprogram to establish some research partnerships in this area.

2. Interaction between nutrition and aquatic animal health.

The Abalone Aquaculture Subprogram and the Rock Lobster Enhancement and Aquaculture Subprogram have both identified nutrition and aquatic animal health as research priorities. In the abalone sector there is a need to establish the role of nutrition in "summer bloat" and research is underway in this area. In rock lobsters, there is a need to develop modes of larval feeding that allow some form of microbial control in propagation systems.

With the above in mind, in feed additives such as probiotics, enzymes, and the role and mode of action of products such as mannan oligosaccharides may prove useful for new and existing aquaculture industries.

3. Basal larval, broodstock and production diets for existing and emerging species.

A number of commercial and public sector research projects involving King George Whiting, rock lobsters, organic barramundi, and sea urchins (to name a few) have recently been undertaken and have experienced difficulties in developing and obtaining extruded basal diets in small quantities, that can be used for subsequent development. In some cases, the inability to produce these products has impeded the progress of existing research projects. An urgent need exists to establish basal extruded diets for use in the development of emerging aquaculture industries across Australia.

All other identified priorities form part of the longer term strategy for the ANS, but can emerge as research projects from the outset subjecting to research investment constraints.

Communication and Training

TRAINING

In keeping with the core objective of improving nutrition research capacity of research providers in and out of Australia, the ANS proposes to offer user-pays training packages that will proceed based on demand. In the first instance, training is proposed in the following areas:

- Practical extrusion of research diets and diets for emerging species (this course will be run in conjunction with and as an extension to the existing "Feed and Food Extrusion Course" which will be relocated to South Australia in 2003);
- Practical nutrition short course for aquaculture producers;
- Feed microscopy for aquaculture;
- Capacity for nutritional control of nutrient outputs from aquaculture systems for policy and planning agencies.

Expressions of interest were sought from both those interested in developing the above courses, and those interested in participating in the above courses. Once course structures were established, and the associated costs of delivery defined, interest was assessed and the course proceeded if there were sufficient numbers to cover the course costs.

ONGOING COMMUNICATION COMMITMENTS

The Australasian Livestock Feed Ingredient (ALFI) database was developed to compile a large amount of information on the chemical composition and nutritional quality of feed grains for pigs, poultry (broilers, layers) and aquaculture species, and can be used by researchers, members of the grains industries, feed manufacturers and plant breeders as a reference for diet formulation, variety comparison and quality control in trading.

Data for pigs, poultry and aquaculture species has been entered into ALFID which now contains more than 22807 sample entries. The ALFI database also incorporates all information contained with the GRDC GRAILE database. The initial beta version has also been reprogrammed so that it is now more user friendly, and run-time versions have been prepared for distribution via CD-ROM. A web-site (www.alfidbase.com) has also been established and a domain name registered to facilitate e-mail submissions for ALFI information, and to promote the benefits of the database. Promotional brochures and presentation formats for the software has been suggested and commercialisation is pending approval from the respective stakeholders in the project (which includes the Fisheries Research and Development Corporation).

A long term objective of the ANS is to continue to provide information arising from research projects associated with the evaluation of feedstuffs for use in aquaculture systems in to the ALFI database and to ensure that the database remains relevant to the aquaculture sector. On-going investment into the development of the ALFI database is being sought from the private and commercial sector.

In addition to the above strategic plan, Annual Operating Plans were presented annually to stakeholders and FRABs through direct mail out and the ANS website. Copies of the Annual Operating Plans are contained in Appendix VI.

Develop a risk management strategy for all aquaculture industries in relation to nutrition to assist the research priority setting process.

- The Subprogram Leader was involved in all FRDC aquaculture subprograms and contributed to the development of their strategic plans in relation to nutrition.
- Direct inputs were provided to private companies during the development of in house strategic plans relating to nutrition, and as part of trouble-shooting processes where nutrition was identified as a potential bottleneck to production efficiency. Specific examples include Pelican Point Aquaculture Pty Ltd and Select Nutrition Pty Ltd.
- With the exception of SBT, most aquaculture sectors view nutrition as a major limitation to production efficiency, but not necessarily a bottleneck to industry development. As a consequence, the level of impact of this objective was minimal.

Forge an agreement between the FRDC and other relevant RDC's and research providers for the management of research relating to aquaculture nutrition under the auspices of an aquaculture nutrition subprogram accounting for variations in priorities between agencies.

After negotiations consistent with this project objective, the Grains Research and Development Corporation invested in this project (\$10,000 per annum) with a view to using the Subprogram as a mechanism for:

- Information on relevant aquaculture nutrition priorities;
- Assessment of aquaculture nutrition-related projects submitted to them for funding;
- Participation in the Expert Working Group, and
- Pursuit of joint initiatives.

GRDC maintained this investment in the subsequent the subsequent Aquaculture Nutrition Subprogram project 2004/235.

Define and facilitate a mechanism for lead agencies for the commercialisation of all relevant nutrition research arising from FRDC funded projects.

During the term of this project, there were no nutrition research outcomes that resulted in commercialisation opportunities. While many outcomes did arise, they were contained within the public sector and utilised without the need for commercialisation negotiations.

It also became clear when developing a mechanism under the terms of this objective that no single approach can be considered appropriate for commercialisation of research outcomes and each must be considered on a case by case basis. It is clear, however, that involvement of potential commercialisation partners at the outset of the project is a far more efficient way to facilitate the commercialisation process than approaching them when the project is complete.

Provide a single point of contact for aquaculture nutrition research in Australia and improve the aquaculture nutrition skills base in Australia.

- The Subprogram Leader provided specific advice to a wide range of private and public proponents of the Australian aquaculture industry during the course of this project. The website generated a large number of requests for information that was provided directly by the Subprogram Leader or delegated to a more appropriate expert in the field.
- The single point of contact concept was successful and the role of the ANS as a provider of information expanded considerably in the subsequent ANS project 2004/235

Facilitate the functions of an aquaculture nutrition "Expert Working Group" to ensure ongoing research programs have a high degree of industry relevance and focus.

An Expert Working Group was established and comprised the following members:

- Robert van Barneveld (Chair)
- Chris Carter (University of Tasmania)
- Craig Foster (Skretting Australia)
- David Smith (CSIRO)
- Brett Glencross (Department of Fisheries, WA)
- Geoff Allan (NSW Fisheries)
- Kevin Williams (CSIRO)
- Mike Taverner (Grains Research and Development Corporation)
- Meegan Vandepeer (PIRSA-SARDI)

The Expert Working Group met on an as required basis, usually in conjunction with an annual workshop. Some of the outcomes from meetings of the Expert Working Group included:

Defining the role(s) of the Aquaculture Nutrition Subprogram

The role of the Aquaculture Nutrition Subprogram was defined based on the FRDC Board's interest in taking research to a high risk area not limited by industry development restrictions. With this in mind the ANS was to take research into a high risk area that is not limited by industry developments restrictions and to go beyond species based projects.

The Roles of the ANS were to include:

- 1 Definition of research standards and methods
- 2 Identification of core research areas and areas of innovation
- 3 Technology transfer
- 4 Act as a linkage and point of contact for aquaculture nutrition in Australia

With the definition of the role of the ANS the following mission statement was drafted for the Subprogram:

- 1 To improve the expertise base
- 2 To combine knowledge to solve common problems

Defining the composition of the Expert Working Group and its functions

It was deemed that the Expert Working Group would strive to be open and flexible in its composition and approach. It would not become an exclusive club with membership restricted to certain individuals. The flexibility in the Expert Working Group meetings composition would see each meeting assembled of groups that represent the task at hand thus ensuring that the roles and functions of the ANS will be upheld.

Assessment of research priorities

The Expert Working Group addressed a range of research priorities associated with aquaculture nutrition in Australia. The following is a list of issues that were raised by various members with those highlighted being those considered as the ones that were most relevant to the ANS, its role and its objectives:

- a) Ingredient evaluation
- b) Feed intake / performance measurement

- c) Fish oil replacement
- d) Modelling tools / requirements / response
- e) Feed attractants / Controlled Release
- f) Feed safety
- g) Regulation of gene expression
- h) Nutrition and environment
- i) Larval feeds species specific
- j) Sources of energy
- k) Nutrition and health
- 1) Processing and ingredient interactions
- m) Production / Manufacturing / Milling (pre-treatment and post pellet)
- n) Feed delivery
- o) Feed strategies
- p) Weaning
- q) Water stability / microencapsulation
- r) Nutrition and genetics
- s) <u>Neutriceuticals</u>
- t) Nutrition research tools
- u) Vitamin and mineral requirements
- v) Nutrition research facilities / capacity

This priority setting process formed the basis of the ANS Strategic Plan for 2002-2007.

Another key outcome from the facilitation of the Expert Working Group was the progression of a methodology workshop that took the place of the third annual ANS workshop in August 2003. The workshop addressed key issues surrounding aquaculture nutrition research methodology, with the outcomes currently being expanded and documented. This document will be presented as a manual for use by the aquaculture sector with the milestone for this outcome now contained with FRDC Project 2004/235. A summary of the issues addressed at this workshop is as follows:

Nutrition and Diet Development for New Aquaculture Species

Task: Define an approach to the development of basal/reference diets for new and emerging aquaculture species.

Summary:

- To ensure that nutrition is not limiting new industry development.
- To limit the amount of research required to establish a reference diet.
- To apply existing knowledge to progress nutritional development.

General considerations:

What are you trying to achieve with nutrition ?

- Identify production objectives eg. Product quality enhancement, growth, reproductive capacity.
- Is a manufactured diet required, a supplement or can natural feeds be utilised ?
- Set some initial performance targets (< = > known natural performance).
- What is a reasonable allocation of resources to this production system.
- What is the primary driver to sustainability feed costs or other system requirements.

Priority is to identify a reference diet and feeding strategy to promote acceptable levels of intake

• Identify existing aquaculture diets/nutritional knowledge that have been shown to be suitable for similar or related species. Once a reference diet has been identified, the capacity exists to fine tune this diet for the target species.

- Assess the cost-benefit of conducting more specific nutrition research for that particular species (eg. ingredient evaluation, nutrient requirements). This may be achieved by undertaking a growth study where you feed diets that vary significantly in nutrient content to assess your capacity to measure a response, and a desktop study to assess the impact of diet cost changes/performance changes on overall enterprise productivity and profitability
- Define trophic feeding habit of the animal (carnivorous, omnivorous, herbivorous, pelagic, benthic) to identify potential feed ingredients and feed forms. Examine anatomy, enzymology and digestive microbiology of the target species. May provide some information, but will only be a guide.
- Examine feeding management regimes and the influence on intake (eg. influence of feeding frequency, time of feeding, light regimes, environmental management, effluent management).

Other considerations:

Attractants:

It is unlikely that an individual attractant will promote superior levels of feed intake or feeding intensity in the absence of good feeding management and sound nutrition. It is suggested that investment in assessment of attractants is less likely to yield results than investment in feeding management practices and optimizing the composition of manufactured feeds.

Detailed requirements studies etc.

It is difficult to justify detailed research of this nature in the early stages of industry development given the potential interactions between feeding management and nutritional outcomes and long term relevance of the outcomes.

Basis For Use Of Surrogates In Nutrition Research

Task: Address the role of surrogates in aquaculture nutrition research.

Summary:

- When is surrogate use appropriate?
- Is there a basis for surrogate selection.
- How do we ensure the selected surrogate produces relevant research outputs.

General considerations:

It is recognized that information derived with surrogates can only be considered as an estimate/model of the target species performance until detailed comparisons between the two can be made (if ever).

If nutritional knowledge is to be advanced, the need for experimental rigor often exceeds any disadvantages associated with working with a surrogate.

When is surrogate use appropriate?

- When the infrastructure available for the conduct of research with the target species is not sufficient to generate statistically measurable responses. There is limited value in pursuing nutrition related research when the responses cannot be measured statistically.
- When the cost of conducting the research is prohibitive with the target species.
- When information relating to an existing aquaculture species is deemed relevant to another species and resources can be used more effectively by focusing on one.
- When translocation and/or quarantine issues restrict the use of the target species.

Basic approach to surrogate use

This approach assumes limited capacity to measure responses in the target species on a routine basis. In the event that limited comparisons can be made with the target species, then as many validations as possible should be undertaken and the surrogate with the greatest compatibility selected.

- Define what you are trying to achieve experimentally to establish the attributes you are seeking in a surrogate (eg digestive similarity, metabolic similarity, ontogenetic compatibility). Is the experimental objective likely to be species specific (eg. preference, feeding habit etc)? This is likely to influence the need for, and choice of a surrogate.
- Identify phyllogenetically linked and trophically similar species that not only resemble specific characteristics of the target species, but can be accommodated more readily in existing, or more cost-effective, infrastructure.
- If key drivers of the nutritional response can be measured in the surrogate species (eg. basic anatomy, enzymes, protein and lipid deposition capacity) they should be defined for comparison with information available on the target species.
- When a surrogate has been selected for a specific task, preliminary data should be generated with a view to applying that data in commercially relevant diets that can be tested against an appropriate benchmark in the target species. Capacity to shift performance commercially is an indicator that the response generated by the surrogate is relevant.
- Assuming the predicted response was measurable, you proceed with use of the surrogate. On-going validation of the surrogate is unnecessary.

Other considerations:

Number of surrogates

It is unlikely that a single surrogate will satisfactorily meet all requirements for all measurements. Surrogate selection should proceed with a view to selecting multiple surrogates for multiple tasks.

Surrogates in feed evaluation systems

In broad terms, a nutritional quality definition system is adequate if it provides a means of discrimination between ingredients, and reflects the level of accuracy that can influence commercial responses in an aquaculture production system. To this end, it is not essential that the values generated by the surrogate are identical to those that would be generated with the target species, providing the ranking of ingredients is the same.

Accuracy in ingredient evaluation and nutrient requirement definition

Consideration must be given to the level of accuracy that can be achieved with a surrogate vs the target species. Surrogate use may be highly justified even when research can be completed with the target species if significant gains can be made in terms of accuracy.

Chemical Analysis Methods for Aquaculture Nutrition Research

Task: Define appropriate methodologies for the analysis of chemical components in ingredients (grains and oilseeds, animal proteins, fats and oils), complete feeds, aquaculture products, faeces and digesta with a view to reducing variation between analysis and nutrition research outcomes derived within Australia.

General considerations:

Several recent reviews have been undertaken identifying the most appropriate techniques for different chemical analyses. Based on the experiences of the members of the Expert Working Group, these methodologies will be assessed and recommendations provided for preferred analysis techniques in addition to sampling requirements, replication and appropriate laboratories.

Nutrient Digestibility Measurements in Aquaculture Species

Task: Define methods for the measurement of nutrient digestibility in aquaculture species.

Summary:

- General summary of why different methods are applied is required. Questionable need to separate digestible energy, metabolisable energy and net energy. Digestible energy is thought to be the most appropriate measure.
- Design should allow the statistical power to detect a 5-10% difference in digestibility at the 5% confidence interval.
- Comparative digestibility is important if we are defining nutritional value for least-cost diet formulation, or if we are trying to assess the influence of a diet component/ingredient on overall digestibility.

General considerations:

- There are differences across the tract in terms of digestion of amino acids and energy. Most methods reflect whole tract digestion.
- Apparent vs true: Apparent often more than adequate. Need to examine enzymically hydrolysed casein as a method for true measurements with amino acids if required.
- Factors affecting nutrient digestibility need to be addressed through the chosen method including age, size, photoperiod, feeding frequency and feeding history.
- While the chosen methodology can influence the results, of greater concern is the way data collected from multiple experiments is pooled, even within a laboratory. Suggestion is to run a random effects model in conjunction with analysis of the data (Random effects ANOVA). Can also look at mixed models. General principle might be to ensure at least one common treatment in subsequent experiments. Allows more flexibility than a common control but does not have as much statistical power. Common controls result in ultra accurate data for one point, which is an inefficient way of undertaking research.
- Direct vs indirect methods: Given issues with labour and water stability, an in-direct method is preferred.
- Markers issues: The chosen marker needs to move at the same rate as the phase being measured, must be inert, fully recoverable, mix homogeniously through the diet, and sample size must permit chemical measurement. Markers include AIA, chromic oxide, ytterbium acetate, ytterbium oxide, titanium oxide, cholestane, n-hexatriacontane. Special notes: n-hexatriacontane not suitable for abalone. Cholestane is excellent for lipid digestibility. Some concerns with chromic oxide influencing carbohydrate digestion and lipid digestion.
- Faecal collection methods: Suitability varies with species (type of pellet excreted), housing and size of fish. Key is to remove representative samples of faeces from the fish as soon as possible. Stripping issue with fish with short digestive tracts and the amount of digestion that occurs in the distal sections of the tract. Settlement retention time in the water. Assisted purging time between anesthesia and purge.

Preferred methodology:

In-direct method.

- Marker must meet basic criteria.
- Acceptable feeding levels: Ration size is important. Most acceptable results will be achieved with high levels of intake. Maintenance feeding may influence digestibility data.
- Acclimation period: As long as possible/necessary. Minimum 5-7 days in most cases. Equilibrium excretion rate of marker.
- Collection method: Representative, minimal influence from leaching etc, representative of whole tract digestion.
- Calculations: Basic approach is (in-out)/in. When using a diet substitution method, use Sugiura or Forster method.
- Experimental conditions need to be documented, and should be consistent across experiments if you wish to compare over time.

- Faecal collection period: Sufficient to collect a representative sample and sufficient for analytical requirements. Given potential digestive differences over the time of a day and time after feeding, collections should attempt to account for these differences (issue with stripping etc).
- Replication: Tank is the unit not the fish within the tank or cage.

Diet formulation principles:

- Vary depending on the objective the experiment.
- Crude protein content in amino acid digestibility should minimize the influences from endogenous losses.
- Minimum of 30% ingredient substitution (minimize accumulation of errors on the test ingredient).
- Additivity In a substitution diet, the test ingredient should not influence the digestibility of the basal diet.
- Basal diet nutrient balanced, some commercial relevance. Inclusion of some test ingredients in the basal diet could be beneficial.
- Lipids intake and feeding rate can affect lipid digestibility. Lipid digestibility should be expressed relative to intake.
- Particle size: Depends on presentation of the commercial diet. Extruded: 250-300 microns. Pelleted diets: 500-600 microns.

Species relevance:

- Settlement: abalone (4), silver perch (3), salmon (smoults) (3-5), prawns (6), snapper (3-5), mulloway (3-5), temperate rock lobsters (?), trout (3-4)
- Stripping: Barramundi (3-5 tanks with 6-10 fish), seawater salmon (?), snapper (3-5), trout (4), yellowtail kingfish (3), flatfish (?)
- Dissection: tuna (5-10 fish), barramundi
- Assisted purging: snapper, trout, barramundi
- Crustaceans: Chromic oxide (typical levels 0.5-1.0%), ytterbium (0.1%) (homogeneity can be a problem), cholestane (0.3%)
- Finfish: AIA (2%), chromic oxide (0.5-1.0%), ytterbium (0.1%), cholestane (0.1%)
- Molluscs: Chromic oxide (0.5-0.75%), AIA (2%) if enough sample.

Nutrient Availability and Utilization in Aquaculture Species.

Task: Define methods for measurement of nutrient availability and utilization in aquaculture species

Summary:

Focus on why the methods are applied – assess nutrient balance, diet suitability, as a check on digestibility methods or step beyond digestibility methods.

General considerations:

- Availability level of accuracy extends beyond the capacity of response measurement in most fish. Methodology does involve slope-ratio type approaches. Could represent a means of obtaining data using growth studies as opposed to faeces collection. Diet formulation flexibility may not facilitate availability experiments.
- Utilization is not used that widely, but if it can add to the nutritional knowledge than there are some basic considerations that need to be taken into account.

Preferred methodology:

Involves a growth study and whole body analysis. Large amount of flexibility in experimental design providing the above constraints are being met.

Nutrient Requirements in Aquaculture Species.

Task: Identify methods for the definition of nutrient requirements.

Summary:

- Nutrient requirement information is necessary for the establishment of diet specifications and optimization of performance. Helps minimize waste excretion.
- Need to be very specific about what the requirement relates to (ie physiological status of the animal, growth, reproduction).

General considerations:

- Significant amount of debate possible in relation to the type of model that is applied to measure requirements.
- Requirement studies involving amino acids must be related to energy.
- Value in measuring a requirement for protein providing it is related to energy because of the contribution of protein to energy. May be influenced by other dietary energy sources.
- Quantity of protein supplied and the capacity of the target fish to utilize non-protein energy will significantly influence the relevance of amino acid balance.
- Ideal protein ratio can have an important role when protein supply is closely matched to protein deposition potential in the fish.
- Survival should be high (>80%) and growth rates on the best diets should be approaching commercially relevant levels.
- Broodstock/Larval viability: Composition of eggs can provide some guide to nutritional status for larvae, but care must be taken to ensure the feeding regimes of broodstock are noted prior to drawing conclusions. Variation in egg composition reflects a capacity to change nutrient content through broodstock nutrition rather than reflecting requirements of the larvae. Survival of larvae on a particular egg of a particular composition does not necessarily reflect optimal specifications.

Preferred methodology:

- As many data points as possible (minimum of 6), but the more data points the better the chance of applying a range of models and defining the actual requirement.
- Dose-response must be measurable and must be attributable to the nutrients being investigated. Duration of the experiment must be sufficient to allow an appropriate response.
- Caution is required with the use of crystalline amino acids given the potential influence of feeding frequency and leaching. Microencapsulated amino acids can also compromise experiments through influence of the encapsulant on responses, some persistent leaching, feeding frequency etc. Some fish can utilize crystalline amino acids, so while there may be problems with their use in requirements studies, they can have a role in fish nutrition.
- When investigating fatty acid requirements, esterified fatty acids (ethyl or methyl) are of limited value due to very poor utilization of these fatty acid forms.
- Covalently bound amino acids can be useful tools in requirements studies.
- Summit dilution: can induce a dose response and may have a role in requirements studies.
- Need to minimize the influence of ingredient substitution on dose responses. Consider the use of blends of a minimal number of carefully formulated diets (eg. high and low energy, high and low protein).
- Must ensure all details associated with the experimental conditions are noted.
- Some preference for curvilinear models so that a potential requirement range can be defined.

Measuring transit time

Task: Measuring transit time in fish.

Summary:

• A difficult area of research requiring some innovation if significant advances are to be made and useful information is to be obtained.

Measuring Feed Intake

Task: Measuring feed intake in fish.

Summary:

- Need to distinguish between feed intake and feed offered.
- Measurement of individual fish feed intakes is difficult.

Preferred methodology:

- Feed in reject out may be suitable for finfish in tanks.
- Need to investigate opportunities for the use of alkane markers.
- Need to examine diet composition studies in addition to intake.
- X-ray methodology.

Measurement of Palatability/Preference/Attractants

Task: Assessment of preference, attractants, and palatability in aquaculture species.

Summary:

• Important to clearly define and distinguish between the terms preference, attractiveness and palatability.

General considerations:

- Important to identify question to be asked otherwise the experimental design may be compromised.
- Need to be clear on the theory behind the measurements. This makes it easier to design testing protocols.
- Important not to provide multiple choices to a target species in a single water space.
- Attractant studies are usually based on 2 choices.

Preferred methodology:

- Testing procedure Static tank (primarily as a rapid screening test (basal responses, concentrations etc) with progression to Y-maze.
- Add to a diet to assess improvement in attractiveness.

Use of growth correlates in aquaculture nutrition research

Task: Potential to use growth and biochemical correlates in nutrition research

Summary:

- Correlates provide information about physiology or nutrition.
- Immunological correlates are not sufficient to provide information on resistance to disease.
- Suggested that correlate information be collected during the course of nutrition research experiments, but should not form the basis of an experiment to assess dietary treatments. Collected information may then have a role in routine assessment of nutritional status of fish in commercial situations.

General considerations:

- Major biochemical correlates include enzyme activities (usually metabolic), RNA, DNA, protein concentrations, hormone concentrations.
- Other correlates Relative organ weights, otoliths, scales.
- Sample should be from representative tissue and from the same site (eg. white muscle).
- Requires rapid sampling and freezing of tissue and transport in liquid N.
- Requires calibration with experimental population of same species with known growth.
- Most laboratory methods are straight forward.
- Growth is the best correlate of growth.
- Growth correlates may be useful in predicting recent growth rates of fish with an unknown growth history.
- Correlates of protein synthesis are more useful than metabolic enzyme activity.
- Correlates of protein synthesis are more useful in the assessment of nutritional status.

Preferred methodology:

RNA:Protein as a measure of capacity for growth and nutritional status.

Feed preparation and measures of ingredient functionality in feed processing systems

Task: Assess requirements for the preparation of experimental feeds and the impact of feed presentation and form on experimental outcomes and their subsequent relevance to commercial diets.

Summary:

Diet choices:

- If the experiment is looking at requirements, then any diet form that allows assessment of nutrient use is adequate (normal caveats of acceptable production levels etc).
- Evaluating ingredients i) comparative nutritional value is less likely to be influenced by the process. Any diet form could be considered adequate ii) ingredient functionality and actual supply of nutrients to an animal, then it needs to be assessed in a form that resembles commercial processing.
- Outcome should not necessarily focus on development of the "perfect diet", but rather information that the feed manufacturer can use to enhance an existing suite of diets. We want more flexibility in diet formulation and enhanced capacity to manipulate diets without compromising processing properties or subsequent diet quality.

Protocol for the basic evaluation of feed ingredients

Initial assessment:

- Gross chemical composition.
- Availability, volume and continuity of supply.
- Complimentary additivity in a diet.
- Variability in composition.
- Potential nutritional value based on gross chemical composition, assumed digestibility and price using parametric analysis.
- Storage requirements.
- Identification of potential anti-nutrients.

Stage I assessment:

- Apparent digestibility of crude protein, energy, organic matter in a representative crustacean, carnivorous and omnivorous finfish.
- Basic functionality in isolation and in a test diet. RVA assessment, rheology measurements.
- Basic assessment of feeding response with a moderate inclusion in a diet.
- Revisit parametrics using actual data.

Stage II assessment:

- Extend digestibility data to amino acids, phosphorus, lipids in target species or a wider range of species.
- Maximum inclusion levels (pellet quality use Evans assessment of pellets, target species intake), restrictions to use (pesticides, accumulation loads, GM).
- Assess the capacity and cost-effectiveness of enhancing/value-adding to the ingredient (expansion, extrusion, enzymes etc).

Improve communication of nutrition research outcomes and current nutrition research between scientists, aquaculturists, ingredient suppliers-and feed manufacturers.

- This project maintained the delivery of annual workshops that facilitated improved communication between scientists, aquaculturalists, ingredient suppliers and feed manufacturers.
- Newsletters and nutrition updates were distributed as appropriate.
- Proceedings from the second annual workshop were published (see Appendix 3). Proceedings from the first annual workshop were not published other than in the form of the strategic plan which was a primary focus of the meeting. The third annual workshop was replaced by a methodology workshop involving the Expert Working Group primarily. A "methodology manual" is currently being prepared for publication and distribution.
- The Subprogram Leader was involved in the development of a number of sessions at Australasian and International workshops on nutrition as a means of further extending research results. In particular, a finfish nutrition section was organised for Australasian Aquaculture 2004.
- The Aquaculture Nutrition Subprogram in association with Food Industry Engineering offered a one day extrusion short course in association with the annual FIE Food and Feed Extrusion Course. This course attracted scientists, commercial aquafeed extruder operators, technicians, and nutritionists. It was supported by Wenger Manufacturing Inc. The paper presented by the Subprogram Leader as part of this short course is presented in Appendix 4.

BENEFITS

The Aquaculture Nutrition Subprogram has benefited the Australian aquaculture sector by providing:

- A more structured approach to aquaculture nutrition research and a more efficient use of limited research funding.
- Clearer definition of the most appropriate methodologies for the conduct of nutrition research.
- An identifiable point of contact for all aquaculture sectors for information on aquaculture nutrition.

FURTHER DEVELOPMENT

As a result of this project, a further submission was made to FRDC for continuation of the Aquaculture Nutrition Subprogram. This project was funded (2004/235) and will be on-going until June, 2007.

CONCLUSIONS

- The Aquaculture Nutrition Subprogram was successful it meeting its contracted objectives and maintained aquaculture nutrition research momentum in Australia.
- It is likely that the scaled-down version of the Subprogram through project 2004/235 will result in some loss of capacity but should still provide adequate resources to ensure aquaculture nutrition research in Australia is well directed and research undertaken represents good value for money for FRDC and associated stakeholders.
- There is still considerable scope to improve the level of innovation associated with aquaculture nutrition research in Australia and the capacity available to undertake that research. There is also scope to focus on non species specific nutrition issues that are limiting the efficiency and profitability of aquaculture enterprises in Australia.

Appendix 1 – Intellectual Property

There is no intellectual property requiring protection arising from this project.

Appendix 2 - Staff

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Appendix 3 – Workshop Proceedings



EXPANDING THE AQUAFEED INGREDIENT BASE

ANS Publication No. 1 Edited by Dr Robert van Barneveld June, 2003



FISHERIES RESEARCH & DEVELOPMENT CORPORATION







Proceedings of the Second Annual Aquaculture Nutrition Subprogram Workshop – Fremantle, 2003

Venue

The Esplanade Hotel

Fremantle, Western Australia

May 29, 2003

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Production and utilisation of vegetable protein sources for aquafeed in

Australia - What are we trying to achieve?

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Given that a wide scope of nutrition research already exists within the FRDC portfolio, as well as a range of other research investors, the Aquaculture Nutrition Subprogram (ANS) aims to take nutrition research into a higher risk area that is not limited by industry development restrictions and is beyond individual species based projects. The vision for this subprogram is the efficient, innovative and sustainable delivery of nutrients to aquaculture production systems to optimise aquatic animal health and production and aquaculture product quality while minimising impacts on the aquatic environment. The subprogram mission is:

"To enhance the Australian aquaculture nutrition expertise base and to utilise combined knowledge to identify and solve common aquaculture nutrition challenges and limitations to development of the Australian aquaculture industries".

The FRDC's Research and Development Plan 2000-2005 lists a number of challenges that exist in the FRDC's business environment. Challenge "5" is reducing the quantity of fish protein fed to terrestrial and aquatic livestock so that it becomes available in the food chain to satisfy environmental and human needs. The FRDC have only made minimal investments in research addressing this challenge since the plan became active and hence this research area remains a very high priority. For this reason, "Production and utilisation of vegetable protein sources for aquafeed in Australia" was the focus for the second annual ANS workshop.

Based on discussions at the workshop, and additional data provided in papers within this proceedings, the following priorities and directions have emerged in relation to expanding the aquafeed ingredient base:

- 1. Expanding the available protein sources for use in aquafeeds in Australia and overseas is clearly the highest research priority for the ANS;
- 2. Feed manufacturers are primarily interested in information on ingredient digestibility (amino acids and energy), maximum inclusion levels, and functionality of ingredients in a feed processing system, rather than development of complete diets using alternative proteins.
- 3. Lupin protein (53-55%) appears to hold the greatest potential as an additional protein source for use in aquafeeds in Australia.
- 4. Development of protein hydrolysates is another priority to reduce reliance on fish meals and oils, and as a means of potentially increasing maximum inclusion of vegetable protein alternatives.
- 5. Research should be undertaken with a representative temperate finfish (salmonid), tropical finfish (eg. barramundi, lower priority) and crustacean (prawn).

In addition to the above, other things that should be considered when progressing the research program include:

- 1. It would be desirable to minimise the amount of processing required to produce a vegetable protein product with a protein content of 53-55% CP.
- 2. It would be desirable to have this processing capacity in Australia.
- 3. If there are co-products produced as a consequence of this processing, it is almost certain that a viable market for these products will be required to make the activity cost-effective.
- 4. We need to involve the expertise of a well-established high volume grain processor in any subsequent research.
- 5. 53-55% CP may not be necessary for use in diets for all aquaculture species (eg. prawns).



- 6. Other protein sources require further investigation as alternatives for use in aquafeeds given the existence of higher protein soybean meals and opportunities for other grain legumes if a lower protein content is required.
- 7. From a grains industry perspective, export markets hold the greatest potential and of these markets, Asia will not only hold the greatest potential in terms of volume, but it is likely to have the greatest variation in requirements. If Asia is a focus, then prawns must be a focus species of this research program.
- 8. Given that a significant proportion of aquaculture expansion in the Asian region is likely to be in species that have a low reliance on manufactured feeds (eg tilapia, catfish etc etc) we should consider the merits of expanding our research program to encompass development of very low cost feeding alternatives based on Australian grains (eg. a blend of cereals and legumes that can be made into doughs and pastes with the addition of water). Some of these products may also have application in lower value Australian aqauculture systems (marron, yabbies, red claw etc).

With all of the above in mind, the following represents a possible way forward for research projects within the ANS:

- 1. Focus on multiple vegetable protein alternatives with varying levels of crude protein and varying origins. This could include lupins, peas, soybean, canola etc.
- 2. Assessment of amino acid and energy digestibility and maximum inclusion levels in a salmonid and a crustacean should be undertaken at the very least.
- 3. A significant focus on the definition of ingredient functionality should be considered.
- 4. Identification of alternative ways to deliver protein and energy from Australian grains to low value, high volume fish species (specific to Asian farming practices).
- 5. Focus on protein sources that can be produced using existing technology, by existing commercial manufacturers in Australia.



Development of vegetable protein sources for finfish

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Introduction

In formulating fin-fish aquaculture diets the alternative options to using fish meals or other marine derived protein meals can generally be summated as those that are from either terrestrial animal meals or plant protein meals. For a variety of technological reasons and social perspectives the aquaculture feeds industry has sought to pursue the preferential adoption of plant protein meals as alternative ingredients for their feed formulations. The composition, one of the key factors in ingredient choice, of plant protein meals varies considerably, usually depending of the variety and level of processing used to make that plant meal (Table 1). Notably, those ingredients that have been more widely used and accepted by the aquaculture industry include those such as soybean meal and more recently lupin kernel meals. Over recent years there has been considerable work examining the utilisation of a wide range of alternative protein ingredients (Allan et al., 2000). To refine this approach my colleagues and I have chosen to rationalise the range of ingredients to be further developed for this feed sector.

| Table 1. | Composition and value (ex. Australia) of ingredients evaluated. Details are on a dry matter |
|----------|---|
| | basis (g/kg DM) unless otherwise specified. |

| | AKM | LKM | SBM | PEA | CAN | GLU | MTM | BLD | FSM |
|--|-------------|-------------|-------------|-------------|-------------|--------------|-------------|-------------|--------------|
| Dry Matter (g/kg) | 885 | 903 | 909 | 903 | 920 | 910 | 920 | 887 | 920 |
| Protein | 415 | 547 | 518 | 257 | 394 | 838 | 600 | 951 | 718 |
| Fat | 53 | 87 | 47 | 12 | 82 | 9 | 110 | 1 | 105 |
| Carbohydrate | 499 | 321 | 365 | 703 | 460 | 146 | 0 | 0 | 0 |
| Ash | 33 | 44 | 69 | 28 | 65 | 8 | 290 | 18 | 152 |
| Organic Matter | 967 | 956 | 931 | 972 | 935 | 992 | 710 | 982 | 848 |
| Phosphorus | 4 | 6 | 8 | - | 11 | 2 | 44 | 2 | 26 |
| Energy (MJ/kg DM) | 20.4 | 20.9 | 19.6 | 18.6 | 20.5 | 22.6 | 18.5 | 23.0 | 21.5 |
| Typical price (\$/tonne) Price (\$) / g Protein | 350 0.84 | 450 0.82 | 450 0.87 | 300 1.17 | 300 0.76 | 3000 3.58 | 500 0.83 | 900 0.95 | 1200 1.67 |

AKM: *L. angustifolius* kernel meal; LKM: *L. luteus* kernel meal; SBM: Solvent-extracted soy bean meal; PEA: Field pea (*Pisum sativum*) meal; CAN: Solvent-extracted canola meal; GLU: Wheat gluten; POU: Poultry meal; MTM: Meat meal; BLD: Blood meal; FSM: Chilean Prime Anchovy meal. Data derived from unpublished data (B. Glencross).

A key constraint in the process of choosing an ingredient to focus on has been the capacity for Australian research benefit to be captured and retained in Australia. To this end we examined the range of grain products produced in Australia and identified that the one grain where we held clear technological and market advantages was in lupin production. Therefore in addition to improving the understanding of the use constraints to simple lupin products, such as lupin whole-seed and kernel meals, we have also undertaken to pursue to the development and evaluation of value-added products and new lupin species and cultivars as they arise. Although the primary focus of this work will be on lupins other grains will be considered where feasible.

To progress the development of lupin products for the aquaculture feeds sector the Department of Fisheries (WA) has undertaken to use rainbow trout, primarily as a "laboratory rat" species, to evaluate and guide product development as it arises. Progress to date in using this species, and the specially designed facilities at the Pemberton Freshwater Research Centre, has been rapid. This is in part due to the capacity to conduct powerful research experiments and the reliable and unrestricted



access to facilities and fish. In progressing the evaluation of grain protein products, three central issues have been at the forefront of the research being undertaken:

- 1. Defining the digestibility of key nutrients from the ingredients.
- 2. Evaluating the palatability of each product when fed to an aquaculture species.
- 3. Defining the influence of ingredient use on the aquaculture species' metabolism or other fundamental limitations to the specific use of that ingredient.

New grain varieties and products

As a precursor to the current grain product development project, since 1999 the Department of Fisheries has had an active research program examining the potential of a variety of grain protein and oil resources when fed to aquaculture species. This has included the evaluation of new grain varieties when fed to rainbow trout (among other fish species). One of the "shining lights" from this work has been the meals from yellow lupins (*Lupinus luteus*) (Glencross et al., 2002).

Yellow lupins, particularly their kernel meals, have proven to be a highly useful feed ingredient when fed to fish (Glencross and Hawkins, 2003). They possess a high digestible protein content (~473 g/kg DM) and a moderate digestible energy content (~13.6 MJ/kg DM). This compares very favourably with solvent-extracted soybean meals (~437 g/kg DM and 14.4 MJ/kg DM) and substantially better than sweet lupin (*L. angustifolius*) kernel meal (~383 g/kg DM and 12.9 MJ/kg DM) and white lupin (*L. albus*) kernel meal (~402 g/kg DM and 14.8 MJ/kg DM). Notably considerable variability of digestible nutrient value within a lupin species, among and within cultivars has been observed (Glencross et al., 2003b). This has important implications for the further development of protein-premiums for grain growers.

Growth studies examining increasing inclusion levels of yellow lupin kernel meal in diets fed to rainbow trout showed a significant deterioration in growth at the 50% inclusion level, but not at 37.5% inclusion. The reduced growth rate was not attributed to decreased feed intake and as such it was concluded that there were no palatability problems with this product. However, reasons for the decline in nutritional value of yellow lupin kernel meal at the 50% inclusion level have not yet been defined, but are suspected to be related to ingredient oligosaccharide levels which have shown to be influential in sweet lupins when fed to fish (Glencross et al., 2003a). Notably some minor aberrations in faecal integrity have been noted with the use of some plant protein meals. However, in contrast to soybean products no histological aberrations of the distal intestine were observed from fish fed even the highest levels of yellow lupin kernel meal (Glencross et al., submitted).

| | LKM | AKM | LPC | APC | LPI | API | SBM | SPC | SPI | EHC |
|-------------------|------|------|------|------|------|------|------|------|------|------|
| Dry Matter (g/kg) | 903 | 885 | 944 | 942 | 924 | 926 | 909 | 939 | 938 | 916 |
| Protein | 547 | 415 | 781 | 690 | 805 | 810 | 518 | 590 | 893 | 839 |
| Fat | 87 | 53 | 78 | 93 | 123 | 125 | 47 | 54 | 13 | 11 |
| Carbohydrate | 321 | 499 | 103 | 186 | 31 | 35 | 365 | 277 | 47 | 80 |
| Ash | 44 | 33 | 37 | 31 | 41 | 30 | 69 | 79 | 47 | 70 |
| Organic Matter | 956 | 967 | 963 | 969 | 959 | 970 | 931 | 921 | 953 | 930 |
| Phosphorus | 6 | 4 | 6 | 5 | 9 | 5 | 8 | 9 | 9 | 9 |
| Energy (MJ/kg | | | | | | | | | | |
| DM) | 20.9 | 20.4 | 22.2 | 22.2 | 22.6 | 22.6 | 19.6 | 20.3 | 23.0 | 21.2 |

 Table 2.
 Composition of ingredients evaluated. Details are on a dry matter basis (g/kg DM) unless otherwise specified.

LKM: *L. luteus* kernel meal; AKM: *L. angustifolius* kernel meal; LPC: *L. luteus* protein concentrate; APC: *L. angustifolius* protein concentrate; LPI: *L. luteus* protein isolate; API: *L. angustifolius* protein isolate; SBM: Solvent-extracted soy bean meal; SPC: Soy protein concentrate; SPI: Soy protein isolate; EHC: Enzymatically-hydrolysed casein.

As part of a major GRDC project, the focus on lupin use in aquaculture feeds has moved towards the development and evaluation of a range of "First-Generation" protein concentrates and isolates.



Presently these are being produced under laboratory conditions, from sweet lupin kernel meal and yellow lupin kernel meal (Table 2). Considerable flexibility exists to manipulate the composition of these ingredients based on micro-management of particular processes involved in the production operations.

Digestible value of ingredients

The determination of the digestible value of the "first-generation" grain protein products was undertaken using the diet-substitution method. In undertaking digestibility evaluation studies, the process used in the collection of faeces has been considered contentious. However, collection of faeces using either settlement or stripping methods is employed widely. Notably both methods have their potential flaws and strengths. In this study both methods were employed to cater for both "schools-of-thought".

High digestibility values of protein and energy for all protein meals and concentrates were observed (Table 3). Notably, the higher digestibility values generally corresponded to decreases in the levels of carbohydrate in specific ingredients. Differences were noted between the two faecal collection methods used, but standardisation to a reference ingredient negated this problem. We chose laboratory grade enzymatically-hydrolyzed casein as that reference. While good digestibility values were evident from the concentrates, inclusion issues are still being resolved.

Table 3. Apparent digestibility coefficients of kernel, protein concentrate and isolate products produced from sweet and yellow lupin varieties when assessed using either of the two faecal collection methods from rainbow trout. Reference and competitor soy products are also included.

| | LKM | AKM | LPC | APC | LPI | API | SBM | SPC | SPI | EHC |
|------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Stripping | | | | | | | | | | |
| Nitrogen/Protein | 0.894 | 0.867 | 1.010 | 0.974 | 0.986 | 0.963 | 0.801 | 0.927 | 1.025 | 0.956 |
| Phosphorus | 0.970 | 1.089 | 0.967 | 0.888 | 0.622 | 0.792 | 0.398 | 0.707 | 0.570 | 0.837 |
| Energy | 0.629 | 0.536 | 0.959 | 0.856 | 0.921 | 0.917 | 0.717 | 0.726 | 0.986 | 0.914 |
| Organic Matter | 0.566 | 0.428 | 0.934 | 0.788 | 0.902 | 0.881 | 0.614 | 0.675 | 0.976 | 0.893 |
| Settlement | | | | | | | | | | |
| Nitrogen/Protein | 0.986 | 0.977 | 1.009 | 0.999 | 0.998 | 1.003 | 0.972 | 1.023 | 1.005 | 0.999 |
| Phosphorus | 0.956 | 0.906 | 0.682 | 0.714 | 0.549 | 0.624 | 0.606 | 0.613 | 0.518 | 0.820 |
| Energy | 0.812 | 0.698 | 0.938 | 0.880 | 0.914 | 0.943 | 0.819 | 0.864 | 0.960 | 0.985 |
| Organic Matter | 0.812 | 0.641 | 0.948 | 0.854 | 0.920 | 0.956 | 0.782 | 0.826 | 0.962 | 0.989 |

LKM: *L. luteus* kernel meal; AKM: *L. angustifolius* kernel meal; LPC: *L. luteus* protein concentrate; APC: *L. angustifolius* protein concentrate; LPI: *L. luteus* protein isolate; API: *L. angustifolius* protein isolate; SBM: Solvent-extracted soy bean meal; SPC: Soy protein concentrate; SPI: Soy protein isolate; EHC: Enzymatically-hydrolysed casein.

In addition to the work with rainbow trout, some of the same diets were also fed to Atlantic salmon and faeces collected using settlement techniques (Table 4). A comparison of the digestibility data between the two fish species identified some subtle differences in their respective capacity to utilise certain ingredients. Notably, Atlantic salmon had poorer capacity to digest nutrients from those ingredients with higher levels of fibre, such as the *L. angustifolius* kernel meal and the soybean meal. However, fewer differences were noted among the higher-processed products when fed to either fish species.

 Table 4.
 Apparent digestibility coefficients of kernel meal, protein concentrate and isolate products produced from sweet lupins and soybean when assessed in rainbow trout and Atlantic salmon.



| | AKM | APC | API | SBM | SPC | SPI | EHC |
|------------------|-------|-------|-------|-------|-------|-------|-------|
| Rainbow trout | | | | | | | |
| Nitrogen/Protein | 0.977 | 0.999 | 1.003 | 0.972 | 1.023 | 1.005 | 0.999 |
| Phosphorus | 0.906 | 0.714 | 0.624 | 0.606 | 0.613 | 0.518 | 0.820 |
| Energy | 0.698 | 0.88 | 0.943 | 0.819 | 0.864 | 0.960 | 0.985 |
| Organic Matter | 0.641 | 0.854 | 0.956 | 0.782 | 0.826 | 0.962 | 0.989 |
| Atlantic salmon | | | | | | | |
| Nitrogen/Protein | 0.910 | 0.961 | 0.998 | 0.873 | 0.898 | 0.987 | 1.020 |
| Phosphorus | 0.419 | 0.309 | 0.390 | 0.313 | 0.064 | 0.373 | 0.762 |
| Energy | 0.653 | 0.884 | 0.990 | 0.808 | 0.855 | 0.998 | 1.038 |
| Organic Matter | 0.551 | 0.829 | 0.975 | 0.802 | 0.784 | 0.976 | 1.033 |
| | | | | | | | |

AKM: *L. angustifolius* kernel meal; APC: *L. angustifolius* protein concentrate; API: *L. angustifolius* protein isolate; SBM: Solvent-extracted soy bean meal; SPC: Soy protein concentrate; SPI: Soy protein isolate; EHC: Enzymatically-hydrolysed casein.

Palatability

Irrespective of how good an ingredient's nutrient and energy digestibility may be, if it has an adverse palatability effect on animals to which it is fed then it may be problematic as a useful feed ingredient. To examine the palatability of the two lupin protein concentrates an experiment was designed in which diets containing increasing levels (up to 40%) of the products were fed to apparent satiety to trout over a six-week period. Two positive controls were included to ensure the experiment had the potential to detect palatability effects. After three weeks an effect of one of the positive controls was evident, but no specific effects that were attributable to inclusion of the protein concentrates.

Preliminary data are presented in Table 5.

| | 0% | 10% L | 20% L | 30% L | 40% L | 10% A | 20% A | 30% A | 40% A | C1 | C2 |
|--|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| Initial weight (g) 3-week weight (g) Feed intake | 35.6 74.1 | 35.6 75.1 | 35.6 75.4 | 35.6 75.1 | 35.6 75.2 | 35.6 72.8 | 35.7 70.5 | 35.5 70.8 | 35.6 73.1 | 35.5 70.2 | 35.8 60.6 |
| (g/fish/d) FCR | 1.76 0.96 | 1.71 0.91 | 1.68 0.89 | 1.68 0.89 | 1.66 0.88 | 1.65 0.93 | 1.54 0.93 | 1.55 0.94 | 1.59 0.89 | 1.52 0.92 | 1.18 1.00 |

Table 5. Growth and palatability experiment preliminary progress.

X%-L: L. luteus protein concentrate at X inclusion level ; X%-A: L. angustifolius protein concentrate at X inclusion level. C1: Positive control 1; C2: Positive control 2. Each Control contains a different level of a palatability inhibitor.

Metabolic value

One of the problems that can result from the use of plant protein resources is the introduction of antinutritional factors. Some of these bioactive compounds can have detrimental problems to fish growth and metabolism, irrespective of digestible nutrient value or palatability of an ingredient. Because of potential interference with effective metabolism of protein and energy from the ingredient, a controlled experiment that eliminates the fish's capacity to regulate its feed intake is required, to more clearly resolve the specific nature of any problem associated with the metabolic value of the ingredient. This approach has been used successfully to differentiate the limited differences in protein (amino acid) value between a transgenic and non-transgenic lupin variety when fed to a fish (Glencross et al., 2003c) and also in evaluating the nutritional value of canola meals produced using different oil extraction methods (Glencross et al., 2004).



The Next Step

As the first phase of the evaluation of the laboratory produced lupin protein concentrates and isolates comes to completion, the grain protein concentrates project is approaching its second phase of progress. It has been identified during project progress that the key process to establishing the protein concentrate production method as a commercially viable one will be the cost of the drying process used in making the product. The next phase of the project will see the project examine key issues such as product drying conditions on ingredient quality and also the first stages of commercial progression of product development and evaluation. In addition to this it is planned to expand the project evaluation base to encompass Atlantic salmon (under both European and Australian conditions) and prawns as defined "target" market species. Work with rainbow trout will continue as a screening mechanism to refine ingredient choice options before getting those products further evaluated in the "target" market species.



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Utilisation of vegetable protein sources in crustacean diets

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Introduction

Fishmeal is one of the most important ingredients in formulated aquafeeds, and particularly in prawn diets where it is typically included at between 200 and 300 g/kg. Its inclusion is primarily for its high quality protein but has the additional benefit of its oil content and associated long-chain, highly unsaturated fatty acids. Around the world there has been widespread interest in the partial replacement of fishmeal in prawn diets, using vegetable protein sources. This has been driven by increased demand for fishmeal whose global production is reported to be either static or decreasing, and which is becoming increasingly more expensive relative to vegetable protein sources. Much of the research has focused on the use of soybean meal but more recent studies have extended to the use of field peas, canola and lupins.

Soybeans

Though soybeans have the most favourable amino acid profile of all plant proteins (Lim and Akiyama, 1991), they also contain high levels of oil and some anti-nutritional factors. The oil in soybeans is a very valuable commodity and hence the soybean meal used in prawn feed is almost entirely comprised of a meal from which most of the oil has been extracted. Apart from reducing the cost of the soybean meal (SBM), this has an additional advantage in that the SBM has little impact on the lipid content and fatty acid composition of the feed. The anti-nutritional factors in SBM, which are predominantly trypsin inhibitors, are rendered essentially inactive by heat treatment that follows the defatting process. Typically, SBM used in aquaculture feeds contains 906 g/kg dry matter (DM), 459 g/kg crude protein (CP), 10 g/kg fat, 63 g/kg ash, 63 g/kg crude fibre and 311 g/kg N-free extract (Hertrampf and Pied-Pascual, 2000).

A great deal of research has been carried out to assess the efficacy of solvent extracted, heat-treated SBM in prawn feeds (reviewed by Akiyama, 1991). Interpretation of much of the work has been made difficult because of the experimental design used, or the way diets have been formulated. However, Pied-Pascual *et al.* (1990) found that with diets containing 400 g/kg CP, a diet containing 350 g/kg defatted SBM and 160 g/kg fish meal performed better than diets with 450 and 550 g/kg SBM and correspondingly lower levels of fishmeal. Both Lim and Dominy (1990) and Akiyama (1990) obtained similar results, suggesting that with a black tiger prawn (*Penaeus monodon*) diet containing 400 g/kg CP (on DM basis), SBM can be included at up to about 350 g/kg without having an adverse effect on performance. In the FRDC Fishmeal Replacement Program, where the apparent digestibility (AD) of SBM was determined for sub-adult black tiger prawns, the DM, CP and energy (E) digestibilities were 64, 92 and 72%, respectively (Smith and Tabrett, 1998). As the soybean protein is highly digestible, it would appear that the markedly reduced ADDM is due to the carbohydrate fraction of the SBM that contains relatively high levels of dietary fibre (233 g/kg) and non-starch polysaccharides (217 g/kg) (Knudsen, 1997).

The effectiveness of soybean protein concentrate (SPC) has been studied in black tiger prawn diets (Paripatananont, 2001). The SPC contained 650 g/kg CP, 3 g/kg fat, 45 g/kg crude fibre and 70 g/kg ash. The results indicated that the inclusion of 175 g/kg of the SPC, at the expense of fishmeal and wheat flour, could support normal growth but when used at 262.5 g/kg, the growth rate decreased significantly. A point to note is that all diets also contained 120 g/kg of SBM in addition to the variable amounts of SPC.



Field peas

Two separate studies have been carried out evaluating field pea products obtained from the same source of supply in Canada, in diets for two different species of prawns, Litopenaeus stylirostris (Cruz-Suarez et al., 2001) and L. vannamei (Davis et al., 2002). The field pea products evaluated were: whole and dehulled peas that were used as raw flour, or as an extruded and re-ground product. The whole meal was also processed using infrared cooking to produce a micronised meal. In the study with L. stylirostris, the field pea meals were used in practical prawn diets at an inclusion rate of 300 g/kg, replacing SBM and wheat (1:3) on an isonitrogenous and isoenergetic basis. Growth was unaffected by dehulling, while extrusion cooking had no effect on growth but improved feed conversion ratio and protein efficiency ratio. The diet containing the micronised meal resulted in the highest feed intake and highest growth rate. In the study with L. vannamei, both extruding and micronising the pea meals resulted in significant improvements in ADCP and ADE. Growth response was evaluated by including 250 g/kg of the meals at the expense of whole wheat in a basal diet containing 360 g/kg of protein. There were no significant differences in weight gain among treatments, suggesting further investigation was warranted (Davis et al., 2002). In the FRDC Fishmeal Replacement Subprogram, with juvenile P. monodon, the AD coefficients of Dunn field peas by were found to be 72, 89 and 83 for DM, CP and E, respectively (Smith and Tabrett, 1998). The ADDM coefficient was lower than obtained by Cruz-Suarez et al. (2001) (89%) (ADDM was not reported by Davis et al., 2002), while the ADCP coefficient was again lower than reported by Cruz-Suarez et al. (2001) (79%) but similar to that obtained by Davis et al. (2002).

Canola

In the FRDC Fishmeal Replacement Subprogram study, canola meal was found to have the lowest AD coefficients of all of the vegetable protein sources evaluated: 42% for ADDM, 78% for ADCP and 49% for ADE (Smith and Tabrett, 1998). Buchanan *et al* (1997) investigated the effect of supplementing diets containing canola with an enzyme mixture (Porzyme, Finnfeeds International). The enzyme treatment did not improve weight gain of *P. monodon* with the "low" canola content diet (200 g/kg) but did significantly improve weight gain with the "high" canola content diet (640 g/kg). Cruz-Suarez *et al.* (2002) used extruded canola meal at 300 g/kg in a practical diet to replace SBM, fishmeal and wheat (1:2:3 parts) on an isonitrogenous and isoenergetic basis in diets for small juvenile *L. stylirostris.* The growth rate of the prawns was not significantly different from those fed the control diet, and FCRs were similar at about 1.8:1. The AD of the extruded canola meal was 80% for ADDM and 80% for ADCP. Davis *et al.* (2002) evaluated the same product with *L. vannamei*, including it at 250 g/kg at the expense of SBM, and also found no significant difference in growth rate. They also found the apparent digestibility of the extruded canola meal to be 73% for ADDM and 96% for ADCP. These two sets of digestibility results suggest marked differences between species or a methodological problem.

Lupins

The apparent digestibility of whole and dehulled lupins (*L. angustifolius* cv. Gungurru) was determined in the FRDC Fishmeal Replacement Subprogram using 10 to 15 g *P. monodon* (Smith and Tabrett, 1998). The dehulled lupin meal (DLM) had higher digestibility coefficients than whole lupins: ADDM 73 and 39%; ADCP 95 and 88%; and ADE 74 and 45%, respectively. The DLM (*L. angustifolius* cv. Warrah) and a transgenic, high-methionine variety, were evaluated in a growth assay in cages in a raceway pond using 3 g *P. monodon*. The lupin meals were included at 250 g/kg of diet, replacing fishmeal and wheat flour, in a diet containing 400 g/kg CP. The results demonstrated that DLM derived from *L. angustifolius* could be included at up to 250 g/kg in commercial prawn feeds without adversely affecting performance. In addition, the results indicate that at this inclusion level, the concentration of the essential amino acid, methionine, in the lupin protein does not limit the performance of 400g/kg CP prawn diets (Smith and Tabrett, 2003).

Sudaryono *et al.* (1999a) evaluated *L. albus* meal in 400 g/kg CP diets for *P. monodon* replacing 0, 25, 50, 75 and 100% of the fishmeal protein with an equivalent of amount of protein supplied as DLM. Re-analysis of the data demonstrated a progressive decline in weight gain when more than 25% of the



dietary fishmeal protein was replaced with dehulled *L. albus* meal. In a second study, Sudaryono and co-workers compared the performance of whole and dehulled *L. albus* meal, dehulled *L. angustifolius* meal, lupin protein concentrate (*L. angustifolius*) and defatted SBM. In this study they concluded that: (a) dehulling seed or concentrating lupin protein did not improve the nutritive value of lupin meal. They also found that the growth rate of prawns fed the diet containing 360 g/kg dehulled *L. angustofolius* meal was significantly greater than prawns fed diets containing lupin protein concentrate; (b) *L. angustifolius* meal generally performed better than *L. albus* meal; (c) *L. angustifolius* meal was comparable to SBM; and (d) *L. angustifolius* meal appeared to provide the feed with greater attractability for prawns than *L. albus* meal (Sudaryono, 1999b).

In a recently completed, GRDC-funded study, Smith (2002) investigated the factors that limit the utilisation of DLM (L. angustifolius cv. Gungurru) in prawn feeds. The study confirmed the relatively high digestibility of crude protein in DLM but demonstrated that growth rate of prawns was adversely affected by DLM when used at dietary inclusion levels greater than 250 g/kg. The ADDM and ADCP of lupin protein concentrate were also found to be significantly greater than that of DLM. In a study on the effect of the endogenous lipid in DLM, they found that the fatty acid composition of the DLM did not have a significant effect on prawn performance when the vegetable to marine lipid ratio in the diet was less than about 1:1. The low level of methionine or methionine + cystine in the lupin protein did not appear to limit the nutritional value of the feed when the dietary lupin inclusion level was less than about 380 g/kg. However, this aspect was not tested adequately and requires the development of an effective method for supplementing the diets with methionine. Lupin kernel fibre, isolated as insoluble NSP, did not have a significant effect on the growth of prawns even at very high levels, equivalent to that of a feed based entirely on DLM. However, it did result in decreases in ADDM and ADCP that were directly related to the inclusion level of the insoluble NSP. The ethanol extraction of oligosaccharides (soluble NSP) from DLM also did not improve the performance of feeds containing 500 g/kg of DLM. However, the performance of prawns fed the basal diet in this experiment was not as good as expected or, alternatively, the performance of the prawns fed the lupin diets was far better than expected for that inclusion level of DLM. This raises the question as to whether the oligosaccharide content of the particular batch of DLM used in the experiment was much lower than for batches used in previous experiments. Further analysis of the oligosaccharide content of the materials and diets are being carried out.

In conclusion, it is essential that the factor or factors limiting the utilisation of DLM in prawn diets be identified. The two factors most likely to be responsible for the lower nutritional value of DLM are its relatively low methionine content and its oligosaccharide content. Tools to test the methionine issue are currently being developed. In addition, further research to test how agronomic factors – lupin variety, soil fertility and locality grown, season etc – affect the nutritional value of lupins in prawn feeds is urgently need. With this information, strategies for the development of lupin products containing higher protein levels can be systematically developed.



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Alternative protein sources in manufactured diets for molluscs

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Abalone aquaculture in Australia

Currently two species of abalone are being cultured in Australia, the blacklip abalone (*Haliotis rubra*) and the greenlip abalone (*Haliotis laevigata*). The greenlip species is cultured in South Australia, Victoria, Tasmania and more recently Western Australia, whilst the blacklip species is only cultured in Victoria and Tasmania. A hybrid of the two species, known as the Tiger abalone, is also being produced in Victoria and Tasmania. In Australia the majority of abalone culture occurs in land based tanks, however a couple of sea cage farms are also in operation. Although the industry has been going for approximately 20 years, production is still relatively small, about 150-200 tonnes per year (total blacklip and greenlip production for Australia). Due to a moratorium on the harvesting of algae from the wild, abalone's natural diet, the farms in Australia feed their abalone a manufactured diet (excluding a couple of sea cage farms) that are produced by two feed companies residing in South Australia. Due to the industry's dependence upon a manufactured diet considerable research has been conducted over the last 7 years on the nutritional requirements of abalone, including the digestibility of alternative protein sources for use in diets.

The assessment of alternative protein sources for abalone

Abalone digestibility experiments

The evaluation of the digestibility of various protein sources for Australian abalone has been conducted using chromic oxide as a marker in the diets (0.5 %) and collection of faeces by the settlement technique. In the experiments juvenile abalone were used, 40-60 mm in shell length. The abalone were housed in conical shaped tanks (approximately 60-80 in each) and fed each night during the experiments. Tanks were cleaned each morning and a collection tube screwed onto the base of the tanks allowing catchment of the faeces during the day.

Protein sources assessed

Using the methodology detailed above the protein and energy digestibility of the following protein sources have been assessed for Australian abalone in projects funded by the Fisheries Research and Development Corporation:

Greenlips - casein, soybean meal, soyflour, vetch, faba beans, field peas, lupins (whole and dehulled *L. angustifolius* and whole *L. luteus*), meat and bone meal, blood meal, sunflower meal, maize meal, canola meal, wheat gluten, fishmeal, mung beans and skim milk powder.

Blacklips – casein, soyflour, fishmeal, mung beans, skim milk powder, lupins (whole and dehulled *L. angustifolius* and whole *L. luteus*).

As can be seen a more extensive range of ingredients has been assessed for greenlip abalone as most of the nutritional work on abalone in Australia has been conducted on this species. Concerns were raised by farmers in Tasmania and Victoria, where the blacklip species are grown, that the digestive capacity and nutritional requirement of the blacklip species may differ from the greenlip species and that they may need differently formulated diets. For this reason a project was funded comparing the nutritional requirements of the blacklips against those of the greenlips.



Results from abalone digestibility studies

Digestibility of various protein sources for greenlip abalone

The protein and energy digestibility of protein sources assessed for greenlip abalone are reported in Table 1. It can be seen that greenlip abalone have a very poor ability to digest the protein and energy from animal based protein sources, particularly terrestrial animal protein sources such as meat and bone meal and blood meal. The milk based animal protein sources, casein and skim milk powder, were an exception to this finding as they both had high protein and energy digestibility. Variable responses were observed in the greenlip abalone's ability to digest the protein from plant based protein sources. The most digestible plant protein sources, in terms of both protein and energy were soyflour and lupins (whole *L. luteus* and dehulled *L. angustifolius*). Although the protein from field peas, vetch and faba beans was highly digestible for greenlip abalone they could not digest the energy from these legumes very well. As for the legumes, the energy from the oilseeds sunflower meal and canola meal was also poorly digested, however, the protein from these oilseeds was lower in digestibility than that from the legumes.

Comparison between abalone species

A comparison of greenlip and blacklip abalone's ability to digest the protein and energy digestibility from several protein sources is reported in Table 2. Significant differences were found between blacklip and greenlip abalone in their apparent faecal digestibility of protein and energy of some of the ingredients evaluated. With respect to gross energy digestibility, blacklip abalone digested the energy from whole *L. angustifolius*, fishmeal and skim milk powder significantly better than greenlip abalone and greenlip abalone digested the energy from dehulled *L. angustifolius* significantly better than blacklip abalone (Table 2). No significant differences were found between the two species in their ability to digest energy from defatted soyflour, casein, mung beans and *L. luteus* (Table 2).

Greater differences were found between the two species in their capacity to digest protein from the ingredients with statistically similar protein digestibility values only being obtained for mung beans and *L. luteus* (Table II). Blacklip abalone digested significantly more protein from defatted soyflour, fishmeal, casein and skim milk than greenlip abalone, whilst greenlip abalone digested significantly more protein than blacklip abalone from dehulled and whole *L. angustifolius* (Table 2).

| Protein source | Protein digestibility | Energy digestibility |
|--------------------------|-----------------------|----------------------|
| Soyflour | 0.82 (0.013) | 0.78 (0.013) |
| Soybean meal | 0.75 (0.016) | 0.75 (0.019) |
| Field peas | 0.89 (0.043) | 0.49 (0.017) |
| Faba beans | 0.95 (0.012) | 0.65 (0.020) |
| Vetch | 0.87 (0.003) | 0.45 (0.015) |
| Canola meal | 0.66 (0.012) | 0.54 (0.011) |
| Wheat gluten | 0.51 (0.024) | 0.13 (0.037) |
| Sunflower meal | 0.75 (0.093) | 0.50 (0.017) |
| Maize meal | 0.49 (0.012) | 0.48 (0.031) |
| Whole L. angustifolius | 0.91 (0.003) | 0.50 (0.005) |
| Dehulled L angustifolius | 0.92 (0.002) | 0.82 (0.008) |
| Whole L. luteus | 0.91 (0.009) | 0.83 (0.018) |
| Mung beans* | 0.91 (0.008) | 0.67 (0.010) |
| Meat & bone meal | 0.34 (0.034) | 0.24 (0.010) |
| Blood meal | 0.08 (0.018) | 0.10 0.029) |
| Fishmeal | 0.46 (0.018) | 0.52 (0.014) |
| Casein* | 0.77 (0.004) | 0.78 (0.003) |
| Skim milk powder* | 0.85 (0.003) | 0.89 (0.001) |

Table 1. Apparent faecal protein and energy digestibility coefficients of various protein sources for greenlip abalone, n = 4 except where indicated by * n = 3, standard errors are in brackets.



 Table 2. Comparison of apparent faecal protein (PD) and energy (GED) digestibility coefficients obtained for different protein sources fed to blacklip and greenlip abalone. Between species comparisons of nutrient digestion of each ingredient are made across rows,

 SEM = standard error of the mean.

| Ingredient | PD blacklip | PD greenlip | F | D | | GED blacklip | GED greenlip | | D | |
|----------------------|----------------|----------------|------------------|-----|-------|-----------------|-----------------|-----------|---------|-------|
| | abalone | abalone | F _{1,4} | Р | SEM | abalone | abalone | $F_{1,4}$ | Р | SEM |
| Defatted soyflour | 0.83 | 0.82 | 18.38 | ** | 0.730 | 0.83 | 0.78 | 0.73 | NS | 1.507 |
| Fishmeal | 0.56 | 0.46 | 27.72 | ** | 1.382 | 0.63 | 0.52 | 48.09 | * | 1.144 |
| Casein | 0.82 | 0.77 | 27.42 | ** | 0.624 | 0.79 | 0.78 | 4.02 | NS | 0.579 |
| Mung beans | 0.89 | 0.91 | 5.13 | NS | 0.630 | 0.65 | 0.67 | 2.40 | NS | 0.986 |
| Skim milk powder | 0.94 | 0.85 | 510 | *** | 0.286 | 0.95 | 0.89 | 1338 | ** * | 0.101 |
| Lupin 1 ^a | 0.91 | 0.91 | 0.03 | NS | 0.804 | 0.79 | 0.83 | 2.83 | NS | 1.780 |
| Lupin 2 ^b | 0.85 | 0.92 | 723 | *** | 0.211 | 0.70 | 0.82 | 66.19 | ** | 1.169 |
| Lupin 3 ^c | 0.84 | 0.91 | 371 | *** | 0.284 | 0.63 | 0.50 | 202 | ** * | 0.682 |

NS, not significant

* p < 0.05

** p < 0.01

*** p < 0.001

^a Whole *L. luteus*

^b Dehulled *L. angustifolius*

^c Whole *L. angustifolius*

Discussion of results from abalone digestibility studies

Digestibility of various protein sources for greenlip abalone

Greenlip abalone's poor digestibility of the energy from the legumes peas, beans and vetch may possibly be due to its inability to digest starches from terrestrial plant feedstuffs, starch being the main energy storage form in these legumes. This may also explain the low gross energy digestibility of maize meal which also contains starch. Although starch is present in red algae (termed floridean starch) it differs from the starch in these legumes as it consists of 99 % amylopectin, similar to waxy maize starch. Extrusion of starch containing legumes and maize meal is likely to improve the digestibility of their energy for greenlip abalone.

The lower digestibility of the oilseeds, sunflower meal and canola meal for greenlip abalone may be due to their antinutrients. Sunflower meal contains tannins, protease inhibitors and an arginase inhibitor (Tacon, 1995) that may have reduced its digestibility. Part of the growth depressing effects of canola meal have been attributed to its high sulfur content (Cheeke, 999). The high sulfur content alters anion-cation balance by increasing the anion fraction (Cheeke, 1999). This may have affected its digestibility in greenlip abalone.

The low protein digestibility of wheat gluten is unusual given the high protein digestibility of other plant protein sources for greenlip abalone. The extremely low gross energy digestibility coefficient obtained is obviously erroneous given that the protein digestibility coefficient was 0.51. The digestibility of wheat gluten for abalone should be re-evaluated at a range of inclusion levels.

Abalone's low digestibility of blood meal and meat meal may be due to the heat involved in processing these feedstuffs. Cheeke (1999) reports that the digestibility of blood meal is often low because of heat damage occurring in the drying process. The quality of meat and bone meal is often variable depending on the proportions of particular by-products included. The relative amounts of collagens and keratins can have a pronounced effect, keratin being a poorly digested protein (Cheeke, 1999).



Discussion on the digestibility of milk products and lupins for abalone is provided below in the comparison between species.

Comparison between abalone species

The results in Table II indicate that blacklip and greenlip abalone differ in their digestive capacity.

With regard to protein digestibility it is interesting to note that blacklip abalone can digest significantly more protein from, in general, non-plant derived protein sources (fishmeal, casein and skim milk powder) than greenlip abalone. In contrast, greenlip abalone can digest significantly more protein from plant derived sources (lupins) than blacklip abalone. This finding is in agreement with that of Wee et al. (1994) who reported that blacklip abalone digested significantly more protein than greenlip abalone from a manufactured diet containing 50 % fishmeal. It appears blacklip abalone may not be able to digest the soluble non-starch polysaccharides found in terrestrial plants as efficiently as greenlip abalone and that soluble non-starch polysaccharides may actually interfere with and reduce blacklip abalone's ability to digest nutrients (both protein and energy) from plant feedstuffs which contain them. As a consequence, use of exogenous enzymes that cleave soluble non-starch polysaccharides may improve the digestive capacity of blacklip abalone.

Dehulling had no effect on the digestibility of protein from *L. angustifolius* when fed to blacklip abalone. Although a significant increase was found in the digestibility of its energy for blacklip abalone after dehulling it was much less than was found for greenlip abalone (0.63 to 0.70 for blacklips compared with 0.50 to 0.83 for greenlips). After removal of the hull the energy from *L. angustifolius* changed from being significantly less to significantly more digestible for greenlip compared with blacklip abalone. The hull of the lupin is composed primarily of cellulose. It appears that blacklip abalone have a greater capacity to digest cellulose than greenlip abalone given that the removal of the hull had a much smaller effect on the capacity of blacklip abalone to digest energy from this lupin compared with greenlip abalone.

Milk based products (casein and skim milk powder) are very digestible sources of protein and energy for both blacklip and greenlip abalone. In particular, the sugar component of milk (lactose) is very digestible for abalone given the extremely high gross energy digestibility coefficient obtained for skim milk powder. Lactose is a disaccharide composed of galactose and glucose. Thus it is a much simpler carbohydrate than those found in many terrestrial plant based feedstuffs such as lupins which are composed of complex structural and storage polysaccharides. β -galactosidase (lactase) activity, needed for the hydrolysis of lactose, has been found in abalone (Oshima, 1931; Bennett et al.,1971). Obviously β -galactosidase activity in wild abalone would not be for the digestion of lactose, but probably for the breakdown of galactose, one of the major components of carrageenan which is found in the cell walls of red algae.

The results from this research demonstrate that greenlip and blacklip abalone have different digestive capacities and thus a different basis should be used for the formulation of manufactured diets.



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International advances in the utilisation of alternative protein sources

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Development and evaluation of grain products for fish

Introduction

Fishmeals are traditionally the major protein ingredients in fish feed. The supply of such feedstuffs is limited, and it is unstable due to over-fishing and fluctuations in important fisheries. Adding to this, fish feed accounts for more than half the total production costs in the fish-farming industry. Thus, novel and cheaper alternative ingredients are imperative to sustain further growth, profitability and sustainability of aquaculture.

In this respect, protein from grains is particularly interesting. The effort to develop vegetable protein feedstuffs for fish is two fold. One approach is to increase the use of inexpensive and crude ingredients, such as meals of leguminous (e.g. soy and lupin), cruciferous (e.g. rape), and sunflower seeds. However, such ingredients are rich in indigestible material (Bach-Knudsen, 1997). Thus, a complimentary approach involves the development of vegetable protein concentrates that meet the requirements by fish.

| Table 1. | Typical composition of commercial fishmeal and vegetable protein concentrates (% of |
|----------|---|
| | dry matter) |

| Protein source | Protein | Oil | Starch | NSP | |
|---|---------|-----|--------|-----|--|
| Fishmeal ^{1,2} | 78 | 12 | - | - | |
| Maize gluten ^{2,3} | 67 | 2 | 21 | 3 | |
| Wheat gluten ⁴ | 85 | 6 | 7 | - | |
| Potato protein concentrate ⁵ | 87 | 3 | - | - | |
| Soy protein concentrate ^{3,6} | 68 | 1 | 7 | 19 | |
| Isolated soy protein ⁶ | 91 | - | - | 3 | |

¹Anderson et al., 1992; ²Anderson et al., 1993; ³Bach-Knudsen, 1997; ⁴Storebakken et al., 2000a; ⁵Refstie and Tiekstra, 2002; ⁶Lusas and Riaz, 1995.

Most vegetable protein concentrates are manufactured from various by products that result from industrial production of starch (e.g. maize gluten, wheat gluten, and potato protein concentrate) or oil (e.g. soy protein concentrate and isolated soy protein). They may substitute for fishmeal without adding substantial indigestible bulk to the diet. If the concentrates contain more protein than does fishmeal, they also make room for cruder and cheap protein meals in lipid rich and energy dense feed formulations.

Antinutritional factors

Exploitation of vegetable protein sources for fish is limited by the presence of antinutritional factors (ANFs) in grains. Among the most potent of such components are enzyme inhibitors, agglutinating glycoproteins (lectins), inositol phopsphates (IP; e.g. phytic acid), non-starch polysaccharides (NSP), and antigenic proteins (reviewed by Storebakken et al., 2000b; Francis et al., 2001). Unless the ANFs can be inactivated or removed, the tolerance by fish restricts the use of such protein sources in fish feeds.

When manufacturing vegetable protein concentrates, proper heating and subsequent extraction procedures inactivate and remove most antinutritional factors (reviewed by Refstie and Storebakken, 2001). Cruder vegetable protein meals (e.g. extracted and toasted oilseeds) might, however, contain



significant quantities of heat-stabile ANFs (e.g. IP, soluble NSP, and allergens). Thus, extensive use of such protein meals in fish feeds requires the development of feasible feed enzymes.

Feed enzymes

It is well established that enzymes may be used to degrade ANFs and to help the fish in digesting its feed, but the development of enzyme-based technology for fish feed has only just begun. Important current research targets concern identification and characterisation of ANFs and development of suitable enzymes by enzyme engineering technology. Optimal enzymes need to withstand the harsh conditions during feed production (e.g. extrusion; heat stability is important), while they at the same time need to be psychrophilic, and thus active at the low temperatures found in the fish intestine. Enzymes that are only used for preconditioning feedstuffs as a part of the feed manufacturing process need to have intermediate stability, high activity, and to be degradable.

Traditional evaluation of feed ingredients

Ingredients for fish feeds must satisfy criteria set by national and/or international authorities. Such criteria include standards for ingredient composition, hygienic quality, and inherent health hazards, which must be determined and specified. Thus, potential fishmeal substitutes must be thoroughly characterised to justify evaluation in fish.

As reviewed by Refstie (2000), substitutes for fishmeal in fish feeds are traditionally evaluated by digestibility estimation, growth study with comparative slaughter, or a combination of these methods. Assuming that digestibility coefficients are additive, and given that coefficients are known for all ingredients, the digestibility, and thus nutritional value of a diet, is often calculated from the diet formula by linear programming. However, nutrient classes and other components (e.g. NSP) in different feed ingredients often interact to affect the overall absorption of nutrients by fish, in particular of lipid (Refstie, 2000). Such non-additive effects are little studied and not quantified in fish. It follows that the nutritional value of a given fish diet formula should actually be based on direct measurements. This is impracticable, and illustrates the need to study nutrient interactions to develop prediction equations for digestibility in farmed fish with adequate correction factors. When developed, it is important that these equations gain general acceptance by the fish feed industry.

For determination of tolerance for potential fishmeal substitutes by fish, dose-response growth studies with incremental replacement of fishmeal have been the preferred method. Fishmeal substitutes may be limiting in one or more indispensable amino acids, but this is overcome by dietary supplementation with crystalline amino acids or combination of ingredients with complementing amino acid profiles (Refstie and Storebakken, 2001). Harder to overcome are active ANFs. Hence, characterisation and determination of tolerance levels for potential ANFs are imperative when evaluating novel vegetable protein sources for fish.

Palatability may be a pitfall when evaluating fishmeal substitutes by growth studies. Fishmeal is palatable to most fishes, and fish used in growth studies are usually pre-adapted to commercial fishmeal based diets. Other ingredients may contain different or lower concentrations of feeding attractants and/or unpalatable compounds, which may reduce the feed consumption. If fish adapt to a new diet, this effect may be temporary. However, even moderate reductions in the daily feed consumption may severely reduce cumulative growth (Einen et al., 1995). Thus, lag periods in feed consumption should be monitored and considered when introducing test fish to new dietary ingredients for biological evaluation.

It must also be stressed that farmed fish are a highly variable group of species. Even closely related fish species might respond differently to vegetable feedstuffs (Refstie et al., 2000). Thus, the nutritional value of a given feedstuff must be evaluated in every species of interest, and cannot be established for fish in general.

New strategies to identify and improve protein sources



The Research Council of Norway has recently initiated a Centres of Excellence (CoE) scheme. The centres will be devoted to long-term basic research, and the Aquaculture Protein Centre (APC) is the only CoE devoted to the field of aquaculture. APC will develop basic nutritional, physiological and technological knowledge needed to optimise the use of protein in feed for farmed fish.

APC is constituted of scientific personnel and resources from three active partners: The Agricultural University of Norway (host institution), the Norwegian School of Veterinary Science, and AKVAFORSK – the Institute of Aquaculture Research AS. Based on the strong points of each partner, APC integrates three main fields of research:

- 1) Protein metabolism and amino acid requirements;
- 2) Digestive physiology and responses to protein quality and antinutritional factors, and;
- 3) Processing to improve the nutritional value of feedstuffs.

The centre relies on close collaboration with an international network of research institutions, as well as on national and international industries that supply and process feedstuffs and fish feed.

The main focus of this work will be on vegetable protein sources. The work will combine traditional experimental approaches with methods in respirometry, molecular biology and gene transcription profiling. The multi-sided approach will determine the need for amino acids by fish, clarify digestive responses by fish to feedstuffs and feedstuff components, and use this information to optimise the exploitation and processing of available sources of protein for farmed fish.



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Aquaculture Diet Development in New Zealand

Michael Bruce

NIWA, New Zealand

Introduction to NIWA

Established in 1992 as one of nine New Zealand Crown Research Institutes (CRI's), NIWA's mission is to provide a scientific basis for the sustainable management and development of New Zealand's atmospheric, marine and freshwater systems and associated resources. As a CRI, NIWA operates as a stand-alone company with its own board of directors and its shares held by the Crown. Our full official name is National Institute of Water and Atmospheric Research Limited.

The company has a staff of around 650 and annual revenue of more than \$75 million derived from competition-based research grants and commercial enterprise.

The majority of NIWA's research funding comes from the Public Good Science & Technology fund, administered by the Foundation for Research, Science & Technology, and from the Ministry of Fisheries. NIWA staff also participate widely in international initiatives, representing New Zealand in such fora as the Intergovernmental Panel on Climate Change (IPCC) and the United Nations Environment Programme (UNEP).

NIWA's commercial clients include New Zealand and overseas governments; regional councils; industries such as energy, fisheries, forestry, dairy, horticulture, agriculture and aquaculture.

Spread throughout New Zealand, NIWA has its corporate headquarters in Auckland, main research campuses in Auckland, Hamilton, Wellington, Nelson, Christchurch and Lauder, and field offices in the smaller centres. Research vessels are maintained in Hamilton, Wellington and Christchurch. Subsidiary companies include NIWA Vessel Management Ltd (in Wellington), NIWA Australia Pty Ltd (in Brisbane), NIWA (USA) Inc and NIWA Environmental Research Institute (also in the USA).

NIWA collaborates in the operation of the Institute of Aquatic and Atmospheric Sciences with Auckland University and Centres of Excellence with Otago University, Canterbury University and Victoria University of Wellington.

NIWA has a project management-based structure, which enables synergies from strong multidisciplinary research and the ability to work in large integrated teams and to shift resources to meet clients' requirements.

The core business for the company falls into 5 main areas; Marine (oceanography, ecology, geomorphology etc), Freshwater (lakes and rivers), Fisheries (stock assessment, modelling and management), Atmosphere and Climate, and finally Aquaculture.

NIWA staff have been at the forefront of aquaculture development in New Zealand since the inception and has invested heavily in facilities and expertise to underpin the commercial success of aquaculture in New Zealand. NIWA's latest development has been the construction in 2002 of a dedicated warmtemperate water marine aquaculture production and research facility, Bream Bay Aquaculture Park in New Zealand's North Island.

The facility has exceeded expectations and continues to attract investors keen to develop new species for aquaculture. The close association with industry allows NIWA to be very commercially focussed and target specific production issues for the development of new ventures. Currently, NIWA has 4 companies on site working in association with NIWA developing the farming techniques for such species as abalone, mussel spat, Yellowbelly flounder, grouper and our own business of kingfish and long and short finned eels. In addition to Bream Bay NIWA has two other aquaculture facilities; Mahanga Bay, Wellington (cold water marine; urchin roe enhancement and seahorse) and Silverstream, Christchurch (salmon smolt production).







Figure 1. NIWA facilities

Aquafeed Development in New Zealand

Due to the upsurge in aquaculture many companies are now showing great interest in the development of aquafeeds. The majority so far have concentrated on the less mainstream species (in global terms) or specific ingredients for the enhancement of growth and/or health. To date commercial investment has come from companies not traditionally connected to the aquafeeds market. The benefit for these companies is the potential for less capital investment. Through collaboration with NIWA these companies can now target new (to them) niche markets or tertiary markets where value and margins are high, making use of the facilities and expertise they already possess.

However, as more companies are now investing in aquaculture production a greater degree of interest and effort is now being devoted to aquafeed development.

Eel

NIWA has for many years been investigating the natural ecology of this species in a bid to understand how to sustainable manage the natural fishery to supply potentially lucrative export industry.

As the life cycle has remained unclosed NIWA has been forced to concentrate on the on-growing of glass eel, newly returned from the sea, and elvers. Work on both life stages has been targeted at ensuring that the greatest proportions of captured animals begin feeding. So far NIWA has concentrated on making use of commercially available diets employing strategies such as starvation and moist transitional diets to coax the animals into feeding under culture conditions

The results so far have been promising with equivalent growth rates and performance to other species of eel investigated.

Abalone

The culture of abalone is another aquaculture industry in New Zealand, which looks set to be a success, and has attracted many investors drawn by the readily available and so far undersupplied markets in Asia. Currently, NIWA is in the process of developing a programme of research and development to investigate the formulation of production diets.

Mussel

Many people, when considering aquaculture in New Zealand, think only of mussels. Indeed the industry has proved to be a great success for the country generating multimillion-dollar revenues from exports expanding at around 10% per annum.

The mussel industry is predominantly situated in two areas. The first and foremost is located in the Marlborough Sound on the top of the South Island. The second area is around the Coromandel peninsula in the North Island. Only 9% of New Zealand's mussel production comes from outside these two areas.



Companies in New Zealand have high hopes and intentions of expanding the industry further. In fact the target over the next ten years would see a 4 fold increase in production accompanied by a trebling of revenues.

However, to achieve this mussel producers in New Zealand must overcome their reliance on wild caught spat as approximately 90% of spat are derived from one location, Ninety Mile Beach in the North Island.

The challenges are not simply due to where and how the spat are sourced, but how many are retained after reseeding onto longlines. Generally, the rule is the larger the animals at reseeding the higher the degree of retention. However, the size of collected spat can be highly variable and resulting in a significant proportion being graded out and discarded to encourage a higher degree of retention.

Hence, a commercial collaboration between NIWA Sealord Shellfish Ltd is currently investigating ways to on-grow spat using artificial diets to guarantee a higher rate of retention. At the moment the most effective and reliable way to on-grow spat is by using algae which is inherently expensive. The current programme of research is geared to significantly reducing or even removing the algal component of the diet and replacing it with artificial diets.

A further component of this project is the production of cultured spat which entails the development of broodstock conditioning diets to maximize spat production and quality.

Sea Horse

Interest for seahorse aquaculture has been high in New Zealand for several years now. Commercial opportunities are available in both the Chinese traditional medicine market and the aquarium trade. However, both require different approaches to be taken. The aquarium trade is generally high value, which allows a more intensive approach could be taken, and still generate large profits. Seahorses destined for the Chinese medicinal market are considerably less valuable and expected to be larger but the numbers demand are very large. To satisfy this market and still make a suitable profit means taking a more extensive approach, possibly cage culture at sea.

Both approaches are beginning investigated by NIWA. However, only the intensive production for the aquarium trade requires any form of diet development as cage culture would rely on natural zooplankton populations. To date work has concentrated on feeding regimes, enrichment combinations and optimisation of intensive *Artemia* production through recirculation technology.

Sea Urchin

Sea urchin in New Zealand are primarily fished for the domestic market. To date attempts to break into the export markets in Asia have been very limited. This lack of success lies in the perception of the New Zealand urchin where the roe of locally fished animals tends to be bitter in taste, small and variable in other factors such as colour. Like salmon, flesh colour is highly important when marketing the product. Therefore, the aim of NIWA's work on sea urchin (*Evechinus chloroticus*) is to enhance the roe of wild caught animals to an acceptable quality for Asian markets through development of a diet that would consistently increase the roe size and enhance roe colour.

Three test diets were assessed against the seaweed Macrocystis pyrifera used as control.

Diet 1 – A moist diet made using the formulation derived from the Norwegian Institute of Fisheries and Aquaculture or NIFA, successfully in Scandinavia and sourced from readily available local raw materials. 60% moisture.

Diet 2 –NIWA's own moist diet formulation fabricated from locally sourced cheap raw materials. 60% moisture.

Diet 3 - Wenger diet semi-moist extruded pellet diet. 30% moisture.

Diets 1 & 2 although semi-moist were water stable for 4-7 days due to binding technology used. Stability is important in urchin culture, which requires that the animals remain relatively undisturbed.



The high stability meant that the uneaten diet could be removed whole despite the time between cage service intervals.

The results of this preliminary trial showed that both the NIWA diet and the Wenger produced the highest gonad yields. However, of these two only the NIWA diet consistently produced the highest percentage of roe of an acceptable colour and remained water stable for 7 days.

Rock Lobster

Rock lobster represents a multi-million dollar export business for New Zealand and although the natural fishery is well managed it still suffers from considerable fishing pressure. Again aquaculture has the capability to relieve that fishing pressure and the potential to expand markets and increase income from additional exports. Industry interest is strong but as the life cycle is proving hard to close, financial support is derived mainly from government via the Foundation for Research Science and Technology (FRST). As in New Zealand the Australian government remains committed to establishing a rock lobster aquaculture industry through the RLEAS programme in which NIWA continues to be a participant. The programmes main emphasis is on the problematic phyllosoma stage of the rock lobster lifecycle.

Phyllosoma

NIWA's contribution to the RLEAS sub-programme has covered several important topics. However, those pertaining to nutrition for phyllosoma include the:

- Assessment of larval feeding behaviour for feed development
- Production of artificial feeds
- Assessment of consumption of artificial feeds
- Assessment of potential attractants for inclusion in artificial feeds
- On-growing

In addition to phyllosoma NIWA continues to develop cage culture in conjunction with commercial partners. As well as cage design NIWA is putting effort into developing diets and diet delivery systems suitable for cage culture, where the emphasis is on low maintenance. Hence diets must be durable and water stable for extended periods. Currently, NIWA is assessing diets developed by the CSIRO in Brisbane as well as diets developed in-house. Topics under investigation include specific components to enhance growth through improving moulting and improved lipid and carbohydrate utilisation.

Bioactives & Probiotics

As aquaculture continues to expand and become more intensive it inevitably leads to a greater risk of disease outbreak. Good farm practice and vaccination can help reduce this but as a last resort antibiotics are the only recourse. However, there are many problems associated with the prolonged use of antibiotics, such as the creation of resistant strains potentially harmful to the cultured species and aquaculturists alike. The NIWA bioactives team in conjunction with two commercial partners is investigating the use of naturally derived bioactives for non-specific immune enhancement and probiotics, for larval diets during the live feed stage of production.

The bioactive combinations tested so far have are presented as a complete enrichment formulation for *Artemia*. The initial trails have proved successful with no difference in survival between test groups challenged with known finfish pathogens, and unchallenged controls. The probiotics identified by NIWA have also performed well against known aquatic pathogens such as *Vibrio harveyi*.

Conclusion



Despite the government moratorium, interest in aquaculture continues to increase in New Zealand. To satisfy demand NIWA continues to be involved in the development of mainstream aquaculture diets. However, the average man in the street today is more aware of food health and safety issues and expects higher standards of food production from every sector particularly aquaculture. In response to this changing public awareness NIWA's approach has been to target the development of functional feeds with attributes designed to improve the health of the culture animal and provide safer foods for a more discerning consumer.

NIWA wishes to thank Dr Robert van Barneveld for the invitation to talk at the Aquaculture Nutrition Sub-programme workshop and FRDC and the participants of the RLEAS programme. Collaborative initiatives such as this one on aquaculture nutrition bring together expertise from both sides of the Tasman covering a broad range of aquaculture issues adding value to Australasian aquaculture efforts.



Increasing the profitability of snapper *Pagrus auratus* farming by improving hatchery practices and diets; collaborative research between NSW Fisheries and the Aquafin CRC

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Industry status in NSW

Farming of marine fish in NSW is developing and is principally based on the seacage growout of snapper, *Pagrus auratus* and mulloway, *Argyrosomus japonicus*. Development of the snapper industry remains in its infancy in NSW and indeed Australia, and expectations for its future development are uncertain. At this time, there are only two commercial operations producing snapper in NSW and their combined total production has climbed from about 24t in 2001/2002 to an estimated 40t in 2002/2003. Initially, the attractiveness of culturing snapper was based on the fact that it was well regarded by Australian consumers and that the same species (Pagrus major = Pagrus auratus) is cultured in Japan where they consistently produce about 60-70 000t per year. Farmed snapper for the Australian east-coast market are mostly sold as 450-550g plate-size fish with markets established in Sydney, Melbourne, Brisbane and Newcastle where they generally retail for about \$9.00 kg⁻¹. More often than not, the market demand in NSW for wild caught and farmed snapper exceeds the available supply and local markets are often supplemented with snapper imported from the commercial catches in Western Australia and New Zealand. However, the commercial catches of Australian snapper are relatively static with only about 249t and 2167t of snapper caught in NSW and Australia each year, respectively.

In NSW, participants in the fledgling snapper industry have identified several major problems that currently hinder the major expansion and viability of this industry. These include a reliable supply of high-quality, cheap fingerlings; reliance on expensive live feeds such as artemia; the high incidence of disease induced mortality due to mostly protozoan infections and the lack of cost-effective, high-performance, species specific diets for both hatchery and grow-out phases of production. Currently in NSW, snapper farmers rely on feeds formulated for species such as barramundi *Lates calcarifer* and Atlantic salmon *Salmo salar*. Other issues of concern to the snapper industry include the dark pigmentation of farmed reared snapper. The solutions to these problems are currently being investigated by NSW Fisheries in collaboration with the Cooperative Research Centre for the Sustainable Aquaculture of Finfish (Aquafin CRC) and a select group of industry participants (Aquafin CRC Annual Report 2001/2002). In keeping with the aims of the Aquaculture Nutrition Subprogram (ANS) workshop, the information we present here relates only to several nutrition experiments conducted for the Aquafin CRC that aim to assist in the development of a specific diet or diets for the aquaculture of Australian snapper.

Experimental approach

Our goal is to develop and formulate diets for snapper that satisfy but do not oversupply essential nutrients. Ideally, these diets should reduce reliance on imported fish meals and fish oils and be manufactured from premium quality, highly digestible and well utilised ingredients. New diet formulations should also aim to maintain the normal fatty acid composition of fish so consumers continue to benefit from eating fish with high levels of HUFA.

Digestibility trials

Our research with snapper commenced with experiments to determine the apparent ingredient digestibility coefficients (ADC) for a selected range of protein and energy sources. These experiments have been conducted using indirect methods of determination. For protein ADC's the experiment was



based on a reference diet composed mainly of low temperature fish meal and extruded wheat. This reference diet was substituted with two different dietary inclusion contents of meat meal, poultry offal meal, haemoglobin meal, blood meal, solvent extracted soybean meal and a solvent free, low allergenic soybean meal (Table 1). All diets contained 0.5% chromic oxide as the inert marker and were fortified with vitamin C. Each diet was randomly allocated to three 170L digestibility

| | Apparent digestibility coefficient (%) | | | | | |
|--------------------------|--|---------|--------------|--|--|--|
| Ingredient & % inclusion | Organic matter | Crude | Gross energy | | | |
| | | protein | | | | |
| Meat meal (30%) | 63.5 | 62.2 | 72.0 | | | |
| Meat meal (50%) | 63.2 | 65.3 | 70.5 | | | |
| Poultry meal (30%) | 88.5 | 84.9 | 91.1 | | | |
| Poultry meal (50%) | 90.6 | 86.9 | 91.4 | | | |
| Haemoglobin powder (15%) | 73.8 | 95.1 | 79.5 | | | |
| Bloodmeal (15%) | 80.4 | 81.6 | 81.3 | | | |
| Soybean meal (30%) | 57.1 | 87.2 | 66.8 | | | |
| Low allergenic soy (30%) | 56.6 | 90.7 | 64.3 | | | |

 Table 1.
 Apparent organic matter, crude protein and gross energy digestibility coefficients for different levels of selected protein sources fed to juvenile Australian snapper *Pagrus auratus*

tanks (Allan et al., 1999) which contained seven fish (c.a. 90g). Fish were fed to excess once daily over a period of 3h using spring loaded belt feeders. Faeces was collected from clean tanks (overnight; 18h) by passive settlement for 23 days. Water temperature was maintained at 24°C. For energy ADC's, experimental protocols were identical to those described above except that in this experiment each digestibility tank was stocked with seven c.a. 70g fish and the experiment was run for 30days. In this experiment we utilised two reference diets. The first was a fish meal based diet used to determine the ADC's for three different dietary inclusion contents of extruded wheat and the second was a fish meal / extruded wheat based reference diet used to determine the ADC's of two different dietary inclusion contents of cod-liver oil and one level of a low temperature fish meal (Table 2).

| Table 2. | Apparent organic matter | , crude protein a | and gross energy | digestibility coefficients for |
|-----------|-----------------------------|-------------------|-------------------|--------------------------------|
| different | t levels of selected energy | sources fed to | juvenile Australi | an snapper Pagrus auratus |

| | Apparent digestibility coefficient (%) | | | | | | |
|--------------------------|--|---------|--------------|--|--|--|--|
| Ingredient & % inclusion | Organic matter | Crude | Gross energy | | | | |
| | | protein | | | | | |
| Extrude wheat (20%) | 75.6 | 100.6 | 80.5 | | | | |
| Extrude wheat (30%) | 73.7 | 105.4 | 76.9 | | | | |
| Extruded wheat (40%) | 69.4 | 100.1 | 74.4 | | | | |
| LT fishmeal (50%) | 98.9 | 94.3 | 99.2 | | | | |
| Cod liver oil (15%) | 106.0 | Na | 100.5 | | | | |
| Cod liver oil (25%) | 100.2 | Na | 98.3 | | | | |

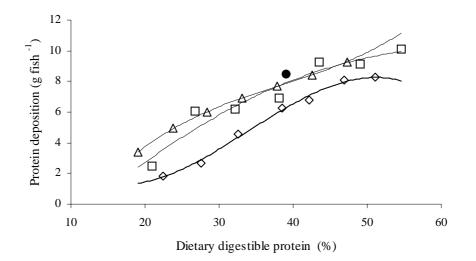
Protein meals were well digested by juvenile snapper and digestibility of meat and poultry meals appeared to be little affected by the inclusion contents we tested. However meat meal was less digestible than poultry meal in all respects. The two soybean meal products exhibited similar digestibility coefficients but their organic matter and energy digestibility was lower than all the other protein meals we tested. Protein digestibility of extruded wheat was extremely high, but as expected organic matter and energy digestibility decreased with increasing inclusion content. Both low temperature fish meal and cod-liver oil were highly digestible (Tables 1 and 2).

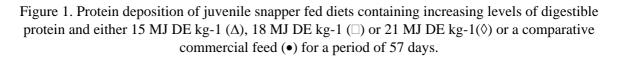
Requirement and utilisation studies

The effects of dietary digestible protein and energy content on the weight gain and performance of juvenile snapper *Pagrus auratus* was evaluated empirically. The experimental design called for seven



dietary digestible protein contents to be formulated at each of three digestible energy contents; 18, 21, and 15MJ DE kg⁻¹. Diets were formulated based on previously determined individual ingredient digestibility coefficients. For each of the three energy levels, a high (summit) and low protein (diluent) diet was formulated. The range of dietary digestible protein contents was then obtained by mixing the summit and diluent diets at the required ratio's. In total, 21 test diets were manufactured. To ensure performance of snapper on the test diets was relative, a commercial barramundi diet was included as an internal control. Diets were manufactured on a dry weight basis using high quality low temperature fish meal, extruded wheat, cod liver oil and two inert fillers. All diets were fortified with 1.5% vitamin / mineral premix including 0.1% vitamin C. Sixty-six experimental units (200L floating cages housed in 10000L tanks) were each stocked with eight juvenile snapper (c.a. 30g) and each dietary treatment was randomly allocated to three experimental units. All fish were acclimated on the commercial control diet for 10 days prior to being switched to experimental diets. Fish in each cage were carefully hand fed twice daily (≈ 0830 and ≈ 1430) to apparent satiation for six days a week. Fish were fed once daily to apparent satiation on Sundays (≈ 0830h). The experiment was conducted at a temperature of approximately 24°C and was completed after 57 days. At completion of this experiment all fish were individually weighed and two fish from each cage were randomly selected and killed to determine chemical composition. The chemical composition of diets and constituent ingredients was also determined.



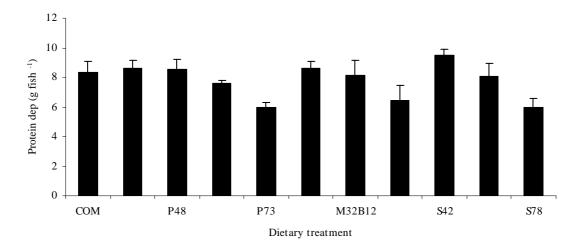


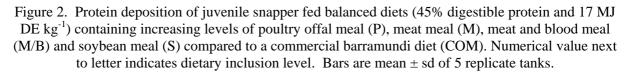
Fish health during this experiment was excellent and 100% survival was recorded for all treatments. Irrespective of digestible energy content, weight gain and protein deposition increased with increasing contents of dietary digestible protein. However, protein deposition in snapper fed diets containing 15 MJ DE kg⁻¹ and 17 MJ DE kg⁻¹ was higher than snapper fed the diet series containing 21 MJ DE kg⁻¹ (Figure 1.). Diets containing about 45% digestible protein and 17MJ DE kg⁻¹ should be sufficient to promote good growth and protein deposition in snapper.

Using the protein and energy requirement values determined in the previous study and ADC's for ingredients already described (Tables 1 and 2), the utilisation by snapper of four locally produced agricultural ingredients was tested using growth assay. These ingredients were poultry offal meal, meatmeal, bloodmeal and solvent extracted soybean meal. All test diets were formulated to a single digestible protein (45%) and energy content (17MJ DE kg⁻¹). The dietary inclusion content of these test ingredients was increased at the expense of fishmeal, extruded wheat or fishoil, with the remainder balanced by small amounts of carboxy-methyl-cellulose or diatomaceous earth. A commercial barramundi diet that had been evaluated in terms of growth with snapper was used as an internal control. Diets were manufactured on a dry weight basis and all diets were fortified with 1.5% vitamin



/ mineral premix including 0.1% vitamin C. In total, 12 diets were evaluated in this trial. Fifty-five juvenile snapper (c.a. 14g) were stocked into each of 55 experimental units (200L floating cages housed in 10000L tanks) and each dietary treatment was randomly allocated to five experimental units. Fish were fed twice daily (0830 and 1500h) to apparent satiation and once on Sundays (0830h). At completion of the experiment (50 days), all fish were individually weighed. Three fish from each cage were randomly selected and killed to determine chemical composition. The chemical composition of diets and constituent ingredients was also determined. Protein deposition for snapper fed diets containing either 36% or 48% poultry meal, 35% meat meal and 42% soybean meal matched the protein deposition of fish fed the commercial control diet (Figure 2). FCR' ranged between 1.4 and 2.0 for all treatments (1.47 for control diet) and tended to increase in response to increasing contents of test ingredients.





Conclusions

The apparent digestibility coefficients (ADC's) for the ingredients we have presented have proved useful in formulating and testing diets to investigate the digestible protein and energy requirements of juvenile snapper. They have also proved useful in formulating diets to investigate the utilisation of promising ingredients at different inclusion contents. These evaluations coupled with further research will assist in the development of commercial diets for this species.



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Commercial production of soy protein concentrates for use in aquafeed

Will Tidswell

Hyfeed Scientific Feeds, Queensland

FISH MEAL REPLACEMENT: Soy Protein Concentrate (SPC)

Why SOY

- Constant supply
- Good average price
- Value add with a variety of quality products

Constant Supply

- Australia is guaranteed to have droughts.
 - Ability to import raw material so as to have continuity of supply for customers.
 - Crushing plants in Australia are currently using imported beans/ not canola.
 - Large oilseed processors have ability to import.
 - Soy beans need to be processed lupins do not.
 - Purchasing competition from other industries based on what they see the feed valve of the ingredient.
 - Need raw material availability .
 - THROUGH PUT TO COVER CAPITAL INVESTMENT.

Value add

- OIL is a HIGH valve commodity
- Food
- Feed
- Manufacturing
- Variety of products and markets

Getting value for the oil helps keep the protein cost down.

Why (SPC)

- Known feed/food value
- Variety of applications
- Feed/food

First Constraints

Price of Soy protein concentrates was too high, limiting use. The traditional process to produce Soy protein concentrates needed to be modified so as to reduce the cost of processing. It is the cost of production that influenced the price far more than the cost of the raw materials.

Cost of production

- Raw materials
- Labor, expertise
- Management
- Plant (capital investment return)
- Maintenance



- Marketing / research
- Taxes / insurance
- Warehousing
- Transport / distribution

How to Process?

Process Considerations

- Variations in process will give different results.
- Every equipment manufacture has the best equipment.

Processing

- Drying / Grading
- Steeping / Hulling
- Oil extraction / cooling / milling
- Protein isolation
- Drying

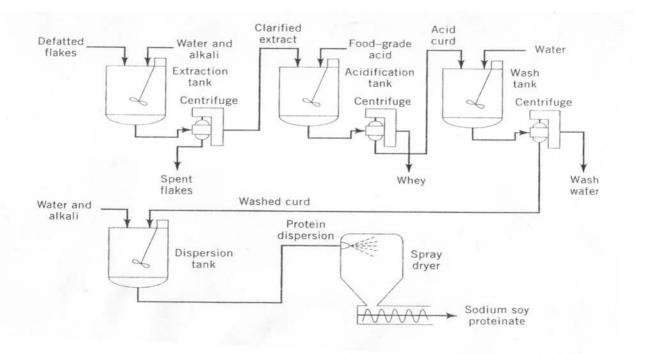


Figure 1. Isolated Soy Protein Process

3 Main Products

- 1. HE Soy (\$100 over solvent) (48% protein, 6% oil, ash)
- 2. LA Soy (\$200 over solvent) (53% protein, 4% oil, ash)
- 3. LA Soy/High Pro (\$1100 /mt) (65% protein, 4 % oil, ash)



Product benefits

- High energy
- Low ash
- Low antigens
- Lecithin
- Oil quality

During the process to produce solvent extracted soy meal, two byproducts are blended back into the meal so as to reduce mill waist and value add these ingredients:

- rancid fats , soaps etc from the oil processing

-Hulls , added back to give a constant protein in the meal.

Protein of soy meals 44% or 48%, traders do not always specify which level of protein they are supplying.

HE Soy

- High energy / Full Fat Soy
- Replace Full Fat Soy
- Cost Effective
- 48% Protein (44% solvent soy)

LA Soy

- High energy / Full Fat Soy
- Low antigens
- Prawn diets
- 50% protein
- Hold oil / low water solubility

LA Soy (65%)

- High Energy /High in protein
- Low antigens
- Prawn diets
- 65%
- Protein
- Hold oil / low water solubility



| | Soybeans Raw | Solvent Soy Meal 47% Protein US | Soy Isolate 65-75% Protein | LA Soy Protei |
|-----------------------|-----------------|---------------------------------------|-------------------------------|---------------|
| Urease Activity | 2.0 | 0.05 | 0.01-0.03 | 0.02-0.03 |
| Trypsin Inhibitor mg | 45-50 | 5.0-8.0 | <1.25 | <1.5 |
| Glycinin Antigenicity | <15 | 13-15 | <1 | <1 |
| | 184,000 | 66,000 | <1 | <30 |
| B-conglycinin | <15 | 13-15 | <1 | <1 |
| antigenicity | 69,000 | 16,000 | <1 | <10 |
| Lectins meg/g | 3600 | 10-200 | <0.1 | < 0.1 |
| Saponins, % | 0.5 | 0.6 | 0 | 0 |
| Oligosaccharides, % | 14 | 15 | <3 | 10 |

Table 1 LA Soy-low levels of anti-nutritional factors.

| | Full Fat soy Expanded | Full fat Soy 36% Protein | Soy flour 48% Protein | Expeller Soy Meal 48% | |
|-----------------------|--------------------------|-----------------------------|--------------------------|--------------------------|--|
| | F | (Baking flour) | (Baking flour) | Protein | |
| Urease Activity | 1.0 | 2.0 | 0.06 | 0.05 | |
| Trypsin Inhibitor mg | 8.0 | 50-55 | 5.0-8.0 | 5.0 | |
| Glycinin Antigenicity | 10-15 | 15-20 | 13-15 | 10 | |
| | 70,000 | 190,000 | 66,000 | 66,000 | |
| B-conglycinin | <15 | 15 | 13-15 | 13 | |
| antigenicity | 20,000 | 69,000 | 16,000 | 16,000 | |
| Lectins meg/g | 11-200 | 3600 | 50-2000 | 10-200 | |
| Saponins, % | 0.6 | 0.5 | 0.6 | 0.6 | |
| Oligosaccharides, % | 15 | 14 | 15 | 15 | |

Soy proteins vary in composition depending upon their intended end use.

Not only do the major constraints change such as protein, oil and fiber but the anti nutritional factors also vary between processor.

| Table 2. | Amino | acid | NIR | V's fish |
|----------|-------|------|-----|----------|
|----------|-------|------|-----|----------|

| SAMPLE | | Protein | Lys | Met | Thr | Try | Val | Ile | Leu | Phe | His | Arg | Digestability |
|-----------|---------|---------|------|------|------|------|------|------|------|------|------|------|---------------|
| | Total | | | | | | | | | | | | |
| LA Soy | protein | 59.62 | 3.33 | 0.87 | 2.31 | 0.68 | 3.24 | 2.72 | 4.81 | 3.00 | 1.56 | 4.37 | 87.80% |
| 2 | Avail | | | | | | | | | | | | |
| LA Soy | Protein | 59.62 | 2.67 | 0.84 | 1.99 | 0.64 | 2.85 | 2.47 | 4.17 | 2.66 | 1.39 | 4.15 | |
| | Total | | | | | | | | | | | | |
| Fish Meal | protein | 57.87 | 3.95 | 1.59 | 2.45 | 0.66 | 2.84 | 2.49 | 4.12 | 2.16 | 1.52 | 3.21 | 96% |
| | Avail | | | | | | | | | | | | |
| Fish Meal | Protein | | 3.74 | 1.52 | 2.28 | 0.65 | 2.97 | 2.39 | 3.58 | 2.1 | 1.52 | 2.97 | |

NIR AAnalyzer Report on LA Soy when compaired with Fish Meal

Cost effective proteins underutilised

Protein Hydroslates

- High in protein
- Low in ash
- Partly digested
- Enhanced flavor
- Cost effective drying



The use of fish hydroslates will increase the palatability of diets that are low in fish meal /oil

Protein Hydrolysates

- Fish based
- Fish market / processors
- Irrigation water storage
- Effluent ponds Mullet, Euro Carp.

Research

- Fish type / feeding system/location
- Hydroslates / process refinement
- Protein quality after drying.

Research (Feeding)

- L.A Soy 65% protein
- L.A Soy 50% protein
- Dry Protein Hydroslates



Commercial aquafeed production priorities for vegetable protein alternatives

Rhys Hauler

Skretting Australia, Tasmania

The diversity of finfish species in Australian Aquaculture presents a challenge for local aquafeed manufacturers. Over recent years marine finfish culture has matured and demanded unique development in feed design. More specifically, with Barramundi (*Lates calcarifer*) and Yellowtail kingfish (*Seriola lalandi*) grown-out to 4kg and Southern Bluefin Tuna (*Thunnus maccoyii*) on-grown to 50kg there is a need to produce pellet sizes that far exceed the requirements set by the salmonid industry. Aquafeed manufacturers now require vegetable protein alternatives that are not only highly digestible and palatable but also demonstrate beneficial functionality in the extrusion process.

Commercial priorities for vegetable protein alternatives

A history of programs has been undertaken to evaluate the nutritional value of alternative protein sources for Australian finfish species. Methodological evaluation has generally included digestibility and palatability evaluation followed by inclusion in complete diets. The outcomes from this approach have always been useful to generate technical specifications and confidence in an ingredient for commercial application. However as commercial formulation becomes more sophisticated there is a greater necessity for standard, commercially applicable data. Commercial formulation now requires digestibility coefficients for amino acid, protein, phosphorus and energy as standard. In addition, there is a specific need for salmonid digestibility coefficients in both freshwater and saltwater due to a subtle differences that are now considered.

Further priorities for vegetable protein alternatives are species specific, particularly when substitution opportunities are considered (Table1). Vegetable protein alternatives for commercial salmonid diets should aim to be cost-effective – and preferably be of Australian origin. For salmonid diets it must be emphasised that commercial manufactures are not necessarily aiming to replace fish meal. It is likely that fishmeal inclusion is at a minimum and vegetable protein alternatives must substitute other vegetable proteins or land animal proteins. The general implication is that vegetable protein alternatives will only be included at a lower cost per unit protein. Vegetable protein alternatives in commercial marine finfish diets should aim to have high utilisation (palatability and digestibility) and a degree of functionality for large pellet manufacturing (discussed later). As the nutritional requirements of marine species still remains to be defined the substitution possibilities of vegetable protein alternatives are more flexible than salmonids. In this case, vegetable protein alternatives could be assumed to replace other vegetable proteins or fish meal.



| Species | Pellet size (mm max.) | Commercial priorities |
|------------|-----------------------|--|
| Salmonids | 9 | <u>Emphasis:</u> Cost-effective <u>Value in:</u> Low-cost, Australian based <u>Substitution opportunity:</u> Vegetable protein replaces vegetable protein or LAP |
| Barramundi | 15 | <u>Emphasis:</u> High utilisation <u>Value in:</u> High palatability/digestibility, improved functionality <u>Substitution opportunity:</u> Vegetable protein replaces vegetable protein or fish meal |
| Yellowtail | 15 | <u>Emphasis:</u> High utilisation <u>Value in:</u> High palatability/digestibility, improved functionality <u>Substitution opportunity:</u> Vegetable protein replaces vegetable protein or fish meal |
| Tuna | 25 | Emphasis: High utilisation and functionality <u>Value in:</u> High palatability/digestibility, improved functionality <u>Substitution opportunity:</u> Vegetable protein replaces fish meal |

Table 1. Commercial priorities for vegetable proteins by finfish species

Functionality of vegetable protein alternatives

Large pellets for marine finfish culture requires a focus on alternative vegetable proteins with favorable functionality. In this case, vegetable proteins are required to display a change in viscosity during the conditioning and forming in the aquafeed extrusion process. The most versatile instrument to identify the cooked viscous properties of ingredients is a Rapid ViscoTM Analyser (RVATM).

A classic RVATM profile assumes a heat-hold-heat cooking cycle for a particular vegetable protein. The viscosity curve produced during the heating and cooling of an ingredient show a similar characteristic pasting curve (Figure 1). Generally unmodified ingredients have a low solubility at low temperature. However, when heated in water beyond a critical temperature they absorb water and swell. Early in the pasting test the temperature is below the critical gel temperature of the ingredient, and the viscosity is low. When the temperature rises above critical and the ingredient begins to swell and viscosity increases on mixing when these swollen granules have to squeeze past each other. The critical temperature provides an indication of the minimum temperature required in cooking a given ingredient.

As the temperature increase further the ingredient undergoes further swelling as it absorbs more water. Subsequent polymer alignment due to the mechanical shear reduces the apparent viscosity of the paste. The peak viscosity occurs at the equilibrium point between swelling that increases viscosity and polymer alignment that decreases viscosity. The peak viscosity indicates the water-binding capacity of the ingredient and it is often correlated with changes in final product quality, and also provides an indication of the viscous load likely to be encountered during extruder conditioning.

During the hold period of the test, the sample is subject to a period of high temperature and mechanical shear stress. This period is generally accompanied by a breakdown in viscosity. The rate of reduction in viscosity depends on the nature of the material itself. The ability of an ingredient to withstand this shear stress is an important stabilising factor in the extrusion process and the holding strength is the viscosity the ingredient manages to retain under a given shear stress. As the mixture is subsequently cooled, re-association between the ingredient molecules occurs, causing a formation of a



gel and the viscosity increases to a final viscosity. Final viscosity commonly indicates the ability of the material to form a viscous paste or gel after cooking and cooling.

As demonstrated in Figure 1, all vegetable proteins initially have a low viscosity at low temperature. However, when heated they behave differently – typically dehulled lupin meal has a lower pasting temperature and shorter pasting time than other vegetable proteins. Importantly, this short pasting time is within the time available in extrusion conditioning and forming and benefits aquafeed manufacture. Dehulled lupin meal also features a higher peak viscosity that is attainable within the extrusion period and demonstrates resistance to mechanical shear (holding strength) adding stability to the process. In comparison, extracted soybean meal and corn gluten meal demonstrates a higher pasting temperature and longer pasting time – typically outside the time available in extrusion conditioning and forming. Hence, is unlikely the extracted soybean meal and corn gluten meal undergo significant changes in viscosity and unlikely to reach the final viscosity indicated in Figure 1.

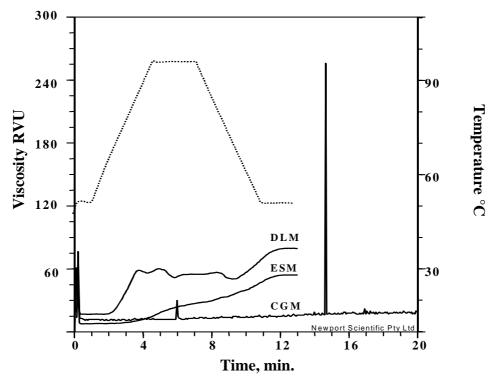


Figure 1. Pasting curve comparing dehulled lupin meal (DLM), extracted soybean meal (ESM) and corn gluten meal (CGM) with a standard temperature profile (dotted lines).

Conclusion

Skretting hold value in safe, nutritionally sound and functional vegetable protein alternatives in commercial aquafeed manufacture – although the substitution opportunity of these ingredients is species specific. Dehulled lupin meal has favorable nutritional and functional properties and will prove particularly useful in Australian marine finfish feeds.



Influence of environment on the diet composition and nutritional

requirements of salmonids

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Temperature is one of the most important factors influencing the growth of ectothermic animals such as fish. Fish will achieve maximum growth over a narrow range of temperatures and this temperature can be defined as the optimum temperature for growth. The optimum temperature for growth varies between species, between sizes within a species and between different strains of a species. When food is unlimited the growth achieved at the optimum temperature is essentially determined by the size of the difference between digested energy and energy lost via metabolism (Jobling, 1994). As temperature increases food intake increases to a peak from where it falls rapidly as temperature increases. Metabolism, in contrast, continues to increase with increasing temperature.

In Australia the most important questions about temperature effects on salmonids, principally Atlantic salmon, relate to how fish perform at high temperatures. High temperatures can be defined as temperatures above the optimum temperature for growth (and within the tolerance zone). Nothing is known about the optimum temperature for growth of Tasmania Atlantic salmon, and there are only a few easily accessible experiments for salmon generally. Optimum temperature for growth of Atlantic salmon have been given as 12-15 °C (Jobling 1981), 16-19 °C for hatchery reared parr , 16 °C for small parr from the English Lake District (Elliott & Hurley, 1997) and 18-19 °C for small parr from northern Norway (Figure 1) (Forseth et al., 2001). The main feature of these studies was the use of small freshwater parr.

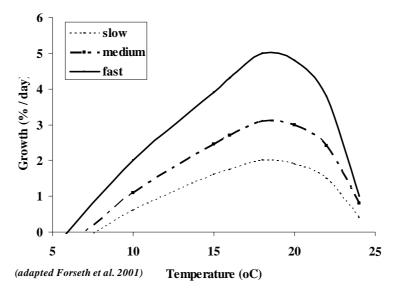


Figure 1. Predicted relationships between temperature and growth of one gram Atlantic salmon divided into slow, medium and fast growing individuals. The optimum temperature is approximately 19 °C.

The decline in growth at temperatures above the optimum is fairly dramatic (Figure 1) and suggests there will be severe restrictions imposed on the growth potential of salmon grown in Tasmania. Furthermore, larger fish usually have lower optimum temperature for growth than smaller fish and they may also be more sensitive to factors such as the decreased oxygen content as water temperature increases. The same data-set also shows that the optimum temperature for feed intake is, as would be



expected, above that for growth and that FCR (growth efficiency) has a more stable response to increasing temperature and is constant over a relatively wide range of temperature (Figure 2).

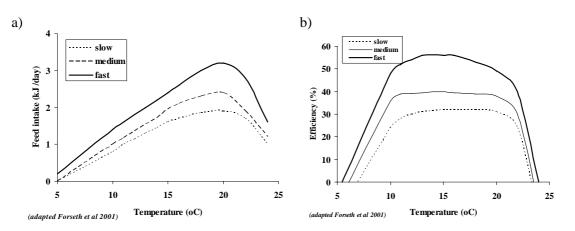


Figure 2. The relationships between temperature and a) maximum feed intake, and b) food conversion ratio (FCR) for Atlantic salmon divided into slow, medium and fast growing individuals.

The primary effect of temperature on nutrient requirements and on diet composition relates to the provision of energy. Above the optimum temperature for growth the exponential nature of the relationship between temperature and metabolism means that energy expenditure more and more rapidly approaches the maximum energy that can be ingested. The consequence is that growth, or the capacity for growth, decreases rapidly (Figure 1). Defining the optimum dietary protein to energy ratio remains a useful parameter and predictions that a higher protein to energy ratio is required at higher temperatures have been made. This is somewhat contradicted by the exponential increase in energy requirement for metabolism and may suggest problems with protein quality (amino acid supply) at higher temperatures. These issues remain to be resolved for Atlantic salmon grown in Tasmania.

The limited literature suggests some other interesting effects of high temperatures on nutrient requirements and diet composition. Recently, a condition named screamer disease, in which there were major bone deformities, was highlighted in Chile (Roberts et al., 2001). The name comes from the mouth being permanently agape. A combination of sea water transfer, low dietary phosphorus and vitamin C and high water temperatures (> 20 °C) caused the problem and highlighted that diets designed for cooler waters in the northern hemisphere may not be suitable for warmer Southern hemisphere waters. Clear evidence that it is a mistake to use nutritional information generated under Norwegian or Scottish conditions.

Carbohydrate digestibility in rainbow trout is higher at higher temperatures (Aguirre et al., 1995). This has the potential to be advantageous when using plant based protein meals in salmon feeds, it suggests that under Tasmanian conditions there may be more potential for using plant protein meals than would be indicated by research in other areas. In addition, salmon appear more able to metabolise carbohydrates at higher temperatures (Hemre et al., 1995). The physiological importance of polyunsaturated fatty acids partly relates to their function in cell membranes at low temperatures. This may make the replacement of fish oils more straightforward under Tasmanian conditions, however this hypothesis remains to be tested especially in view of disease challenge data (Carter et al., 2003).

In conclusion, our understanding of effects of high temperatures on nutritional requirements and diet composition is limited for salmon. Areas of immediate concern are determining the optimum protein energy ratios, ensuring vitamin and mineral requirements are met and testing the feasibility of alternative ingredients.

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Progress in the development of manufactured diets for larval species

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Microdiets for marine fish larvae - current development and progress

The Problem

Marine fish larvae fed microdiets have not, at this stage, matched the growth and survival performances demonstrated by larvae fed live feeds.

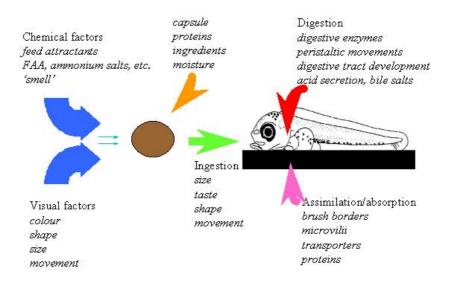
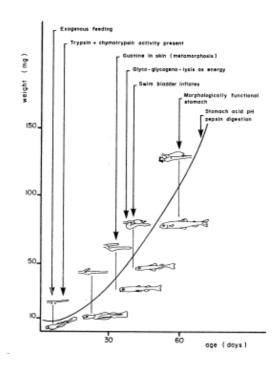
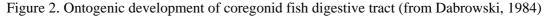


Figure 1. Factors affecting food particle utilisation







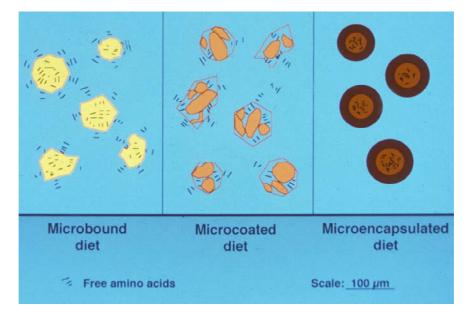


Figure 3. Micro diet types

Microbound and Microcoated diets

- Easy to prepare
- Cheap (relatively, depends on food ingredients)
- Easier digestion by the larvae (compared to microencapsulated diets)
- Amino acids and short peptides leaching (70% 90% lost in the first few minutes) to the water
- Disintegrating relatively fast in the water causing environmental problems
- Sinking fast

Microencapsulated diets

- Reduces and controls amino acids and short peptides leaching
- Retains as a whole capsule in the water for long period of time
- Controlled buoyancy
- Prevents lipids and other substance degradation
- Controlled digestion (can be adjust to digest at acid or basic pH)
- Poor digestibility (protein cross-linking technology)
- Expensive and complicated preparation

Hypothesis

Fish larvae utilise enzymes from their prey to facilitate the process of digestion.

- Direct donation of digestive enzymes
- Activation of zymogens and induction of endogenous enzyme secretion
- Live food autolysis
- Stimulating of pancreas enzymes and zymogens activation by neuropeptides factors resulting from live food autolysis



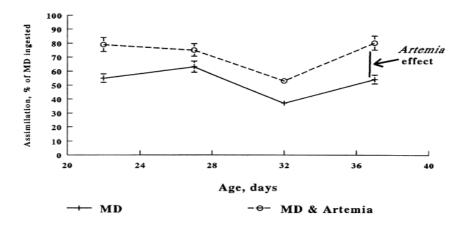


Figure 4.Assimilation rates of seabass *Dicentrarchus labrax* larvae co-fed microdiet (MD) and/or *Artemia* (from Kolkovski et al. 1997)

| Species | Live food organism | Findings | Authors |
|---|---|--|---|
| Carp Cyprinus carpio, Grass carp Ctenophayngodon idella, Salmon Salmo gairdneri, whitefish Coregonus lavaretus | Copepods, Cladocera, rotifer, Artemia | 10%-98% of proteolytic activity is due to the food organisms | Dabrowski and Glogowski (1977a) |
| Turbot Scophtalmus maximus | Artemia, rotifers, copepods | Exogenous digestive enzymes contribution: proteases 43-60% esterase 89-94% exonuclease 79-88% amylase 15-27% | Munila-Moran et al. (1990) |
| Herring Clupea herrengus | copepods | 0.5% of total trypsin content in intestine is derived from the live food | Pedersen et al. (1987), Pedersen and Hjelmeland (1988) |
| Whitefish Coregonus sp. | Monia sp. | 70% of the trypsin activity in intestine derived from the live food | Lauff and Hoffer (1984) |
| Japanese sardine Sardinops melanotictus | Rotifer protease | 0.6% of total protease activity in larvae | Kurokawa et al. (1998) |

Table 1. Digestive enzyme contribution by live food organisms

Do fish larvae possess enough enzymes to digest microdiets ?

- Proteins and other ingredients are hard to digest by larvae
- Binders used for microbond diets such as alginate and zein
- Proteins and synthetic polymers used for cross-linking with encapsulation methods
- Microdiets contains 60-90% dry matter compared to only 10% in live food organisms



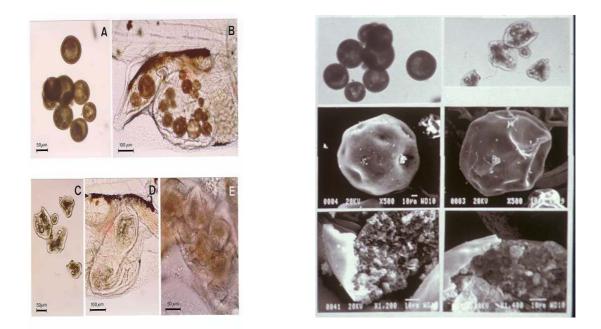
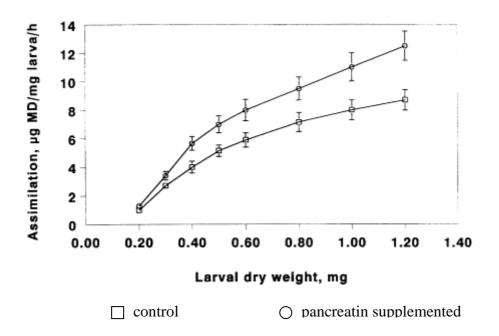
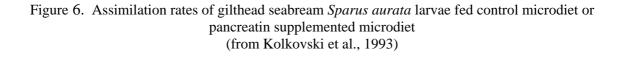


Figure 5. Microcapsules in 8 d old S. aurata larvae digestive system

Possible solution for the low digestion and assimilation efficiency in larval guts:



• Dietary Digestive enzymes





| Species | Enzyme supplementation | Findings | Authors |
|---|---|---|--|
| Carp Cyprinus carpio | bovine trypsin | increased proteolytic activity | Dabrowski and Glogowski (1977b) and Dabrowska et al. (1979) |
| Salmon Salmo salar | Dietary amylase | No effect on growth or protein utilization | Carter et al. (1992) |
| Salmon Salmo salar | Dietary mixture of pancreatic enzymes | Positive effect on growth and protein utilization in soybean based diet | Carter et al. (1994) |
| Carp Cyprinus carpio | polyzyme mixture | increased weight gain | Bogut et al. (1995) |
| Gilthead seabream Sparus aurata | pancreatin (porcine pancreatic extract) | 30% increase in MD assimilation, double growth rates | Kolkovski et al. (1993) |
| SeaBass Dicentrarchus labrax | pancreatin | no effect | Kolkovski et al. (1997c) |
| Gilthead seabream lipase Sparus aurata | | 300% increase in glycerol trioleate absorption in 45 day old juvenile. No effect on younger larvae | Koven et al. (1993) |
| Yellow perch Perca flavescens | pancreatin | no effect | Kolkovski et al. (1999a) |

Table 2. Dietary digestive enzymes supplementation in microdiets

Alternative Strategy

Supplementation microdiets with:

- Pre-digested protein sources (hydrolysates) Free Amino Acids ٠
- •



| Species | Hydrolysate or free amino acids supplemented | Findings | Authors |
|-----------------------------------|--|--|---------------------------------------|
| Atlantic salmon Salmo salar | Fish meal and mixture of free amino acids (<40%) | double weight gain | Espe and Lied (1994) |
| Rainbow trout Oncorhynchus mykiss | 54% mixture of free amino acids | no effect | Rodehutscord et al. (1995) |
| Atlantic salmon Salmo salar | 100% free amino acids | reduced weight gain | Espe and Njaa (1991) |
| Gilthead seabream Sparus aurata | 50% and 100% squid protein hydrolysate | reduced growth | Kolkovski and Tandler (1999) |
| Seabass Dicentrarchus labrax | 20 and 40% fish meal hydrolysate | Increase final weight with 20% hydrolysate | Zambonino-Infante et al. (1997) |
| Seabass Dicentrarchus labrax | 40% fish protein hydrolysate (CPSP-G) | double final weight compared oto control without hydrolysate | Cahu et al. (1998) |
| Seabass Dicentrarchus labrax | Casein hydrolysate (partially hydrolysed) | survival improvement | Cahu and Zambonino- Infante (1995) |
| Goldfish Carassius auratus | casein hydrolysate | survival improvement | Szlaminska et al. (1991) |
| Dover sole Solea solea | 20-80% hydrolysed fish protein concentrate | No correlation between growth rates and levels of HFPC. Positive correlation between survival and HFPC percentages | Day et al. (1997) |

Table 3. Protein Hydrolysates and FAA in microdiets

Hydrolysates and FAA

FAA and hydrolysates can only be partial replacement for protein source in microdiet for fish larvae.

Possible explanation:

- Fast flow of short peptides and FAA through the gut, a flow that the larvae can not handle in terms of FAA absorption. As a result, most of these metabolites are flushed out of the digestive system (Kolkovski and Tandler, 1999).
- High levels of FAA in fully hydrolysed protein (or a high percentage of FAA in diet) changed the rate of amino acid absorption in the gastrointestinal tract of the fish, resulting in premature absorption of certain essential amino acids present in the free form relative to the absorption of other essential amino acids, present as polypeptides or intact proteins (Hardy, 1991).
- Increasing nutrient availability needs to be coupled with increased absorption of these nutrients.
- As a general recommendation, the level of the hydrolysate or FAA in the microdiet should not exceed 30% of the total protein levels.
- Partially hydrolysed protein may be more suitable as protein source in microdiets than fully hydrolysed protein or a mixture of free amino acids.



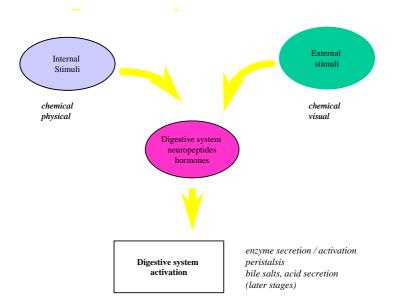


Figure 7. Digestive System Activiation

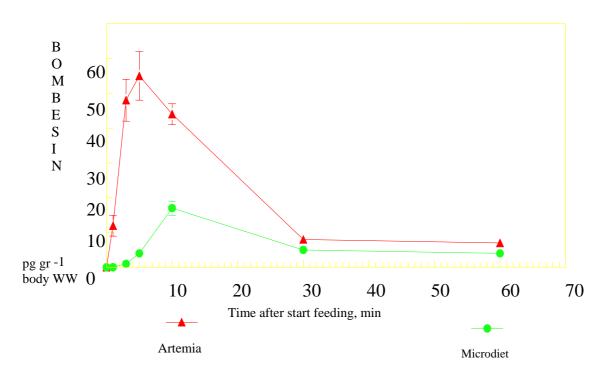


Figure 8. Effect of food type on bombesin activity in 24 day old gilthead seabream larvae (from Kolkovski et al., 1997)

Stimulation of neuropeptides by live food organisms

- The movement of the live organisms (usually, still alive in the oesophagus) may cause movement of the gut walls and microvilii, stimulating neuropeptides release
- Chemical stimuli (taste)
- Physical stimuli (shape, texture)

Indirect digestive system activation



Chemical stimuli

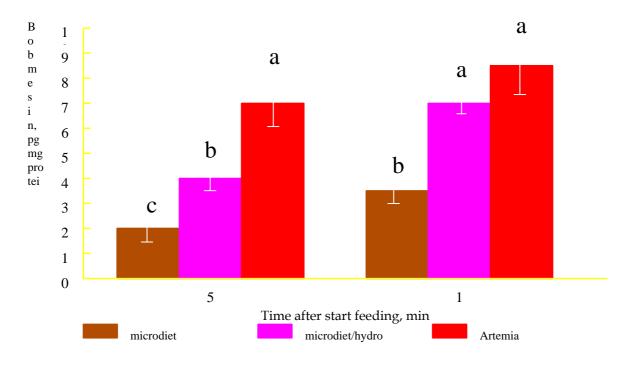
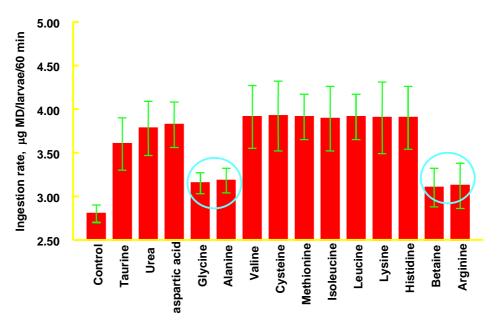


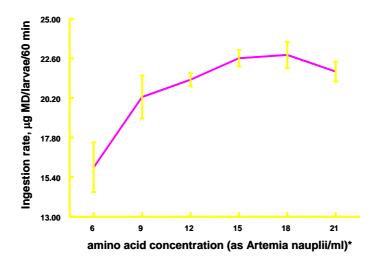
Figure 9. Effect of krill hydrolysate supernatant on bombesin activity in barramundi *Lates calcarifer* larvae (from Kolkovski et al., 1999)



Metabolites were given at a concentration equal to 9 nauplii/ml

Figure 10. The effect of Artemia rearing medium metabolites on microdiet ingestion in 20-day old gilthead seabream larvae (from Kolkovski et al. 1997)





Amino acid mixture concentration was equal to the concentration in Artemia nauplii rearing medium

Figure 11. The effect of amino acid mixture (glycine, alanine, arginine and betaine) on MD ingestion rates of 34-day old gilthead seabream larvae (from Kolkovski 1995)

| Rainbow trout Salmo gairdineriMixture of L-amino acidsAdron and Mackie, 1978Atlantic salmon Salmo salarGlycineHughes, 1990Sea bass Dicentrarchus labraxMixture of L-amino acidsMackie and Mitchell, 1982Pig fish OrthopristisGlycine, BetaineCarr et al. 1977, 1978chrysopterusGlycine, BetaineGoh and Tamura, 1980Red sea bream ChrysophrysGlycine, BetaineGoh and Tamura, 1980majorGlycine, Glutamic acid and ArginineIna and Matsui, 1980Gilthead sea breamGlycine, Betaine, Alanine, ArginineKolkovski et al., 1981Valine, Glutamic acid and ArginineIna and Matsui, 1980Gilthead sea breamGlycine, BetaineKolkovski et al., 1997Sparus aurataInosine and IMPMackie and Adron, 1978Dover sole Solea soleaGlycine, BetaineMetaillet et al., 1980Glycine, Inosine, BetaineMetaillet et al., 1983Puffer Fugu pardalisGlycine, Arginine, Alanine, ProlineYoshii et al., 1978Japanese eel Anguilla japonicaGlycine, Arginine, Alanine, ProlineYoshii et al., 1979Cod Gadus morhuaArginineDamsey, 1984Glusine, Glycine, Histidine , ProlineKaastra and Heinsbrock, 1991Lobster Homarus AmericanusButanoic acid, Carboxylic acid, Trehalose, carbohydrates, Homarine, AsparagineTrott and Robertson, 1984Vestern Atlantic ghost crab Ocypode quadrataButanoic acid, Carboxylic acid, Trehalose, carbohydrates, Homarine, AsparagineTrott and Robertson, 1984Freshwater prawn Macrobrachium rosenbergiiTaurine, | | | |
|---|--------------------------------|--------------------------------------|------------------------------|
| Sea bass Dicentrarchus labraxMixture of L-amino acidsMackie and Mitchell, 1982Pig fish Orthopristis chrysopterusGlycine, BetaineCarr et al. 1977, 1978Red sea bream Chrysophrys majorGlycine, BetaineGoh and Tamura, 1980Red sea bream Chrysophrys majorGlycine, BetaineGoh and Tamura, 1980Gilthead sea breamGlycine, Glutamic acid and ArginineIna and Matsui, 1980Gilthead sea breamGlycine, Betaine, Alanine, ArginineKolkovski et al., 1997Sparus aurataInosine and IMPMackie and Adron, 1978Dover sole Solea soleaGlycine, BetaineMetaillet et al., 1980Glycine, Inosine, BetaineMackie et al., 1980Japanese eel Anguilla japonicaGlycine, Arginine, Alanine, ProlineYoshii et al., 1979Cod Gadus morhuaArginineDoving et al., 1974Herring Clupea herangusGlycine, Arginine, Alanine, ProlineDamsey, 1984Glass eel Anguilla anguillaGlycine, Arginine, Alanine, ProlineMackie and Mitchell, 1983Lobster Homarus AmericanusGlutamate, Betaine, Taurine, Ammonium chlorideCoroto et al., 1992Western Atlantic ghost crab Ocypode quadrataButanoic acid, Carboxylic acid, Trehalose, carbohydrates, Homarine, AsparagineTrott and Robertson, 1984Freshwater prawn Macrobrachium rosenbergiiTaurine, Glycine, Trimethylamine, BetaineHarpaz et al., 1987 | Rainbow trout Salmo gairdineri | Mixture of L-amino acids | Adron and Mackie, 1978 |
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Table 4. Amino Acids and other metabolites used as feed attractants in marine organisms



Amino acids, types and combinations

- Only the L-isomers found to be active as feed attractants.
- Various combinations of amino acids found to have positive effect on different fish species.
- Synergistic effects were found with many mixtures of amino acids and other substances such as ammonium salts.
- Concentrations of amino acids (when added to the water), which were found to have positive effects on feeding, range between 10^{-8} to 10^{-2} M.

Extracts from marine organisms

- Concentrations of extracts and/or hydrolysates from aquatic animals are harder to quantify than amino acids. However, concentrations that are found to have a positive effect on feeding, range between 10⁻² to 10⁻¹⁰ g/l (when added to the water).
- In most cases, when incorporated into the diet, the concentration of hydrolysates and extracts released into the water was not determined.
- As a 'thumb role' protein fraction weight between 1000 10,000 Dalton found to have a positive effect on feeding.

The 'Holistic' Approach

- Integrative approach is needed to be taken in the development of microdiets for fish larvae.
- Different aspects of research need to be addressed and should incorporate:
 - digestive system development including, enzymes, hormones, neuropeptides, transporters etc.
 - digestive system activation, external and internal stimuli.
 - ingestion vs. digestion vs. assimilation and absorption.

Ingestion

- feed attractants
- taste (pH)
- shape, colour etc.

Digestion

- 'easy to digest' proteins
- binders and capsules
- liquid or semi-moist particles
- dietary supplementation

Assimilation and absorption

• transporters and carriers



Using terrestrial carbon sources to enhance productivity and sustainability in

high intensity prawn farming

Kevin Williams

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Introduction

Prawn farming is a major global aquaculture industry with current production estimated to be 1,000,000 t/year (Rosenberry, 2002). However, there are increasing concerns about the environmental sustainability of prawn farming, including discharge of nutrient-rich water originating from prawn feed into coastal waterways (Briggs and Funge-Smith, 1994; Naylor et al., 1998). These concerns are increasingly placing pressure on the prawn farming industry to improve their environmental credentials while remaining viable and profitable. In Australia, strict enforcement of government regulations on nutrient levels in prawn discharge waters has maintained a clean and green image for prawn farming for the country, but production and profitability have languished. Australian prawn production (mostly *Penaeus monodon*) has been static for the past 4 to 5 years, amounting to a modest 2800 t in 2000/01 (O'Sullivan and Dobson, 2003): pond productivity has also stagnated at about 4 t/ha/crop (Lobegeiger, 2003), often with just one crop per year, while the cost of production remains high at about AUD\$9-11/kg prawn. By comparison, Australian importers can source white shrimp, Litopenaeus vannamei from China for AUD\$7/kg and P. monodon from Thailand at AUD\$9/kg. Clearly, the Australian prawn farming industry will decline unless farming costs can be lowered so that production remains competitive with other producing nations and at the same time, ensuring that nutrient thresholds for prawn discharge waters are not exceeded.

One radical new approach to improving prawn farming sustainability has been the development of high-intensity, zero water exchange systems. In addition to the obvious environmental benefits, this system has the added advantages of increased biosecurity, higher per unit productivity and hence, greater profitability. This technology was developed commercially at Belize Aquaculture in Central America for the white shrimp *L. vannamei* (McIntosh et al., 1999). This paper outlines the Belize farming system and discusses its applicability to Australian prawn farming.

Characteristics of Australian prawn farms

The predominant prawn species farmed in Australia is the black tiger prawn, P. monodon. While one of the most sought after species in the market place, a major disadvantage is that it has not yet been commercially domesticated and as a result, farmed stock are derived from wild-caught broodstock. A consequence of this is that viral diseases endemic in wild stocks are often carried through to the progeny with subsequent catastrophic farm losses. Moreover, selective breeding for improved growth rate, greater disease resistance and/or better tolerance to intensive farming conditions is not possible while progeny continue to be sourced from wild broodstock. The most common prawn farming system in Australia is to stock postlarval prawns into aerated 1 ha earth ponds at densities of 30 to 40 per m² surface area. Prawns are fed pelleted feeds, which typically contain 40 to 42% crude protein (CP), and the water quality is managed by water exchange. On well managed prawn farms, pond discharge water is subjected to on-farm bioremediation processes so as to reduce particulate and nutrient loads and permit some recirculation of the treated water back to the prawn ponds. Under these conditions, prawn survival is typically better than 75% (unless a disease outbreak occurs) and 4 to 9 t per ha of prawns are produced per 140 d crop (about 4 t/ha /crop on average; Lobegeiger, 2003). Studies on the wholefarm, whole-season nitrogen (N) budget of this system of prawn farming show that more than 90% of the input N arises from the added feed but that only about 21-22% of this N is actually recovered as harvested prawns (Briggs and Funge-Smith, 1994; Burford and Williams, 2001; Jackson et al., 2003). Most of the N (57%) is contained in the pond effluent and a small proportion, about 14-16%, is retained in the pond sediment.



The effect of reducing the CP content of the pelleted diet on water quality and prawn productivity under simulated pond conditions has recently been investigated by Burford et al. (2003a,b). They stocked juvenile 3.1 g *P. monodon* at a density of 25/m² into sand-bottom and aerated 2.5 t outdoor tanks, which were filled with water drawn from a commercial prawn pond. A microalgal bloom was maintained in the tanks and water was periodically exchanged to stabilise the bloom. Prawns were fed one of three protein diets that varied serially at 5% increments between 30 and 40% CP. At the conclusion of the 8-week study, the total N content of the tank water was 12.6, 10.3 and 7.3 mg/L for dietary CP contents of 40, 35 and 30%, respectively while prawn growth rate was unaffected other than for the lowest protein diet: 1.50, 1.48 and 1.34 g/d, respectively. Thus, a reduction in the dietary CP content of 5% (from 40 to 35%) resulted in a 20% reduction in the total N content of the tank water and had no effect on the prawn growth.

Characteristics of the Belize prawn farming system

The technology for a high-intensity prawn grow-out with no water discharge during the crop cycle was developed experimentally at, amongst others, the Waddell Mariculture Centre in USA (Sandifer and Hopkins, 1996) and adopted, with modifications, by Belize Aquaculture, Ltd (BAL) in the mid 1990s (McIntosh et al., 1999; McIntosh and Bowen, 1999). BAL developed an integrated approach to farming shrimp using high health, selectively bred L. vannamei stocks, low-protein feed input, high stocking densities (150/m²) in fully-lined ponds, no water exchange over the crop cycle and recirculation of water through treatment ponds at harvest time. One key aspect of the high-intensity BAL system is the addition of grain/legume products and molasses to ponds to promote the growth of flocculated clumps of microorganisms, which in turn improve nutrient processing and provide a food source for the prawns. This results in improved prawn productivity (as high as 20 t/ha but averages 15 t/ha) and reduced feed wastage. Additionally, the use of grain/legume products and molasses in prawn ponds at BAL has resulted in a reduction in the protein levels of feed added to ponds from >30% to 20%, hence improving water quality and decreasing the cost of production. Total feed input for a 15 t crop of prawns comprised 12 t of grain/legume products (wheat, corn and soybean meal; 18% CP; C:N ratio of 20:1), 1 t of molasses and 20 t of a low protein prawn feed (fishmeal based) (McIntosh and Bowen, 1999). Overall feed conversion ratio averages 1.8 with 2.4 crops/year, 38% of the input N is retained in the harvested prawns and cost of production in 2000 was US\$2.95/kg (about AUD\$4.75/kg, allowing for 3% inflation) (McIntosh and Bowen, 1999; McIntosh, 2001). CSIRO researchers visited BAL in 2001 and studied the nutrient cycling and microbial community in the ponds in detail (Burford et al., 2003b). Using stable ¹⁵N labeling procedures, they confirmed that prawns ranging in size from 1 to 9 g consumed the bacterial flocs, which contributed from 18 to 29% of the total assimilated N, and that the flocs played an important role in improving water quality.

Benefits of adopting Belize prawn farming technology

There are a number of clear benefits for the farmer: a higher production per hectare of pond – up to four-times higher productivity than the current Australian farming system; a lower feed cost; increased biosecurity and attendant higher prawn health statu; and reduced environmental effects by lowering water discharge. All add to increased farm profitability and sustainability. Potential downsides are: higher capital set-up costs – particularly for lined ponds and increased aerator capacity as required for the Belize technology; the need to farm fully domesticated species, which have been selected for high-intensity culture compatibility and high health status; and perhaps the risk of a catastrophic production failure may be greater with increasing intensity of production. The Belize technology, and even more intensive variations of the same, is beginning to be adopted throughout SE Asia and China with *L. vannamei* specifically being imported for this purpose. Unfortunately, there appears to be insufficient attention being paid to the viral disease status of these importations and a calamitous failure may be looming.

Adoption of high-intensity culture systems equally has clear benefits for global prawn production provided stocks free of viral diseases are used. Prime benefits would be a more sustainable system of prawn farming; a reduced demand for fish meal as an ingredient in feeds fed to prawns; and a greater capacity for increased prawn production as operating margins increasingly come under supply and demand forces. The environmental and profitability benefits of high-intensity prawn farming are readily apparent. The impact on fish meal supplies requires further explanation. If it is assumed that farm FCRs are about 1.8 for conventional (eg Australian) and high-intensity (eg Belize) systems, then



the quantity of fish meal and grain/vegetable protein meals needed for each tonne of prawn production can be calculated. For conventional culture systems feeding a 40-42% CP feed, about 950 kg of marine protein meals (predominantly fish meal) and 690 kg of grain/vegetable protein meals would be consumed; for the high-intensity system where the average CP content of the total feed input is about 23%, some 220 kg of marine protein meal and 1,460 kg of grain/vegetable protein meals would be consumed. If the high-intensity technology was used to produce just 10% of current global prawn production, this would spare about 90,000 t of fish meal, i.e. 3% of the global amount of traded fish meal. The quantity of grain/vegetable protein meal required would increase by a similar amount of 90,000 t but this represents less than 0.03% of traded global grain/vegetable protein meals. Thus, considerable savings are achieved in fish meal, which is already under heavy demand pressures as an ingredient for other aquafeeds, particularly for salmonids, while the required additional grain is easily accommodated from existing agricultural production.

Application of Belize technology to Australian prawn farming

Perhaps the greatest obstacles to Australian prawn farmers adopting the high-intensity technology are: their preference to culture *P. monodon*, which may not be suitable because of uncertain viral disease status; the high capital investment necessary for adopting this technology; and high pond water salinity problems in areas where evaporation exceeds precipitation and fresh water is not readily available. However, there is enormous interest amongst Australian prawn farmers in adopting the technology and an eagerness of some farmers to experiment with other species that are fully domesticated, including the white banana prawn *Penaeus merguiensis* and the indigenous brown tiger prawn *Penaeus esculentus*. Clearly, prawn farming in Australia is unlikely to remain profitable and competitive in the market place unless Australian prawn farmers increasingly move towards higher intensity production systems.

Conclusions

As pressure increases to intensify production and reduce environmental effects, there is increasing interest worldwide in using the Belize technology for a range of prawn species. Adoption of this technology will result in new markets for grain/vegetable protein products and sugars with Australian agriculture being well placed to share in meeting this demand. Additionally, the use of terrestrial carbon sources in Australian prawn farming, coupled with high intensity production of prawns, has the potential to reduce waste N and to improve prawn productivity and profitability. However, a number of key research questions relating to the use of these products in high intensity systems in Australia remain to be answered. What kinds of terrestrial carbon sources will be the most effective in promoting bacterial growth and can the system be better managed to deliver the best sources of nutrients for the prawns? As P. monodon domestication becomes closer to commercial reality and the prospects for selectively bred high health stocks are realized, the question remains as to whether this species is suited to high intensity production. Furthermore, would enclosed systems such as tanks or raceways under glasshouses be more suitable for high-intensity prawn production in Australia than pond-based culture? Researchers must quickly address these issues. Unless suitable high-intensity prawn production systems are developed for Australian conditions, prawn production in this country will rapidly become an industry of the past!



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Commercial aquaculture perspectives on diet and protein requirements - How

to get a large manufacturer such as CP to use Australian vegetable protein

Doug Pearson

Proaqua, Queensland

Feed constitutes the largest portion of the costs of production in prawn farming. In Australian farms, feed represents 30% to 40% of the costs of production. Therefore it can be seen that feed and its quality are a very important factor in the economic success of a prawn crop.

There are a number of key attributes that a farmer requires in the feed. These are:

Good attractants

Prawns locate their food via chemo-receptors on their antennae. Feeds containing good attractants allow the prawns to locate it quickly before to its nutritional value is compromised through leaching.

Palatable

Once the feed is located the ingredients need to be palatable to the prawn so it continues to feed. Unlike fish where the feed is swallowed almost immediately, prawns pick up individual pellets and pull them apart, chewing small portions at a time.

Good water stability

Feed that has been broadcast into the pond may remain on the bottom uneaten for 2 to 4 hours. If the stability of the feed is low it will swell and fall apart before the ration is totally consumed. This will reduce the feeding efficiency of the prawns, increase the FCR and compromise the water quality.

Good digestibility

The digestive system of a prawn is quite short therefore the feed needs to be highly digestible. To aid in the digestibility of a feed the ingredients need to be finely ground.

Good growth

As with any animal production facility reduction in growing times have a large effect on the economics of a crop.

Low FCR

As mentioned previously feed constitutes are large portion of the cost of production. Reducing the FCR not only helps the bottom line but will also help in the reduction of nutrient discharge levels.

Issues Impacting Australian Farmers

Over the last few years, global production of farm-raised prawn has grown considerably. It is estimated that over 1 billion metric tonnes will be produced this year compared to 700,000 tonnes in 1999. This is mainly due to the cultivation of domesticated *P. vannamei* in intensive pond systems. In contrast to this, the Australian industry has had relatively little to no increase in productivity levels. Due to the global increase in production, as well as reduced cost of production of *P. vannamei*, the global price and the domestic price of prawns has dropped considerably. Australian farms currently operate with costs of production between \$8 and \$12 per kg. Chinese *P. vannamei* are being landed on our shore for \$9 per kg. To remain viable, Australian farmers need to lower their costs of production as well as increase their productivity (kg/hectare produced).

In addition to this Australian farmers are required to operate under some of the strictest discharge limits of any prawn producing country. In fact many countries do not operate under limits at all. There are however, initiatives underway to change this. The Global Aquaculture Alliance recently published a list of suggested water quality standards for prawn farm effluents. The suggested initial standard was <5mg/l Total Ammonia Nitrogen with a suggested target level of <3mg/l. Prawn farms in South East



Queensland are already being asked by the EPA to achieve <1mg/l Total Ammonia Nitrogen. To continue to operate under these conditions Australian farmers need to develop ways to more efficiently use the nitrogen in their systems and feed is the major contributor of nitrogen to the systems.

Currently there are a number of options a farmer may choose from to try to reduce their effluent nutrient levels:

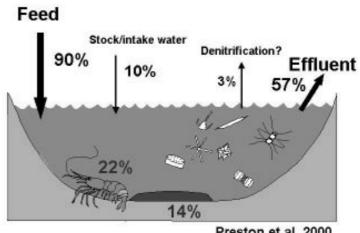
Extensive farming

Stocking rates are less than $20/m^2$ and greater reliance is placed on the natural productivity of the ponds with less feed being used. Larger prawns are also grown to target more lucrative niche markets. With Australia's relatively high costs of land and labour this option has some issues attached. It is also out of the question to many of the existing farms due to the levels of investment they have on existing infrastructure.

Modify Existing Systems

Some of the existing farms have the option to incorporate settling ponds to treat their effluent water. This water may also be recycled back through the farm to further improve nutrient efficiencies. Aeration can be improved and increased to oxidize excess nutrients. Water quality management overall can be improved. Husbandry practices can be improved and modified, such as improving feed management strategies. Overall Best Management Practices can be implemented to improve efficiencies. Improvements may also be possible on the utilisation efficiencies of the diets to reduce nitrogen wastes. Lower protein feeds incorporating a higher vegetable protein content may enable higher nitrogen retention. As can be seen from Figure 1, the prawns retain only 22% of the nitrogen entering into a standard pond system, with 57% leaving as effluent.

Nitrogen budget



Preston et al. 2000

Figure 1. Nitrogen budget of a typical Australian prawn pond.

Develop New High Intensive Systems

Belize Aquaculture Ltd (BAL) in Belize is an example of such a system. At BAL no water is exchanged through the entire crop, water is merely added to replace evaporation. The water quality is maintained through high aeration application and promotion of bacterial flocs. Typically in Australia, ponds are aerated at a rate of 16Hp per hectare. In the BAL, ponds aeration is applied at a rate of 50Hp per hectare. The ponds are also lined to reduce erosion and to eliminate any negative from the soil. Bacterial floc formation is achieved through the addition of carbon via a grain-based feed in addition to a typical low protein prawn diet. The bacterial flocs, not only enhance the nitrification and denitrification processes within the pond, but also become an additional food source to the prawns. Analysis has shown that the flocs have a protein content of up to 40%. Upon harvest the pond water is held in a settling pond for 7 days and then returned to another pond to begin a new crop. As can be



seen from Figure 2, this allows BAL to achieve nitrogen retentions of 39% in the prawns. As the water is re-used very little effluent leaves the farm.

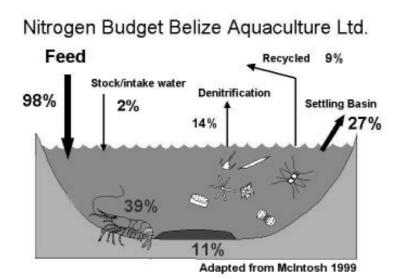


Figure 2. Nitrogen budget of Belize Aquaculture Ltd

How to Get a Large Manufacturer Such As CP to Use Australian Vegetable Proteins Charoen Pokphand Fish & Shrimp Feeds

Charoen Pokphand Feeds (CP) has aquaculture feed mills in Thailand, Indonesia, Malaysia, India, Vietnam and China. Their annual production of prawn and fish feeds combined is approaching 1 million metric tonnes. To do this they consume from 300,000 to 400,000 metric tonnes of fish meal. Global annual fish meal production is approximately 6,500,000 metric tonnes (FAO 2001). On these figures alone CP's aquafeed production potentially accounts for up to 6% of the world's fish meal. CP are also very large producers of terrestrial animal feeds. Combine the two and you have a company with very substantial buying power. This then raises the question. Is CP interested in replacing fish meal with vegetable protein? The short answer is yes. CP recognises that the global trend is to do so and they are following the market demands. In fact a lot of vegetable protein is used already, such as soybean meal, peanut meal, corn gluten meal, etc. The amount and type of inclusion is very much dependant on the country, price and availability. Predominately more vegetable proteins are used in fish feeds where then total feed protein requirement is less. Examples of typical Shrimp and Sea Bass diets are shown below in Tables 1 and 2.

| Ingredient | Inclusion Rate | Source |
|---------------------|----------------|--------------------|
| Fish meal | 47% | Thailand |
| Squid meal | 5% | Thailand |
| Wheat flour | 26% | Australia |
| Soybean meal | 15% | Thailand |
| Fish oil | 2% | Japan, Peru, Chili |
| Vitamins & minerals | 5% | Switzerland, Japan |



| Ingredient | Inclusion Rate | Source |
|--------------------|----------------|---------------------------------|
| Fish meal | 35% | Thailand, Chili, Peru |
| Soybean meal | 20% | Thailand |
| Full fat soybean | 10% | Thailand |
| Corn | 11% | Thailand |
| Fresh rice brain | 10% | Thailand |
| Broken rice | 5% | Thailand |
| Coconut oil meal | 5% | Thailand |
| Fish oil | 2% | Japan, Peru, Chili, New Zealand |
| Vitamin & minerals | 2% | Switzerland, Japan |

CP has a very strong R&D focus, budgeting 3% of sales to this area. They also have strong ties to a number of universities. Annually they recruit over 200 people from these universities and give them further training for areas of marketing, production, technical development and extension. They do their own assessments on new products and systems, conducting trials on the company owned farms. CP produces over 3000 metric tonnes of prawns from these farms per year. Once they are comfortable with the performance of a new product they will promote it to their contract growers and other customers.

They do not need or want to see any more "Feed and Measure" studies. They consider these to be useless in terms of usable information. For them to consider a product it needs to be competitive on a cost per unit of "available nutrient". Fish meals are not always more expensive than vegetable proteins. It will depend on the country the feed is being manufactured in and the type of feed being produced. For any new product to be considered by CP, they will first need to be satisfied that there is a reliable, consistent supply of the product in the order of thousands of tonnes. They will want to see nutrition data on the products digestible energy values. As well as the availability of essential nutrients such as amino acids, fatty acids, phosphorus, etc...

Details of any anti-nutritional compounds in the product that could affect growth or increase mortalities and the FCR are also required. Compounds such as trypsin inhibitors, aflatoxins, mycotoxins, oligosaccarides, etc...

In summary the steps to take to get CP to use Australian vegetable proteins in my opinion are:

- Acquire and provide good nutritional data on the products.
- Ensure a consistent supply of large volumes of the product can be maintained.
- Invest time in convincing key people in CP that the product is worth trying.
- Supply product and allow CP to conduct their own research and testing, in both the laboratory and the field. This may take 2 to 3 crops.

If the product works you can be sure it will be used.

I would like to acknowledge the following for their candid comments:

Dr Dean M Akiyama - Senior Vice President, Aquafeed Technology, CP Indonesia

Dr Ming Dang Chen - Senior Vice President, Aquafeed Nutrition, CP Bangkok

Robins McIntosh - Senior Vice President, Shrimp Production Technology, CP Bangkok

Dr. Sujint Thammasart - Senior Vice President, Aquatic Research & Development, CP Bangkok

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Can modeling help define the protein requirements of key aquaculture

species

Brett Glencross

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Introduction

The specifications for dietary energy and protein are the key nutritional parameters in defining any aquaculture diet. However, the means traditionally used to define these requirements have been variable to say the least, and sometimes unreliable. Typically, empirical experiments examining dose-response requirements to either nutritional parameter have been standard (Mercer, 1982). However, using such an approach often assumes key factors such as uniformity of requirement across a varying size-range of animals. Clearly the use of variable-parameter modeling techniques can circumvent some of these problems, such as the assumption of constancy of protein/energy demand. However, there are numerous considerations in the development of a potentially useful bio-energetically based model.

Outlining the basis for a bio-energetic model

The use of bio-energetic models to predict energy and subsequently food demand is not a new concept (Ursin, 1967; Cuenco et al., 1985; Machiels and Henken, 1986; Cacho, 1990; Cho and Bureau, 1998). The development of a bio-energetic model for estimation of dietary energy and subsequently nutrient demands by fish depends on the determination of a range of key relationships (Lupatsch et al., 1998). Principal of these relationships is an understanding of:

- The relationship between body size (live-weight) and metabolic energy demand
- The relationship between body size (live-weight) and growth rate
- The relationship between growth rate and water temperature
- The relationship between body size and body composition, and
- The relationship between dietary digestible energy intake and energy retention.

The determination of these relationships is the primary empirical basis to the development of any subsequent models. Studies with a wide range of species have also indicated that many of these relationships are species specific (Lupatsch et al., 2003). Once the basis of these key relationships is defined for a particular species it becomes possible to derive a series of inter-related mathematical equations. These inter-related equations allow the calculation of a range of parameters based on inputs such as initial live-weight, water temperature, time and diet composition. From these inputs it becomes possible predict growth and feed utilisation, based primarily on dietary energy demand.

It has been shown that the metabolic requirement for energy in most animals, at a constant temperature, is generally a function of body mass (Withers, 1992). Fish are also similar in this regard, although it has been noted that there appear to be some species-specific aspects to their metabolic weight exponents, though even this is widely debated (Hepher, 1988; Lupatsch et al., 1998). Similarly the importance of body composition in defining gross energy requirements for these animals demands that each specific species is at least compared to other similar species to determine the complementarities of such data. For defining an energy budget generally it can be surmised that energy requirements = $M \cdot BW(kg)^b + G \cdot growth$, where $M \cdot BW(kg)^b$ is the metabolic body weight and M and G are coefficients describing the efficiencies of energy utilisation of maintenance and growth respectively.

Dictating the demand for dietary protein

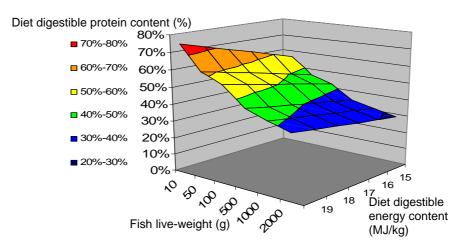


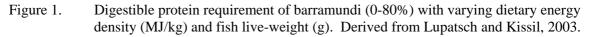
From any given diet energy density, the required feed intake is relatively defined. Based on knowledge of dietary protein (or other nutrient) utilisation efficiencies and maintenance requirements for protein (or other nutrient), the required dietary protein concentration to sustain the required growth is also largely dictated by that level of feed intake or more specifically the diet energy density. In contrast to the vagaries seen in the energy use parameters between species, the efficiencies of protein use seem relatively conserved across species provided adequate dietary energy is provided (Lupatsch et al., 2003).

However, the energy density of diets used in any particular fish farming enterprise is not necessarily a fixed parameter. Notably as diet energy density changes, changes are also seen in feed utilisation. Typically as diet energy density increases, feed intake requirement diminishes. Because of the influence of diet energy density on feed intake this also has implications on the utilisation of other dietary nutrients, primarily through its influences on gross nutrient intake (Lupatsch et al., 2001).

Dietary composition implications

Based on a dictated level of diet energy density, the required level of digestible dietary protein intake can also be defined. In theory, this principle can also be applied to many other dietary nutrients. The other important factor, than diet energy density, determining diet nutrient composition requirements is the fish's live-weight (Figure 1). This relationship is primarily based on the influence that somatic energy demand places on dietary energy intake use efficiency.





In using this nutritional research approach it becomes possible to clearly demonstrate that the demands for protein and indeed perhaps most nutrients are a highly dynamic (Table 1). These findings are largely consistent with what is also known of the nutrient demands by other animals, which also show a higher protein/amino acid demand at a younger age.

This finding has substantial implication to the dietary formulation process in that specific gains should be possible by better catering to actual nutrient demand at any particular point of the animal's growth phase. In addition, by utilising the fishe's capacity to consume proportionally larger amounts of food when young, a dynamic strategy of changing diet energy density and diet protein levels allows for better catering to specific growth requirements over a production cycle of any fish species.

Table 1.Changes to digestible protein and lysine demand with varying barramundi size as
determined using an iterative approach. (Based on data derived from Lupatsch and
Kissil, 2003 and Glencross 2003 unpublished).



| Fish weight (g) | 10 | 50 | 100 | 500 | 1000 |
|-----------------|------|------|------|------|------|
| Protein (g/MJ) | 38 | 31 | 28 | 23 | 21 |
| Lysine (g/MJ) | 1.53 | 1.26 | 1.15 | 0.93 | 0.85 |

Conclusions

Practical use of this technology can be made on several different levels. Functional models of growth, energy demand and, by inference, feed demand can be devised and used as a basis for prescriptive feed management for fish production. On another level, the data can be used to iteratively define protein requirements based on a defined dietary energy density. The two processes described can, of course, be used inter-relatedly. Discrete assessment of the nature of diet specification use and how it changes with changing fish size and live-weight energy density also provides a mechanism to better assign diet types and also suggest at which point diet specifications should be changed (Figure 2).

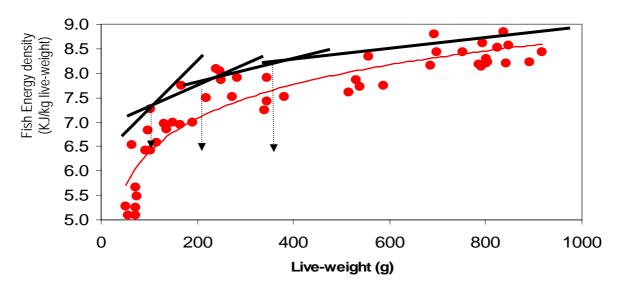


Figure 2. Stylised assignment of diets to barramundi production. Intercept of lines on energy curve indicating were diet choice (diet energy density and accordingly protein content) should change. Angle of line is indicative of the lysine to energy ratio required in the diet, while the Y-intercept of the line would be indicative of the respective energy density of the diet suggested. Arrows indicate fish size were diet change should take place. Figure derived from Glencross et al., 2002.

This approach to nutritional investigations also confers numerous secondary benefits. In the process of defining dietary energy and protein demands a defined ration structure is also determined, allowing some basis to feed allocation to maximise production whilst minimising feed wastage. Because the amount and composition of feed fed can be defined, the resultant nutrient losses can also be estimated thereby allowing estimations of environmental loading to also be undertaken (Kaushik, 1998; Glencross et al., 2002).

Perhaps one of the greatest strengths in using such modeling approaches to nutritional research is the capacity to construct robust hypotheses that can be simply answered using limited experimental treatments. Essentially this allows the testing of certain assumptions to determine level of confidence in a specific parameter estimation.

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Appendix I: Workshop Agenda





Aquaculture Nutrition Subprogram 2nd Annual Workshop Thursday, 29th May 2003



Esplanade Hotel Cnr Marine Tce & Essex St Fremantle, Western Australia

Agenda

Workshop focus: Expanding the aquafeed ingredient base.

| 8.30 | Introduction and welcome | (Patrick Hone) |
|-------|--|------------------------|
| 8.45 | Production and utilisation of vegetable protein sources for aquafeed in Australia – What are we trying to achieve ? | (Robert van Barneveld) |
| 9.15 | Development of vegetable protein sources for finfish | (Brett Glencross) |
| 9.45 | Utilisation of vegetable protein sources in crustacean diets | (David Smith) |
| 10.15 | Morning tea | |
| 10.30 | Alternative protein sources in manufactured diets for molluscs | (Meegan Vandepeer) |
| 11.00 | International advances in the utilisation of alternative protein sources | (Stahle Refstie) |
| 11.30 | Developments in aquaculture diet development in NZ | (Michael Bruce) |
| 12.00 | Alternative proteins in snapper diets – Recent research within the Aquafin CRC. | (Mark Booth) |
| 12.30 | Lunch | |
| 1.15 | Commercial production of soy protein concentrates for use in aquafeed | (Will Tidswell) |
| 1.45 | Requirements for alternative proteins by Australian aquafeed manufacturers. | (Rhys Hauler) |
| 2.15 | Influence of environment on the diet composition and nutritional requirements of salmonids | (Chris Carter) |
| 2.45 | Progress in the development of manufactured diets for larval species | (Sagiv Kolkovski) |
| 3.15 | Afternoon tea | |
| 3.30 | Using terrestrial carbon sources to enhance productivity and sustainability in high intensity prawn farming | (Kevin Williams) |
| 4.00 | Commercial aquaculture requirements for vegetable protein sources and adoption of alternative proteins by international aquafeed producers | (Doug Pearson) |
| 4.30 | Can modeling help define the protein requirements of key aquaculture species | (Brett Glencross) |
| 5.00 | Summary and close | (Robert van Barneveld) |



Appendix 4 – Additional Publications

1. van Barneveld, R.J. (2003). Aquafeed Ingredient Selection – What needs to be considered? In preoceedings of the Food Industry Engineering workshop on food and feed extrusion convened at the Australasian Experimental Stockfeed Extrusion Centre.

Aquafeed Ingredient Selection – What needs to be considered ?

Prepared by Dr Robert van Barneveld

Leader, FRDC Aquaculture Nutrition Subprogram

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Introduction

Selection of ingredients for use in aquaculture diets can be a complex process. Ingredients need to contribute to the nutritional needs of the target species (which are wide and varied between species), as well as being complimentary to the feed type and presentation, the proposed manufacturing process, and the feeding habits of the fish. Because of the comparatively large number of constraints that must be applied when formulating aquatic feeds, access and appreciation of the characteristics and composition of a broad range of feed ingredients can simplify the diet formulation process.

Basic considerations

Feeding habits and preferences

Even before considering the nutritional requirements of the target species, ingredient selection can be influenced by some basic diet requirements. Aquafeed ingredient selection will be influenced by the feeding habits of the target species, the requirements for water stability, the preferred presentation of the feed and the proposed mode of production (Table 1). For example, it is difficult to produce floating feeds if the diets do not contain any starch, or aquafeeds designed for carnivorous species generally need to restrict the quantity of dietary carbohydrate.

| Target species | Feeding | Sink rate | Stability | Presentation | Production |
|--|---|---|--------------------------|----------------------------|--|
| Carnivorous Herbivorous Omnivorous | Pelagic Benthic Opportunistic Frequent | Fast sinking Slow sinking Neutrally buoyant Floating | Days Hours Minutes | Moist Semi-moist Dry | Paste/dough Formed Steam-pressed Extruded Vacuum infused |

Table 1. Factors that influence the formulation of aquatic feeds and the ingredients selected.

Because of the wide range of potential target aquaculture species, and the resulting range in diet requirements, selection of complimentary ingredients is as important as selection of individual ingredients. When formulating aquafeeds, emphasis should never be placed on selection of a single "perfect" ingredient. Rather, efforts should be directed towards identifying and understanding how suites of ingredients relate when combined and manufactured using a specific process. Examples of some aquaculture species cultured in Australia and their diet requirements are presented in Table 2. If we consider prawns and sea urchins, both are benthic feeders with a requirement for a diet with a high water stability and fast sink rate. However, as the prawn diets are presented dry following steam-pressing, they will contain a significantly different range of ingredients to the sea urchin diets that are presented semi-moist following extrusion. Similarly, if the same ingredient base was used to produce a steam-pressed and extruded rock lobster diet, it is unlikely that both diets would confer the same water stability due to differences in the way the ingredients are bound during the manufacturing process.

| Species | Feeding | Sink rate | Stability | Presentation | Production |
|--------------|---------|--------------------|-----------|--------------|------------------------|
| | | | | | |
| Prawns | Benthic | Fast sinking | 24 hours | Dry | Steam-pressed |
| Tuna | Pelagic | Slow sinking | Minutes | Semi-moist | Extruded/formed |
| Barramundi | Pelagic | Floating/Slow sink | Minutes | Dry | Extruded/Steam-pressed |
| Sea urchins | Benthic | Fast sinking | 48 hours | Semi-moist | Extruded |
| Abalone | Benthic | Fast sinking | 48 hours | Dry | Formed |
| Lobster | Benthic | Fast sinking | 48 hours | Dry | Steam-pressed/Extruded |
| Salmon | Pelagic | Slow sinking | Minutes | Dry | Extruded/Infused |
| Crocodiles | Pelagic | Floating | 72 hours | Moist | Formed |
| King-George | Pelagic | Floating | Minutes | Dry | Extruded |
| Whiting | C | C C | | - | |
| Snapper | Pelagic | Slow sinking | Minutes | Dry | Steam-pressed |
| Silver perch | Pelagic | Slow sinking | Minutes | Dry | Steam-pressed |
| • | C | Ū. | | • | * |

Table 2. Range in diet forms for some Australian aquaculture species.

Production logistics

In addition to the requirements of the target aquaculture species, the raw materials selected will be heavily influenced by the logistics of commercial production. For example, there is little point basing an aquaculture diet on a feed ingredient that is in short or interrupted supply, is costly to source and transport, requires additional storage (such as freezing), and cannot be handled by the existing production facilities. In general, consideration must be given to the following:

| Raw material form: | Fresh, wet, preserved (ensiled), rendered, dried. |
|--------------------------|--|
| Raw material source: | Cost constraints will usually dictate a maximum distance from the mill where transport costs become prohibitive. |
| Raw material storage: | Ingredients may have special storage requirements prior to use which can add significantly to the overall cost of producing the diet. |
| Production requirements: | Fresh, preserved and wet ingredient addition is usually limited to extruded, formed or paste type diets and often require special modes of delivery. |
| Product quality: | While many waste streams have potential for use in aquafeed diets, it is important that their quality in terms of consistency and microbiological integrity is maintained. |

Influence of natural diet and basic digestive anatomy and physiology

When developing diets for new and emerging aquaculture species, attention is often directed towards the natural diet of this species. Indeed, this is one of the reasons why fish meal features so heavily in most aquafeeds. In fact, the higher the use of fish meal in an aquatic feed, the less knowledge we have on the nutritional requirements of that aquaculture species. It is rare for any animal to have an obligate requirement for a specific ingredient (koalas and eucalyptus leaves are a good example of an exception), and most ingredients can be replaced by a combination of alternative ingredients.

In general, examination of natural diets offers little assistance in the selection of ingredients for use in aquafeeds. Firstly, aquatic organisms have no access to the huge diversity of terrestrial feed ingredients in their natural state, so this approach will always exclude these products. Secondly, aquaculture species are not "production" animals in the wild, and hence their primary source of nutrients is more likely to sustain existence rather than maximum growth. For example, in the wild, abalone graze algae and seaweeds. Globally, the majority of abalone aquaculture production systems are based on algae and seaweed as the primary source of nutrients. In countries such as Australia, however, where these natural diets cannot be

legally harvested and fed, alternative manufactured diets had to be developed and now produce growth rates that far exceed those that will ever be achieved with natural diets.

The common exception to the examination of natural diets vests with live feeds for larval diets.

In contrast to natural diet examination, an understanding of the basic digestive physiology and anatomy of the target aquaculture species can provide useful insights into ingredient selection. For example, an assessment of the digestive physiology and anatomy of southern bluefin tuna reveals that the tract is short (so nutrients need to be readily available), there is an absence of any form of fermentation capacity in the tract, there is no appreciable enzymic capacity to digest any form of carbohydrate or starch, protease is higher in the pyloric caeca whereas lipase activity is highest in the intestine and particle size may influence movement of feed out of the blind stomach sac. Armed with this knowledge, tuna diets need to be well bound to control movement through the tract, ingredients cannot contain any form of carbohydrate, ingredients should be highly digestible, and manufactured diets should not be fat coated.

Ingredient categories

Protein sources

In general, aquatic species have a high requirement for protein, both for muscle deposition and as an energy source. Proteinaceous ingredients can comprise between 25 and 60% of a typical aquatic feed. In addition to supplying nutrients, proteins are functionally important because they can influence water absorption, elasticity and binding of the diet.

Protein ingredients are characterized by origin – plant or animal. Plant proteins include products such as wheat gluten, corn gluten, oilseed meals (soybean, canola, sunflower, peanut), legumes (lupins, peas, beans, vetches). Animal proteins include fish meals, meat and bone meals, blood meals, poultry offal meals, eggs, and feather meals. The use of terrestrial animal proteins in aquafeeds has diminished since outbreaks of BSE have generated concern amongst export markets.

A clear need has been identified for the development of a vegetable protein isolate available for use in Australian aquafeeds as an alternative to fishmeal. Lupins, particularly new cultivars such as *L. luteus*, hold the greatest potential for the production of a high protein isolate in Australia. Research and development is required to identify the most appropriate process for the production of such an isolate, the efficiency of such a process, the need to identify multiple end-users for the graded isolates, and the suitability of such an isolate for aquaculture. This process holds potential to produce a range of additional co-products such as non-starch polysaccharide isolates, isoflavones and oils that would require further investigation by multiple end-users.

When producing semi-moist diets, extruded diets, formed diets or pastes then fresh bait fish, processed fish wastes, fish solubles and fish silage can be very valuable protein sources. Inclusion rates can be limited by their moisture content and level of bacterial contamination. An example of some processing waste streams and their chemical composition is presented in Table 3. When using these waste streams, diligence in required in relation to monitoring microbiological contamination (Table 4).

Carbohydrate sources

Starch is included in aquafeeds as an energy source, but has an important function as a binder. Starch content can have a significant influence on the capacity of an extruded aquafeed to sink or float, or to absorb significant quantities of fat during processes such as vacuum infusion post-extrusion. In general, a minimum of 10% starch is recommended in sinking aquatic feeds while a minimum of 20% is recommended as bound starch.

Wheat and wheat co-products are the most common source of starch in aquafeeds, however, starch can be contributed via grain legumes, rice, corn, and tropical root crops.

Carbohydrates in the form of non-starch polysaccharides (NSP) often have a limited role in aquafeeds. With the exception of some benthic feeders such as abalone and sea urchins, which can tolerate very high levels of dietary fibre, most aquatic species have a limited capacity to utilize high levels of dietary NSP.

| Sample | Moisture | Crude protein | Crude fat | Ash | Fibre |
|------------------------|----------|---------------|-----------|-----|-------|
| Crab pereipoda | 673 | 103 | 5 | 177 | 33 |
| Crab claw | 521 | 90 | 5 | 310 | 32 |
| Crab carapace | 701 | 88 | 7 | 152 | 13 |
| Grouper head and frame | 718 | 128 | 57 | 53 | 0 |
| Snapper gut | 757 | 131 | 56 | 27 | 0 |
| Grouper gut | 741 | 92 | 95 | 22 | 0 |

 Table 3. Proximate analysis of potential fish processing waste streams (g/kg, as received).

Table 4. Microbial analysis of fresh grouper and snapper guts with potential use in aquafeeds

| Test | Unit | Snapper | Grouper | Standard |
|-----------------------|---------------|----------|----------|----------|
| Total plate count | cfu/ml | 45,000 | 700,000 | <500,000 |
| Coliform count | MPN/ml | 93 | 43 | <100 |
| E.coli | MPN/ml | 43 | 4 | Negative |
| Salmonella | Colonies/gram | Negative | Negative | Negative |
| Staphylococcus aureus | Colonies/gram | 10 | 50 | <5000 |

Fats and oils

Supply of fatty acids is important in most aquatic diets, but there is significant variation in the quantity of fat required to optimize growth rates. For example, high energy salmonid diets with oil contents' exceeding 35-40% are common for the supply of essential fatty acids and energy, whereas in prawn diets, there are specific requirements for essential fatty acids, but the total oil content of the diet may only be around 4%. Abalone, have a minimal requirement for fatty acids, and oil levels exceeding 3% can negatively affect the digestion of other ingredients in the diet.

Fat can be applied during the extrusion process or following extrusion using processes such as vacuum infusion. Fat levels up to 12% in the diet will have little influence on the extrusion process. For every 1% of fat above 12%, the bulk density of the final product will decrease by 16 g/l. When fat levels are between 17 and 22%, the extruded product will have little or no expansion, but will remain durable. Above 22% fat, the extruded product durability will be very poor.

Like fishmeal, there is an urgent need to find alternatives to fish oil in aquafeeds. Vegetable oils in particular have potential for blending to produce fish oil replacements. For example, crude and refined canola oil and refined soybean oil have been assessed as replacements for added dietary fish oil in diets for juvenile red seabream (B.D. Glencross, personal communication). These oil resources were evaluated for their potential to replace added fish oil (40 g/kg) in fishmeal based (600 g/kg) diets, with 100 g/kg of total lipids. Each of the three plant oils was used to replace 25%, 50%, 75% or 100% of the added dietary fish oil. Each of the three plant oils showed potential as a replacement for dietary fish oil, though significant reductions in growth and feed utilisation were observed with the complete replacement of fish oil by crude canola oil. No other significant effects of oil type or inclusion level on growth were apparent. Notably, few effects of the alternative oils were seen on the proximate composition of the fish. However, the influence of the alternative oils on the tissue fatty acid composition was considerable, irrespective of plant oil type or processing grade. Particularly notable was the overall increase in the level of polyunsaturated fatty acids in the tissues of the fish fed the plant oil diets, with these trends becoming more apparent with the greater levels of fish oil replacement. Minimal reductions in the levels of the long-chain polyunsaturated fatty acids of eicosapentaenoic (20:5n-3) and docosahexaenoic (22:6n-3) acid were observed from any of the plant oil

treatments. Sensory assessment, by an Australian taste panel, of the fish fed the fish oil reference, or the 100% replacement by refined canola or refined soybean diets showed a preference in order of canola oil > soybean oil > fish oil fed fish. Clearly, both canola and soybean oils have considerable potential as replacements of fish oils in diets for this species.

Additives

Additives include vitamins and minerals, pigments, preservatives, exogenous enzymes, anti-oxidants, emulsifiers, flavours and attractants. Vitamin and mineral addition is based on some research, but most addition rates account for possible losses during extrusion (15%) or leaching prior to consumption. Some typical vitamin and mineral specifications for aquatic diets are presented in Table 5.

| Vitamin | Finfish | Crustaceans |
|-----------------------------------|---------------|-------------|
| Fat-soluble vitamins | | |
| Vitamin A, IU/kg | 5,000 (2,500) | 5,000 |
| Vitamin D, IU/kg | 3,000 (2,400) | 0.1 mg/kg |
| Vitamin E, IU/kg | 250 (50) | 100 |
| Vitamin K, mg/kg | 5(1) | 5 |
| Water-soluble vitamin, mg/kg | | |
| Riboflavin | 20 (4) | 25 |
| Pantothenic acid | 40 (20) | 75 |
| Niacin | 50 (10) | 40 |
| Vitamin B12 | 0.04 (0.01) | 0.2 |
| Biotin | 0.6 (0.15) | 1 |
| Folate | 5 (1) | 10 |
| Thiamin | 10(1) | 60 |
| Vitamin B6 | 10 (3) | 50 |
| Vitamin C | 150 (50) | 200 |
| myo-Inositol | 150 (300) | 400 |
| MINERALS, MG/KG | | |
| Magnesium (as magnesium sulphate) | 500 | 3,000 |
| Iron (as iron sulphate) | 60 | |
| Zinc (as zinc sulphate) | 50 | 100 |
| Manganese (as manganese sulphate) | 15 | |
| Copper (as copper sulphate) | 5 | 40 |
| Iodine (as potassium iodide) | 4 | |
| Selenium (as sodium selenite) | 0.4 | 0.4 |
| Cobalt (as cobalt sulphate) | 0.5 | |

Table 5. Generic vitamin and mineral specifications for aquatic diets.

*Values in brackets represent NRC (1993) recommendations for salmonids.

Anti-oxidants, such as tocopherol compounds or commercial anti-oxidants such as Banox, should be added aquatic feeds to stabilize lipids when the dietary level is high. Lipid oxidation can have the greatest influence on the shelf life of aquatic feeds, particularly in hotter climates.

When producing moist or semi-moist aquatic feeds, then some form of preservative must be incorporated into the diet formulation. One strategy is to reduce the pH to 4.5-5.0 using phosphoric acid or lactic acid, add mould inhibitors such as potassium sorbate, and to utilize humectants such as glycerine or propylene glycol to reduce water activity to below 0.75.

Feed additives such as silicas and clays can be used to alter the degree of slip in the extruder barrel and hence influence product density and final appearance, but to also increase the oil and water absorption capacity of the diet.

Exogenous enzymes have been assessed for some aquatic species based on the reduced capacity of these species to digest carbohydrate components of the diet. Exogenous enzymes in aquatic feeds hold the greatest potential when the carbohydrate component of the diet influences digesta viscosity and subsequent action of endogenous enzymes.

Ingredient characterization

Nutrient digestibility and availability as a means of describing the nutritive value of feed ingredients can be applied in many aquaculture systems. Definition of nutritive value on this basis will also facilitate the most accurate description of nutritional requirements for the target aquaculture species. Based on terrestrial nutrition research, protein and energy requirements must take priority in the development of aquaculture diets and should be defined by first assessing the response of the aquaculture species to protein and energy intake, followed by assessment of amino acid requirements and energy requirements. When these factors have been defined, further information on lipid requirements and vitamin and mineral requirements may prove beneficial in improving production efficiency and product quality.

In some aquaculture systems, *in vivo* assessment of ingredients can be difficult. In this case, an *in vitro* system or surrogate system can be employed, as long as the system provides a basis for comparison between ingredients and is representative of the target aquaculture species. Table 6 displays some comparative assessments of plant-based protein meals for use in Southern Bluefin Tuna diets using *in vitro* and surrogate analysis systems.

In addition to characterising the digestible or available nutrient supply from an ingredient, it is also important to establish maximum inclusion levels. Maximum inclusion levels are generally species specific and will be influenced by the levels of anti-nutritional factors in the ingredient, the carbohydrate and moisture content of the ingredient, the ingredient palatability, and the comparative ability of the ingredient to supply protein and energy for a set cost.

Other factors worth characterizing when assessing the nutritional quality of ingredients for use in aquafeeds include:

- Nitrogen solubility index;
- Hydration capacity;
- Binding capacity;
- Microbial load;
- Heavy metal contamination.

When combining ingredients in aquafeeds, characteristics such as hydration rate can have a significant influence on final product quality. If the combination of ingredients used varies widely in hydration rate, then product binding, mixing and degree of cook can be influenced. Ingredient particle size distribution is also an important consideration if post-mix grinding is not possible.

Table 6. *Plant meals:* Apparent dry matter and nitrogen (crude protein) digestibility coefficients for *in vivo* (*Salmo salar* at 2 and 4 weeks at 30 % ingredient inclusion) and *in vitro* analyses using commercial enzymes (Enzyme commercial) or *S. salar* crude enzymes (Enzyme salmon) for selected plant meals. All data are % mean ± standard deviation.

| Ingredient (%CP) | In vivo | | In vitro | |
|---------------------------|------------------|-------------------|----------------------|------------------|
| | 2 weeks | 4 weeks | Enzyme commercial | Enzyme salmon |
| Dry matter | | | commercial | Saiiii0ii |
| Lupin-meal(35.1%) | | | 57.40 ± 0.43 | 42.55 ± 0.85 |
| Lupin-dehulled(27.9%) | | | 50.34 ± 1.60 | 49.12 ± 2.34 |
| Pea-conc. A(37.5%) | | | 56.71 ± 1.08 | 33.50 ± 2.50 |
| Pea-conc. B(41.0%) | | | 64.94 ± 1.10 | 39.49 ± 2.39 |
| Pea-conc.C(40.6%) | | | 63.86 ± 1.51 | 32.12 ± 2.14 |
| Pea-Ex.conc. $(41.0\%)^d$ | 55.91 ± 1.79 | 60.39 ± 8.19 | 81.54 ± 0.05 | 66.02 ± 0.22 |
| Pea-Ex.conc.(41.0%) | 67.00 ± 9.90 | 80.76 ± 1.30 | 81.54 ± 0.05 | 66.02 ± 0.22 |
| Pea-dehulled(21.8%) | | | 45.02 ± 3.54 | 22.10 ± 1.76 |
| Soybean-meal A(41.1%) | | | 72.93 ± 0.89 | 43.57 ± 1.88 |
| Soybean-meal B(40.4%) | 67.19 ± 8.49 | 66.01 ± 0.98 | 80.43 ± 0.26 | 47.05 ± 0.32 |
| Soybean-full fat(32.4%) | | | 71.23 ± 0.07 | 50.95 ± 1.99 |
| Soybean-fat | | | 80.64 ± 0.02 | 44.77 ± 0.09 |
| extracted(43.8%) | | | | |
| Wheat gluten(67.1%) | 84.38 ± 13.11 | 86.40 ± 0.96 | 78.52 ± 0.78 | 70.19 ± 0.92 |
| Nitrogen | | | | |
| Lupin-meal(35.1%) | | | 91.05 ± 0.49 | 58.23 ± 1.00 |
| Lupin-dehulled(27.9%) | | | 87.34 ± 0.56 | 80.20 ± 1.80 |
| Pea-conc. A(37.5%) | | | 88.91 ± 0.35 | 50.27 ± 1.14 |
| Pea-conc. B(41.0%) | | | 94.48 ± 1.23 | 51.33 ± 1.14 |
| Pea-conc.C(40.6%) | | | 89.34 ± 0.63 | 50.51 ± 0.91 |
| Pea-Ex.conc.(41.0%) | 88.85 ± 0.57 | 92.03 ± 1.20 | 94.61 ± 0.28 | 87.83 ± 0.64 |
| Pea-Ex.conc.(41.0%) | 90.84 ± 0.58 | 96.71 ± 0.25 | 94.61 ± 0.28 | 87.83 ± 0.64 |
| Pea-dehulled(21.8%) | | | 88.12 ± 0.96 | 53.45 ± 1.64 |
| Soybean-meal A(41.1%) | | | 89.97 ± 0.54 | 37.46 ± 0.98 |
| Soybean-meal B(40.4%) | 92.79 ± 1.24 | 93.38 ± 0.24 | 93.46 ± 0.48 | 53.25 ± 0.14 |
| Soybean-full fat(32.4%) | | | 88.33 ± 1.33 | 57.38 ± 0.75 |
| Soybean-fat | | | 93.28 ± 0.04 | 48.50 ± 0.80 |
| extracted(43.8%) | | | | |
| Wheat gluten(67.1%) | 96.33 ± 9.43 | 101.04 ± 0.90 | 94.64 ± 0.13 | 94.55 ± 3.09 |

When selecting ingredients for use in aquafeeds, guidelines and standards such as those published by the National Agricultural Commodities Marketing Association (NACMA) can form a useful basis for selection.

Conclusions

Ingredient selection for use in aquafeeds is generally more complex than that for traditional stockfeeds. The greater the understanding of the wide number of feed ingredients that can potentially be used in aquafeeds, coupled with an understanding of the nutrient requirements of the target species, the easier it will be to formulate and manufacture a suitable diet.

Appendix 5 – Annual Operating Plans



Aquaculture Nutrition Subprogram Annual Operating Plan - 2002

Prepared by **Dr Robert van Barneveld** Subprogram Leader

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A) Activity description for 2000-2001

i) Sector Progress

Aquaculture nutrition research is a fundamental component in the establishment of new aquaculture industries and in the further development of existing aquaculture industries for the following reasons:

- 6. Feed costs represent a significant proportion (30-50%) of the production costs in most aquaculture systems. The most effective way to reduce feed costs is to improve the feed conversion efficiency of the target species which can only be accomplished by improving definition of feed ingredients, defining the nutritional requirements for different production phases, improving diet form and feed stability and increasing the range of feed ingredients available for use all of which require significant research inputs.
- 7. Development of cost-effective manufactured feeds for aquaculture species is a difficult process because the research has to be conducted underwater. This makes collection of information on fundamental parameters such as feed intake difficult. In addition, most aquaculture diets needs to be extruded which is a research discipline in itself and requires highly specialised equipment.
- 8. Many aquaculture diets have a heavy reliance on fresh fish, fish meals and fish oils. To provide long term stability to new and established aquaculture industries and to reduce the reliance on these ingredients, there is a need to identify alternative nutrient sources that facilitate maintenance of similar production levels and product quality.
- 9. Improved nutrition of aquaculture species is one of the most effective ways to reduce any environmental impacts from aquaculture by reducing nutrient loads in effluent.
- 10. Some aquaculture sectors, such as prawns, still rely heavily on imported feeds due to a lack of alternatives in Australia.

Given the large number of unknowns often associated with the nutrition of new aquaculture species and the difficulties associated with conducting the research, significant improvements in the efficiency of conducting this research can be achieved by developing a coordinated approach to the definition of research priorities and

research methods, such as that achieved within a managed subprogram. To this end, the Aquaculture Nutrition Subprogram was established in September, 2001.

ii) Major research outputs of the Subprogram

The Aquaculture Nutrition Subprogram was established in September, 2001. To date there have been no research outputs specific to this Subprogram.

iii) Related projects and research linkages

There is a significant investment in aquaculture nutrition research in Australia. Since 1990, FRDC has funded more than 50 projects that focus on nutrition or have components of research that focus on nutrition. In addition, a range of species specific FRDC Subprograms have, or have had, nutrition research as a high priority within their research portfolio including the Abalone, Rock Lobster Enhancement and Aquaculture and Southern Bluefin Tuna Aquaculture subprograms. Current investment in nutrition research projects by the FRDC, the AQUAFIN CRC and research providers will exceed \$AUD5 million over the next 3 years.

iv) Role the Subprogram has played in industry development

To date FRDC has invested in two nutrition specific subprograms – the Fishmeal Replacement (FMR) and Aquaculture Diet Development (ADD) Subprograms. The FMR and ADD Subprograms were successful in meeting the objectives of managed subprograms on the basis that they:

- Promoted a high level of collaboration between scientists working within a common discipline;
- Successfully delivered nutrition research expertise to infant aquaculture industries that otherwise would have not had access to this level of nutritional skill;
- Reduced the level of duplication of research effort towards a common goal;
- Applied outcomes were delivered to industry improving the profitability and viability of these industries;
- Facilitated a coordinated delivery of research funding submissions and research reports to the FRDC.
- Advanced the overall international knowledge base for aquaculture nutrition.

The smaller aquaculture industries benefiting from research conducted within the FMR and ADD Subprograms (ie. barramundi, salmon, prawns, silver perch) have suggested they would value the continuation of further coordinated research in the area of aquaculture nutrition. Industries such as the barramundi industry rate nutrition as the highest research priority above all other disciplines, and believe that nutrition research on behalf of their industry would be severely compromised in the absence of a nutrition specific subprogram. Research providers and researchers operating within the FMR and ADD Subprograms valued their involvement as the Subprograms, through the Subprogram Leader, managed to breakdown many institutional boundaries that previously existed. The Subprogram workshops also represented a valuable form of peer review for research results. Research providers and researchers conducting nutrition research projects within species-based subprograms found the FMR and ADD to be a valuable resource. Workshops conducted as part of these subprograms provided an outlet for related research results and a valuable forum for critical review of the research. Many researchers operating outside the FMR and ADD used these subprograms as their reporting vehicles to FRDC.

The infrastructure (ie collaborations, industry contacts, mailing lists etc) developed as part of the FMR and ADD Subprograms represents a valuable resource to the Australian aquaculture industries, and this resource will be maintained through the Aquaculture Nutrition Subprogram.

v) Operating procedures

The Aquaculture Nutrition Subprogram is a new subprogram that represents an evolution from the highly successful FMR and ADD Subprograms, both managed with great success by Dr Geoff Allan. Following Geoff's decision not pursue a leadership role of a further subprogram, Robert van Barneveld has undertaken to develop the model established by Geoff, but with some new strategic directions relevant to all aquaculture species.

The objectives of the Aquaculture Nutrition Subprogram are outlined below:

- 1. Establish what the fundamental non species specific nutritional constraints are to aquaculture production (eg. diet form, nutritional requirements, feeding strategies, feed evaluation or a combination of several factors) and then establish and manage a collaborative core research program to address this.
- 2. Develop a risk management strategy for all aquaculture industries in relation to nutrition to assist the research priority setting process.
- 3. Forge an agreement between the FRDC and other relevant RDC's and research providers for the management of research relating to aquaculture nutrition under the auspices of an aquaculture nutrition subprogram accounting for variations in priorities between agencies.
- 4. Define and facilitate a mechanism for lead agencies for the commercialisation of all relevant nutrition research arising from FRDC funded projects.
- 5. Provide a single point of contact for aquaculture nutrition research in Australia and improve the aquaculture nutrition skills base in Australia.
- 6. Facilitate the functions of an aquaculture nutrition "Expert Working Group" to ensure ongoing research programs have a high degree of industry relevance and focus.
- 7. Improve communication of nutrition research outcomes and current nutrition research between scientists, aquaculturists, ingredient suppliers and feed manufacturers.

As the Subprogram evolves, further information on operating procedures will be made available.

vi) Meetings and workshops

ANNUAL AQUACULTURE NUTRITION WORKSHOP

A core service of the Aquaculture Nutrition Subprogram will be the organisation and delivery of an annual aquaculture nutrition workshop. This workshop will provide a forum for the delivery of research results from all FRDC-funded aquaculture nutrition projects. Where possible, invited speakers will be utilised to address issues relevant to nutrition research. The workshop will also provide an opportunity for feed manufacturers and end users to present their views in relation to aquaculture nutrition research priorities.

Further details pertaining to the first annual workshop will be distributed by the Subprogram Leader as they become available. At this point, the workshop will be held in March or April, 2002.

PROVISION OF SHORT COURSES

As a service to end users, researchers and feed manufacturers, the Aquaculture Nutrition Subprogram plans to offer a number of short courses each year based on interest and on a full cost recovery basis. Under the guidance of the Expert Working Group, a number of courses will be designed and advertised in January of each year. Promotion will include the cost of attendance, the minimum number of participants required for the course to proceed and an outline of the course content. If there is insufficient interest, the course will not proceed. Courses may be run by members of the Expert Working Group who have specific areas of expertise, or they may utilise the inputs of others. Suggestions for courses that could be developed and delivered via the Subprogram include:

- 1. Short course in practical feed microscopy;
- 2. Practical extrusion of experimental diets;
- 3. Aquaculture nutrition principles for end users;
- 4. Practical aquaculture diet formulation;
- 5. Practical assessment of aquaculture diets on-farm.

If you have any suggestions relating to short courses that may be of interest to you, please contact the Subprogram Leader.

vii) Summary of current project status

At this point, the Aquaculture Nutrition Subprogram has no core projects, but obviously has links with all other aquaculture nutrition projects currently funded by FRDC.

B) SUMMARY OF STRATEGIC PLAN OR DIRECTIONS

A strategic plan for the Subprogram will be developed during the first year of operation.

C) COMMUNICATION AND TECHNOLOGY TRANSFER ACTIVITIES

The Aquaculture Nutrition Subprogram will undertake the following communication and technology transfer activities:

- 1. Maintenance and annual upgrading of an Aquaculture Nutrition Subprogram strategic plan on CD-ROM for distribution to research agencies, FRABs, and industry.
- 2. Publication of an annual operating plan for the subprogram for delivery to FRABs in December each year to assist deliberations on projects related to aquaculture nutrition..
- 3. Publication of an aquaculture nutrition newsletter for distribution to researchers and industry via the subprogram mailing list. This mailing list has recently been updated and now contains 125 contacts.
- 4. Publication of workshop proceedings from the annual aquaculture nutrition workshop for distribution to workshop participants, industry and educational institutions.
- 5. Coordinated delivery of progress and final reports to FRDC from core projects managed within the aquaculture nutrition subprogram.
- 6. Development and maintenance of an aquaculture nutrition website. The Subprogram Leader will also provide regular contributions to Aquafeed.com to promote the activities of the Subprogram.
- 7. Where sufficient interest exists, the subprogram will provide short courses to improve the practical nutrition knowledge base that exists in the Australian aquaculture sector.

D) PROPOSED NEW RESEARCH

Until the Expert Working Group has been convened and the first annual workshop held, it is not possible to identify potential new research projects that will form core components of the Subprogram.

E) WORKPLAN FOR 2001-2002

The following core activities will be undertaken during the first year of the Aquaculture Nutrition Subprogram:

ESTABLISHMENT OF A RECOGNISED "EXPERT WORKING GROUP"

A core function of the Aquaculture Nutrition Subprogram will be the facilitation of an Expert Working Group that will undertake the following:

- Develop a 5 year research and development plan for generic Australian aquaculture nutrition research priorities.
- When requested by FRDC, scrutinise nutrition research planned within the species based subprograms.
- Provide advice to the FRDC Board and other Australian research and development investors on aquaculture nutrition research priorities.
- Act as an identifiable point of contact as an expert working group on nutrition for all Australian stakeholders in aquaculture.
- Provide industry feedback and views.
- Review existing nutrition research based on contractual obligations when requested by FRDC.

- Ensure nutrition research outcomes are commercially focused;
- Identify those research providers and researchers best equipped to address defined research priorities;
- Commission tendered research to address defined priorities;
- Define an appropriate basis for the distribution of intellectual property on a case by case basis.
- Facilitate extension and technology transfer of nutrition research outcomes.

The Expert Working Group will meet face to face once per year in conjunction with the annual workshop. Teleconferences will be convened as required during the year. Membership of the Expert Working Group will be skills based, and will include scientists, feed manufacturers, aquaculturists, and others as appropriate. This project will support a membership of 8-10.

STRATEGIC PLANNING AND ESTABLISHMENT OF A CORE PROJECT BASE

Based on the inputs of the Expert Working Group, consultation with the species-based subprograms and consultation with industry, a strategic plan will be developed for the Subprogram to address aquaculture nutrition research issues that are common to all species. This strategic plan will be distributed on CD-ROM.

Based on the strategic plan, the Subprogram will commission a number of core research projects as appropriate and will manage these projects on behalf of FRDC in a similar way to species-based subprograms. If a research project exists that is consistent with the strategic plan for the Subprogram, a recommendation will be made to FRDC to make it a core subprogram project. New research proposals will be developed through the use of facilitated strategic planning meetings. Using priorities published in the Aquaculture Nutrition Subprogram Strategic Plan, the Subprogram Leader will convene meetings with relevant researchers and research institutions to:

- 1. Define the planned outcomes of the new proposal;
- 2. Manage an indicative budget for the research;
- 3. Identify which researchers/institutions are best placed to undertake the research;
- 4. Promote collaboration between researchers and institutions where appropriate;
- 5. Seek external expertise and inputs as required.
- 6. Ensure the new proposal meets the objectives of the subprogram and that the research remains relevant and focused.

Development of formal relationships between FRDC and other investors

The Subprogram will forge an agreement between the FRDC and other relevant RDC's (such as ACIAR and GRDC) and research providers for the management of research relating to aquaculture nutrition under the auspices of the Aquaculture Nutrition Subprogram. This process will account for variation in priorities between agencies. This mechanism will be for providing information to other RDC's rather than making decisions on their behalf.

IDENTIFICATION AND PROCUREMENT OF ADDITIONAL FUNDING

Utilising the relationship between FRDC and other investors, the Aquaculture Nutrition Subprogram will identify and procure additional funding for the conduct of core aquaculture nutrition projects.

COMMERCIALISATION OF AQUACULTURE DIETS

In the past, difficulties have been encountered commercialising outcomes from nutrition projects, and this was highlighted in the review conducted by the BECAN Consulting Group. This can be partly attributed to the fact that most of the outcomes from nutrition projects are intangible (eg. digestibility values, diet formulation). Heavy reliance was placed on the feed manufacturers to adopt these outcomes, to use the outcomes to develop commercial diets, and to produce sufficient quantities of diet for commercial-scale experiments - a process that was not always successful.

The Aquaculture Nutrition Subprogram could act as the conduit between research providers and feed manufacturers to facilitate the commercialisation of technology arising from aquaculture nutrition research. This could include establishing a basic procedure for the commercialisation of nutrition research. In addition, the Subprogram could facilitate the pre-commercial production of extruded aquatic feeds in association with the newly established "Australasian Experimental Stockfeed Extrusion Centre".

F) BUDGET

No information is available at this time.

Recommendations/Variations

The Subprogram will forward recommendations to FRDC on any nutrition related projects that may be reviewed in 2001-2002.



Aquaculture Nutrition Subprogram Annual Operating Plan - 2003

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A) ACTIVITY DESCRIPTION FOR 2001-2002

viii) Sector Progress

Aquaculture nutrition research is a fundamental component in the establishment of new aquaculture industries and in the further development of existing aquaculture industries for the following reasons:

- 1. Feed costs represent a significant proportion (30-50%) of the production costs in most aquaculture systems. The most effective way to reduce feed costs is to improve the feed conversion efficiency of the target species which can only be accomplished by improving definition of feed ingredients, defining the nutritional requirements for different production phases, improving diet form and feed stability and increasing the range of feed ingredients available for use all of which require significant research inputs.
- 2. Development of cost-effective manufactured feeds for aquaculture species is a difficult process because the research has to be conducted underwater. This makes collection of information on fundamental parameters such as feed intake difficult. In addition, most aquaculture diets needs to be extruded which is a research discipline in itself and requires highly specialised equipment.
- 3. Many aquaculture diets have a heavy reliance on fresh fish, fish meals and fish oils. To provide long term stability to new and established aquaculture industries and to reduce the reliance on these ingredients, there is a need to identify alternative nutrient sources that facilitate maintenance of similar production levels and product quality.
- 4. Improved nutrition of aquaculture species is one of the most effective ways to reduce any environmental impacts from aquaculture by reducing nutrient loads in effluent.
- 5. Some aquaculture sectors, such as prawns, still rely heavily on imported feeds due to a lack of alternatives in Australia.

Given the large number of unknowns often associated with the nutrition of new aquaculture species and the difficulties associated with conducting the research, significant improvements in the efficiency of conducting this research can be achieved by developing a coordinated approach to the definition of research priorities and

research methods, such as that achieved within a managed subprogram. To this end, the Aquaculture Nutrition Subprogram was established in September, 2001.

ix) Major research outputs of the Subprogram

The Aquaculture Nutrition Subprogram was established in September, 2001. During 2002 the subprogram prepared a strategic plan and has assessed a range of preliminary research proposals relevant to this plan. Full proposals have been prepared where appropriate and if successful, a number of core projects will exist within the ANS from July 1, 2003. From this point, the subprogram can claim research outputs.

Given that a wide scope of nutrition research already exists within the FRDC portfolio, as well as a range of other research investors, the ANS aims to take nutrition research into a higher risk area that is not limited by industry development restrictions and is beyond individual species based projects. The vision for this new subprogram is the efficient, innovative and sustainable delivery of nutrients to aquaculture production systems to optimise aquatic animal health and production and aquaculture product quality while minimising impacts on the aquatic environment. The subprogram mission is to:

"To enhance the Australian aquaculture nutrition expertise base and to utilise combined knowledge to identify and solve common aquaculture nutrition challenges and limitations to development of the Australian aquaculture industries".

x) *Related projects and research linkages*

The FRDC's Research and Development Plan 2000-2005 lists a number of challenges that exist in the FRDC's business environment. Challenge "5" is reducing the quantity of fish protein fed to terrestrial and aquatic livestock so that it becomes available in the food chain to satisfy environmental and human needs. The FRDC have only made minimal investments in research addressing this challenge since the plan became active and hence this research area remains a very high priority.

Dr Brett Glencross has successfully attracted research funding from the Grains Research and Development Corporation to investigate the development of value-added plant protein products for the aquaculture feeds sector. This project is due to commence on January 1, 2003 and has the following objectives:

- 1. Development of value-added lupin protein products for use in the animal feeds sector;
- 2. Evaluation of the nutritional value of a range of value-added lupin protein products when fed to fish;
- 3. Commercial transfer of intellectual property for development of new-product(s).

As a broad outline, the ANS recommends that the FRDC invest in research aimed at developing commercial processes for the production of protein isolates or concentrates from a variety of sources and assessment of these proteins in major Australian species including salmon, southern bluefin tuna, barramundi and prawns. With this in mind, formal linkages with commercial partners is an important component of the project. It is also important to remember that the FRDC challenge encompasses the reduction in fish protein use by the terrestrial livestock sectors, so some involvement from the pig, poultry and ruminant industries may also be required.

At this stage, a lot of further work is required to develop a broader project that would attract co-investment from the FRDC. This project would also need to involve other FRDC subprograms such as the SBT Aquaculture Subprogram and the Atlantic Salmon Aquaculture Subprogram. A review of this GRDC project in the form of a workshop in late April, 2003 would also be an important step in the development of a revised program that included FRDC co-investment. Details of how the ANS plans to develop this project are presented in the subprogram workplan for 2003 presented later in this operating plan.

Examining other projects, there is a significant investment in aquaculture nutrition research in Australia. Since 1990, FRDC has funded more than 50 projects that focus on nutrition or have components of research that focus on nutrition. In addition, a range of species specific FRDC Subprograms have, or have had, nutrition research as a high priority within their research portfolio including the Abalone, Rock Lobster Enhancement and Aquaculture and Southern Bluefin Tuna Aquaculture subprograms. Current investment in

nutrition research projects by the FRDC, the AQUAFIN CRC and research providers will exceed \$AUD5 million over the next 3 years.

xi) Role the Subprogram has played in industry development

To date FRDC has invested in two nutrition specific subprograms – the Fishmeal Replacement (FMR) and Aquaculture Diet Development (ADD) Subprograms. The FMR and ADD Subprograms were successful in meeting the objectives of managed subprograms on the basis that they:

- Promoted a high level of collaboration between scientists working within a common discipline;
- Successfully delivered nutrition research expertise to infant aquaculture industries that otherwise would have not had access to this level of nutritional skill;
- Reduced the level of duplication of research effort towards a common goal;
- Applied outcomes were delivered to industry improving the profitability and viability of these industries;
- Facilitated a coordinated delivery of research funding submissions and research reports to the FRDC;
- Advanced the overall international knowledge base for aquaculture nutrition.

The smaller aquaculture industries benefiting from research conducted within the FMR and ADD Subprograms (ie. barramundi, salmon, prawns, silver perch) have suggested they would value the continuation of further coordinated research in the area of aquaculture nutrition. Industries such as the barramundi industry rate nutrition as the highest research priority above all other disciplines, and believe that nutrition research on behalf of their industry would be severely compromised in the absence of a nutrition specific subprogram. Research providers and researchers operating within the FMR and ADD Subprograms valued their involvement as the Subprograms, through the Subprogram Leader, managed to breakdown many institutional boundaries that previously existed. The Subprogram workshops also represented a valuable form of peer review for research results. Research providers and researchers conducting nutrition research projects within species-based subprograms found the FMR and ADD to be a valuable resource. Workshops conducted as part of these subprograms provided an outlet for related research results and a valuable forum for critical review of the research. Many researchers operating outside the FMR and ADD used these subprograms as their reporting vehicles to FRDC.

The infrastructure (ie collaborations, industry contacts, mailing lists etc) developed as part of the FMR and ADD Subprograms represents a valuable resource to the Australian aquaculture industries, and this resource will be maintained through the Aquaculture Nutrition Subprogram.

xii) Operating procedures

The ANS was established to develop and implement a core research program based on fundamental, non species-specific nutritional constraints to aquaculture production. In developing this core research program, the ANS will develop a risk management strategy for all aquaculture industries in relation to nutrition to assist the research priority setting process. In developing a core research program, the ANS applies five criteria against potential areas of core research and innovation including:

- 6. Must be of national significance/benefit.
- 7. Must have multi-species significance.
- 8. Must require multiple knowledge inputs to achieve an outcome.
- 9. Must be limiting the development and/or viability of aquaculture industries.
- 10. Must not duplicate existing research efforts.

In addition, the ANS will:

- Establish basic research standards and preferred methodologies for nutrition research;
- Facilitate technology transfer in the field of aquaculture nutrition;
- Develop and provide training for research investors, research providers, and end-users associated with aquaculture nutrition;
- Act as a point of contact for aquaculture nutrition in Australia; and

• Develop links between research investors, research providers and end-users associated with aquaculture nutrition.

xiii) Meetings and workshops

ANNUAL AQUACULTURE NUTRITION WORKSHOP

A core service of the Aquaculture Nutrition Subprogram will be the organisation and delivery of an annual aquaculture nutrition workshop. This workshop provides a forum for the delivery of research results from all FRDC-funded aquaculture nutrition projects. Where possible, invited speakers will be utilised to address issues relevant to nutrition research. The workshop also provides an opportunity for feed manufacturers and end users to present their views in relation to aquaculture nutrition research priorities.

The First Annual ANS Workshop was held on February 25th 2002 at CSIRO, Cleveland, Qld. The 2003 ANS workshop will be held in April / May 2003 and the proposed venue is Adelaide, SA or Fremantle, WA.

TRAINING

In keeping with the core objective of improving nutrition research capacity of research providers in and out of Australia, the ANS proposes to offer user-pays training packages that will proceed based on demand. In the first instance, training is proposed in the following areas:

- Practical extrusion of research diets and diets for emerging species (this course will be run in conjunction with and as an extension to the existing "Feed and Food Extrusion Course" which will be relocated to South Australia in 2003 and a commercial course being offered by Wenger Manufacturing in Australia at the Australasian Experimental Stockfeed Extrusion Centre);
- Practical nutrition short course for aquaculture producers;
- Feed microscopy for aquaculture;
- Capacity for nutritional control of nutrient outputs from aquaculture systems for policy and planning agencies.

xiv) Summary of current project status

At this point, the Aquaculture Nutrition Subprogram has no core projects, but obviously has links with all other aquaculture nutrition projects currently funded by FRDC.

B) SUMMARY OF STRATEGIC PLAN OR DIRECTIONS

Core Research and Development

A range of aquaculture nutrition research programs are underway within the following aquaculture sectors (some with established Subprograms):

- Southern Bluefin Tuna
- Abalone
- Prawns
- Rock Lobster
- Snapper
- Salmon
- Barramundi
- Other (Silver Perch, Yellowtail Kingfish, Sea Urchin, King George Whiting, Crabs).

A collation of aquaculture nutrition research and development issues faced by these aquaculture sectors together with inputs from related sectors (facilitated through the first annual ANS workshop held in February, 2002) is summarised as follows (in no specific order):

FEED EVALUATION

- Poor capacity to accurately measure feed intake (*All*).
- Capacity to manipulate dietary amino acid supply without utilising crystalline amino acids (*Prawns, Abalone*).
- Influence of manufactured aquatic feeds on aquaculture product quality and food safety hazards (All).
- Influence of nutrition on aquatic animal health (*Abalone, Salmon, Tuna, Snapper, Rock Lobster*).
- Basal larval, broodstock and production diets for existing and emerging species (All).

NUTRITIONAL REQUIREMENTS

- Application of inappropriate research methodologies in some cases resulting in inaccurate or misleading results (particularly in the definition of nutritional requirements) (*Prawns, Tuna, Abalone*).
- Poor capacity to accurately measure feed intake (*All*).
- Influence of feed form, feeding strategies and feeding regime on the surrounding aquatic environment (*All*).
- Alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils (*All*).
- Suitable larval, broodstock and production diets for existing and emerging species (*All*).

FEED PROCESSING

- Basal larval, broodstock and production diets for existing and emerging species (All).
- Fat stability in extruded pellets (*Salmon*).
- Capacity to manufacture extruded pellets with a diameter less than 1.5 mm (*All*).
- Effects of mechanical energy inputs during diet manufacture on water stability (All).
- Influence of feed production processes on micro-ingredient losses and stability (All).
- Starch and protein losses through degradation during feed processing (All).
- Interaction between ingredient source and type and extrusion processing parameters (*All*).
- Production of shelf stable semi-moist pellets (*Tuna, Sea urchins*).

An Expert Working Group was convened by the Aquaculture Nutrition Subprogram in 2002 to identify additional nutritional constraints to aquaculture production in Australia. Aquaculture nutrition constraints and/or requirements in addition to those listed above include:

- Routine evaluation of feed ingredients suitable for use in aquatic diets.
- Availability/reliability of energetic and other models for use in managing feeding systems and aquatic animal responses.
- Assessment of feed attractants.
- Strategies for feed delivery to aquaculture systems.
- Techniques to facilitate rapid weaning of aquaculture species onto manufactured diets.
- Feed formulation approaches to meet both the nutritional needs of the target species and the manufacturing requirements.
- Nutriceuticals (eg. probiotics, enzymes) suitable for use in aquaculture feeds.
- Availability of nutrition research infrastructure and the nutrition research capacity of research providers in and out of Australia.

Based on the five criteria defined for core research and development projects suitable for inclusion within the auspices of the Aquaculture Nutrition Subprogram (ie national significance/benefit, multi-species significance, multiple knowledge inputs required, limiting development and/or viability of aquaculture industries, no duplication of existing research efforts) the collation of research and development issues can be reduced and grouped into the following categories:

NUTRITION RESEARCH TOOLS

• Capacity to accurately measure feed intake in aquaculture species.

- Development of energetic and production models for simulating the responses of aquaculture species to nutritional inputs.
- Availability of nutrition research infrastructure and the nutrition research capacity of research providers in and out of Australia.

AQUATIC FEED PROCESSING

- Basal larval, broodstock and production diets for existing and emerging species.
- Interaction between ingredient source and type and extrusion processing parameters.

FEED EVALUATION

• Alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils.

NUTRITION AND AQUATIC ANIMAL HEALTH

- Interaction between nutrition and aquatic animal health.
- Nutritional prevention of disease and maintenance of animal health.

NUTRITION AND THE ENVIRONMENT

• Influence of feed form, feeding strategies and feeding regime on the surrounding aquatic environment.

NUTRITION AND PRODUCT QUALITY

• Influence of manufactured aquatic feeds on aquaculture product quality and food safety hazards.

Short and Long Term Research Needs

From the above list of ten priority research areas, three can be declared as having an urgent research requirement and will therefore represent target research areas within the ANS from 2003-04.

4. Alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils.

A clear need has been identified for the development of a vegetable protein isolate available for use in Australian aquafeeds as an alternative to fishmeal. Critical to achieving this goal is the involvement of industry with the technical expertise to produce such an isolate, end-users of both mid-and high protein components of the isolate and any additional co-products, other research investors, and research providers.

Lupins, particularly new cultivars such as *L. luteus*, hold the greatest potential for the production of a high protein isolate in Australia. Research and development is required to identify the most appropriate process for the production of such an isolate, the efficiency of such a process, the need to identify multiple end-users for the graded isolates, and the suitability of such an isolate for aquaculture. This process holds potential to produce a range of additional co-products such as non-starch polysaccharide isolates, isoflavones and oils that would require further investigation by multiple end-users.

The Grains Research and Development Corporation have also expressed an interest in this research area and could work together with the Aquaculture Nutrition Subprogram to establish some research partnerships in this area.

5. Interaction between nutrition and aquatic animal health.

The Abalone Aquaculture Subprogram and the Rock Lobster Enhancement and Aquaculture Subprogram have both identified nutrition and aquatic animal health as research priorities. In the abalone sector there is a need to establish the role of nutrition in "summer bloat" and research is underway in this area. In rock lobsters, there is a need to develop modes of larval feeding that allow some form of microbial control in propagation systems.

With the above in mind, in feed additives such as probiotics, enzymes, and the role and mode of action of products such as mannan oligosaccharides may prove useful for new and existing aquaculture industries.

6. Basal larval, broodstock and production diets for existing and emerging species.

A number of commercial and public sector research projects involving King George Whiting, Rock Lobsters, Organic Barramundi, and Sea Urchins (to name a few) have recently been undertaken and have experienced difficulties in developing and obtaining extruded basal diets in small quantities, that can be used for subsequent development. In some cases, the inability to produce these products has impeded the progress of existing research projects. An urgent need exists to establish basal extruded diets for use in the development of emerging aquaculture industries across Australia.

All other identified priorities form part of the longer term strategy for the ANS, but can emerge as research projects from the outset subjecting to research investment constraints.

D) COMMUNICATION AND TECHNOLOGY TRANSFER ACTIVITIES

The Aquaculture Nutrition Subprogram undertakes the following communication and technology transfer activities:

- 1. Maintenance and annual upgrading of an Aquaculture Nutrition Subprogram strategic plan available via the FRDC web-site for use by research providers, industry, other subprograms and FRAB's. Between September and November, 2002, the ANS strategic plan was downloaded from the FRDC website more than 292 times number 6 on the FRDC web-sites most popular download list !
- 2. Publication of an annual operating plan for the subprogram for delivery to FRABs in December each year to assist deliberations on projects related to aquaculture nutrition.
- 3. Publication of an aquaculture nutrition newsletter for distribution to researchers and industry via the subprogram mailing list. This mailing list has recently been updated and now contains 125 contacts.
- 4. Publication of workshop proceedings from the annual aquaculture nutrition workshop for distribution to workshop participants, industry and educational institutions.
- 5. Coordinated delivery of progress and final reports to FRDC from core projects managed within the aquaculture nutrition subprogram.
- 6. Development and maintenance of an aquaculture nutrition website. The Subprogram Leader will also provide regular contributions to Aquafeed.com to promote the activities of the Subprogram.
- 7. Where sufficient interest exists, the subprogram will provide short courses to improve the practical nutrition knowledge base that exists in the Australian aquaculture sector.

ONGOING COMMUNICATION COMMITMENTS

The Australasian Livestock Feed Ingredient (ALFI) database was developed to compile a large amount of information on the chemical composition and nutritional quality of feed grains for pigs, poultry (broilers, layers) and aquaculture species, and can be used by researchers, members of the grains industries, feed manufacturers and plant breeders as a reference for diet formulation, variety comparison and quality control in trading.

Data for pigs, poultry and aquaculture species has been entered into ALFID which now contains more than 22807 sample entries. The ALFI database also incorporates all information contained with the GRDC GRAILE database. The initial beta version has also been reprogrammed so that it is now more user friendly, and run-time versions have been prepared for distribution via CD-ROM. A web-site (www.alfidbase.com) has also been established and a domain name registered to facilitate e-mail submissions for ALFI information, and to promote the benefits of the database. Promotional brochures and presentation formats for the software has been suggested and commercialisation is pending approval from the respective stakeholders in the project (which includes the Fisheries Research and Development Corporation).

A long term objective of the ANS is to continue to provide information arising from research projects associated with the evaluation of feedstuffs for use in aquaculture systems in to the ALFI database and to ensure that the database remains relevant to the aquaculture sector. On-going investment into the development of the ALFI database is being sought from the private and commercial sector.

D) PROPOSED NEW RESEARCH

Based on distribution of the ANS strategic plan, the following preliminary research proposals were submitted to the subprogram in 2002 for comment.

 Using grain/legume products to enhance productivity of high intensity prawn farming. Principal Investigator: Michelle Burford Priority: High

Proposed Objectives

- 1. Improve prawn pond productivity by the addition of grain/legume products to contribute to prawn nutrition and the maintenance of water quality through the promotion of bacterial populations by:
- 2. Establishing which grain/legume products are the most effective in promoting bacterial growth in high intensity prawn systems.
- 3. Identifying which form of addition of grains/legumes (e.g. pellets, whole grain) would result in the maximum benefit.
- 4. Determining the direct and indirect contribution of grain/legume products to prawn nutrition in high intensity culture systems.

The ANS provided the following comments in relation to this project:

- The FRDC's Research and Development Plan 2000-2005 lists a number of challenges that exist in the FRDC's business environment. Challenge "5" is reducing the quantity of fish protein fed to terrestrial and aquatic livestock so that it becomes available in the food chain to satisfy environmental and human needs. The FRDC have only made minimal investments in research addressing this challenge since the plan became active and hence this research area remains a very high priority.
- The ANS Strategic Directions 2002-2007 lists the identification of alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils as a high priority. Your proposal addresses this priority and is supported by the Subprogram. The suggested research contributes to fish meal replacement strategies in a novel way and helps broaden the Subprogram's approach to this issue.
- It is recommended that you prepare a full research proposal for consideration by the FRDC Board using the guidelines supplied by the FRDC. Full application details can be found on the FRDC website <u>www.frdc.com.au</u>. A supporting letter outlining the priorities and recommendations of the ANS will be forwarded directly to FRDC. If you would like further comments on your application I would be happy to provide them prior to submission.
- When preparing your full application, it is suggested that you address the following issues:
 - How do you control the composition of the flocs ?
 - How do you know that incorporation of the flocs into pellets is a meaningful comparison ? Presentation as live organisms may have a significant effect on their efficicacy.
 - The proposal should give extra emphasis to the potential use of carbon sources other than grains and legumes. The composition of the pond nutrients used by Belize Aquaculture was fairly fundamental. Ultimately, the most cost-effective carbon source is required.
 - You should ensure that the high salary:operating cost ratio outlined in your pre-proposal is thoroughly justified.

- If there is potential to reduce the scope of this project in terms of budget then it is more likely to be successful given the current funding constraints faced by the FRDC.
- Any full proposal will need to be considered by the relevant FRAB's when it is complete.
- Assessment of nutritional status under limiting environmental conditions. Principal Investigator: Assoc. Prof Chris Carter Priority: High

Proposed Objectives

- 1. To develop an approach to assessing the relationships between nutrient supply and nutritional status under limiting conditions using two main aquaculture species: Atlantic salmon and Barramundi.
- 2. Establish factorial models to describe nutrient supply and nutrient retention under limiting temperatures (high for salmon and low for barramundi) and in combination with other key environmental factors (such as low DO).
- 3. Refine the use of disease challenge protocols to test the effect of fish nutritional status under limiting temperatures.
- 4. Combine nutrient supply-retention model and disease challenge protocol to test potential ingredients or changed feed formulations under limiting conditions.

The ANS provided the following comments in relation to this project:

- The PRP addressed a research priority identified by the ANS and is consistent with the research needs of both the beneficiaries and the Subprogram. The potential negative influences of Tasmanian water temperatures on nutritional responses of salmon and the relevance of nutritional research completed in Northern Europe has been questioned by the Australian salmon industry for some time.
- The ANS is aware that this application was not endorsed by the TasFRAB, and while supported in principal by the Atlantic Salmon Aquaculture Subprogram, it was deemed more appropriate for management under the auspices of the ANS.
- The ANS endorse progression of this PRP to a full proposal for consideration by the FRDC Board with some suggested amendments to the project objectives, including:
 - The proposal should focus on objectives 1 and 3 unless significant extra justification can be made for inclusion of objective 2. Work underway within the Abalone Aquaculture Subprogram investigating interactions between nutrition and disease has proved particularly difficult due to problems associated with inducing disease conditions and symptoms in a laboratory environment that are consistent with those experienced in the field. For this reason, even if the objective could be justified, it may be logistically difficult to complete experimentally.
 - Involvement of industry partners in this project would be viewed very favorably.
- If there is potential to reduce the scope of this project in terms of budget then it is more likely to be successful given the current funding constraints faced by the FRDC.
- Any full proposal will need to be considered by the relevant FRAB's when it is complete.
- Aquaculture nutrition subprogram preliminary research proposal. Principal investigator: professor sena de silva Priority: low

Proposed Objectives

1. Modify the DU Diet, with a view to further reducing its cost through partial and/or complete replacement of the fish oil component with n-3 rich oils of plant origin, and/ or n-6 rich oils of plant origin, and evaluate the performance of Murray cod in response to the dietary changes.

- 2. To determine whether any of the above modifications would result in desirable amounts of n-3 PUFA in marketable size fillets, with out compromising growth and feed utilisation efficacy, and if not determine the optimal time period which the modified diet could be used in grow out operations before being transferred on to the original diet with equal amounts of fish and vegetable oils.
- 3. Evaluate cost benefits of the use of the diets in Murray cod grow out operations.

This proposal was assessed against the published strategic plan for the ANS. The ANS offered the following comments and suggestions in relation to this proposal:

- The proposed project (that does not have a title) has the potential to make only minor contributions to the ANS strategic objectives and is not supported for development into a full proposal for consideration by the FRDC Board.
- There is limited evidence that the application of linseed or sunflower oil will make Murray Cod diets more cost-effective, even if the project was successful.
- 4. Tuna nutrition project: Development of predictive models of tuna metabolism, growth and excretion. Principal Investigator: Dr Brett Glencross Priority: Medium

Proposed Objectives

- 1 To review the literature to identify information gaps and limitations to the development of bio-energetic growth models for tuna.
- 2 To empirically determine the key bio-energetic and synthetic constraints influencing growth in tuna.
- 3 To develop a computer simulation model to describe the growth, feed utilization and nutrient excretion of tuna.

The above preliminary research proposal was forwarded to the Aquaculture Nutrition Subprogram (ANS) for comment. The proposal was assessed against the published strategic plan for the ANS together with supporting information arising from SBT Aquaculture Subprogram meetings held in Port Lincoln in November, 2002.

The ANS offered the following comments and suggestions in relation to this proposal:

- The proposal addresses a specific issue that was conveyed as a priority by the tuna industry at their recent industry workshops.
- The proposal has significant overlaps with aspects of the revised tuna nutrition research program currently being developed by Dr Robert van Barneveld on behalf of the SBT Aquaculture Subprogram. A rough outline of the revised program is attached for your information.
- If you are agreeable, it is suggested that a budget be allocated within the revised tuna nutrition program for you to develop the desktop aspects of the proposed bio-energetics model. You will also be included as a full collaborator on this project. The bulk of the experimental work that you outline in your PRP will be covered within revised aspects of the tuna nutrition project (Note: This was subsequently done as part of the revisions of the tuna nutrition research program).
- With the above in mind, it is not necessary for you to prepare a full proposal for consideration by the FRDC Board. I will forward you drafts of the revised tuna nutrition proposal as they are prepared.
- Food safety risk profile for manufactured Australian aquafeed. Principal Investigator: Mr David Padula Priority: Medium/High

Proposed Objectives

- 1. Identify public health hazards that may enter the food chain from aquaculture species grown in Australia and rank them in terms of risks to the consumer and capacity to disrupt trade.
- 2. Identify priority hazards where insufficient information exists to confidently rank risks for fish.

- 3. Identify potential risk management approaches for the identified hazards and potential hazards.
- 4. Identify information poor areas where additional research may be necessary to adequately assess or manage public health risks.
- 5. Identify hazard: commodity combinations where further risk assessment may need to be undertaken.

This proposal was assessed against the published strategic plan for the ANS. The ANS offered the following comments and suggestions in relation to this proposal:

- The ANS has identified the influence of manufactured aquatic feeds on aquaculture product quality and food safety hazards as a high priority. Your proposal addresses this priority and is supported by the Subprogram.
- The ANS is aware that the southern bluefin tuna aquaculture industry regards the research proposed in your application as being extremely important for their industry. This information was conveyed via the SBT Aquaculture Subprogram industry workshop held recently.
- It is recommended that you prepare a full research proposal for consideration by the FRDC Board using the guidelines supplied by the FRDC. Full application details can be found on the FRDC website <u>www.frdc.com.au</u>. A supporting letter outlining the priorities and recommendations of the ANS will be forwarded directly to FRDC. If you would like further comments on your application I would be happy to provide them prior to submission.
- When preparing your full application, it is suggested that you address the following issues:
 - The project application needs to place emphasis on additional species as well as SBT (apart from the estimated flow of benefits section). In particular, the project should examine the risks posed by importation of complete feeds and should extend to include prawn feeds.
 - The project should place a heavy emphasis on fish meals and oils.
 - At a minimum, the project should address food safety risk profiles associated with prawn, barramundi, salmon and tuna feeds.
- If there is potential to reduce the scope of this project in terms of budget then it is more likely to be successful given the current funding constraints faced by the FRDC.
- Any full proposal will need to be considered by the relevant FRAB's when it is complete.
- Aquaculture Nutrition Subprogram Preliminary Research Proposal: Lupin And Canola Processing Products In Aquaculture Feeds For Prawns. Principal Investigator: Mr David Smith Priority: High

Proposed Objectives

- 1. To compare the performance of meals with increased protein content derived from selected varieties of lupins and canola, with that of fishmeal and solvent extracted soybean meal in prawn feeds (P. *monodon*).
- 2. To determine the cause of any negative responses attributable to the lupin and canola products and to investigate methods to address the problem in a cost-effective manner.
- 3. To define specifications for total lipid and essential fatty acids that will enable the effective minimisation of fish oil content of prawn feeds.

This proposal was assessed against the published strategic plan for the ANS. The ANS offered the following comments and suggestions in relation to this proposal:

• The FRDC's Research and Development Plan 2000-2005 lists a number of challenges that exist in the FRDC's business environment. Challenge "5" is reducing the quantity of fish protein fed to terrestrial and aquatic livestock so that it becomes available in the food chain to satisfy environmental and human needs. The FRDC have only made minimal investments in research addressing this challenge since the plan became active and hence this research area remains a very high priority.

- The ANS Strategic Directions 2002-2007 lists the identification of alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils as a high priority. Your proposal addresses this priority and is supported by the Subprogram.
- The ANS is aware of the GRDC research that you have conducted previously demonstrating the potential value of lupins for prawns and the relevance of this research to the Subprogram objectives.
- The ANS is attempting to establish a collaborative research program with the GRDC. The research will target the development of commercial processes for the production of protein isolates or concentrates from a variety of sources and assessment of these proteins in major Australian species including salmon, southern bluefin tuna, barramundi and prawns. With this in mind, formal linkages with commercial partners would be viewed as an important component of the project. It is also important to remember that the FRDC challenge encompasses the reduction in fish protein use by the terrestrial livestock sectors, so some involvement from the pig, poultry and ruminant industries may also be required.
- While this project could potentially contribute to the above program, there is a lot of further work required to develop a broader project that would attract co-investment from the GRDC. This project would also need to involve other FRDC subprograms such as the SBT Aquaculture Subprogram and the Atlantic Salmon Aquaculture Subprogram. As you are aware, full proposals are required by the FRDC by December 1, 2002. While the ANS would like you to participate in the development of this project with GRDC, it is unreasonable to expect you to prepare this full proposal in the required time frame given the number of assumptions that need to be made at this stage and the additional linkages that will be required. The ANS will coordinate the development of a broader project with your involvement. The Subprogram will seek out of session funding with an anticipated project commencement of January 1, 2004. This is to coincide with the beginning of year 2 of the current GRDC project.

E) WORKPLAN FOR 2003

Development of a formal relationship between frdc, grdc and commercial investors to produce a vegetable protein alternative to fish meal for use in australian aquafeeds.

- The ANS will use it's annual workshop in 2003 to participate in a review of an existing GRDC project focussing on the development of lupin proteins isolates or concentrates being undertaken by Dr Brett Glencross, and to involve potential commercial partners and other research providers in the project.
- The ANS will develop a co-investment and research strategy for FRDC with the outcome being the production of a viable vegetable protein alternative to fish meal in Australia. This strategy will need to be available by August 2003.
- The ANS will invite submissions from appropriate research providers that address the defined research strategy.
- The ANS will develop a memorandum of understanding with the GRDC in relation to co-investment in this research area, with a view to expanding the existing project being undertaken by Dr Glencross.
- The ANS will seek out-of session funding for co-investment with GRDC to coincide with Year 2 of the current GRDC project due to commence on **January 1, 2004**. The ANS attempted to submit a full proposal to the FRDC for the current funding round, but there were too many unknowns to make this possible.

DELIVERY OF TRAINING COURSES

• The ANS will develop and deliver training courses in practical extrusion in 2003.

ESTABLISHMENT OF AN EXPERT WORKING GROUP

• With the establishment of some core ANS projects in 2003, the subprogram will re-examine the role of an Expert Working Group based on the projects that are ultimately approved.

COMMERCIALISATION OF THE ALFI DATABASE

• Where possible, the ANS will contribute to the commercialisation processes involved with the ALFI database in 2003, to ensure this valuable resource is delivered to the aquafeed sector in Australia.

F) BUDGET

No information is available at this time.

Recommendations/Variations

The Subprogram will forward recommendations to FRDC on any nutrition related projects that may be reviewed in 2002-2003.

Dr Robert van Barneveld ANS Leader December, 2002



Aquaculture Nutrition Subprogram Annual Operating Plan - 2004

Prepared by **Dr Robert van Barneveld** Subprogram Leader

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A) ACTIVITY DESCRIPTION FOR 2002-2003

xv) Sector Progress

The Fisheries Research and Development Corporation established the Aquaculture Nutrition Subprogram (ANS) in September, 2001. Given that a wide scope of nutrition research already exists within the FRDC portfolio, as well as a range of other research investors, the ANS aims to take nutrition research into a higher risk/market failure area that is not limited by industry development restrictions and is beyond individual species based projects. The vision for ANS is the efficient, innovative and sustainable delivery of nutrients to aquaculture production systems to optimise aquatic animal health and production and aquaculture product quality while minimising impacts on the aquatic environment. The subprogram mission is to:

"To enhance the Australian aquaculture nutrition expertise base and to utilise combined knowledge to identify and solve common aquaculture nutrition challenges and limitations to development of the Australian aquaculture industries".

WHY IS AQUACULTURE NUTRITION RESEARCH IMPORTANT ?

Aquaculture nutrition research is a fundamental component in the establishment of new aquaculture industries and in the further development of existing aquaculture industries for the following reasons:

- 1. Feed costs represent a significant proportion (30-50%) of the production costs in most aquaculture systems. The most effective way to reduce feed costs is to improve the feed conversion efficiency of the target species which can only be accomplished by improving definition of feed ingredients, defining the nutritional requirements for different production phases, improving diet form and feed stability and increasing the range of feed ingredients available for use all of which require significant research inputs.
- 2. Development of cost-effective manufactured feeds for aquaculture species is a difficult process because the research has to be conducted underwater. This makes collection of information on fundamental parameters such as feed intake difficult. In addition, most aquaculture diets needs to be extruded which is a research discipline in itself and requires highly specialised equipment.

- 3. Many aquaculture diets have a heavy reliance on fresh fish, fish meals and fish oils. To provide long term stability to new and established aquaculture industries and to reduce the reliance on these ingredients, there is a need to identify alternative nutrient sources that facilitate maintenance of similar production levels and product quality.
- 4. Improved nutrition of aquaculture species is one of the most effective ways to reduce any environmental impacts from aquaculture by reducing nutrient loads in effluent.
- 5. Some aquaculture sectors, such as prawns, still rely heavily on imported feeds due to a lack of alternatives in Australia.
- 6. Given the large number of unknowns often associated with the nutrition of new aquaculture species and the difficulties associated with conducting the research, significant improvements in the efficiency of conducting this research can be achieved by developing a coordinated approach to the definition of research priorities and research methods, such as that achieved within a managed subprogram.

xvi) Major research outputs of the Subprogram

Between 2001 and 2003, the ANS addressed the following core objectives with associated outcomes:

Objective 1: Implement a core research program based on fundamental, non-species specific nutritional constraints to aquaculture production.

The ANS convened an industry workshop and facilitated a planning meeting to identify and prioritise key nutritional limitations to aquaculture production. The outcomes from these meetings culminated in the publication of the ANS Strategic Directions 2001-2005. The development and production of a vegetable protein alternative to fish meal and alternatives to fish oil still exists as the highest research priority in this field. In response to this, the ANS has acted to develop collaborative research projects with the Grains Research and Development Corporation with due consideration for the following:

- a. Feed manufacturers are primarily interested in information on ingredient digestibility (amino acids and energy), maximum inclusion levels and functionality of ingredients in a feed processing system, rather than development of complete diets using alternative proteins.
- b. Lupin protein (53-55%) appears to hold the greatest potential as an additional protein source for use in aquafeeds in Australia.
- c. Development of protein hydrolysates is another priority to reduce reliance on fish meals and oils, and as a means of potentially increasing the maximum inclusion of alternative vegetable protein sources.
- d. Research should be undertaken with a representative temperate finfish (salmonid), tropical finfish (eg. Barramundi, lower priority), and crustacean (prawns).
- e. It would be desirable to minimise the amount of processing required to produce a vegetable protein product with a protein content of between 53 and 55%.
- f. It would be desirable to have this processing capacity in Australia.
- g. If there are co-products produced as a consequence of this processing, it is almost certain that a viable market for these products will be required to make the process cost-effective.
- h. We need to involve the expertise of a well-established, high volume grain processor in any subsequent research.
- i. 53-55% crude protein may not be necessary for use in diets for all aquaculture species. (eg. Prawns).
- j. Other protein sources require further investigation as alternatives for use in aquafeeds given the existence of higher protein soybean meals and opportunities for other grain legumes if a lower protein content is required.
- k. From a grains industry perspective, export markets hold the greatest potential, and of these markets, Asia will not only hold the greatest potential in terms of volume, but it is likely to have the greatest variation in requirements. If Asia is a focus, then prawns must be a focus species of this research program.
- 1. Given that a significant proportion of aquafeed expansion in the Asian region is likely to be in species that have a low reliance on manufactured feeds (eg. Tilapia, catfish etc.) we should consider the merits of expanding our research program to encompass development of very low cost feeding alternatives

based on Australian grains eg. A blend of cereals and legumes that can be made into doughs and pastes with the addition of water). Some of these products may also have application in lower value Australian aquaculture systems (eg. Marron, yabbies, red claw etc.).

With all of the above in mind, the ANS is progressing project development with the following focus:

- 1. Focus on multiple vegetable protein alternatives with varying levels of crude protein and varying origins. This could include lupins, peas, soybean, canola etc.
- 2. Assessment of amino acid and energy digestibility and maximum inclusion levels in a salmonid and a crustacean diet should be undertaken at the very least.
- 3. A significant focus on ingredient functionality should be considered.
- 4. Identification of alternative ways to deliver protein and energy from Australian grains to low value, high volume fish species (specific to Asian farming practices).
- 5. Focus on protein sources that can be produced using existing technology, by existing commercial manufacturers in Australia.

Objective 2: Establish basic research standards and preferred methodologies for nutrition research.

In August 2003, the ANS convened a Methodology Forum involving key aquaculture nutrition scientists from across Australia. The forum aimed to develop a standardised approach to a range of nutrition research issues including:

- 1. Nutrition and diet development for new aquaculture species.
- 2. Basis for the use of surrogates in nutrition research.
- 3. Chemical analysis methods for aquaculture nutrition research.
- 4. Nutrient digestibility measurements in aquaculture species.
- 5. Nutrient availability and utilisation in aquaculture species.
- 6. Nutrient requirements in aquaculture species.
- 7. Measuring transit time.
- 8. Measuring feed intake.
- 9. Palatability/preference/attractants.
- 10. Growth correlates.
- 11. Feed preparation and measures of ingredient functionality in feed processing systems.
- 12. Basic evaluation of feed ingredients.
- 13. Growth performance, ingredient evaluation and diet formulation.

Outcomes from the forum will be published as a standards manual for use by Australian scientists, the FRDC and aquaculturalists.

Objective 3: Facilitate technology transfer in the field of aquaculture nutrition.

The ANS has convened themed workshops to identify priorities for aquaculture nutrition research and to promote collaboration and communication between scientists and stakeholders in the sector. A third annual workshop is planned for April, 2004 with a focus on larval feeds. In addition, the ANS in collaboration with the GRDC are planning a delegation to Asia to promote Australian aquaculture nutrition research and the potential for Australian grains in aquaculture diets.

Practical publications are pending from the ANS in collaboration with the Abalone Aquaculture Subprogram and the Southern Bluefin Tuna Aquaculture Subprogram on the nutrition of abalone and tuna respectively. The ANS will develop standardised formats for the delivery of these manuals, and Dr Robert van Barneveld will be the principal author on both.

The Australian Livestock Feed Ingredient Database (ALFI) has been developed with co-investment from FRDC and remains the primary means of collating data arising from FRDC nutrition-related projects. Commercial release of this database is anticipated in early 2004. In addition, through the SBT Aquaculture Subprogram, Dr van Barneveld has developed a feed formulation package that has been released as "Formu-

bait". Potential exists through the ANS to modify this Excel-based package for other aquaculture sectors under a free license agreement.

Objective 4: Develop and provide training for research investors, research providers, and end-users associated with aquaculture nutrition.

The ANS collaborated with Food Industry Engineering and the South Australian Research and Development Institute to deliver a short-course in aquafeed extrusion in 2003. Further courses are planned in feed microscopy and basic nutrition for aquaculturalists depending on demand.

In addition to the above, the ANS has acted as a point of contact for aquaculture nutrition in Australia., and has actively developed links between research investors, research providers and end-users associated with aquaculture nutrition.

xvii) Related projects and research linkages

The FRDC's Research and Development Plan 2000-2005 lists a number of challenges that exist in the FRDC's business environment. Challenge "5" is reducing the quantity of fish protein fed to terrestrial and aquatic livestock so that it becomes available in the food chain to satisfy environmental and human needs. The FRDC have only made minimal investments in research addressing this challenge since the plan became active and hence this research area remains a very high priority.

Dr Brett Glencross has successfully attracted research funding from the Grains Research and Development Corporation to investigate the development of value-added plant protein products for the aquaculture feeds sector. This project is due to commence on January 1, 2003 and has the following objectives:

- 4. Development of value-added lupin protein products for use in the animal feeds sector;
- 5. Evaluation of the nutritional value of a range of value-added lupin protein products when fed to fish;
- 6. Commercial transfer of intellectual property for development of new-product(s).

The ANS is actively seeking to coinvest in this existing research program and an application is currently pending with FRDC.

In addition to the above, all research projects within the FRDC portfolio and those within the AQUAFIN CRC are relevant to and related to the ANS.

xviii) Role the Subprogram has played in industry development

To date the FRDC has invested in three nutrition specific subprograms – the Fishmeal Replacement (FMR), Aquaculture Diet Development (ADD) and Aquaculture Nutrition (ANS) Subprograms. All of these subprograms have been successful in meeting the objectives of managed subprograms on the basis that they:

- Promote or promoted a high level of collaboration between scientists working within a common discipline;
- Successfully delivered nutrition research expertise to infant aquaculture industries that otherwise would have not had access to this level of nutritional skill;
- Reduced the level of duplication of research effort towards a common goal;
- Applied outcomes were delivered to industry improving the profitability and viability of these industries;
- Facilitated a coordinated delivery of research funding submissions and research reports to the FRDC;
- Advanced the overall international knowledge base for aquaculture nutrition.

The smaller aquaculture industries benefiting from research conducted within these Subprograms (ie. barramundi, salmon, prawns, silver perch) have suggested they would value the continuation of further coordinated research in the area of aquaculture nutrition. Industries such as the barramundi industry rate nutrition as the highest research priority above all other disciplines, and believe that nutrition research on behalf of their industry would be severely compromised in the absence of a nutrition specific subprogram.

Research providers and researchers operating within the subprograms value their involvement as the Subprograms, through the Subprogram Leader, manage to breakdown many institutional boundaries that previously existed. The Subprogram workshops also represent a valuable form of peer review for research results. Workshops conducted as part of these subprograms provided an outlet for related research results and a valuable forum for critical review of the research.

xix) Operating procedures

The ANS was established to develop and implement a core research program based on fundamental, non species-specific nutritional constraints to aquaculture production. In developing this core research program, the ANS will develop a risk management strategy for all aquaculture industries in relation to nutrition to assist the research priority setting process. In developing a core research program, the ANS applies five criteria against potential areas of core research and innovation including:

- 11. Must be of national significance/benefit.
- 12. Must have multi-species significance.
- 13. Must require multiple knowledge inputs to achieve an outcome.
- 14. Must be limiting the development and/or viability of aquaculture industries.
- 15. Must not duplicate existing research efforts.

In addition, the ANS will:

- Establish basic research standards and preferred methodologies for nutrition research;
- Facilitate technology transfer in the field of aquaculture nutrition;
- Develop and provide training for research investors, research providers, and end-users associated with aquaculture nutrition;
- Act as a point of contact for aquaculture nutrition in Australia; and
- Develop links between research investors, research providers and end-users associated with aquaculture nutrition.

xx) Meetings and workshops

ANNUAL AQUACULTURE NUTRITION WORKSHOP

A core service of the Aquaculture Nutrition Subprogram is the organisation and delivery of an annual aquaculture nutrition workshop. This workshop provides a forum for the delivery of research results from all FRDC-funded aquaculture nutrition projects. Where possible, invited speakers are utilised to address issues relevant to nutrition research. The workshop also provides an opportunity for feed manufacturers and end users to present their views in relation to aquaculture nutrition research priorities.

The First Annual ANS Workshop was held on February 25th 2002 at CSIRO, Cleveland, Qld. The 2003 ANS workshop was held on May 29th, 2003 in Fremantle, WA in conjunction with a GRDC funded workshop.

TRAINING

In keeping with the core objective of improving nutrition research capacity of research providers in and out of Australia, the ANS offers user-pays training packages that proceed based on demand. The ANS has collaborated with Food Industry Engineering and the South Australian Research and Development Institute to deliver a short-course in aquafeed extrusion in 2003. Further courses are planned in:

- 1. Practical nutrition short course for aquaculture producers;
- 2. Feed microscopy for aquaculture;
- 3. Capacity for nutritional control of nutrient outputs from aquaculture systems for policy and planning agencies.

xxi) Summary of current project status

At this point, the Aquaculture Nutrition Subprogram has no core projects based on its strategic plan and apart from the management component of the subprogram. The subprogram does provide advice on milestones arising from all nutrition-related FRDC projects.

B) SUMMARY OF STRATEGIC PLAN OR DIRECTIONS

Core Research and Development

A range of aquaculture nutrition research programs are underway within the following aquaculture sectors (some with established Subprograms):

- Southern Bluefin Tuna
- Abalone
- Prawns
- Rock Lobster
- Snapper
- Salmon
- Barramundi
- Other (Silver Perch, Yellowtail Kingfish, Sea Urchin, King George Whiting, Crabs).

A collation of aquaculture nutrition research and development issues faced by these aquaculture sectors together with inputs from related sectors (facilitated through the first annual ANS workshop held in February, 2002) is summarised as follows (in no specific order):

FEED EVALUATION

- Poor capacity to accurately measure feed intake (*All*).
- Capacity to manipulate dietary amino acid supply without utilising crystalline amino acids (*Prawns, Abalone*).
- Influence of manufactured aquatic feeds on aquaculture product quality and food safety hazards (All).
- Influence of nutrition on aquatic animal health (Abalone, Salmon, Tuna, Snapper, Rock Lobster).
- Basal larval, broodstock and production diets for existing and emerging species (All).

NUTRITIONAL REQUIREMENTS

- Application of inappropriate research methodologies in some cases resulting in inaccurate or misleading results (particularly in the definition of nutritional requirements) (*Prawns, Tuna, Abalone*).
- Poor capacity to accurately measure feed intake (*All*).
- Influence of feed form, feeding strategies and feeding regime on the surrounding aquatic environment (*All*).
- Alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils (*All*).
- Suitable larval, broodstock and production diets for existing and emerging species (All).

FEED PROCESSING

- Basal larval, broodstock and production diets for existing and emerging species (All).
- Fat stability in extruded pellets (Salmon).
- Capacity to manufacture extruded pellets with a diameter less than 1.5 mm (*All*).
- Effects of mechanical energy inputs during diet manufacture on water stability (All).
- Influence of feed production processes on micro-ingredient losses and stability (All).
- Starch and protein losses through degradation during feed processing (All).
- Interaction between ingredient source and type and extrusion processing parameters (All).
- Production of shelf stable semi-moist pellets (*Tuna, Sea urchins*).

An Expert Working Group was convened by the Aquaculture Nutrition Subprogram in 2002 to identify additional nutritional constraints to aquaculture production in Australia. Aquaculture nutrition constraints and/or requirements in addition to those listed above include:

- Routine evaluation of feed ingredients suitable for use in aquatic diets.
- Availability/reliability of energetic and other models for use in managing feeding systems and aquatic animal responses.
- Assessment of feed attractants.
- Strategies for feed delivery to aquaculture systems.
- Techniques to facilitate rapid weaning of aquaculture species onto manufactured diets.
- Feed formulation approaches to meet both the nutritional needs of the target species and the manufacturing requirements.
- Nutriceuticals (eg. probiotics, enzymes) suitable for use in aquaculture feeds.
- Availability of nutrition research infrastructure and the nutrition research capacity of research providers in and out of Australia.

Based on the five criteria defined for core research and development projects suitable for inclusion within the auspices of the Aquaculture Nutrition Subprogram (ie national significance/benefit, multi-species significance, multiple knowledge inputs required, limiting development and/or viability of aquaculture industries, no duplication of existing research efforts) the collation of research and development issues can be reduced and grouped into the following categories:

NUTRITION RESEARCH TOOLS

- Capacity to accurately measure feed intake in aquaculture species.
- Development of energetic and production models for simulating the responses of aquaculture species to nutritional inputs.
- Availability of nutrition research infrastructure and the nutrition research capacity of research providers in and out of Australia.

AQUATIC FEED PROCESSING

- Basal larval, broodstock and production diets for existing and emerging species.
- Interaction between ingredient source and type and extrusion processing parameters.

FEED EVALUATION

• Alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils.

NUTRITION AND AQUATIC ANIMAL HEALTH

- Interaction between nutrition and aquatic animal health.
- Nutritional prevention of disease and maintenance of animal health.

Nutrition and the Environment

• Influence of feed form, feeding strategies and feeding regime on the surrounding aquatic environment.

NUTRITION AND PRODUCT QUALITY

• Influence of manufactured aquatic feeds on aquaculture product quality and food safety hazards.

Short and Long Term Research Needs

From the above list of ten priority research areas, three can be declared as having an urgent research requirement and will therefore represent target research areas within the ANS from 2004.

7. Alternative sources of protein and lipids capable of sustaining aquatic animals exclusive of fresh bait or trash fish, fish meals and fish oils.

A clear need has been identified for the development of a vegetable protein isolate available for use in Australian aquafeeds as an alternative to fishmeal. Critical to achieving this goal is the involvement of industry with the technical expertise to produce such an isolate, end-users of both mid-and high protein components of the isolate and any additional co-products, other research investors, and research providers.

Lupins, particularly new cultivars such as *L. luteus*, hold the greatest potential for the production of a high protein isolate in Australia. Research and development is required to identify the most appropriate process for the production of such an isolate, the efficiency of such a process, the need to identify multiple end-users for the graded isolates, and the suitability of such an isolate for aquaculture. This process holds potential to produce a range of additional co-products such as non-starch polysaccharide isolates, isoflavones and oils that would require further investigation by multiple end-users.

The Grains Research and Development Corporation have also expressed an interest in this research area and could work together with the Aquaculture Nutrition Subprogram to establish some research partnerships in this area.

8. Interaction between nutrition and aquatic animal health.

The Abalone Aquaculture Subprogram and the Rock Lobster Enhancement and Aquaculture Subprogram have both identified nutrition and aquatic animal health as research priorities. In the abalone sector there is a need to establish the role of nutrition in "summer bloat" and research is underway in this area. In rock lobsters, there is a need to develop modes of larval feeding that allow some form of microbial control in propagation systems.

With the above in mind, in feed additives such as probiotics, enzymes, and the role and mode of action of products such as mannan oligosaccharides may prove useful for new and existing aquaculture industries.

9. Basal larval, broodstock and production diets for existing and emerging species.

A number of commercial and public sector research projects involving King George Whiting, Rock Lobsters, Organic Barramundi, and Sea Urchins (to name a few) have recently been undertaken and have experienced difficulties in developing and obtaining extruded basal diets in small quantities, that can be used for subsequent development. In some cases, the inability to produce these products has impeded the progress of existing research projects. An urgent need exists to establish basal extruded diets for use in the development of emerging aquaculture industries across Australia.

All other identified priorities form part of the longer term strategy for the ANS, but can emerge as research projects from the outset subjecting to research investment constraints.

E) COMMUNICATION AND TECHNOLOGY TRANSFER ACTIVITIES

The ANS has defined a policy for the distribution of information arising from research conducted within the Subprogram that forms the basis of a communication plan for this project. This project distributes information on behalf of all projects within the subprogram with the following objectives:

- 1. To distribute research outputs (technologies and knowledge) that has a net benefit for the Australian industry and to distribute that information in a timely manner to achieve rapid adoption by industry.
- 2. To disseminate information about the subprogram's role, activities and achievements relevant to stakeholders.
- 3. To disseminate information to the general public when it contributes to a positive perception of the sector and/or the FRDC and contributes to the public good.
- 4. To disseminate information to international partners when there is a two-way flow of information

Target audiences:

1. The Australian aquaculture industries, aquafeed manufacturers, infrastructure manufacturers and FRDC stakeholders.

2. General public.

Key messages:

- 1. Research outputs from the projects managed under the subprogram.
- 2. Role, activities and achievements of the subprogram.
- 3. Positive image of rock lobster aquaculture (clean and green, environmentally sustainable, economically beneficial for Australia, provides employment in regional Australia etc)

The Aquaculture Nutrition Subprogram undertakes the following communication and technology transfer activities:

- 1. Facilitation of an annual workshop aimed at disseminating the latest nutrition research results from Australia and New Zealand and identifying core research priorities for pursuit within the subprogram.
- 2. Publication of workshop proceedings from the annual aquaculture nutrition workshop for distribution to workshop participants, industry and educational institutions.
- 3. Maintenance and annual upgrading of an Aquaculture Nutrition Subprogram strategic plan available via the FRDC web-site for use by research providers, industry, other subprograms and FRAB's. Between September and November, 2002, the ANS strategic plan was downloaded from the FRDC website more than 292 times number 6 on the FRDC web-sites most popular download list !
- 4. Publication of an annual operating plan for the subprogram for delivery to FRABs in December each year to assist deliberations on projects related to aquaculture nutrition.
- 5. Publication of an aquaculture nutrition newsletter as required for distribution to researchers and industry via the subprogram mailing list. This mailing list has recently been updated and now contains 125 contacts.
- 6. Publication of workshop proceedings from the annual aquaculture nutrition workshop for distribution to workshop participants, industry and educational institutions.
- 7. Coordinated delivery of progress and final reports to FRDC from core projects managed within the aquaculture nutrition subprogram.
- 8. Maintenance of an aquaculture nutrition website. The Subprogram Leader will also provide regular contributions to Aquafeed.com to promote the activities of the Subprogram. The ANS website can be found at <u>www.frdc.com.au/research/programs/ans</u>.
- 9. Where sufficient interest exists, the subprogram will provide short courses to improve the practical nutrition knowledge base that exists in the Australian aquaculture sector.

ONGOING COMMUNICATION COMMITMENTS

The Australasian Livestock Feed Ingredient (ALFI) database was developed to compile a large amount of information on the chemical composition and nutritional quality of feed grains for pigs, poultry (broilers, layers) and aquaculture species, and can be used by researchers, members of the grains industries, feed manufacturers and plant breeders as a reference for diet formulation, variety comparison and quality control in trading.

Data for pigs, poultry and aquaculture species has been entered into ALFID which now contains more than 22807 sample entries. The ALFI database also incorporates all information contained with the GRDC GRAILE database. The initial beta version has also been reprogrammed so that it is now more user friendly, and run-time versions have been prepared for distribution via CD-ROM. A web-site (<u>www.alfidbase.com</u>) has also been established and a domain name registered to facilitate e-mail submissions for ALFI information, and to promote the benefits of the database. Promotional brochures and presentation formats for the software has

been suggested and commercialisation is pending approval from the respective stakeholders in the project (which includes the Fisheries Research and Development Corporation).

A long term objective of the ANS is to continue to provide information arising from research projects associated with the evaluation of feedstuffs for use in aquaculture systems in to the ALFI database and to ensure that the database remains relevant to the aquaculture sector. On-going investment into the development of the ALFI database is being sought from the private and commercial sector.

D) PROPOSED NEW RESEARCH

No preliminary research proposals were forwarded to the subprogram for consideration, however, the ANS has been actively involved in the development of a number of full proposals currently being considered by the FRDC. These include (other full proposals relevant to the ANS may have been received by FRDC, but only those developed with inputs from the ANS are mentioned here):

2004/236: Aquaculture Nutrition Subprogram: Evaluation of value-added grain protein products for Atlantic salmon and Black tiger prawns Principal Investigator: Dr Brett Glencross Priority: High

Objectives:

- 1. To determine the nutritional value of selected grain products, developed as part of the linked CLIMA-GRDC project, when included in feeds for Black tiger prawns and Atlantic salmon
- 2. To evaluate any potential nutritional limitations of the grain products in aquaculture feeds
- 3. To provide grain producers, grain processors, aquaculture feed manufacturers and the prawn and salmon aquaculture industries with information about the nutritional characteristics and quality assurance criteria of grain products so that they can be marketed and used with confidence in aquaculture feed formulations

This project represents a major collaboration between FRDC and the GRDC and addresses the highest research priority associated with aquaculture nutrition in Australia. It is a cost-effective way of meeting multiple industry needs.

2004/238: Aquaculture Nutrition Subprogram: Further development towards commercialization of marine fish larvae feeds Principal Investigator: Dr Sagiv Kolkovski Priority: High

Objectives:

- 1. To optimize formulated marine fish larvae diets and to foster commercialisation
- 2. To develop, with industry, intensive and extensive production methods of Artemia cysts and biomass
- 3. To serve as service centre for any larvae problems and product development

Larval feeds represent a major bottleneck in aquaculture production. A significant amount of FRDC research is focussing on propagation of key aquaculture species, and this project represents a very useful adjunct to these programs (eg. rock lobster). The project addresses a high priority research area identified by the ANS.

204/235: Aquaculture Nutrition Subprogram: Maintenance of strategic directions, project and infrastructure development, and facilitation of nutrition research and extension across all Australian aquaculture sectors Principal Investigator: Dr Robert Van Barneveld Priority: High

Objectives:

1. Maintain existing, and promote further, collaborative and co-funded research into the fundamental nutritional constraints to aquaculture development in Australia.

- 2. Coordinate aquaculture nutrition research in Australia to ensure optimum use of limited resources and funds and maximum outputs from research projects.
- 3. Promote capacity building and human capital development through the provision of training and resources in aquaculture nutrition and aquafeed manufacturing.
- 4. Develop risk management strategies as required for all aquaculture industries in relation to nutrition to assist the research priority setting process.
- 5. Provide a single point of contact for aquaculture nutrition research in Australia and improve the aquaculture nutrition skills base in Australia.
- 6. Improve communication of nutrition research outcomes and current nutrition research between scientists, aquaculturists, ingredient suppliers and feed manufacturers utilizing annual workshops, facilitated planning meetings, and conferences.

The first phase of the Aquaculture Nutrition Subprogram (ANS) has demonstrated a strong need for the national coordination of the nutrition components of both species and non-species based research programs. Without a coordinated subprogram approach to this research portfolio, the FRDC would have been unable to develop a collaborative program with the Grains Research and Development Corporation to characterise and produce vegetable protein alternatives to fish meal, nor would it have been able to standardize the methodologies and research approach for aquaculture nutrition in Australia with involvement from all relevant scientists. The subprogram is also addressing "market failure" in the areas of training and communication in the field of aquaculture nutrition, with a firm emphasis on empowering end-users of aquafeeds with basic nutritional and feed processing knowledge. The ANS needs to be maintained in a similar format to the first phase in order to capitalize on many of the initiatives that were implemented during the first 3 years of the project.

The need for on-going research into aquaculture nutrition and the need for a coordinated approach to this research is emphasized by the fact that Australian aquaculture industries have a heavy reliance on imported nutrition technologies, feeds and ingredients for the supply of nutrients to target species. This includes feed manufacturing technologies, ingredients such as bait fish, fish meals, crustacean meals and fish oils, and complete feeds such as those utilised by the prawn industry. Not only does this create issues in relation to imported disease risks, continuity of supply and cost, but it means that many local products are being under utilised. Given the broad range of issues associated with the provision of aquaculture diets, the real cost of aquaculture diets in Australia will only be reduced through a coordinated research approach such as that proposed within the ANS. The infancy of the Australian aquaculture industries also means that a coordinated research approach is required to maximise the outcomes from research investment in the area.

E) WORKPLAN FOR 2004

During 2004, the ANS will undertake the following:

Subprogram Management:

1. Re-establish a management component for the subprogram through project 2004/235.

TRADE DELEGATION

2. Convene a trade delegation to Asia in collaboration with GRDC to promote Australian aquaculture nutrition research, the potential for Australian grains in aquafeeds and to identify core research areas that may assist the development of the Australian aquaculture sector and markets for aquafeeds in Asia.

COLLABORATIVE RESEARCH

• Establish a collaborative research program with GRDC into the development of vegetable protein alternatives to fishmeal.

COMPILATION OF MANUALS

• The ANS will develop and deliver manuals in nutrition research methodology, abalone nutrition, and SBT nutrition.

DELIVERY OF TRAINING COURSES

• The ANS will develop and deliver further training courses in 2004.

ESTABLISHMENT OF AN EXPERT WORKING GROUP

• With the establishment of some core ANS projects in 2003, the subprogram will re-examine the role of an Expert Working Group based on the projects that are ultimately approved.

COMMERCIALISATION OF THE ALFI DATABASE

• Where possible, the ANS will contribute to the commercialisation processes involved with the ALFI database in 2004, to ensure this valuable resource is delivered to the aquafeed sector in Australia.

F) Budget

Apart from the management component of the ANS, there is currently no core project budget for this Subprogram.

Recommendations/Variations

The Subprogram will forward recommendations to FRDC on any nutrition related projects that may be reviewed in 2003-2004.

Dr Robert van Barneveld ANS Leader December, 2003