AQUACULTURE NUTRITION SUBPROGRAM

EXPANDING THE AQUAFEED INGREDIENT BASE

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AQUACULTURE NUTRITION SUBPROGRAM

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Table of contents

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 ($/tonne)	350	450	450	300	300	3000	500	900	1200
Price ($) / g Protein	0.84	0.82	0.87	1.17	0.76	3.58	0.83	0.95	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).

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

	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

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.

Table 5. Growth and palatability experiment preliminary progress.

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

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 anti-nutritional 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).

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.

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 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 abalone	PD greenlip abalone	$F_{1,4}$	P	SEM	GED blacklip abalone	GED greenlip abalone	$F_{1,4}$	P	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

[1]Anderson et al., 1992; [2]Anderson et al., 1993; [3]Bach-Knudsen, 1997; [4]Storebakken et al., 2000a; [5]Refstie and Tiekstra, 2002; [6]Lusas 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 warm-temperate 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).

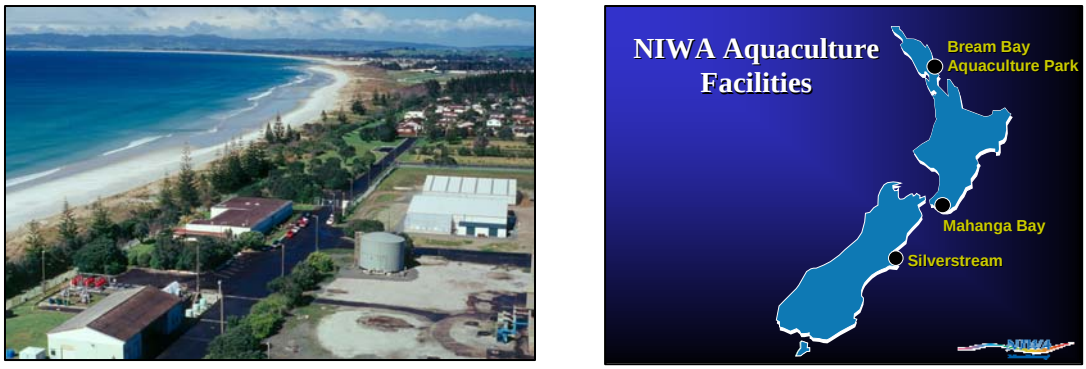

Figure 1. NIWA facilities

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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Port Stephens Fisheries Centre, New South Wales& [2]University of Queensland

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

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*

	Apparent digestibility coefficient (%)		
Ingredient & % inclusion	Organic matter	Crude protein	Gross energy
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

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 and gross energy digestibility coefficients for different levels of selected energy sources fed to juvenile Australian snapper *Pagrus auratus*

	Apparent digestibility coefficient (%)		
Ingredient & % inclusion	Organic matter	Crude protein	Gross energy
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 ≈ 1430h) 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.

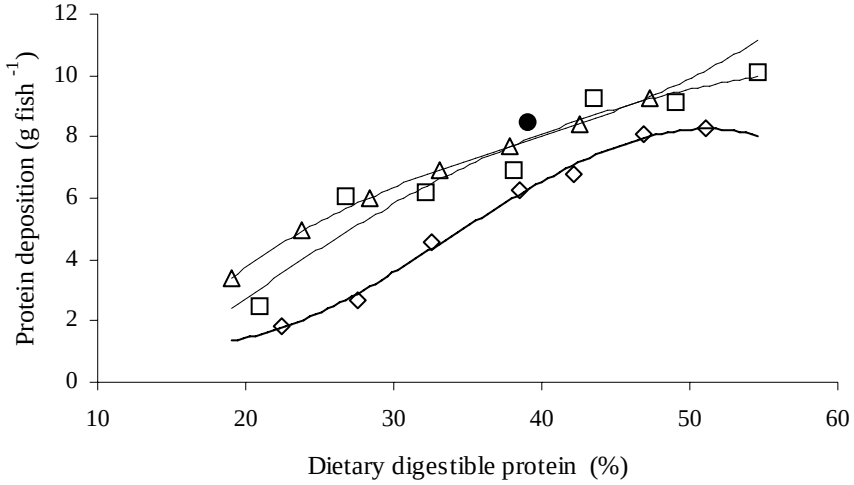

Figure 1. Protein deposition of juvenile snapper fed diets containing increasing levels of digestible protein and either 15 MJ DE kg-1 (Δ), 18 MJ DE kg-1 (□) or 21 MJ DE kg-1(◊) or a comparative commercial feed (•) for a period of 57 days.

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.

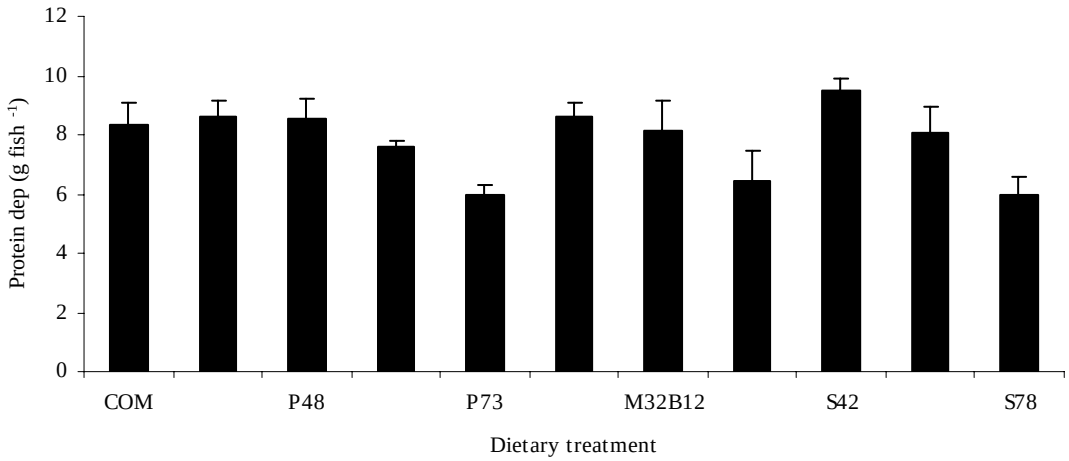

Figure 2. Protein deposition of juvenile snapper fed balanced diets (45% digestible protein and 17 MJ DE kg^{-1}) containing increasing levels of poultry offal meal (P), meat meal (M), meat and blood meal (M/B) and soybean meal (S) compared to a commercial barramundi diet (COM). Numerical value next to letter indicates dietary inclusion level. Bars are mean ± sd of 5 replicate tanks.

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

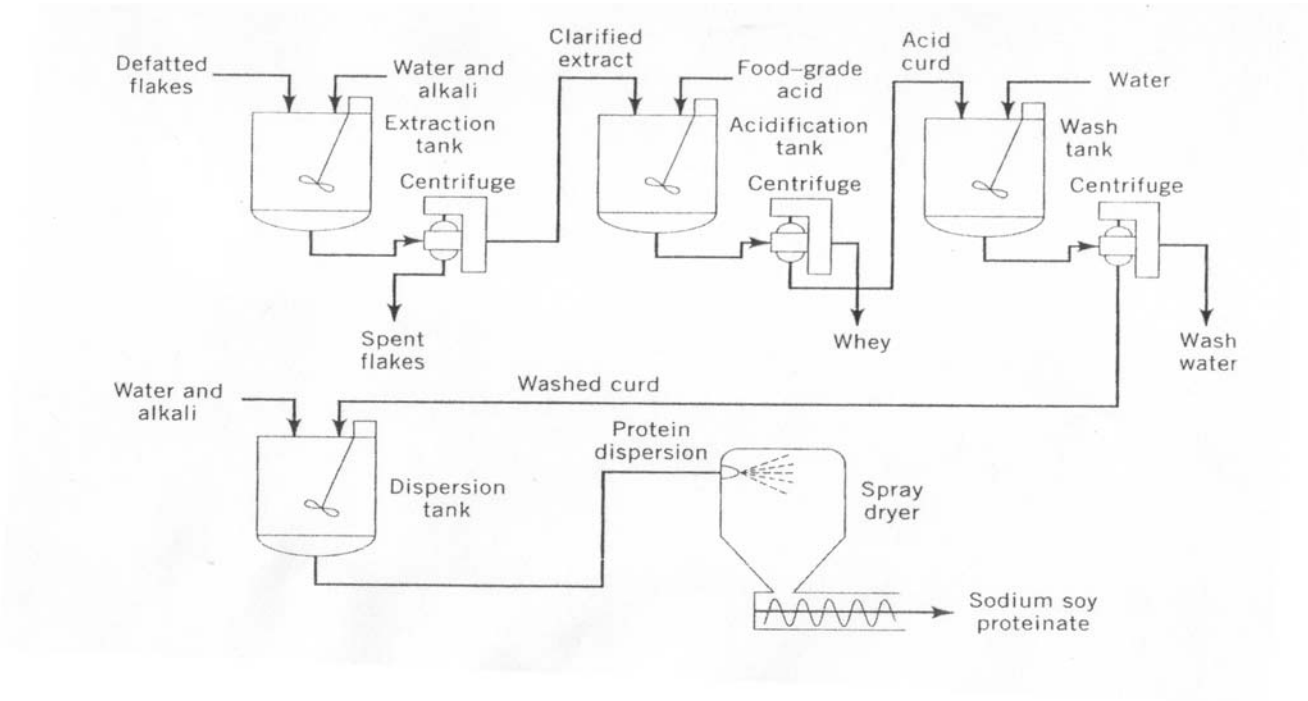

Figure 1. Isolated Soy Protein Process

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

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

	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

	Full Fat soy Expanded	Full fat Soy 36% Protein (Baking flour)	Soy flour 48% Protein (Baking flour)	Expeller Soy Meal 48% 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

NIR AAnalyzer Report on LA Soy when compaired with Fish Meal

SAMPLE		Protein	Lys	Met	Thr	Try	Val	Ile	Leu	Phe	His	Arg	Digestability
LA Soy	Total 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%
LA Soy	**Avail Protein**	**59.62**	**2.67**	**0.84**	**1.99**	**0.64**	**2.85**	**2.47**	**4.17**	**2.66**	**1.39**	**4.15**	
Fish Meal	Total protein	57.87	3.95	1.59	2.45	0.66	2.84	2.49	4.12	2.16	1.52	3.21	96%
Fish Meal	**Avail Protein**		**3.74**	**1.52**	**2.28**	**0.65**	**2.97**	**2.39**	**3.58**	**2.1**	**1.52**	**2.97**	

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.

Table 1. Commercial priorities for vegetable proteins by finfish species

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

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 Visco^{TM} Analyser (RVA^{TM}).

A classic RVA^{TM} 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 at the onset of this rise in viscosity is known as the pasting temperature. The pasting 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.

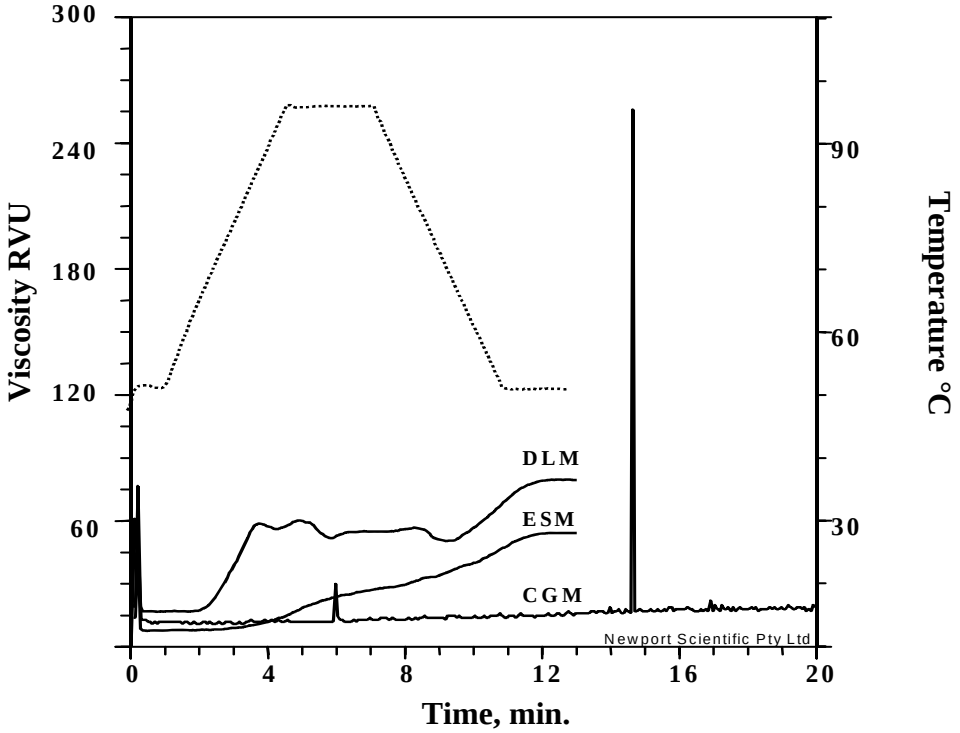

Figure 1. Pasting curve comparing dehulled lupin meal (DLM), extracted soybean meal (ESM) and corn gluten meal (CGM) with a standard temperature profile (dotted lines).

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.

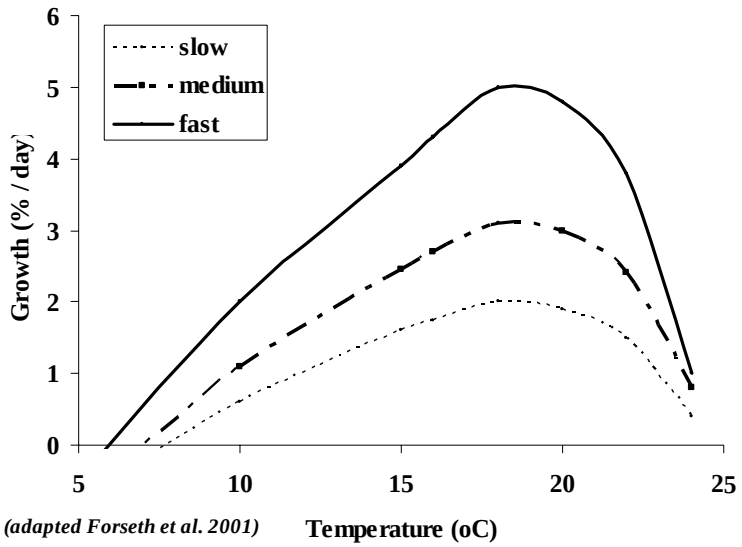

Figure 1. Predicted relationships between temperature and growth of one gram Atlantic salmon divided into slow, medium and fast growing individuals. The optimum temperature is approximately 19 ˚C.

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).

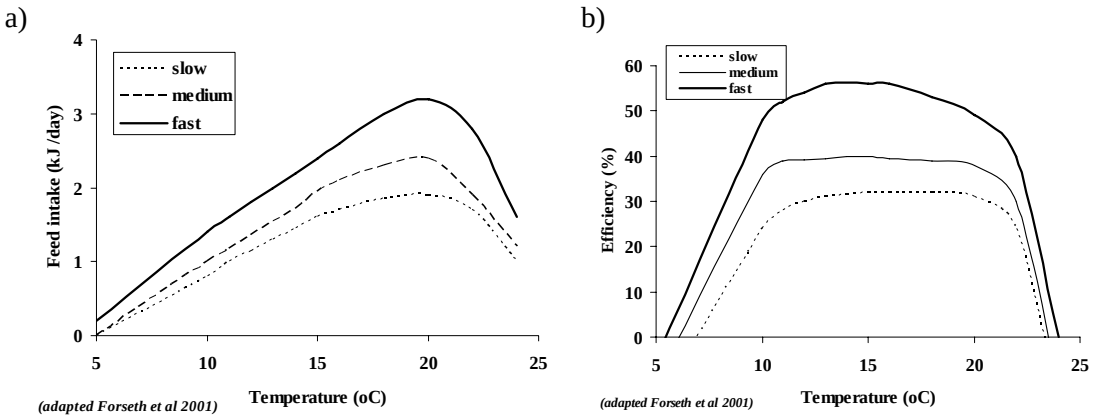

Figure 2. The relationships between temperature and a) maximum feed intake, and b) food conversion ratio (FCR) for Atlantic salmon divided into slow, medium and fast growing individuals.

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.

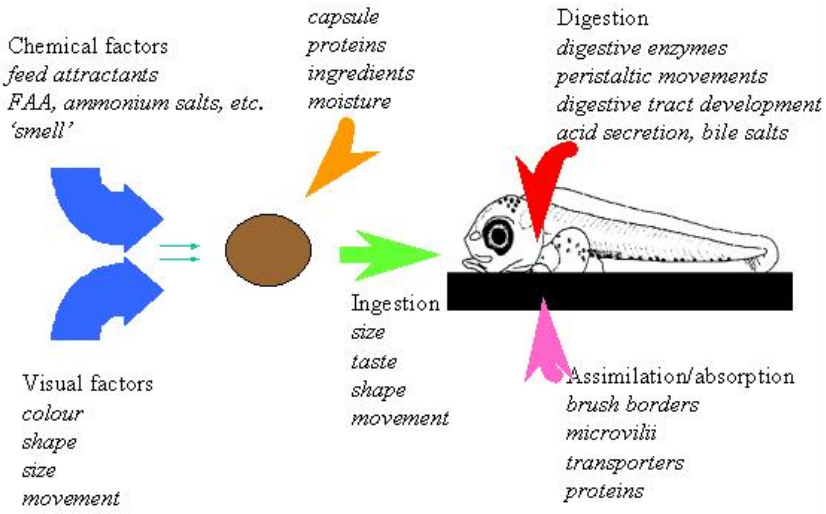


Figure 1. Factors affecting food particle utilisation

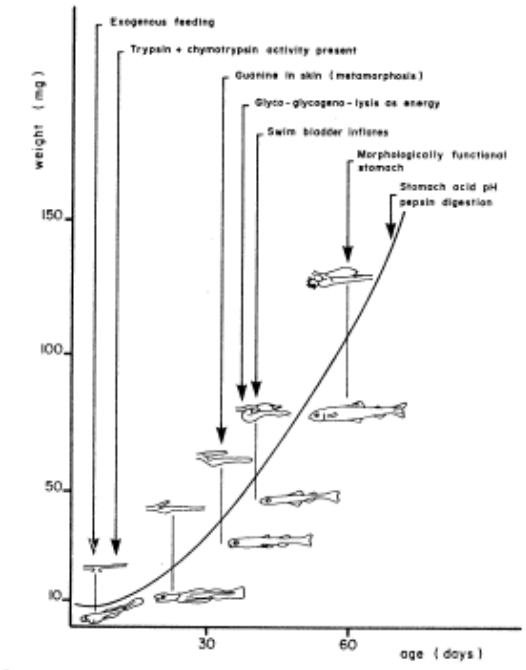


Figure 2. Ontogenic development of coregonid fish digestive tract (from Dabrowski, 1984)

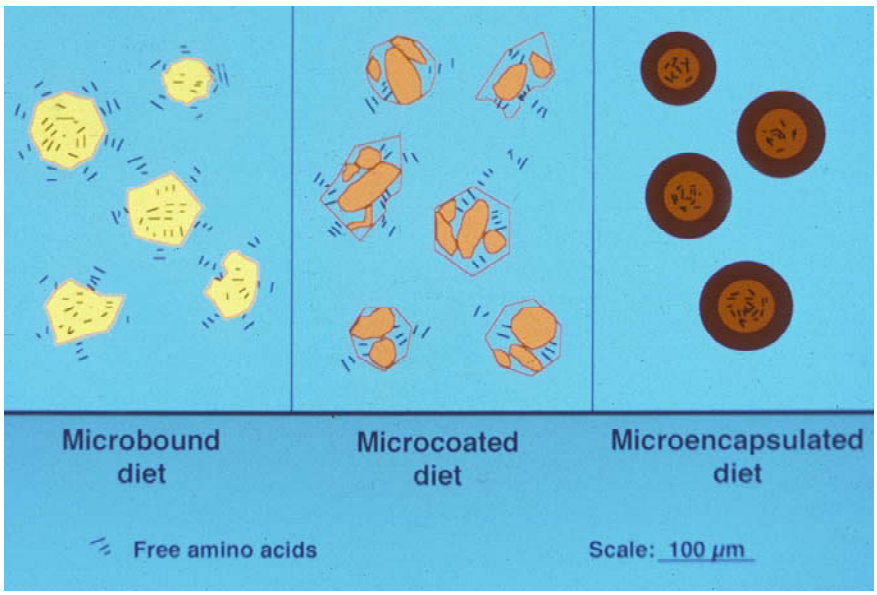


Figure 3. Micro diet types

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

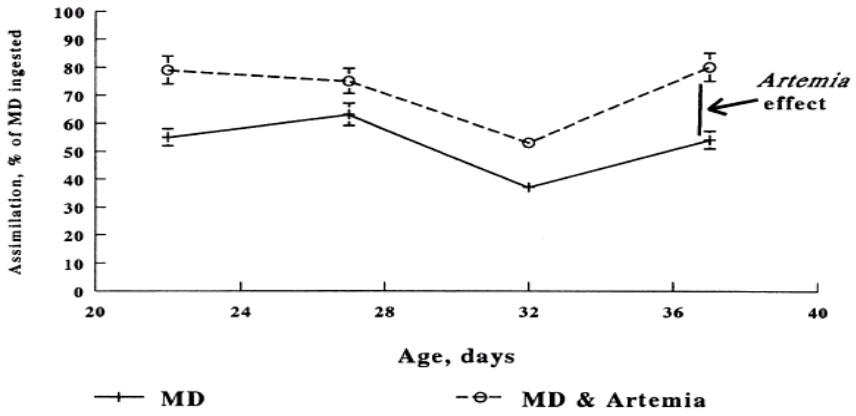


Figure 4.Assimilation rates of seabass *Dicentrarchus labrax* larvae co-fed microdiet (MD) and/or *Artemia*
(from Kolkovski et al. 1997)

Table 1. Digestive enzyme contribution by live food organisms

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)

Do fish larvae possess <u>enough</u> 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

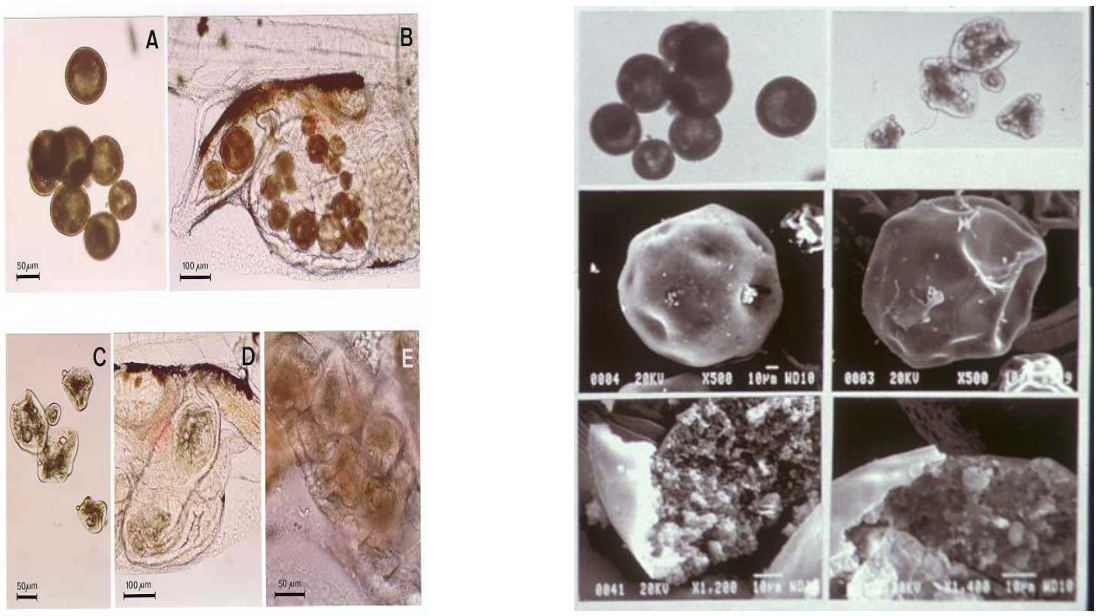


Figure 5. Microcapsules in 8 d old *S. aurata* larvae digestive system

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

- Dietary Digestive enzymes

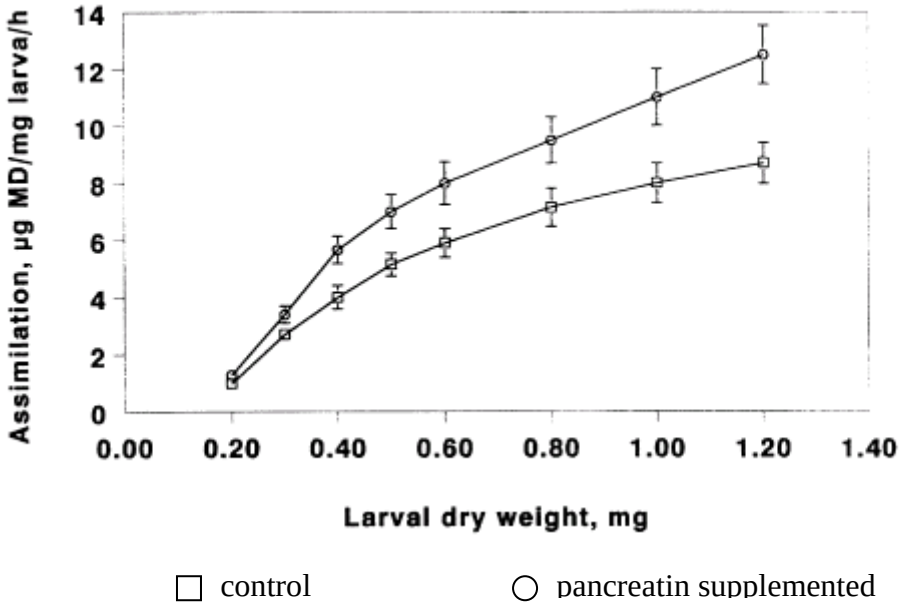


Figure 6. Assimilation rates of gilthead seabream *Sparus aurata* larvae fed control microdiet or pancreatin supplemented microdiet
(from Kolkovski et al., 1993)

Table 2. Dietary digestive enzymes supplementation in microdiets

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 *Sparus aurata*	lipase	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)

Alternative Strategy

Supplementation microdiets with:

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

Table 3. Protein Hydrolysates and FAA in microdiets

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)

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.

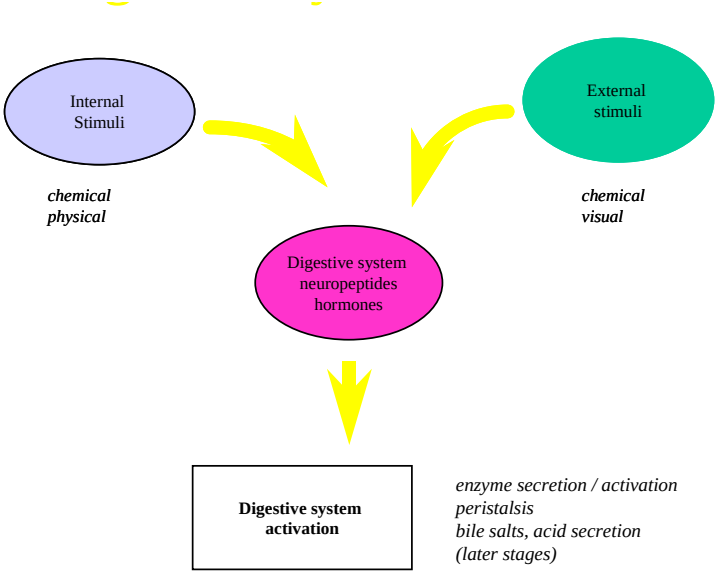


Figure 7. Digestive System Activiation

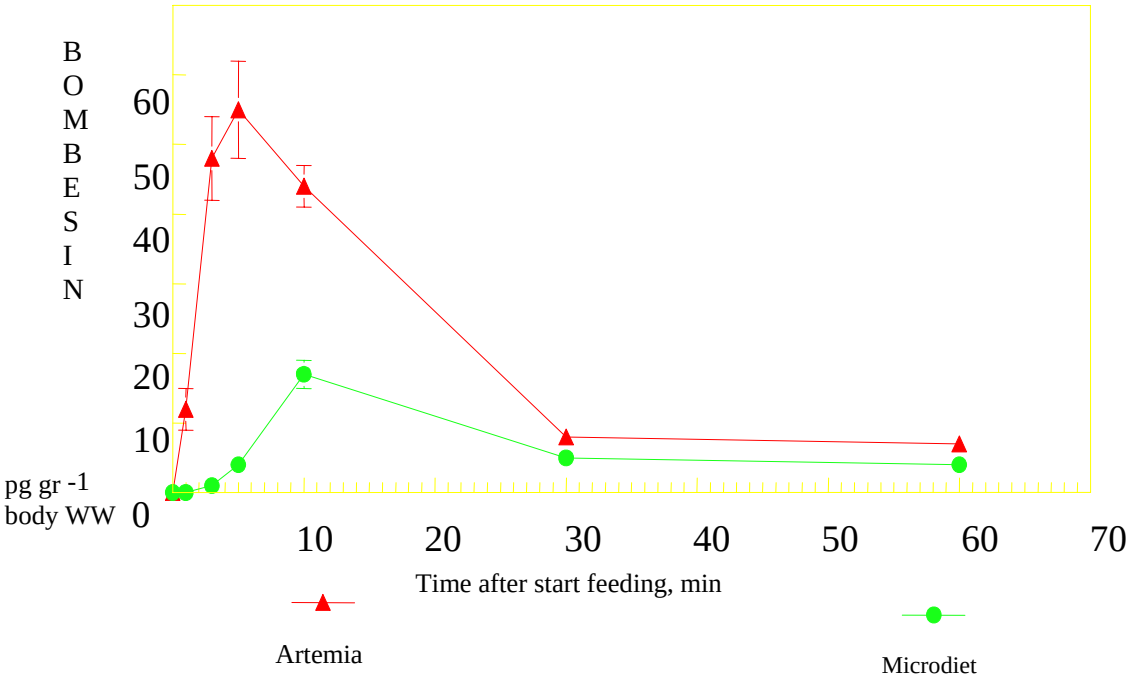


Figure 8. Effect of food type on bombesin activity in 24 day old gilthead seabream larvae (from Kolkovski et al., 1997)

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

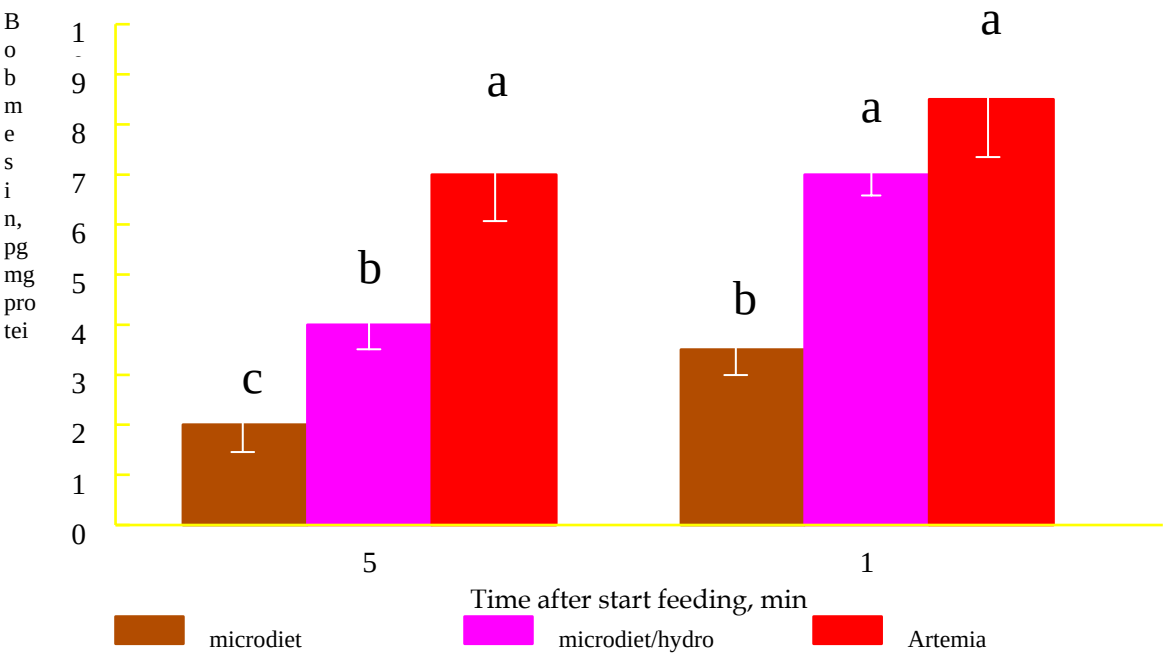
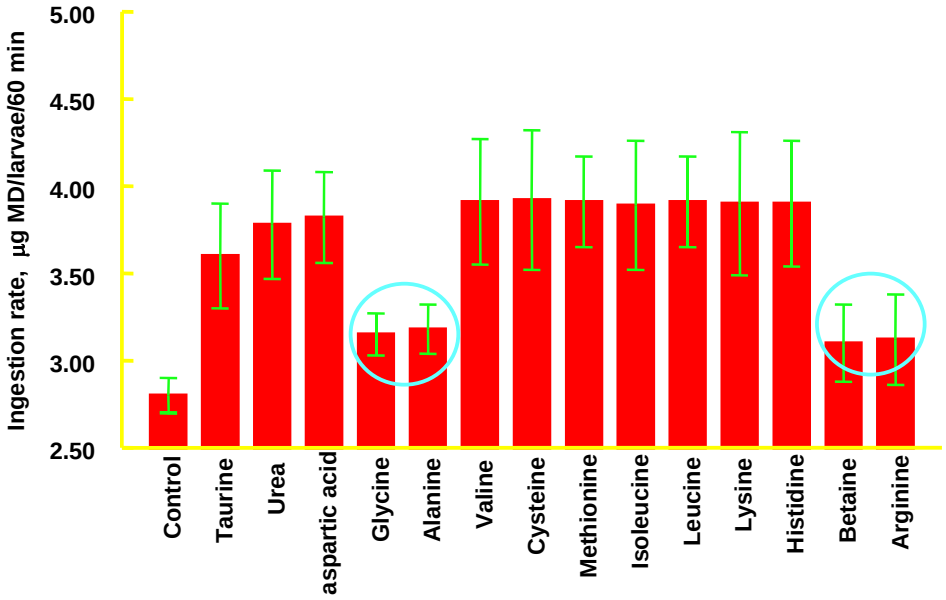
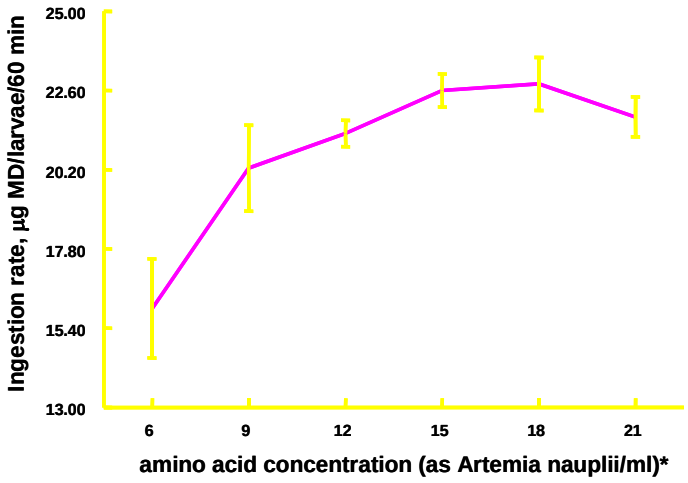


Figure 9. Effect of krill hydrolysate supernatant on bombesin activity in barramundi *Lates calcarifer* larvae (from Kolkovski et al., 1999)



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)

Table 4. Amino Acids and other metabolites used as feed attractants in marine organisms

Rainbow trout *Salmo gairdineri*	Mixture of L-amino acids	Adron and Mackie, 1978
Atlantic salmon *Salmo salar*	Glycine	Hughes, 1990
Sea bass *Dicentrarchus labrax*	Mixture of L-amino acids	Mackie and Mitchell, 1982
Pig fish *Orthopristis chrysopterus*	Glycine, Betaine	Carr et al. 1977, 1978
Red sea bream *Chrysophrys major*	Glycine, Betaine	Goh and Tamura, 1980
	Glycine, Alanine, Lysine	Fuke et al., 1981
	Valine, Glutamic acid and Arginine	Ina and Matsui, 1980
Gilthead sea bream *Sparus aurata*	Glycine, Betaine, Alanine, Arginine	Kolkovski et al., 1997
Turbot *Scophthalmus maximus*	Inosine and IMP	Mackie and Adron, 1978
Dover sole *Solea solea*	Glycine, Betaine	Mackie et al., 1980
	Glycine, Inosine, Betaine	Metaillet et al., 1983
Puffer *Fugu pardalis*	Glycine, Betaine	Ohsugi et al., 1978
Japanese eel *Anguilla japonica*	Glycine, Arginine, Alanine, Proline	Yoshii et al., 1979
Cod *Gadus morhua*	Arginine	Doving et al., 1994
Herring *Clupea herangus*	Glysine, Proline	Damsey, 1984
Glass eel *Anguilla anguilla*	Glycine, Arginine, Alanine, Proline	Mackie and Mitchell, 1983
	Alanine, Glycine, Histidine , Proline	Kamstra and Heinsbroek, 1991
Lobster *Homarus Americanus*	Glutamate, Betaine, Taurine, Ammonium chloride	Corotto et al., 1992
Western Atlantic ghost crab *Ocypode quadrata*	Butanoic acid, Carboxylic acid, Trehalose, carbohydrates, Homarine, Asparagine	Trott and Robertson, 1984
Freshwater prawn *Macrobrachium rosenbergii*	Taurine, Glycine, Trimethylamine, Betaine	Harpaz et al., 1987
Abalone Ha*liotis discus*	Mixture of L-amino acid and lecithin	Harada et al., 1987

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

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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 m^2 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 whole-farm, 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/m^2 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/m^2) 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 ^{15}N 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

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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.

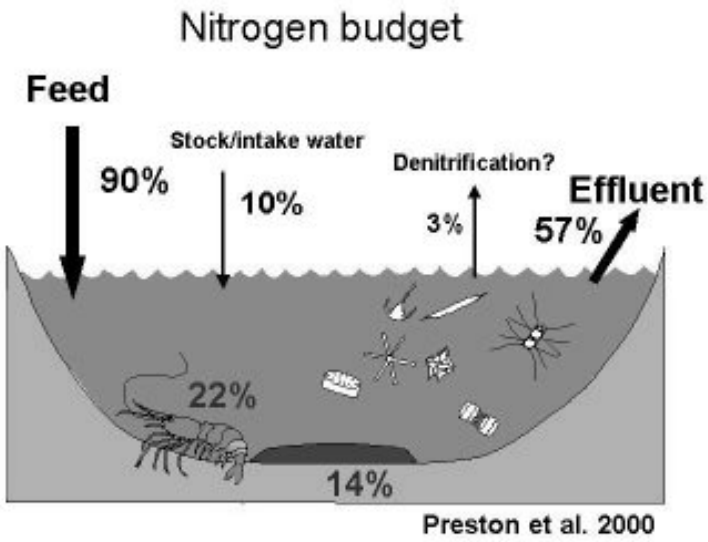

Figure 1. *Nitrogen budget of a typical Australian prawn pond.*

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 de-nitrification 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.

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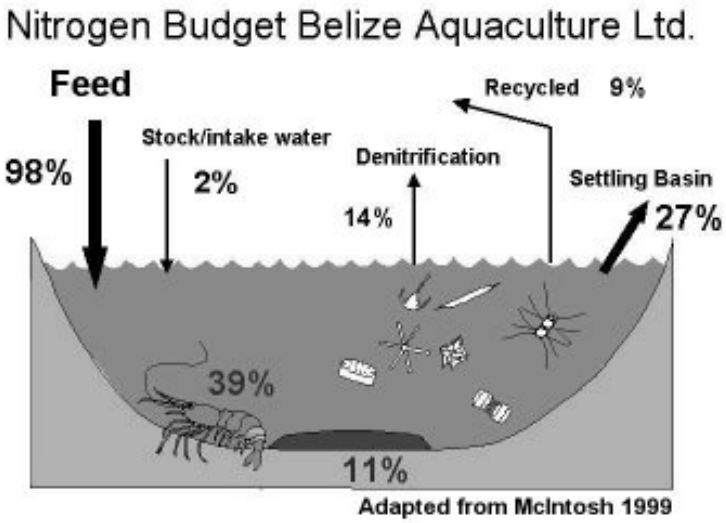

Figure 2. *Nitrogen budget of Belize Aquaculture Ltd*

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.

Table 1. *Typical Shrimp Feed Formulation*

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

Table 2. *Typical Sea Bass Formulation*

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.

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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

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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 = $M \cdot \text{BW(kg)}^b + G \cdot \text{growth}$, where $M \cdot \text{BW(kg)}^b$ 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.

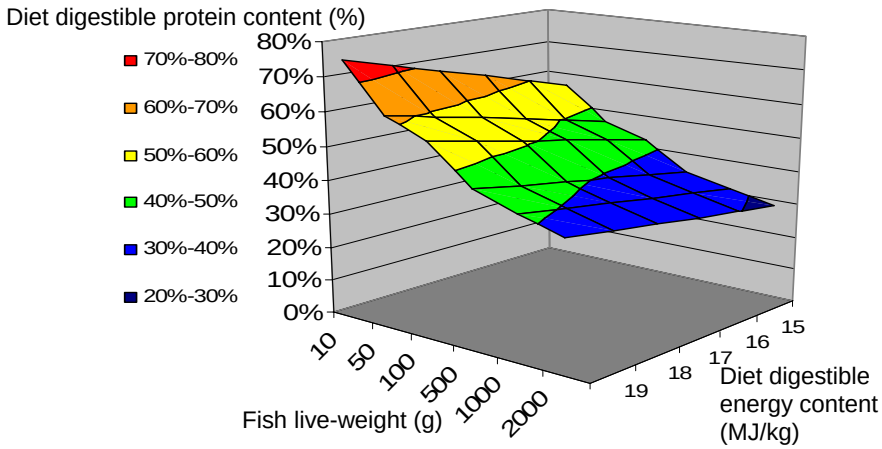

Figure 1. Digestible protein requirement of barramundi (0-80%) with varying dietary energy density (MJ/kg) and fish live-weight (g). Derived from Lupatsch and Kissil, 2003.

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).

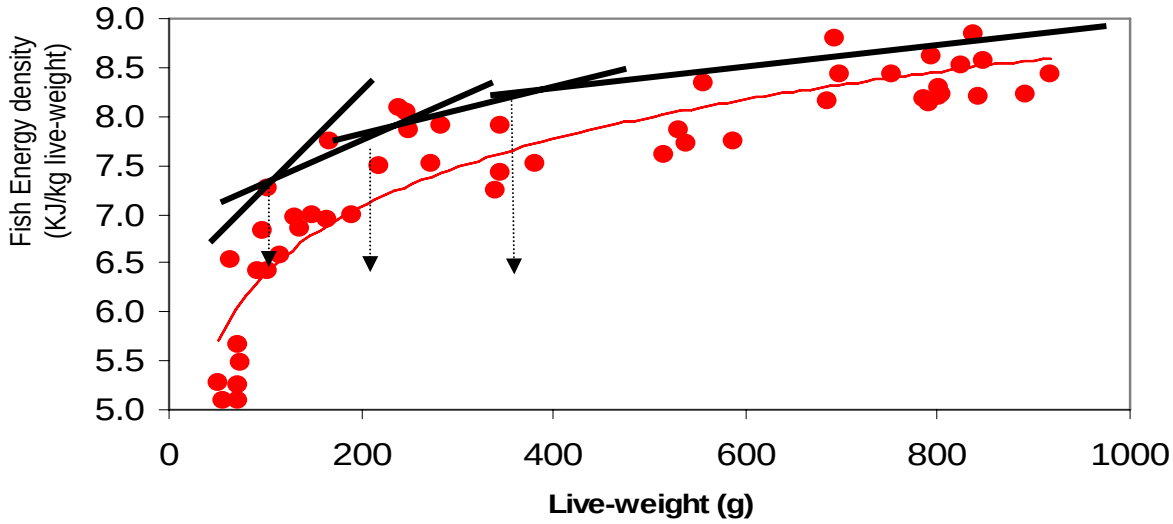

Figure 2. Stylised assignment of diets to barramundi production. Intercept of lines on energy curve indicating were diet choice (diet energy density and accordingly protein content) should change. Angle of line is indicative of the lysine to energy ratio required in the diet, while the Y-intercept of 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)*