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Critical requirements for aquaculture feeds

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Introduction

The meal from rendered bait-fish, commonly referred to as fish meal, has long been a central ingredient in compounded aquaculture diets. As an ingredient it has several attributes favourable for use in aquaculture diets, such as high protein and energy levels, good processing characteristics for producing pellets and is highly palatable to virtually all aquaculture species. However, it is now generally regarded that the stocks of fish suitable for producing these meals are at maximum sustainable harvest capacity or in some cases in decline. Furthermore, the influence of El nino events on the fish stocks in the major bait-fish fisheries in the Eastern Pacific is such that unfavourable conditions can have major impacts on global supplies of this ingredient.

The risk therefore, with being too dependent on fish meal, is clear and initiatives have been in progress since the 1980's to identify and evaluate alternative protein resources. However, not all protein resources are suitable for aquaculture feeds and distinct differences even exist between the different types of aquaculture feeds produced.

Alternative protein resources

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. Terrestrial animal meals are typified by moderate to high protein levels, low to moderate fat levels and moderate levels of ash. No appreciable levels of carbohydrate are found in terrestrial animal meals (Table 1). By contrast plant protein meals have considerable quantities of carbohydrates, not all of which are utilisable by fish. Protein and fat levels in plant protein meals vary considerably, usually depending of the variety and level of processing used. Notably, those ingredients that have been more widely used and accepted by the aquaculture industry include those such as soybean meal and meat meals.

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

INGREDIENTS	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

LKM: L. luteus kernel meal; AKM: L. angustifolius 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).

Feed specifications and formulation flexibility

The term “aquafeed” is somewhat of a generalisation, as there are numerous types of diets, depending on species and age of the animals being fed (Table 2). Typically, modern feeds designed for younger, smaller fish tend to be high protein (>500 g/kg) and are moderately energy dense (< 20 MJ/kg), while feeds for larger and older fish tend to be lower in protein (400 to 450 g/kg) and are more energy dense (> 21 MJ/kg) (Webster and Lim, 2002). Typically such feeds have a high fat content to maximise the dietary energy intake. These types of feeds are often referred to as high-nutrient-dense (HND) diets.

By contrast there is also a range of diets for species that are either unable to deal with high dietary levels of lipids, or their large gustatory capacity makes it practical to feed them on lower-cost, less energetically dense diets. For example, a prawn diet has a protein level not dissimilar to that of a salmon or barramundi diet, but because they are unable to deal with high dietary lipid levels the total dietary lipid content must be restricted to less than 100 g/kg (Glencross et al., 2002). Abalone diets also have similar limitations (van Barneveld et al., 1998). Tilapia are a species that has a large gustatory capacity and can compensate the use of low protein diets by consuming sufficient amounts of a low-energy dense diet to satisfy its demand for protein for growth. These types of diets are often referred to as low-nutrient-dense diets (LND).

One of the fundamental constraints to HND diets is the limited formulation flexibility that exists. The capacity to use ingredients that do not contribute useful nutritional material is limited in these diets. In contrast, LND diets have considerably more capacity to accommodate ingredients with additional non-useful nutritional content. The capacity that each of the different diets have to accommodate this non-useful nutritional content is estimated in table 2 under the term of “space”, with the higher the amount of “space” the greater the capacity to accommodate non-useful nutritional content.

Table 2. Generalised composition (g/kg as-fed) of diets for various species, including an indication of the typical amount of formulation “space” available.

	Salmon 1	Salmon 2	Salmon 3	Barra 1	Barra 2	Prawns	Tilapia	Abalone	Marron
Dry Matter	920	920	920	920	920	920	920	920	920
Protein	400	450	550	450	500	450	300	300	250
Fat	300	250	200	200	130	90	80	50	50
Other “essentials”	50	50	50	50	50	50	50	50	0
Energy (MJ/kg)	24.0	22.5	22.0	21.5	20.5	19.0	18.0	17.5	17.0
“Space”	170	170	120	220	240	330	490	520	570

What characteristics should a protein concentrate or isolate have?

It is recognised that the higher the protein content of an ingredient then the higher it’s potential value. In addition, protein sources with functional properties are also likely to command premiums. A plant derived protein concentrate for aquaculture feed use though doesn’t necessarily have to have specific functional properties, but its use is likely to be highly price sensitive. Accordingly, keeping the cost/price of such an ingredient to an effective level will depend on many things. One important step is the determination of prospective protein levels at which the ingredient is likely to be cost-effective to both produce and use. This issue becomes further complicated by the fact there are two key strategies that can be used to increase the use of alternative ingredients. One uses the basis of sole substitution and the other, dual substitution, requires the complimentary use of an accessory low-value ingredient.

In considering the first option, optimising the protein level (and by default the non-useful content) is the key to defining the most useful product. The determination of an “ideal” protein level can be determined using a variety of methods and is also likely to be somewhat formulation dependent. However, it is this first option, of defining what characteristics a single inclusion ingredient should have to be an optimal product.

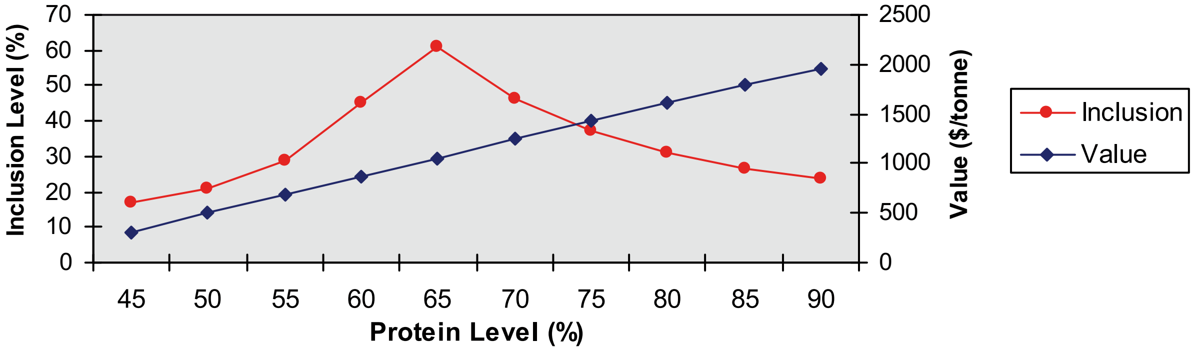

Figure 1. Influence of protein level of hypothetical protein concentrates on inclusion level and ingredient value ($/tonne) when included in a single type of HND diet (450 g protein/kg and 22.5 MJ).

Although a somewhat simplistic evaluation, least-cost linear formulation with hypothetical ingredients can show the relationship between diet formulation, ingredient composition, potential ingredient value and likely inclusion level (Figure 1). The limitations of this evaluation are that the inclusion levels and price of the hypothetical ingredients are highly dependent on the price and composition of fishmeal. Many of these issues are discussed more fully in Appendix II. For this reason, this is why the hypothetical ingredients are secondarily evaluated at fixed inclusion levels of 20% and 30% (Table 3). What these two approaches do define is that the "ideal" protein level is from 500 g/kg to 700 g/kg. Within those options, the issue then becomes predominantly a price sensitive one.

Table 3. Influence of a fixed inclusion level of hypothetical protein concentrates when included in a single type of HND diet (450 g protein/kg and 22.5 MJ). Ingredient prices were determined using a fixed-cost sensitivity analysis.

		20% inclusion			30% inclusion		
	Fishmeal	LPC500	LPC600	LPC700	LPC500	LPC600	LPC700
Diet cost	1101	1101	1101	1101	1101	1101	1101
Ingredient Cost		1011	1215	1425	1015	1218	1420
FORMULATION							
Pre-mix vitamins	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Fish oil ($1000/t)	19.10	19.60	19.50	19.40	19.90	19.80	19.60
Wheat flour ($240)	13.50	9.50	13.80	18.10	7.60	13.90	20.30
LPC 700	0.00	0.00	0.00	20.00	0.00	0.00	30.00
LPC 600	0.00	0.00	20.00	0.00	0.00	30.00	0.00
LPC 500	0.00	20.00	0.00	0.00	30.00	0.00	0.00
Fish meal ($1200)	66.90	50.40	46.20	42.00	42.00	35.80	29.60

Formulations based on fishmeal composition of 65% protein and 9% fat.

The dual use option

The dual use option works on the principle of a single high-protein ingredient creating formulation "space" so as to enable a cheap, lower protein ingredient to be used. The potential value for such an ingredient is dependent on its protein content. Interestingly though an examination of the potential of high protein-high fat (e.g. 80% protein – 9% fat) isolates identified that these would potentially be worth more than protein isolates that were higher in protein content, but lower in fat content (87% protein – 2% fat). This "bargaining" aspect of such an isolate is derived primarily from its ability to draw in greater use of lower cost ingredients. While there is clearly potential for such an ingredient in the aquaculture feeds sector, its use would never be high volume and it has limited potential to minimise fish meal inclusion compared to the other ingredient development option proposed in this paper.

Table 4. Influence of a fixed inclusion level of hypothetical protein isolates when included in a single type of HND diet (450 g protein/kg and 22.5 MJ). Ingredient prices were determined using a fixed-cost sensitivity analysis.

	Reference	High Protein - High Fat (800-90)		High Protein - Low Fat (870-20)	
Diet Cost	1101	1101	1101	1101	1101
Ingredient value ($/tonne)		2300	2020	2275	2000
Ingredient Inclusion					
Pre-mix vitamins	0.5	0.5	0.5	0.5	0.5
Fish oil	19.1	19.1	19.1	19.5	19.9
Wheat flour	13.5	10.0	10.0	10.0	10.0
Sweet lupin kernel	0.0	10.4	13.6	10.8	14.0
Fish meal	66.9	55.0	46.8	54.2	45.6
LPI-(800-90)	0.0	5.0	10.0	0.0	0.0
LPI-(870-20)	0.0	0.0	0.0	5.0	10.0

References

Glencross, B.D., Smith, D.M., Thomas, M.R. and Williams, K.C. (2002). The effects of dietary lipid amount and fatty acid composition on the digestibility of lipids by the prawn, *Penaeus monodon.* Aquaculture 205, 157-169.

van Barneveld, R.J, Fleming, A.E., Vandepeer, M.E., Kruk, J.A., Hone, P.W. (1998). Influence of dietary oil type and oil inclusion level in manufactured feeds on the digestibility of nutrients by juvenile greenlip abalone (*Haliotis laevigata*). J. Shellfish Res. 17, 649-655.

Webster, C.D. and Lim, C.E., (2002). Nutrient Requirements and Feeding of Finfish for Aquaculture. CAB International. Wallingford, UK. pp 418.

Protein concentrates and isolates

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Introduction

The growing need for protein (food & animal feed including fish) and of protein enriched products has resulted in an intensive search for new protein sources. Vegetable protein preparations primarily based on soybeans have been used widely in the food and feed sectors. Due to their high protein content, good nutritional value, and lower price compared with animal proteins, soybeans were the first source of protein concentrates and isolates as food ingredients, and more recently feed ingredients.

In this study we are investigating the production of protein enriched products (concentrates and isolates) from lupins. There are several species in the genus Lupinus. The economically significant species include *Lupinus albus* (albus) the 'European lupin', *L. luteus* (yellow lupin YL) mainly grown in Germany and Eastern Europe, and *L. angustifolius* (narrow leafed lupin NLL), the main lupin grown in Australia and in particular Western Australia. Recent investigations into the potential of *L. mutabilis* in the West Australian cropping system are looking very promising.

The gross chemical composition of these four lupin species are shown in Table 1. Both the whole seed values as well as the dehulled kernels (cotelydons) are reported. Dehulling is the first step in the process of producing a 'protein enriched lupin product'. The kernel values for protein, polysaccharide and oligosaccharide will influence which (if any) processing pathway the grain should take.

The most outstanding point is the kernel protein level of both *L. luteus* and *L. mutabilis*. However it needs to be noted that the seed coat accounts for 27% of the *L. luteus* seed and 16% of the *L. mutabilis* seed, therefore the theoretical maximum yield for dehulling is 73% and 84%, respectively. The other points to note in Table 1. include the kernel non-starch polysaccharide (NSP) values, mostly insoluble fibrous material, and the oligosaccharide.

Table 1. Gross chemical composition (%) of the four lupin species *L. albus, L. luteus, L. angustifolius, L. mutabilis.*

Species	*L. angustifolius*		*L. albus*		*L. luteus*		*L. mutabilis*	
	Seed	Kernel	Seed	Kernel	Seed	Kernel	Seed	Kernel
Seed Coat	24	0	18	0	27	0	16	0
Moisture	9	12	9	11	9	12	?	?
Protein	32	41	36	44	38	52	44	52
Fat	6	7	9	11	5	7	14	17
Ash	3	3	3	4	3	4	3	4
Lignin	1	1	1	1	1	1	1	1
Polysaccharides	22	28	17	21	8	10	9	10
Oligosaccharides	4	6	7	8	9	12	?	?
Minor Components	0.5	1	0.6	1	0.9	1	?	?

Protein concentrates

Protein concentrates are considered to have greater than 50% protein, (although the soy industry has set the benchmark at 65% dry basis) and are essentially flour (dehulled kernels) from which the carbohydrates (free sugars and oligosaccharides) and other soluble materials have been removed. These concentrates are commercially produced by various processes including:

- separation of the sugar fractions (oligosaccharides) by extracting with 20-60% ethanol
- washing away the carbohydrates at the isoelectric point (based on the fact that the major part of the native protein is insoluble in acidic (pH 4.5–5.0) aqueous solutions
- by denaturing the flour with moist heat and then washing with water

Table 2 shows that the theoretical value of protein concentrates produced from kernel flours from NLL and YL, was 59% and 70%, respectively. It is apparent that this method favours Wodjil which has more aqueous extractable material such as the soluble oligosaccharides and it is also relatively low levels of insoluble fibrous material.

Table 2. A theoretical comparison of protein concentrates produced starting from Myallie *(L. angustifolius)* and Wodjil *(L. luteus)* kernel flours, using the traditional ethanol wash method.

Species	*L. angustifolius (cv. Myallie)*					*L. luteus (cv. Wodjil)*				
	Seed as is	Seed DM	Kernel DM	Ethanol washed	Fat Removed	Seed as is	Seed DM	Kernel DM	Ethanol washed	Fat Removed
Seed Coat	23	23	0			18	0	0		
Moisture	9	0	0			9	11	0		
Protein	34	38	49	59	64	36	44	59	70	76
Fat	5	6	7	8		9	11	7	8	
Ash	3	3	4	5	5	3	4	5	6	6
Lignin	1	1	1	1	1	1	1	2	2	2
Polysaccharides	21	24	31	27	30	17	21	12	15	16
Oligosaccharides	4	5	6			7	8	13		
Aqueous extractables	6	7	9			*assumed to be nil*				
Minor Components	1	1	1			0.6	1	2		

Protein isolates

Protein isolates are defined as the major protein fraction of soybean prepared by removing most of the non-protein components. They contain not less than 90% protein on dry basis (N x 6.25). This definition was approved for soy by the USFDA in 1961 and is commonly accepted for other legumes for the 'food industry'. These are the benchmark levels required for a protein isolate or concentrate in the soybean industry but these levels may vary between 50%-90% protein depending on end use.

The technology associated with protein isolates is well known. The protein is extracted with water at alkaline pH to yield a soluble protein and a protein exhausted residue (fibre). The fibre is removed, and the soluble protein is then precipitated at pH 4.5-5.0 to yield a protein curd and a legume whey. The curd is then washed (may be neutralized) and dried (usually spray dried) and is then called an isolate.

The theoretical yields of the three products (protein isolate, fibre and whey solids) after applying the isolate processing mechanism to NLL and YL are demonstrated in Table 3. In these calculations there is an assumption that we achieve 100% efficiency at separation of the major components and there are no protein losses in the whey solids.

Table 3. A theoretical comparison of protein isolates starting from Myallie *(L. angustifolius, NLL)* and Wodjil *(L. luteus)* kernel flours, using the traditional isoelectric precipitation method.

Species	*L. angustifolius (cv. Myallie)*				*L. luteus (cv. Wodjil)*			
	Kernel DM	Protein Isolate	Fibre Residue	Whey	Kernel DM	Protein Isolate	Fibre Residue	Whey
Recovery from start								
Protein	49	49			59	59		
Fat	7	7			7	7		
Ash	4	2	2		5	3	2	
Lignin	1		0.5	0.5	2		0.5	0.5
Polysaccharides	31		22		12		13	
Aqueous extractables	9			9				
Oligosaccharides	6			6	13			13
Minor Components	1			1	2			2
Percent composition								
Protein	49	84			59	86		
Fat	7	13			7	10		
Ash	4	3	8		5	4	15	
Lignin	1		4	3	2		3	4
Polysaccharides	31		88		12		82	
Aqueous extractables	9			52				
Oligosaccharides	6			36	13			86
Minor Components	1			9	2			10

In theory NLL yields protein isolates and fibre accounting for 58% and 25%, respectively, of the kernel flour which compares to 69% and 16%, respectively in YL kernel flour. While the processing of YL yields higher levels of protein isolate if you take into account the commercial value of the fibre fraction (considered equal to the protein isolate) then both species produce products of equal value. In practicality however the efficiency of separation is never 100%, and losses of material occur. The success of a lupin processing industry will in large be dependent on the ability to effectively achieve maximum separation and minimum losses.

Grain varieties and species potential

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Introduction

Australia is the largest producer of low alkaloid (sweet) lupins in the world. The production of Australian Sweet Lupins in Western Australia began in the early 1970's and has progressed rapidly to a stage where on average about 1 million hectares of lupins are grown every year. Lupins have become a vital component of the sand-plain farming system because it is well adapted to those infertile, sandy soils; fixes nitrogen which is accessible by the following cereal crop; assists in disease breaks; the deep roots open up the soil and the stubble prevents wind erosion. The major breeding centre for lupins is in Western Australia with a smaller program at Wagga Wagga in NSW concentrating on mid season narrow-leafed lupins and albus lupins.

Current production

There are three species of lupin produced commercially in Australia:

- Narrow-leafed lupins (NLL) or *L. angustifolius*.
- Albus lupins (*L. albus*)
- Yellow lupins (*L. luteus*)

The majority of the production is NLL and a record crop was harvested in 1999 with an estimated production of 1.5 million tonnes. Production in the eastern States is not insignificant and amounts to about 200,000 tonnes.

Albus lupin production is currently centred in New South Wales approximately 50-80,000 tonnes are produced. An albus industry in Western Australia was producing about 30,000 tonnes annually until a fungal disease (Anthracnose) wiped it out in 1996. Production is planned to recommence in 2005 with the release of an anthracnose resistant variety next year. A significant proportion of the current production is exported to the Middle East for human consumption. The price paid for albus lupins has been about $30 /tonne above the NLL price.

Yellow lupin industry was initiated with the release of the variety Wodjil in 1997 but has not reached greater than 2000 tonnes. While yellow lupins have great potential on the sandy soils of Western Australia it has not been well accepted by farmers because of the aphid susceptibility of the current variety and the lower yield compared to narrow-leafed lupins. The breeding program is addressing both of these. Current yellow lupins receive a price premium above NLL price of approximately $30/tonne

Species potential

Narrow-leafed lupins

The NLL industry has been well supported by the breeding program based in Western Australia. The breeding program has concentrated on increasing yield, maintaining protein and low alkaloid levels and decreasing the risks from diseases and insect attack. We have seen the industry peak in 1999 with production up to 1.5 m tonnes and we expect this to level off at approximately 800,000 tonnes. Yield has been the greatest consideration for selection of variety for farmers as the market was only interested in quantity. There is now a modest premium paid for protein, so the emphasis in the breeding program has also shifted to lifting the protein levels above the current average of 32%. There are two new varieties being released in the next two years one of which is higher yielding and more robust in the farming system while the other is similar to current varieties in yield but has higher levels of protein. These varieties will give farmers the choice to chase income through yield or income through protein. It will also create opportunities in the end user market to tap into this higher protein variety for protein sensitive markets such as the aquaculture feed market.

New initiatives in the breeding program include

- Development of thin seedcoat varieties that are easier to dehull and will produce more concentrated protein
- Development of varieties with higher protein content (up to 36%) more tailored to specific feed markets
- Development of varieties with higher concentration of specific proteins of functional and feed value

NLL is a well established industry that is robust enough to support a value adding industry of moderate size.

Albus lupins

Albus lupins have been used as a traditional snack food, after debittering, in the Mediterranean and Middle East countries for many centuries. Albus lupins have been grown commercially in Australia since the early1980s. They are adapted to the heavier neutral to alkaline soils typical of the Avon River valley and the red loamy soils around Morawa. Production on these soils does not compete with the NLL or yellow lupin production. Production Australia wide could exceed 200,000 tonnes with WA capable of producing 60-100,000 tonnes annually given the appropriately priced market.

A large effort has been put into developing disease resistant varieties that are needed to re establish the industry in Western Australia and to protect the industry in the eastern States. There has been no attempt to increase either the oil content (9-12%) or the protein content (38-42%)

Yellow lupins

Yellow lupins have been grown as a companion crop with cork trees in Portugal and as a green manure crop in eastern Europe for many years. The grain is higher in protein and has higher sulphur amino acid levels. It is only after collaboration with some Polish breeders were we able to find varieties that flowered early enough for our environment. This led to the release of the first Australian yellow lupin variety (cv. Wodjil) which receives a $30/tonne premium over NLL. In general yellow lupins grow well on acid to neutral sandy soils, including those currently being used to grow NLL. Yellow lupins are not competitive in yield with NLL on good deep sand plain country and the price differential doesn’t compensate for the yield difference, however on some soils yellow lupins are very competitive. Those soils are acidic soils high in aluminium content of the eastern wheatbelt, the manganese deficient soils on the south coast and possible transient waterlogging duplex soils of the lower Great Southern district.

The breeding program is concentrating on improving the yield as well as the resistance to insect attack. New variety will be released in 2004 or 2005 which will be 30% higher yielding than the current variety and with improved resistance to aphids. The release of this variety will stimulate the industry and produce grain that is high in demand. Without further improvement in the price premium the production is estimated to reach about 50,000 tonnes in 5 years. If the price premium increased, making it more competitive with NLL then a greater area will be grown using ground currently occupied by NLLs. Under these circumstances I could see production reaching 200,000 tonnes if required.

Pearl Lupins

The pearl or Andean Lupin originates from South America where it has been cultivated for centuries by the indigenous peoples of Ecuador, Peru and Bolivia and is consumed as a traditional food after leaching the bitter alkaloids. Grain protein and oil levels rival and in some cases exceed soybean (protein = 38-50% and oil = 13-24%). The oil is high in unsaturated fatty acids and low in erucic acid. The grain has a thin seed coat (13%) similar to soybean making it highly suitable for dehulling. With increasing interest in legume protein concentrates and isolates for the human food ingredients and animal feeds, pearl lupin has come under renewed interest. A new GRDC funded project aims to:

- Develop a range of domesticated breeding material with appropriate grain quality and agronomic characteristics that could form the basis of the first pearl lupin variety release for Australia.
- Determine pearl lupin genotype performance on a range of soil-types; waterlogging, pH and herbicide tolerances, and reaction to the major lupin pests and diseases.
- Analyse whole grain quality on a range of genotypes grown in a range of environments; including protein concentrate and genistein levels and basic feed performance data for fish (trout)

Given the results from the first year of testing we are particularly excited about the potential of this species although there seems to be a lot of work to do if a low alkaloid variety is required. If alkaloids are not a problem or can be removed in the production of concentrates then a variety could be released within 3 –4 years. As yet we don’t know enough about its adaptation to give a precise idea of production but an estimated potential of 40,000 tonnes I’m sure would be achievable.

Conclusion

Production of high protein diets using any of the four lupin species is possible and WA has the breeding programs to develop the varieties and the farming systems to accommodate production of all four species.

Critical control points in processing

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Introduction

To date lupin breeders have concentrated on maximising yield and disease resistance with little attention to the quality or properties of the proteins present. Our current study has concentrated on the comparison of the proteins present in various lupin cultivars rather than optimising the extraction conditions. However, this has made us aware of the large number of factors to be considered in developing processing processes. The work to date has demonstrated that whilst the vast amount of work done on soy processing can give some guidance, lupins are not soy and lupin proteins are markedly different from soy proteins.

A schematic representation of the critical control points in the preparation of the protein concentrates and isolates is shown in Figure 1.

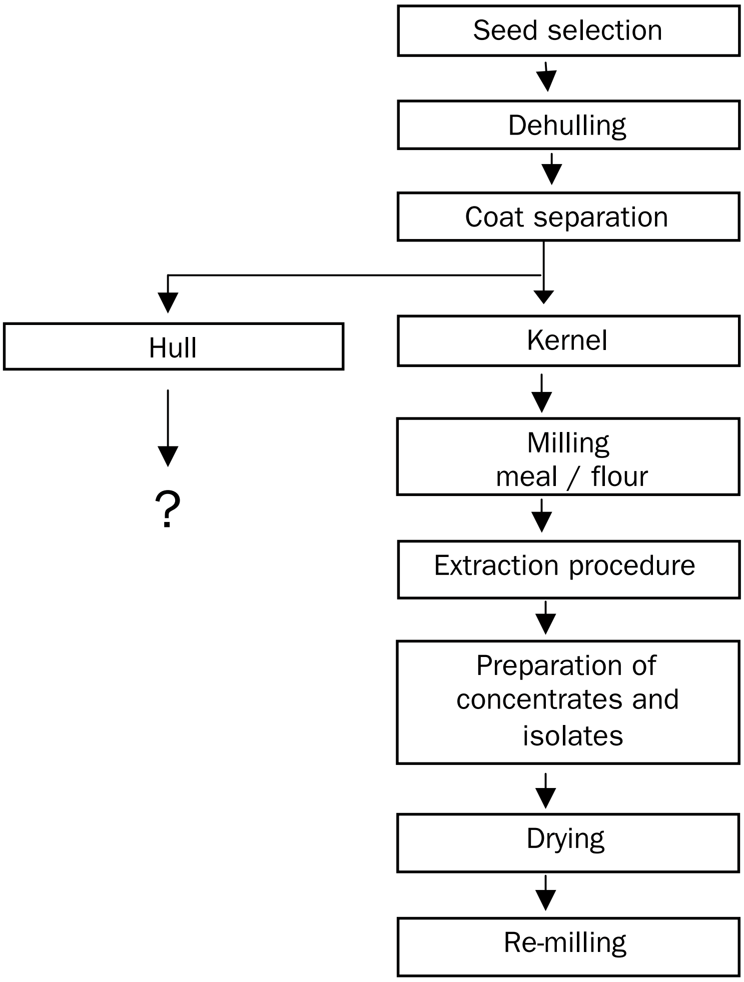

Figure 1. A schematic representation of the critical control points in the preparation of the protein concentrates and isolates.

The parameters used in this study to compare the proteins of the different cultivars include:

- The dry weights of the various fractions.
- The nitrogen content of the various fractions
- The amino acid content and profiles of the various fractions.
- The electrophoretic behaviour, both agarose and PAGE, of the various fractions.
- The titration profiles of the various meals.
- The functional properties of the various fractions.

Critical Control Points

1. Seed Selection

To date we have examined 21 cultivars of lupins from the three domesticated species available (18 Angustofolius 2 Luteus and 1 Albus). For comparison a soy and field pea (Alma) have also been examined. There are quite marked differences between the legume types in terms of all the parameters measured. Between the lupin species, Albus is quite similar to Angustifolius in terms of yield and amino acid composition. Whereas the 2 Luteus cultivars were very similar to each other but are markedly different to Angustofolius in terms of all the parameters measured. Luteus yielded more total protein with a better amino acid profile particularly with regard to the sulphur amino acid, cysteine. Based on the amino acid profiles the Angustifolius cultivars essentially fell into two groups one of which the profiles were similar to the old white lupins, eg. Uniwhite, and those that had profiles similar to Gungurru.

2. Dehulling

Some groups advocate soaking or steaming prior to dehulling. They claim it makes it easier and is cost effective in terms of the power saved. However, we do not have any direct evidence to support this. We have used dry mechanical dehulling throughout our study.

3. Milling

There are those who advocate wet milling because it is easier and does not cause heating of the meal. However, the only equipment we had available was considered unsuitable since it resulted in excessive foaming of the suspension and an emulsion, presumably composed of oil, protein and water formed which hindered subsequent extraction of the proteins. Basically there are two types of dry mill, a hammer mill or a shearing mill. Most commercial mills tend to be hammer mills which generally produce a flour whilst the shearing type mill used in this study (Newport) produced a considerably coarser meal. As a general rule the finer the flour/ meal the more rapid the extraction and the greater the yield. However a fine flour can be difficult to process, as it is more difficult to separate the soluble material from the insoluble and may limit options in the further processing.

4. Protein extraction

If the meal has a high oil content (>10%) it may be necessary to solvent (hexane) extract the oil prior to aqueous extraction of the proteins. Solvent extraction is not necessary for most of the lupin varieties used in this study but would probably required for species such as *L. mutabilis*.

When using aqueous extraction there are a number of factors to be considered. These include:

- The ratio of water to meal.
- pH to be used.
- Ionic strength.
- Temperate.
- Time.

Routinely we have used a ratio of 10:1 water to meal with a pH of 9 for 90 minutes at room temperature. The extraction time was based on the titration behaviour of the suspension. These may well not be the optimium conditions, which are currently being examined. Also the possibility of using enzymic digestion is under investigation.

5. Separation of soluble and insoluble materials

For the separation of soluble and insoluble materials we considered 3 choices:

- Filtration.
- Centrifugation.
- Settling.

Routinely we used centrifugation (5,000g, 20 minutes). However the precipitate was loose and occupied approximately one third of the volume. So the precipitate was washed with 5 volumes of water, centrifuged and the two superannuants combined.

The fibre rich precipitate was then dried (precipitate 1). If a reasonable coarse meal is used filtration can be used in place of centrifugation. The supernatant contains the soluble protein but the concentration is low (<2%) which makes it unsuitable to spray dry and expensive and time consuming to freeze dry or oven dry. So we have used the widely used procedure of adjusting the pH to 4.5 to precipitate the majority of the protein present. The mixture is then centrifuged (5.000g,15minutes). The precipitate was then dried. The supernatant contains a low concentration of protein(<1%) which is potentially value. The protein can be recovered ultrafiltration if the equipment is available. We have used trichloroacetic acid (5%) precipitation followed by centrifugation the recover this fraction.

6. Protein concentrate drying

The method we have routinely used, freeze drying, would be prohibitively expensive for the commercial scale production of product for aquaculture feeds. Laboratory evaluation of oven (air) drying results in the protein fractions having unattractive brown appearance which would limit their use in human foods. However this appearance issue is unlikely to be problem in aquaculture as the amino acid profiles of various fraction are identical for the freeze dried and oven dried samples. However, the heating process is likely to involve some degree of Maillard type reactions which will ultimately limit the nutritional value of the protein. However, the extent of this aspect of protein concentrate drying, on product quality, has yet to be further studied.

Economic considerations for Lupin Protein Concentrate production

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Introduction

Since their commercial incorporation in Australian farming systems, lupins have become accepted as a feed supplement for ruminants. Their ease of handling, storage and feeding, along with their nutritional characteristics, has made them useful as a sheep feed and a key ingredient in cattle, poultry and pig rations, and most recently a nutritional ingredient in aquaculture feeds (Glencross 2002). Lupins contain low levels of anti-nutrients (Petterson and Mackintosh, 1994; Petterson *et al*., 1997) and unlike whole soybeans and some other grain legumes, do not require heat treatment to destroy the lectins and protease inhibitors that reduce protein digestion and availability.

Although lupins have possible human consumption end-uses, lupins are primarily sold as a feed grain. If lupins remain as a feed grain then several key R&D questions arise such as:

(i) what comparative advantage does lupins offer that will either protect its market share or stimulate a price premium for lupins in various feed markets?

(ii) which of those advantages can be further enhanced by R&D?

(iii) who is best-placed to undertake the required R&D?

(iv) which of those advantages will be difficult for competitors to duplicate or overcome?

For the particular case of lupins in aquaculture feeds, evidence is emerging that some lupin species, particularly yellow lupins (*Lupinus luteus*), have characteristics that establish for that specie a comparative advantage. Identifying and understanding the nature and size of that advantage has the potential to translate to an increased price premium for *L. luteus* production.

Current profitability of lupins

Various analyses in the 1990s supported the view that lupins have a profitable place in farming systems in some regions (Kingwell, 1994; Pannell, 1995; Schilizzi and Kingwell, 1999). The analyses identified that the rotational benefits of lupins were a major component of their profitable inclusion in farm plans. The degree to which farmers grew lupins was a practical verification of farmers' perceptions of the value of lupins in farming systems.

Most lupins were and continue to be grown on sandy soils in rotation with wheat. For much of the 1990s on these soils, particularly in the northern and eastern wheatbelt, rotation options that included lupins were generally more profitable than those that excluded lupins. Further, in the mid-1990s wheat prices were at an historical high while wool and sheep prices remained mostly depressed. These conditions favoured retention of lupins rather than traditional legume pastures. Because pasture phases typically last a few years, in contrast to the single year of the lupin phase, rotations that included wheat and lupins offered a greater frequency of wheat than rotations with pasture and wheat. Hence, the wheat:lupin rotation offered a greater opportunity to capitalise on relatively high wheat prices.

However, towards the end of the 1990s and in recent years lupin production has been challenged by disease outbreaks, poor seasons, herbicide resistant weeds, new phase pastures and much higher prices for wool and sheep. Current analyses of narrow-leaf lupin (*Lupinus angustifolius*) in low rainfall farming systems show they remain as a profitable option on good sandplain soil, but often on a diminished area. By contrast analyses of yellow lupins (*Lupinus luteus*) conclude that at current relative yields, costs and prices, the planting of yellow lupins is not part of profitable farm plans. Even farms with Wodjil soils that suit yellow lupins, have other land use options on these soils that are more profitable than yellow lupins. So there is an economic challenge for those serving lupin growers to improve the relative prosperity of lupin production.

Improving returns to farmers

In species like *L. angustifolius* and *L. luteus* that have not been subject to many decades of intensive breeding effort, often large improvements are possible from breeding and agronomic management. Yield improvement, yield stability, pest and disease resistance, herbicide tolerance and grain quality enhancement are all feasible improvements. The investment dilemma is knowing which of these traits, or which combinations, are most limiting farmers' adoption of the species.

Boosting the profitability of growing lupins by altering its agronomic traits has been the traditional and legitimate on-farm focus of delivering value to farmers. However, another way of delivering value to farmers is through off-farm processing or industrial organisation, including supply chain alliances, that enable some of the off-farm value-adding profits to be shared by farmers. It is mostly through this avenue that farmers may potentially profit from *L. luteus* production, as outlined in the following sub-sections.

Sources of market value for lupins

As a feed grain lupins are sold between farms or delivered to marketers for on-selling in major national and international feed grain markets. As a feed grain the valuation of lupins depends on its nutritional and anti-nutritional characteristics, the level of demand for those characteristics, the cost of delivering the grain and the availability of competing and complementary feed stuffs.

In general, a key component of the value of many feed grains like lupins is their protein content. Market analysis of prices paid for feeds of different protein content reveals that the marginal value of protein content in feeds is an increasing function of protein content (see Figure 1). In other words, low protein feeds attract small premiums for any increases in their protein content (e.g. A$6 per % protein improvement) while high protein feeds receive large premiums for their further improvement in protein content (e.g. A$22 per % protein improvement).

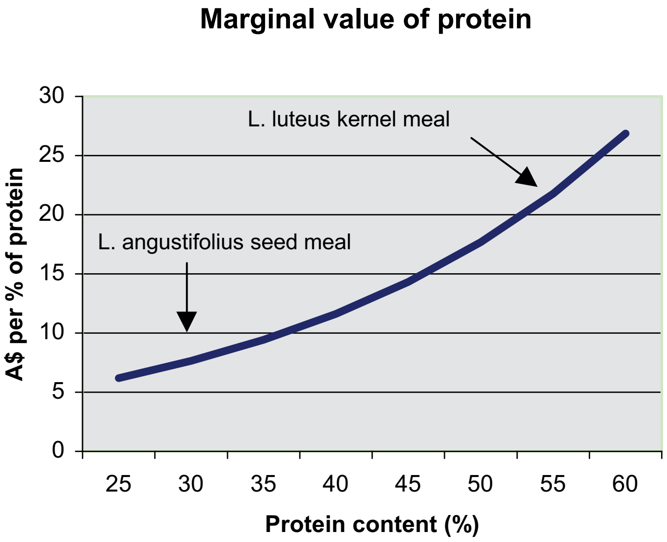

Figure 1. Marginal value of protein content of feeds.

Source: A best-fit curve to data on 14 different feeds; $R^2 = 0.90$

There are various reasons for the increasing marginal value of protein content. For example, there are fewer sources of and competitors for the supply of high protein feeds, so prices are bid up. Another explanation is that high protein feeds are often produced through a value-added process (e.g. de-hulling) whose costs are imbedded in the higher price paid for the high protein feed. The increasing marginal value of protein poses some interesting R&D questions.

For example, is it technically feasible to boost the protein content of lupins and receive increasing price rewards without having off-setting reductions in yield or production input requirements? Also, is it preferable to market the nutritional components of lupin seed rather than the whole seed? In short, are the profits from isolating and selling nutrient components greater than the profits from traditional grain marketing?

This latter issue can be subject to a preliminary analysis by comparing the nutritional components of *L. angustifolius* and *L. luteus*, their associated isolation costs and prices received. Relevant data are presented in Table 1. The data show firstly that the major sources of value in *L. luteus* are its protein components whereas in *L. angustifolius* fibre and protein components are of main importance.

The revenue and cost data reveal that for *L. luteus*, there could be profitable opportunities for component isolation. The same cannot easily be said for *L. angustifolius*. The data also show that drying costs are a major component of the cost structure of component isolation. Accordingly, any innovation that lessens the cost of drying will greatly improve the profit margin from component isolation. Also in *L. luteus* a reduction in seed coat thickness that reduces the hull proportion by 3 percent (33% down to 30%) increases the overall revenue per tonne of derived fractions by $20. Hence, increasing the profit margin, for example by altering the nutritional mix or reducing processing costs, increases the capacity to pay growers more for their lupins.

Table 1. Costs, returns and margins associated with nutritional component isolation in *L. angustifolius* and *L. luteus.*

Isolation Margins – *L. luteus*

Fraction	Amount in whole grain	Protein content	Protein share in whole grain	Price of fraction	Value of Component
Hulls	30%	10%	3%	$150	$45
Fibre	19%	5%	1%	$1,000	$190
LPC	32%	65%	21%	$1,000	$318
Albumin	4%	90%	4%	$2,500	$100
LPI	13%	90%	11%	$2,000	$254
	98%		40%	Revenue per tonne of isolates	*$907*
				Variable costs per tonne of isolates	
				Dehulling	$20
				Milling	$15
				Sieving and Extraction	$50
				Drying LPC	$32
				Drying LPI & Fibre	$250
				Repairs&Maintainence	$5
					$372
				Profit margin (15% of revenue)	$136
				Overheads (10% of revenue)	$91
				Margin for seed payment	**$399**

Table 1. Cont'd

Isolation Margins – *L. angustifolius*

Fraction	Amount in whole grain	Protein content	Protein share in whole grain	Price of fraction	Value of Component
Hulls	28%	8%	5%	$150	$42
Fibre	28%	3%	1%	$1,000	$280
LPC	27%	55%	14%	$800	$216
Albumin	4%	90%	4%	$2,500	$100
LPI	11%	85%	9%	$1,800	$194
				Revenue per tonne of isolates	
	98%		32%		$832
				Variable costs per tonne of isolates	
				Dehulling	$20
				Milling	$15
				Sieving and Extraction	$50
				Drying LPC	$27
				Drying LPI & Fibre	$300
				Repairs&Maintainence	$5
				Profit margin (15% of revenue)	$125
				Overheads (10% of revenue)	$83
				Margin for seed payment	***$291***

It appears from the data in table 1 that nutrient isolation would allow a larger price premium to be paid for *L. luteus* than is currently the case. This is a finding also reported by Glencross (2002). He reports the effects on rainbow trout (*Oncorhynchus mykiss*) of feeding kernel meals of three species of lupin (*Lupinus albus*, *L. angustifolius* and *L. luteus*), compared against a reference ingredient of solvent extracted soybean meal. He concludes that, based on digestible protein value, *L. luteus* kernel meals should command at least a 26% premium compared with a 21% premium when determined on crude protein content of the kernel meal. He notes that *L. luteus* seed usually commands only an 18% premium.

Implications for Lupin Isolate R&D

Although not shown in Table 1, there are different methods of isolating nutritional components. Dehulling seed is the simplest nutritional partition. However, there are several more advanced techniques that allow much more complete separation of nutritional isolates. Comparison of different methods of nutritional component isolation often reveals potentially greater returns from more advanced methods.

An interesting R&D issue is establishing which isolation techniques for *L. luteus* potentially generate the greatest profit and which components of the isolation process are most amenable to cost reduction and/or improvements in their technical efficiency. Data in Table 1 point to a need to reduce drying costs. Low-cost drying techniques would significantly boost profits from isolate production.

Implications for Lupin Growers

The creation of a successful commercial venture of lupin isolate production is predicated on attracting sufficient supplies of *L. luteus*. The supplies can be generated in various ways. Some of the options are:

(i) raise the price offered to growers sufficient to make *L. luteus* production attractive. This is the most simple option.

(ii) offer long term production contracts to lupin growers that include price conditions sufficient to make *L. luteus* production attractive.

(iii) encourage growers to form either an investor-owned firm or a new generation co-operative based on shares of delivery rights of *L. luteus*. The growers as shareholders can then directly benefit from the production and processing of *L. luteus*.

(iv) manufacturers of lupin isolates purchase or lease tracts of lands suitable for *L. luteus* production and employ specialist crop managers to supply *L. luteus.*

What might be the next step?

A pre-condition of commercial involvement by farmers or others in isolate production will be further investigation of processing efficiencies and commercial-scale tests based on actual commercial scale equipment. A sound business case for investment would need to draw on the findings of such R&D and demonstration activity.

References

Glencross, B. (2002) Feeding plant protein meals and oils to fish : A review of the nutritional and biological value of legume and oilseed resources in aquaculture feeds, Appendix 1 in Final Report to the Grains Research and Development Corporation, Pilot assessment of the potential for canola meal and oil use in aquaculture feeds. (Available as Glencross, B. 2003, *Pilot assessment of the potential for canola meal and oil use in aquaculture feeds*, Fisheries Research Contract Report No. 5, Department of Fisheries, Western Australia, 132p.

Kingwell, R. (1994) Effects of tactical responses and risk aversion on farm wheat supply. Review of Marketing and Agricultural Economics 62:29-42.

Pannell, D.J. (1995) Economic aspects of legume management and legume research in dryland farming systems of southern Australia. Agricultural Systems 49:217-236.

Petterson, D.S. and Mackintosh, J.B. (1994) *The chemical composition and nutritive value of Australian grain legumes*, Grains Research and Development Corporation, pp. 68.

Petterson, D.S., Sipsas, S. and Mackintosh, J.B. (1997) *The chemical composition and nutritive value of Australian pulses 2nd Edition*, Grains Research and Development Corporation, pp. 64.

Schilizzi S. and Kingwell R. (1999). Effects of climatic and price uncertainty on the value of legume crops in a Mediterranean-type environment. Agricultural Systems 60: 55-59.

International development and evaluation of grain products for fish

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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.

References

Anderson, J.S., Lall, S.P., Anderson, D.M., Chandrasoma, J., 1992. Apparent and true availability of amino acids from common feed ingredients for Atlantic salmon (*Salmo salar*) reared in sea water. Aquaculture 108, 111-124.

Anderson, J.S., Lall, S.P., Anderson, D.M., McNiven, M.A., 1993. Evaluation of protein quality in fish meals by chemical and biological assays. Aquaculture 115, 305-325.

Bach-Knudsen, K.E., 1997. Carbohydrate and lignin contents of plant material used in animal feeding. Anim. Feed Sci. Technol. 67, 319-338.

Einen, O., Holmefjord, I., Åsgård, T., Talbot, C., 1995. Auditing nutrient discharges from fish farms: theoretical and practical considerations. Aquacult. Res. 26, 701-713.

Francic, G., Makkar, H.P.S., Becker, K., 2001. Antinutritional factors in plant-derived alternate fish ingredients and their effects in fish. Aquaculture 199, 197-227.

Lusas, E.W., Riaz, M.N., 1995. Soy protein products: Processing and use. J. Nutr. 125, 573S-580S.

Refstie, S., 2000. Evaluating soybean meal as a fish meal substitute in feeds for Atlantic salmon and rainbow trout. Doctor Scientiarum Theses 2000: 12, 196 pp.

Refstie, S., Storebakken, T., 2001. Vegetable protein sources for carnivorous fish: potential and challenges. Recent Advances in Animal Nutrition in Australia 13, 195-203.

Refstie, S., Tiekstra, H.A.J., 2002. Potato protein concentrate with low content of solanidine glycoalkaloids in diets for Atlantic salmon (*Salmo salar*). Aquaculture 216, 283-298.

Refstie, S., Korsøen Ø.J., Storebakken, T., Baeverfjord, G., Roem, A.J., 2000. Differing nutritional responses to dietary defatted soybean meal in rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*). Aquaculture 190, 49-63.

Storebakken, T., Shearer, K.D., Baeverfjord, G., Nielsen, B.G., Åsgård, T., Scott, T., De Laporte, A., 2000a. Digestibility of macronutrients, energy and amino acids, absorption of elements and absence of intestinal enteritis in Atlantic salmon, *Salmo salar*, fed diets with wheat gluten. Aquaculture 184, 115-132.

Storebakken, T., Refstie, S., Ruyter, B., 2000b. Soy products as fat and protein sources in fish feeds for intensive aquaculture. I: Drackley, J.K. (Red.), Soy in Animal Nutrition. Fed. Anim. Sci. Soc., Savoy, IL, USA, s. 127-170.

Evaluating the use of lupin products in diets for Rainbow trout

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Introduction

To progress the development of grain protein concentrates and isolates 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 high capacity to conducted 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.

New grain varieties and products

As a precursor to the current grain product development project, since 2000 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. Among this work has been the evaluation of new grain varieties when fed to rainbow trout (among other fish species). One of the "shining lights" through this work has been the protein 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).

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.

In addition to the work on new lupin varieties the focus has moved to evaluating the nutritional value of a range of "First-Generation" protein concentrates and isolates, produced under laboratory conditions, from sweet lupin kernel meal and yellow lupin kernel meal. The composition of these products varies depending on grain source and processing method used (Table 1). Considerable flexibility exists to manipulate the composition of these ingredients based on micro-management of particular processes involved in the operation.

Table 1. Composition of ingredients evaluated. Details are on a dry matter basis (g/kg DM) unless otherwise specified.

Ingredients	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
Ash	44	33	37	31	41	30	69	79	47	70
Organic Matter	956	967	963	969	959	970	931	921	953	930
Carbohydrates	321	499	103	186	31	35	365	277	47	80
Phosphorus	6	4	6	5	9	5	8	9	9	9
Energy (MJ/kg DM)	20.9	20.4	22.6	22.2	22.2	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. NFE: Nitrogen-Free Extract (approximates carbohydrate content)

Digestible value

The determination of the digestible value of the "first-generation" grain protein products was undertaken using the diet-substitution method (Aksnes et al., 1998). 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. Notably both methods have their potential flaws and strengths. In this study both methods were employed to cater for both "schools-of-thought".

High digestible value of protein and energy for all protein meals and concentrates was observed (Table 2). 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 digestible value was evident from the concentrates, inclusion issues remained to be resolved.

Table 2. Apparent digestibility coefficients of protein concentrate and isolate products produced from sweet and yellow lupin varieties when assessed using either of the two faecal collection methods. Reference and competitor soy products are also included.

Ingredients	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.

Palatability

Irrespective of how good an ingredient may be nutritionally (digestible nutrient value), 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. 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.

Presently this experiment is still in progress, with only preliminary data presented (Table 3).

Table 2. Growth 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: Control 1; C2: 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 protein (amino acid) value between a transgenic and non-transgenic lupin variety when fed to a fish (Glencross et al., 2003c).

References

Aksnes, A., Hjertnes, T., Opstvedt, J. (1996). Comparison of two assay methods for determination of nutrient and energy digestibility in fish. Aquaculture 140, 343-359.

Glencross, B.D. Curnow, J.G., Hawkins, W.E. and Felsing, M. (2002). Preliminary evaluation of yellow lupin (*Lupinus luteus*) kernel meal as an alternative protein resource in the diet of sea-cage reared rainbow trout, *Oncorhynchus mykiss*. Journal of the World Aquaculture Society 33, 287-296.

Glencross, B.D., Boujard, T.B. and Kaushik, S.J. (2003a). Evaluation of the influence of oligosaccharides on the nutritional value of lupin meals when fed to rainbow trout, *Oncorhynchus mykiss*. Aquaculture 219, 703-713.

Glencross, B.D., Curnow, J.G. and Hawkins, W.E. (2003b). Evaluation of the variability in chemical composition and digestibility of different lupin (*Lupinus angustifolius*) kernel meals when fed to rainbow trout (*Oncorhynchus mykiss*). Animal Feed Science and Technology 107, 117-128.

Glencross, B.D., Curnow, J.G., Hawkins, W.E., Kissil, G.Wm. and Petterson, D.S. (2003c). Evaluation of the feed value of a transgenic strain of the narrow-leaf lupin (*Lupinus angustifolius*) in the diet of the marine fish *Pagrus auratus*. Aquaculture Nutrition. IN PRESS.

Glencross, B.D., and Hawkins, W.E. (2003). A comparison of the digestibility of several lupin (*Lupinus* spp.) kernel meal varieties when fed to either rainbow trout (*Oncorhynchus mykiss*) or red seabream (*Pagrus auratus*). Aquaculture Nutrition. ACCEPTED.

Evaluating the use of lupin products in diets for Atlantic salmon

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Introduction

Research into the utilisation of grain products by Atlantic salmon, rainbow trout, short-finned eel and southern rock lobster (Engin & Carter, 2002; Farhangi & Carter, 2001); (Carter, 1998) has progressed at TAFI through support from FRDC, GRDC and TAFI. Potential ingredients that have been tested include commercially available soybean, lupin, field pea and canola products as well as specially prepared protein concentrates (Carter & Hauler, 2000). Approaches to evaluation have included assessment of digestibility and growth (Carter, et al., 2002), metabolism (Ward et al. in prep) and resistance to disease challenge (Bransden, et al., 2001; Carter, et al., 2003). Recent developments have involved collaboration with WA Fisheries to test new lupin products. This report focuses on feed trials with Atlantic salmon.

Ingredients and digestibility

Digestibility measurements have used a Guelph-system to collect settled faeces. The standard approach of adding 30% of the test ingredient to a feed has been followed and digestibility values for protein, energy and other nutrients calculated (Table 1).

Table 1. Summary of ingredients evaluated with aquaculture species and evaluation: D, digestibility; G, growth; R, Disease resistance. (ADC, apparent digestibility coefficient)

Ingredients	AKM	APC	API	SBM	SPC	SPI	CAW	CAM	CG	WG
Atlantic salmon	D,G,R	D,G	D	D	D,G	D	D	D,G	D	D
Rainbow trout	D,G			D,G						
Short-finned eel	D			D				D	D	
Southern rock lobster	D$^{L.albus}$			D						D
Evaluation:										
Atlantic salmon										
ADC protein (%)	96.7			100	97.1		96.1	68.0	88.1	99.0
ADC energy (%)	63.1			89.4	79.4		80.0	42.3	83.5	90.0
ADC amino acids	✓			✓	✓		✓	✓	✓	✓
ADC protein (%)[1]	91.0	96.1	99.8	87.3	89.8	98.7				
ADC energy (%)[1]	65.3	88.4	99.0	80.8	85.5	99.8				
ADC phosphorus[1]	41.9	30.9	39.0	31.3	6.4	37.3				

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; CAW: Whole canola meal; CAM, Dehulled solvent extracted canola meal; CG, Corn gluten; WG, Wheat gluten.

*From Carter et al (2002) except for [1](Glencross *et al. in prep*).

Digestibility showed a range of values for the different grain products but also some variation between values for similar products. Of the high protein products protein digestibility of the soy and lupin isolates and of wheat gluten was very high and nearly 100% where as it was lower for corn gluten.

Mineral digestibility

There is relatively little data available on mineral and trace element digestibility from grains. It is important to understand micro-nutrient availability especially in view of recent reports of mineral deficiencies, phosphorus being one example. Mineral and trace-element digestibility was compared between a soy protein concentrate and dehulled *L. albus* and *L. angustifolius* products. There were few significant differences in mineral or trace element digestibility. Diets containing 35% of the two lupin products out performed the fish meal control and had significantly higher digestibility for phosphorus, sulphur, copper and zinc.

Digestive tract processing

Current research is assessing the effects of lupin carbohydrates on gastric and digestive tract evacuation rates. Gastric evacuation will be measured using X-radiography to follow X-ray opaque glass beads, calibrations will be made by comparison with digestive tract contents from serial slaughter. Rates will be compared between base diets, containing fish meal or fish meal plus lupin concentrate (APC), to which different carbohydrate fractions are added. This research will develop the approach for further assessment of grain products in order to understand their effects on digestive processing.

Phosphorus dynamics and phytase use

Plant meals contain considerable amounts of phytate that reduces the amount of available phosphorus and can also impact on the availability of other minerals and nutrients such as amino acids. Phytase has been used with varying degrees of success with aquafeeds. Reasons for the variability include the use of different feeding regimes since fish may increase feed intake and consequently growth. The relationship between available phosphorus and the phosphorus requirement will also influence whether phytase has an effect. In salmon we showed that in low phosphorus diets phytase had no effect independent of phytate. Phytase significantly increased whole-body phosphorus, bone phosphorus and bone ash in diets containing 35% canola meal.

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References

Bransden, M.P., Carter, C.G., Nowak, B.F., 2001. Effects of dietary protein source on growth, immune function, blood chemistry and disease resistance of Atlantic salmon (*Salmo salar* L.) parr. Animal Science 73, 105-113.

Carter, C.G., 1998. Fish Meal Replacement in Aquaculture Feeds for Atlantic Salmon. Fisheries Research and Development Corporation, Deakin, Australia.

Carter, C.G., Hauler, R.C., Foster, C., 2002. Aquaculture Feed Development for Atlantic Salmon (*Salmo salar*). Fisheries Research and Development Corporation, Deakin, Australia, 188 pp.

Carter, C.G., Bransden, M., Lewis, T., Nichols, P., 2003. Potential of thraustochytrids to partially replace fish oil in Atlantic salmon feeds. Mar. Biotechnol. *in press*.

Engin, K., Carter, C.G., 2002. Ingredient apparent digestibility coefficients for the Australian short-finned eel (*Anguilla australia australis*, Richardson). Animal Science 75, 401-413.

Farhangi, M., Carter, C.G., 2001. Growth, physiological and immunological responses of rainbow trout (*Oncorhynchus mykiss*) to different dietary inclusion levels of dehulled lupin (*Lupinus angustifolius*). Aquacult. Res. 32 (Suppl. 1), 329-340.

Factors affecting the use of lupins in prawn feeds

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Introduction

CSIRO Marine Research has recently completed a study, supported by the Grains Research and Development Corporation (GRDC), to improve the efficacy of lupins as fishmeal replacements in aquaculture diets for prawns (GRDC Project Number CSM1). This project extended the work carried out in the Fisheries Research and Development Corporation (FRDC) Fishmeal Replacement Sub-program. In the Fishmeal Replacement Sub-program, the Australian sweet lupin, *Lupinus angustifolius* cv Gungurru*,* was evaluated as a whole lupin meal, as a dehulled lupin meal (DLM) and, in a few experiments, as a lupin protein concentrate (LPC) that was prepared by air-classification. These studies showed that the dehulling significantly improved the apparent digestibility of the dry matter, protein and energy of the meal (Smith, 1998). In addition, it was found that when DLM was included in a practical black tiger prawn (*Penaeus monodon*) diet at 250 g/kg, replacing fishmeal and flour, there was no significant difference in the growth rate, survival or feed conversion ratio (FCR) of prawns. Other studies were carried out at Curtin University (WA) by Sudaryono and co-workers who investigated the usefulness of DLM as a replacement of fishmeal in feeds for the black tiger prawn (Sudaryono *et al.,* 1999a, 1999b). They examined the performance of prawns fed diets containing either ground whole or dehulled lupin of both *L. albus* and *L. angustifolius* types and compared these performances to those fed a soybean meal-based feed. Their findings were: (a) dehulling seed or concentrating lupin protein did not improve the nutritive value of lupin meal; (b) *L. angustifolius* meal generally performed better than *L. albus* meal; (c) *L. angustifolius* meal was comparable to soybean meal; and (d) *L. angustifolius* meal appeared to provide the feed with greater attractability for prawns than *L. albus* meal.

In our GRDC study, we investigated the maximum practical inclusion level of *L. angustifolius* cv. Gungurru DLM in feeds for *P. monodon* and studied its effect on feed pellet stability and the attractiveness of the feed. In addition we investigated the effects of the fatty acid, amino acid and carbohydrate composition of the DLM on its nutritional value for black tiger prawns.

Effect on Pellet Stability

Increasing the amount of DLM in the feed, at the expense of fishmeal and wheat flour, and when the protein content was maintained at a constant level, resulted in a progressive decrease in the amount of dry matter retained in the pellets after 4 h immersion in water. This effect of legume meals on feed pellet water stability has also been reported with *L. albus* meal (Sudaryono *et al.*, 1999c) and with soybean meal (Lim and Dominy, 1990). Increasing the amount of water added to the dry ingredients during processing improved the pellet stability of the feeds with high lupin meal content, but the extruded strands were too sticky and very difficult to separate. Under commercial pelleting conditions, it is likely that the effects of DLM on pellet stability could be managed effectively to provide an acceptable level of pellet stability.

Effect of lupin inclusion level in feed

Using DLM at increasing levels in a prawn feed, at the expense of fishmeal and flour in feeds containing 400 g/kg crude protein, resulted in a progressive decrease in the growth rate of prawns. This became quite marked at inclusion levels of 300 g/kg and above. However, this decrease does not appear to be due to low attractability or palatability as the prawns' feed intake increased progressively with increasing DLM content of the feed. This result clearly shows that the nutritional value of DLM is much lower than that of high quality fishmeal. The key research objective was to identify the component or characteristic of the DLM that was responsible for its low nutritional value.

Fatty acid composition

The DLM used in this study contained 84 g/kg of lipid and had a fatty acid profile that was similar to other vegetable oils. In particular, it lacked the nutritionally important, long chain omega-3 polyunsaturated fatty acids, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are characteristic components of marine oils. Fishmeal used in aquaculture feeds generally contains between 50 and 100 g/kg of lipid (essentially fish oil). The replacement of some of the fishmeal in a practical prawn feed with DLM results in a decrease in the fish oil and an increase in the vegetable oil content of the feed. Changing the fatty acid composition of the feed potentially could have an adverse effect on the performance of prawns (Glencross *et al.*, 2002). This hypothesis was tested by defatting DLM and using this product to prepare feeds that contained the equivalent of either 200, 400 or 600 g/kg of normal DLM. To these feeds, supplemental oil in the form of either fish oil or lupin oil and in an amount equivalent to the oil extracted from the DLM was added so that all feeds had a total lipid content of 94 g/kg.

In a 6-week growth experiment with these feeds, the major observed effect was a decline in growth as the inclusion level of DLM was increased. However, there was no significant effect of the altered fatty acid composition on prawn growth. It should be noted that in all the feeds containing defatted DLM plus lupin oil, there was a significant amount of marine oil such that even at the highest inclusion level of DLM, the EPA + DHA content was 17% of the total fatty acid content, compared with 27% in the Basal diet. This result clearly indicates that the fatty acid composition of DLM will not adversely affect the performance of prawns fed practical diets when about half of the dietary lipid, from all sources, is of marine origin.

Amino acid composition

Prawn feeds containing 400 g/kg of crude protein (as fed) will typically contain at least 360 g/kg of digestible protein. Though prawns grow at a faster rate on feeds containing 400 to 450 g/kg of crude protein than on those with less protein, the essential amino acid content of such feeds appear to be well in excess of the prawn's requirements for tissue protein synthesis. Amino acids in excess of tissue protein synthesis requirements are catabolized and appear to be the preferred substrate for energy production. This probably explains the high performance of these feeds. However, the essential amino acid requirement of prawns has not yet been clearly defined.

If the 360 g/kg of digestible protein in prawn feed had an amino acid profile that closely matched the amino acid composition of whole prawns, the feed, on an 'as fed' basis would contain (g/kg): arginine, 20.9; lysine, 19.1; methionine + cystine, 13.0; and threonine, 13.0. Under these circumstances, the essential amino acid content of a lupin-based prawn feed would not becoming limiting until about 380 g/kg of DLM was included in a practical feed. At this inclusion level, methionine + cystine would appear to be the first limiting amino acid. However, the performance of diets containing DLM has been found to decrease at inclusions levels above 250 g/kg. This suggests that either the requirement for essential amino acids is greater than that provided by 360 g/kg of the 'ideal' protein, or some other factor in the DLM is responsible for the decrease in performance. An experiment to determine the benefit of adding supplementary methionine to diets with high DLM content produced inconclusive results, mainly because of the inability of preventing a significant leaching loss from the feed of the supplementary methionine.

Carbohydrate composition

Lupins contain very low levels of starch (<10 g/kg), with the carbohydrate component comprising mainly non-starch polysaccharide (NSP). The NSP consists of both soluble and insoluble material with free sugars and oligosaccharides making up the soluble fraction (van Barneveld, 1999).

Insoluble NSP

An experiment was carried out in which the wheat flour in a basal diet (360 g/kg digestible protein) was incrementally replaced with insoluble NSP (fibre) isolated from lupin kernels. Including lupin 'fibre' at up to 300 g/kg of feed (equivalent to the fibre contained in a diet solely made up of DLM), did not significantly affect the growth rate of prawns. The incremental increase in lupin fibre content led to a progressive decrease in the digestibility of dry matter, protein and energy in the feed, which the prawn compensated for by increasing voluntary feed intake. The digestibility of these diets was lower than would be expected from an equivalent inclusion of DLM (on a fibre content basis), suggesting that the isolation process had changed the physical characteristics of the fibre. Notwithstanding the change in expected digestibility, the result shows that lupin fibre, *per se*, does not have an adverse effect on the growth of prawns.

Soluble NSP and oligosaccharides

Oligosaccharides in lupin meals have been identified as reducing the digestibility and nutritional value of feeds containing them. A prawn growth experiment was carried out with diets containing a high inclusion of DLM (500 g/kg) in which the oligosaccharide content of the DLM was either extracted or left *in situ*. Oligosaccharides were extracted by steeping DLM with 80% ethanol, filtering off the ethanol and drying the extracted DLM at 40°C (Olsen, van Barneveld & Choct, in prep). The digestibility of dry matter or crude protein of the diet was not significantly affected by the extraction of the oligosaccharides and neither was the growth rate of the prawns affected. However, the inclusion of 500g/kg of DLM in the feed, at the expense of fishmeal and starch, did result in a significant reduction in the apparent digestibility of dry matter and a significant increase in the apparent digestibility of crude protein. Despite the adverse effect that the high inclusion of DLM had on apparent digestibility, the absence of any effect on prawn growth rate compared to the non-lupin containing control feed, indicate the prawn's capacity to regulate voluntary feed intake. Nonetheless, these were unexpected results, which might be due to the particular batch of DLM that was used in the experiment. This batch had not previously been used and may have contained a much lower level of the factor/s that are responsible for the low nutritional value of the DLM used in earlier experiments.

Conclusions

In this study into the factors that limit the utilisation of DLM in prawn feeds, we have confirmed the relatively high digestibility of crude protein in DLM but found that growth rate of prawns was adversely affected by DLM when used at dietary inclusion levels greater than 250 g/kg. We have established that the fatty acid composition of the DLM does not have a significant effect on prawn performance when the vegetable to marine lipid ratio is less than about 1:1. The low level of methionine or methionine + cystine in the lupin protein also does not appear to be limiting the nutritional value of the feed when the dietary lupin inclusion level is less than about 380 g/kg. However, this aspect has not been tested adequately. 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. 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 these feeds in that experiment 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 the batches used in previous experiments. Research to test how agronomic factors – lupin variety, soil fertility and locality grown, season etc – affect the nutritional value of lupins as a replacement of fish meal protein in prawn feeds is an urgent need.

References

Glencross, B.D., Smith, D.M., Thomas, M.R., Williams, K.C., 2002. Optimising the essential fatty acids in the diet for weight gain of the prawn, *Penaeus monodon*. *Aquaculture* 205, 85-99.

Lim, C., Dominy, W., 1990. Evaluation of soybean meal as a replacement for marine animal protein in diets for shrimp (*Penaeus vannamei*). Aquaculture 87, 53-63.

Smith, D.M. 1998. Fishmeal replacement in aquaculture feeds for prawns. Final Report to Fisheries Research and Development Corporation, (FRDC) Project 93/120-02. 168 pp.

Sudaryono, A., Tsvetnenko, E., Evans, L.H., 1999a. Replacement of soybean meal by lupin meal in practical diets for juvenile *Penaeus monodon*. J. World Aquaculture Soc. 30, 46-57.

Sudaryono, A., Tsvetnenko, E., Evans, L.H., 1999c. Evaluation of potential of lupin meal as an alternative to fish meal in juvenile *Penaeus monodon* diets. Aquacult. Nutr. 5, 277-285.

Sudaryono, A., Tsvetnenko, H., Hutabarat, J., Supriharyono, Evans, L.H., 1999b. Lupin ingredients in shrimp (*Penaeus monodon*) diets: influence of lupin species and types of meals. Aquaculture 171, 121-133.

van Barneveld, R.J. 1999. Understanding the nutritional chemistry of lupins (*Lupinus* spp.) seed to improve livestock production efficiency. Nutr. Res. Rev. 12, 203-230.

Appendix 1 – Feeding plant protein meals to fish : A review of the nutritional and biological value of key legume and oilseed meals in aquaculture feeds

i Summary of the generic value of plant protein resources to aquaculture species

- A range of plant protein and oil resources have been evaluated for use in aquaculture diets. There is a notable dominance of soybean meal and product information in the literature, which is also consistent with these products being the dominant plant protein commodities being used in this feed sector.
- In all aquaculture species for which a nutritional assessment has been made on the value of soybean products, they have generally been shown to be a well accepted and nutritionally useful ingredient. The extent of this value varies marginally between fish species.
- Processing of soybean meals has been shown to improve the overall value of these grain resources. Notably, the greatest protein value has been shown from protein concentrates and isolates.
- Limitations to the use of soybean meals have been suggested through anti-nutritional factors present in the meal such as protease inhibitors, saponins, oligosaccharides and cellulose/fibre content.
- Assessment has been made of several other plant protein meal commodities that are of relevance to Australia. Notable among these assessments are those of lupins, canola/rapeseed and field peas.
- The nutritional value of the protein content of lupins has consistently been shown to be equal, if not superior to that of soybean meals. The energy value of lupins tends to be slightly less than that of soybean, reflecting the overall lower protein content of most lupin meals.
- The nutritional value of the protein content of field pea meals has also consistently been shown to be equivalent to that of soybean meals. The energy value of field pea meal tends to be slightly less than that of soybean, reflecting the lower protein content of field pea meals.
- The nutritional value of the protein content of canola meals has also been shown to be consistent to that of soybean meals. The energy value of canola meals tends to be somewhat less than that of soybean, reflecting the lower protein content of these meals.
- The maximum inclusion levels of each of these ingredients varies between study reported, fish species and most likely ingredient source. Both soybean and lupin meals show the greatest acceptability, with the level of acceptability improving with concentration of protein through secondary processing.

A1.1 Introduction

The identification and development of alternatives to the use of fishmeal and fish oil in aquaculture diets remains a high priority for improving the sustainability of aquaculture. Modern intensive aquaculture is still perceived as a net fish user rather than producer (Naylor et al., 2000). This practice questions both the reliability of aquaculture as a food provider, and also the long-term sustainability of these industries. To improve resource security and reliability for aquaculture feeds, one option has been to increase the use of plant protein meals and oils as feed ingredients in diets for aquaculture species.

Both plant protein meals and oils have considerable potential to supply the required dietary nutrients for fish. These resources have generally been shown to provide promising levels of digestible and available nutrients and energy. Clearly the optimisation of the use of these resources in aquaculture diets depends on a detailed understanding of the chemical composition of these products and the consequences of feeding these product and their influence on each specific species being fed

Notably though, the use of plant protein resources in fish diets can also introduce a suite of problems. Not only does the use of high-levels of plant proteins increase the potential for inducing an essential amino acid limitation, most plant protein resources also contain a variety of anti-nutritional (biologically active) factors (ANF). The influence of these ANF on fish can be considerable and varied. In assessing the value and potential of a range of plant protein resources there has been considerable research on the use these resources in the diets of aquaculture species (Arnesen et al., 1989; Gomes et al., 1995; Booth et al., 2001). However, despite this, there still remains considerable need for more targeted research on identifying key attributes and limitations to the use of plant meals in aquaculture diets.

Soybean meal is one plant protein resource that has been widely used in aquaculture diet formulations, with considerable success (Arnesen et al., 1989; Kaushik et al., 1995; Refstie et al., 1998; Storebakken et al., 1998b; Vielma et al., 2000). However, in Australia there is limited production of soybeans, but substantial production of lupins, canola and field peas. Each of these grains has been shown to provide some value as a potential aquaculture feed ingredient (Gomes et al., 1995; Burel et al., 2000a; Booth et al., 2001).

While the replacement of fishmeal in aquaculture diets, with such alternatives as plant protein meals, has received much attention, alternatives to the use of fish oils in aquaculture diets have been comparatively neglected. In some respects, with the present trend towards using high-nutrient-dense diets in aquaculture, there is a more urgent need to identifying and adopt alternatives to the use of fish oils than there is for fish meals. Although some research on the inclusion of plant oils, such as canola or soybean oil in aquaculture diets has been undertaken, consolidation of the information on this issue remains a high priority for the aquaculture industry (Naylor et al., 2000).

This review examines the present state of knowledge of the use of plant protein and oil resources in diets for aquaculture species. First, an examination of the composition of the key plant protein meal and oil commoditees is presented, detailing the physical chemistry of each and the variations that occur between the different varieties and processing forms. The second part of the review examines some of the work published to date where some of these plant protein meal and oil resources have been fed as an ingredient in a compound feed to an aquaculture species.

A1.2 Nutrient composition of plant protein meals

Table A1.1 Proximate specifications of key plant protein meal resources and fishmeal (all values g/kg DM unless otherwise specified).

	Dry matter content	Crude protein	Crude Fat	Ash	Nitrogen-free extractives	Gross energy (MJ/kg DM)
Fish meal	925	703	79	216	4	21.8
Sweet Lupin kernel meal [a]	910	411	60	32	497	20.7
Yellow lupin kernel meal [b]	937	496	55	38	410	21.0
White lupin kernel meal [c]	922	455	137	36	405	23.1
Field pea meal	903	257	12	28	703	18.6
Expeller Canola meal	938	380	136	59	425	22.0
SE Canola meal	889	432	30	74	464	19.7
SE Soybean meal	890	503	12	88	397	19.2
Full-fat Soybean meal	909	416	196	53	336	23.4

[a] *L. angustifolius*, [b] *L. luteus*, [c] *L. albus*, SE: solvent-extracted. Data derived from Petterson et al. (1998) and Glencross (2001, unpublished).

A1.2.1 Protein and amino acids

The crude protein content of the plant protein resources considered in this review varies markedly between each resource (Table A1.1). Notably, the level of protein in soybean meals also varies according to whether the grain is hulled or dehulled, full-fat or extracted and even according to the method of oil extraction used (Tacon, 1991). A similar level of variability is also observed of canola meals between oil extraction method used.

Solvent extracted soybean meal has among the highest crude protein content of the plant protien meals though this is only marginally greater than that of Yellow lupin kernel meals. The protein content of the Field pea meal was among the lower of the potential plant protein meals considered for use in aquaculture diets.

Table A1.2 Amino acid composition (g amino acid / 16 g N) of key plant protein resources and fishmeal.

Amino acid	Soybean	Lupin [a]	Field Pea	Canola	Fishmeal
Arginine	5.42	11.62	10.05	3.10	6.14
Histidine	2.46	2.57	2.40	2.37	4.44
Isoleucine	4.51	3.91	3.86	4.51	4.50
Leucine	6.81	6.61	6.64	5.84	7.94
Lysine	5.66	4.66	6.87	4.43	8.20
Methionine	1.28	0.72	0.85	2.32	2.93
Phenylalanine	3.60	3.65	4.24	3.98	4.26
Threonine	3.56	3.54	3.44	5.58	4.90
Tryptophan	1.35	1.00	0.78	1.04	1.29
Valine	4.44	3.78	4.29	4.38	5.60

[a] *L. angustifolius*. Data derived from Tacon (1990); Petterson et al. (1997); van Barneveld (1999).

The amino acid composition of the plant protein resources also varies considerably among each of the resources (Table A1.2). In comparison to fishmeal, which is usually considered as the ideal amino acid source for fish, most plant protein meals are relatively limited in their lysine and methionine content. Of the plant protein resources considered in this review the lysine content was highest in field pea protein and lowest in canola protein. However, the relative level of methionine was highest in canola and lowest in lupin protein.

It should be noted that these relative levels can be somewhat misleading, in that they are representative only of the amino acid composition of the protein, not the complete plant protein meal, in that the absolute amount of each amino acid is not accounted for. Indeed, when examined on an absolute basis soybean meal has the highest levels of both lysine and methionine and field pea the lowest.

A1.2.2 Lipids

The amount of lipids in the plant protein resources considered also varies considerably among each of the resources (Table A1.2). Full-fat soybean meal has considerably greater levels of lipid than most other plant protein meals. Both White lupin kernel meal and Canola meal, when processed by expeller extraction, also have reasonably high lipid contents at about 13 to 14% on a dry matter basis. Both pea meal and the solvent extracted soybean meal had the lowest levels of residual fat in the meal.

Table A1.3 Fatty acid composition (% of total fatty acids) of the key plant protein resources and fish meal.

Fatty Acids (% of total)	Fishmeal	Lupin	Field pea	Canola	Soybean
C14:0	6.4	0.1	0.3	0.0	1.0
C16:0	22.8	11.0	12.5	5.5	7.8
C16:1n-7	6.9	0.1	0.0	0.5	1.5
C18:0	5.8	3.8	1.2	2.4	3.0
C18:1n-9	13.8	38.2	27.6	54.1	19.1
C18:2n-6	2.0	37.1	42.3	19.5	56.3
C18:3n-3	1.0	5.3	9.7	12.7	7.5
C20:0	0.4	0.9	0.7	0.6	0.6
C20:1n-9	3.3	0.3	0.5	1.4	1.6
C20:2n-6	0.3	0.4	0.0	0.1	0.2
C20:3n-9	0.3	0.2	0.0	0.0	0.0
C20:5n-3	12.7	0.0	0.0	0.0	0.0
C22:0	0.0	1.9	0.3	0.0	0.0
C22:1n-11	0.6	0.0	0.2	0.3	0.4
C22:6n-3	20.2	0.0	0.0	0.0	0.0
Total saturates	35.3	17.7	15.0	8.5	12.4
Total monounsaturates	24.5	38.6	28.3	56.4	22.5
Total polyunsaturates	36.5	43.0	52.0	32.3	64.0
n-3 (omega-3)	33.9	5.3	9.7	12.7	7.5
n-6 (omega-6)	2.3	37.5	42.3	19.6	56.6

All other fatty acids had levels less than 0.5% in all species presented. Data derived from Tacon (1990); Petterson et al. (1997); van Barneveld (1999) and Glencross (2001, unpublished).

The fatty acid composition of the lipid content of each of the meals also varies considerably among each of the resources (Table A1.3). As with the amino acids the fatty acids too are examined on proportional basis of the total fatty acids. Consideration needs to be given to not only the levels of the fatty acids, but also the total amount of lipid in each meal.

Notably each of the plant proteins has relatively low levels of saturated fatty acids in contrast to fish oil. The levels of monounsaturated and polyunsaturated fatty acids vary considerably. The highest levels of monounsaturates was found in the canola meals, with the highest levels of polyunsaturated fatty acids found in the soybean meals. Predominant of the polyunsaturated fatty acids of each of the meals was linoleic acid (18:2n-6), though appreciable levels of linolenic acid (18:3n-3) are also found in canola meals.

A1.2.3 Carbohydrates

Plant protein meals are typified by possessing considerably higher levels of carbohydrates than most other feed ingredients. Of those resources examined in this review the carbohydrate content is also quite different to that of many other feed grain resources in that the meals are charactersied by possessing high levels of both soluble and non-soluble non-starch polysaccharides (NSP). This group of carbohydrates forms primarily the structural polysaccharides of the seed, though some are considered as non-structural. Indeed, with the exception of the field peas, starch is essentially non-existent in each of these resources (Petterson, 2000).

The precise composition of the NSP differs between the plant protein resources. Typically, though the NSP levels of lupin kernel meals are about 400 g/kg DM, essentially double that of soybean meal (217 g/kg DM), field peas (180 g/kg DM) and canola meals (solvent-extracted; 190 g/kg DM) (van Barneveld, 1999). The hemicellulose content of the crude fibre was shown to be proportionally greater in lupin kernel meals than in other resources such as field peas, canola and soybeans in which the cellulose content comprised a greater proportion of the fibre (van Barneveld, 1999).

A further group of polysaccharides, the pectins are comprised primarily of β-(1,4)-galactan, which itself is comprised of sub-units of L-rhamnose, L-arabinose, D-galactose and galacturonic acid (Carre et al., 1985). The polysaccharide group of the lignins is prevalent in soybean meals, with considerably lower levels in the lupin kernel and field pea meals (van Barneveld, 1999).

A1.2.4 Anti-nutrients

Anti-nutrients, also referred to as biologically active substances, are essentially evolutionary developments by plants to enable some level of defense against being eaten. In this sense these anti-nutrients are essentially a variety of chemical defense mechanisms being employed by plants. However, the variety of anti-nutrients found in the different plant species, let alone their seeds, varies quite widely, both in diversity of anti-nutrient type and relative concentration (Table A1.4).

A1.2.4.1 Alkaloids

Alkaloids are generally bicyclic, tricyclic or tetracyclic derivatives of the molecule quinolizidine (Petterson, 2000). Although there is no reported data on the influence of alkaloids on fish, alkaloids are generally considered a feeding deterrent because of their bitter taste. While the alkaloids are found primarily in the legumacae family (peas and beans), high levels were traditionally found in lupins. Present levels of alkaloids in lupin varieties, such as *L. angustifolius* are usually less than 200 mg/kg. Wild-type varieties still found in their countries of origin may contain from 5,000 to 40,000 mg/kg of alkaloids (Harris and Jago, 1984).

Table A1.4 Nutrient levels in the key plant protein resources.

	Soybean [a]	Lupin [b]	Canola	Field pea
Alkaloids (mg/kg DM)	n.d.	200	n.d.	n.d.
Glucosinolates (μmol/kg DM)	n.d.	n.d.	9000	n.d.
Lectins (dilutions)	n.d.	n.d.	n.d.	4
Oligosaccharides (g/kg DM)	50	40	30	35
Phytate (g/kg DM)	15	5	40	5
Protease Inhibitors (g/kg DM)	3.1	0.2	n.d.	2.9
Saponins (mg/kg DM)	5000	573	n.d.	n.d.
Tannins-total (g/kg DM)	n.d.	n.d.	1.8	3.7

n.d. : no data identified. Data derived from Petterson et al. (1997), van Barneveld (1999), and Glencross (2001, unpublished). [a] Soybean meal data is that of dehulled and defatted meal. [b] Lupin data is based on the whole-seed characteristics of *L. angustifolius*.

A1.2.4.2 Glucosinolates

Glucosinolates in their own right have little biological activity. The actual anti-nutritional components are actually the breakdown products of glucosinolates, such as isothyocyanates, nitriles, thiocyanate anions and vinyloxazolidinethiones all have some goitrogenic activity. Effectively these compounds induce hypothyroidism in most vertebrate animals and lead to reduced levels of the thyroid hormones triiodothyronine (T_3) and thyroxine (T_4). The breakdown of glucosinolate to these products is achieved via the enzyme myrosinase which cleaves the glucosinolate to produce glucose and a variety of subsequent bioactive compounds. The modes of action of these bioactive compounds is closely involved with the synthesis of T_3 and T_4, notably thiocyanate anions compete with the active transport of iodine to the thyroid while vinyloxazolidinethiones blocks the coupling of sub-units of the precursors to T_4. The consequence of hypo-thyroidic response in fish is usually manifested by a reduced metabolic rate leading lethargy, low appetite and subsequently poor growth.

A1.2.4.3 Lectins

Lectins, also known as haemaglutenins, are proteins that possess as specific affinity for carbohydrates. These proteins cause the agglutination of erythrocytes, hence their other name. The primary mode of the anti-nutritional action of lectins is their ability to reduce the absorption of nutrients from the gastro-intestinal tract. Some action of gastric enteritis and internal haemorrhaging has also been suggested. Notably, lectins being proteins are heat labile and can be inactivated by precooking of meals.

A1.2.4.4 Oligosaccharides

The oligosaccharides are generally α-galactosyl homologues of sucrose. Oligosaccharides also contain significant amounts of the raffinose, stachyose, verbascose and sucrose families. Of these raffinose has a single galactose moiety linked to a sucrose molecule, while stachyose has two and verbascose three (Petterson, 2000). High levels of raffinose oligosaccharides have been reported to present some negative nutritional effects, some of which may be applicable to fish. These include; (a) interference with the digestion of other nutrients, (b) osmotic effects of oligosaccharides in the intestine and (c) anaerobic fermentation of the sugars resulting in increased gas production (van Barneveld, 1999). The utilisation of these nutrients has not been well defined in fish, although studies with pigs and poultry have shown that oligosaccharides are indigestible in the stomach or small intestine, primarily due to a lack of the enzyme -galactosidase (EC 3.2.1.23) (Gdala et al., 1997). Ultimately the influence of the oligosaccharides on the nutritional value of lupins appears to vary on a species specific basis. What influence oligosaccharides are likely to have on fish is presently unknown, though studies examining ethanol soluble carbohydrates (most likely to be oligosaccharides) from soybean meals on Atlantic salmon, have shown some antagonistic effects (Arnesen et al. 1989; Refstie et al., 1998).

A1.2.4.5 Phytate

The molecule inositol hexaphosphate and salt ions of this molecule are commonly referred to as phytate. Phytate is strongly negatively charged at all pH values usually encountered in feeds. Consequently, phytate has been known to complex with proteins at acidic pH values and also polyvalent ions, such as zinc, at intestinal pH values. This has been reputedly attributed to a reduced availability of these nutrients to animals when fed diets with a significant phytate content. It has also been suggested that high dietary calcium levels can exacerbate the complexation of zinc with phytate. Other significant effects that have been attributed to phytate include depressed growth, depressed feed intake, reduced protein utilisation and depressed thyroid function (Petterson, 2000). The commercial use of exogenous enzyme supplements has made considerable improvements to the utilisation of phytates by both pigs and poultry. The key to this is the use of the enzyme phytase (EC 3.1.3.8) which cleaves the phosphate units from the inositol base. Recent work has indicated that there may be potential for phytase use with fish diets (Carter and Hauler, 1999; Storebakken et al., 1998). Interestingly, improved feed intakes have also been observed of Atlantic salmon when fed diets containing phytase (Carter and Hauler, 1999).

A1.2.4.6 Protease inhibitors

Protease inhibitors are specific substances that have the ability to inhibit the proteolytic activity of certain digestive enzymes. A range of protease inhibitors have been identified from a variety of plant meals. Notably, soybean is a prominent plant meal with a substantial protease inhibitor content. Five different types of protease inhibitors have been identified in the seeds of this plant, which has trypsin inhibitor levels of about 60,000 mg/kg DM in unprocessed seed and about 3,400 mg/kg DM in a processed meal (White et al., 2000). The mode of action of protease inhibitors is primarily through either the competitive or allostearic binding of the molecule to the digestive enzyme to render it in active. Similar to lectins, being proteins, protease inhibitors are heat labile and can be inactivated by precooking of meals.

A1.2.4.7 Saponins

Saponins are plant glycosides with a steroid or triterpenoid structure as part of the molecule. Similar to alkaloids, saponins are also a bitter tasting molecule. This means that their primary anti-nutritional basis is as a feeding deterrent. An additional effect attributable to saponins is an increase in the permeability of the small intestine mucosal cells (Fenwick et al., 1991).

A1.2.4.8 Tannins

Tannins are a group of polyphenolic compounds that bind to other proteins to either inhibit their activity in the case of digestive enzymes or to prevent their digestion, in the case of most other proteins. There are two tannin sub-groups, those being either the hydrolysable or condensed (non-hydrolysable) forms. The condensed tannins have been reported to be able precipitate proteins, particularly the digestive enzymes. Tannins can also form cross-linkages between proteins and other macro-molecules and render them unavailable for digestion (Griffiths, 1991). These inhibitory facets, in conjunction with an astringent taste constitute the anti-nutritional characteristics of tannins (Petterson, 2000).

A1.4 Nutritional and biological value of plant protein meals to aquaculture species

A wide range of plant protein commodities have been reported in the literature as being used as alternative to fish meal in aquaculture diets for virtually all aquaculture species. Key among those ingredients used have been the soybean meals. More recently, this has further progressed to t he development and evaluation of protein concentrates and isolates from soybean meals and some other ingredients. From an Australian perspective there are primarily three other plant protein meals/resources that are of key interest as potential alternative, for both use in the domestic aquaculture industries and also for the development of export opportunities. These resources are lupins, canola and field peas.

The approach taken in this review to evaluating each of these feed grain commodities has been to identify the nutritional value of the primary plant protein resources and also the variety of resources of that grain available. In addition, maximum inclusion limits and factors influencing their inclusion have also been identified where possible along with some details on anti-nutritionals and recent findings on each respective plant protein resource.

A1.4.1 Soybean

Soybean meals constitute clearly the largest volume of plant protein meal available in the world (USDA, 2001). Not only is the volume (some 100 million tonnes per annum) the largest, but a considerable diversity of products is also available. In addition to this several processed soybean products have also entered the market place and have not only been evaluated by in many cases are also extensively used by the aquaculture feeds industry. Some of the products include protein concentrates and protein isolates.

Kaushik et al. (1995) evaluated the digestible value of a wide range of soybean meals of various forms (Table A1.6). From this study it was shown that all of the soybean meals had very high protein/nitrogen digestibilities. The extrusion of the meals had little influence on their digestible energy value, though notably an unextruded full-fat soybean meal was not evaluated. It could be argued that a single extrusion has some benefit, but that there is little additional benefit with further extrusion.

The comparison of soybean meal and soy flour showed a minor improvement in the digestibilities of both protein and energy. This was concomitant with the decrease in particle size of the ingredient being examined. This was attributed to an increased availability of both the protein and carbohydrate content of the meals being achieved. The concentration of the protein content was also clearly seen to improve the overall value of soybean meals with an increase in digestible energy content without any loss in protein digestibility.

Table A1.6 Digestibility values of a range of soybean resources fed to rainbow trout. Data derived from Kaushik et al. (1995).

Apparent Digestibility	Soybean meal	Full-fat Soybean meal (single extrusion)	Full-fat Soybean meal (double extrusion)	Soy Flour	Soybean Protein Concentrate
Nitrogen (%)	92.8	97.7	97.2	95.1	96.1
Energy (%)	76.8	85.1	86.7	80.7	83.3

Work by Allan et al., (2000) as part of Australia's Fishmeal Replacement Subprogram of the Fisheries Research and Development Corporation also examined the nutritional value three commonly available soybean meals used in the feeds industries, when fed to the silver perch (*Bidyanus bidyanus*). This species is atypical of most other aquacultured fin-fish species in the western world in that it is considered omnivorous. However, the work of Allan et al. (2000) provides a good robust comparison of some key Australian plant protein resources.

There were key composition differences between each of the soybean meals examined in this study (Table A1.7). Interestingly the protein content of the solvent and expeller-extracted meals was essentially the same. The protein content of the full-fat soybean meal was considerably lower (350 g/kg cf. 475 g/kg). The lower protein content of the full-fat meal was concomitant with a considerably higher fat level in this meal (195 g/kg cf. 40 to 60 g/kg). The higher fat levels of the full-fat soybean meal also dominate the gross energy content of the soybean meal in this form.

In this work Allan et al. (2000) showed that each of the soybean meals evaluated had better dry matter and energy digestibilities than either whole-seed lupins (*L. angustifolius*) or field peas. This was most likely linked to the fact that the soybean meals had lower levels of NFE than the other meals. With the majority of NFE in these ingredients (excepting Field peas) being non-starch polysaccharides and therefore relatively indigestible. The protein digestibility of the soybean meals was equivalent to that lupins and better than that of the field pea meal. This observation of protein digestibility was also consistent with the observations of some key amino acid digestibilities.

Table A1.7 Nutrient digestibility of soybean meals fed to silver perch. Data derived from Allan et al. (2000).

Nutrient	Soybean			*L.angustifolius*	Field pea (Dunn)
	solvent extr.	expeller extr.	full-fat	whole-seed	whole-seed
Ingredient composition					
Protein (g/kg DM)	478	475	358	341	255
Fat (g/kg DM)	37	64	195	57	11
Ash (g/kg DM)	80	63	55	28	34
NFE (g/kg DM)	405	398	392	574	700
Gross energy (MJ/kg DM)	17.0	20.9	23.3	17.9	17.0
Digestibility (%)					
Dry matter	73.1	81.4	74.9	50.3	62
Nitrogen	95.3	97.2	92.1	96.6	83.3
Energy	81.5	85.2	78.7	59.4	67
Lysine	98.1	97.3	95.3	98.1	86.3
Methionine	96.4	97.6	95.5	83.9	87.5

NFE: Nitrogen-free extractives

Gomes et al., (1995) also evaluated the nutritional value of a suite of plant protein meals when fed to rainbow trout. Included in this study were full-fat toasted soybean meal and full-fat micronised soybean meal. Additional plant proteins included lupin (*L. angustifolius)* whole-seed meal and field pea meal (Table A1.8). Of the plant protein meals, full-fat micronised soybean meal had the highest apparent dry matter digestibility (86.4%) and the lupin meal the lowest (63.3%). The apparent dry matter digestibility value of field pea meal (66.6%) was similar to that of the lupin meal.

Apparent protein digestibility of the plant protein meals was also highest in full-fat micronised soybean meal (96.3%) strongly supporting the value of further processing of plant protein meals to improve their nutritional value. The apparent protein digestibility of the lupin meal was the highest of the unprocessed whole-seed meals (85.5%). It was notable that in this study the soybean meals had significantly higher protein digestibilities than most other ingredients.

The apparent energy digestibilities of the plant meals ranged from 59.2% to 90.7%. The highest was that of the full-fat micronised soybean meal (90.7%) and the lowest, that of the pea seed meal (59.2%). The apparent energy digestibility of the lupin seed meal was similar to the other whole-seed legume meals (61.2%). No significant differences were evident between the three whole-seed legume meals, though the soybean meals had significantly higher apparent energy digestibilties.

Table A1.8 Digestibility values of a range of protein resources, including *L. angustifolius* whole-seed meal, fed to rainbow trout. Data derived from Gomes et al. (1995).

	Dry matter digestibility (%)	Protein digestibility (%)	Energy digestibility (%)
Fishmeal	78.0	86.6	69.7
Full-fat toasted soybean meal	75.4	86.4	80.2
Full-fat micronised soybean meal	86.6	96.3	90.7
Lupin seed meal	63.3	85.5	61.2
Field pea meal	66.6	80.4	59.2
Meat meal	94.1	90.8	92.1

Table A1.9 Growth performance of rainbow trout fed a range of soybean resources. Data derived from Kaushik et al. (1995).

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Ingredients						
Fishmeal	650	420	215	-	440	300
Soy Protein Concentrate	-	220	420	620	-	-
Soyflour	-	-	-	-	240	420
L-Methionine	-	-	0.3	0.4	-	0.3
Gelatinised Wheat Starch	260	250	235	230	215	175
Fish oil	60	80	100	120	75	85
Remains (common to all diets)	60	60	60	60	60	60
Diet Proximate Specifications						
Dry matter (g/kg)	896	904	890	884	909	894
Protein (g/kg DM)	465	458	445	427	446	443
Fat (g/kg DM)	122	124	133	125	127	126
Ash (g/kg DM)	109	90	73	55	93	84
Gross Energy (MJ/kg DM)	20.9	21.1	21.7	21.7	21.1	21.5
Fish Performance Criteria						
Initial weght (g)	83.0	83.0	83.0	83.0	83.0	83.0
Final weight (g)	223.6	215.1	224.2	222.4	216.1	205.7
Gain (g)	140.6	132.1	141.2	139.4	133.1	122.7
DGC (%/d)	2.03	1.94	2.04	2.02	1.95	1.83
FCR	1.04	1.11	1.10	1.08	1.17	1.24
PER	1.95	1.90	2.02	2.07	1.89	1.77
Nitrogen retention (%)	35.2	33.8	36.9	36.9	33.7	32.9
Energy retention (%)	41.7	41.8	46.1	45.6	37.2	33.5

The work of Kaushik et al., (1995) demonstrated that up to 62% of the diet could be comprised of a soy protein concentrate without loss of feed intake or performance of the fish (Table A1.9). In contrast, the inclusion of soyflour at 42% of the diet resulted in a deterioration of growth, though food intake was relatively uninfluenced. When included at 24% of the diet the soyflour proved to be an adequate ingredient, though notably there were no negative controls in this study. It was suggested that at the higher inclusion level of soyflour, that the NSP content of this ingredient was causing problems with nutrient absorption by the fish. In addition the influences of oligosaccharides and saponins were also suggested.

A study by Robaina et al., (1995) examined the incremented inclusion of either soybean meal (proximate specifications not detailed) or whole-seed lupins (*L. angustifolius* whole-seed meal proximate specifications or cultivar used not detailed) in diets fed to sea bream (*Sparus auratus*). (Table A1.10). Prior to incorporation of the grain meals, these workers also examined the solubility and trypsin inhibitor activity of the soybean meal to ensure it had been heat-treated and soaked the lupin seeds in water for 24 h in an attempt to remove excess alkaloids.

Table A1.10 Utilisation of soybean and *L. angustifolius* whole-seed meals by the gilthead sea bream (*Sparus auratus*). Data derived from Robaina et al. (1995).

	Control	*S10*	*S20*	*S30*	*L10*	*L20*	*L30*
Diet ingredients							
Fishmeal	766	690	613	536	690	613	536
Soybean meal	-	101	202	302	-	-	-
L. angustifolius whole seed meal	-	-	-	-	115	231	346
Fish oil	60	66	72	78	41	23	4
EPA 42 (enriched fish oils)	-	-	-	-	9	17	25
Cellulose	91	60	30	-	62	33	4
Remains (uniform across treatments)	83	83	83	83	83	83	83
Diet proximate specifications							
Dry matter (g/kg)	940	941	913	906	937	922	898
Crude protein (g/kg DM)	593	607	606	612	601	606	589
Crude fat (g/kg DM)	141	135	145	152	134	128	127
Gross ash (g/kg DM)	159	146	131	134	128	130	127
Gross energy (MJ/kg DM)	19.28	20.13	20.22	18.70	17.97	18.19	20.12
Fish performance criteria							
Initial weight (g)	38.3	40.3	39.4	37.0	38.5	38.7	39.6
Final weight (g)	60.1	64.5	62.0	56.5	60.3	59.1	61.5
DGC (%/d)	0.91	0.97	0.93	0.84	0.91	0.85	0.90
FCR (g fed / g gain)	1.64	1.59	1.64	1.82	1.59	1.89	1.79
Nitrogen retention (%)	24.9	22.4	19.7	21.9	19.0	24.9	27.7
Apparent Protein Digestibility (%)	92.9	93.6	86.2	87.4	95.5	94.5	93.0
Apparent Lipid Digestibility (%)	92.6	93.2	95.9	97.5	97.2	93.9	95.3

S10, S20, S30: Diets containing 10%, 20% or 30% soybean meal respectively. L10, L20, L30: Diets containing 10%, 20% or 30% *L. angustifolius* whole-seed meal respectively

After a ten week growth study, there were mixed results for both the soybean and lupin series of diets. Notably a decline in performance of the fish was observed with the highest inclusion level of soybean (30%) and this was consistent with a concomitant decline in digestibility of protein in the diet. In contrast, the lupin series of diets had no significant declines in performance, though fish fed the 20% inclusion treatment did have a reduced growth rate. However it was noted that this was not consistent with any changes in digestibility parameters of the protein or energy content of the diet.

A histological examination was also made of the influence on the fish of each of the dietary treatments. This showed that fish fed the soybean meal based diets had an increased amount of lipid droplets around their pancreatic tissue in the liver. Eccentrically located cell nuclei were also observed in the hepatocytes from fish fed diets containing 20% or 30% of soybean meal. High levels of hepatocyte vacuolisation and disorganisation were observed from fish fed the diet containing 30% soybean meal. Only minor histological differences were also observed between fish fed the control diet and the diets containing the *L. angustifolius* whole-seed meal. However, in comparison to the effects seen with the inclusion of soybean meal, those from fish fed the *L. angustifolius* whole-seed meal diets were considered minor.

Table A1.11 Nutrient digestibility soybean meals and products fed to Atlantic salmon. Data derived from Refstie et al. (1999).

	Protein Isolates	Protein Concentrate	Oligosaccharide Reduced	Solvent Extracted
Ingredients				
Isolated Soya Protein	248	-	-	-
Soya Protein Concentrate	-	320	-	-
Reduced Oligosaccharide Soyabean Meal	-	-	370	-
Solvent Extracted Soyabean Meal	-	-	-	475
Fishmeal	295	295	295	295
Fishoil	170	170	170	170
Pre-cooked corn starch	25.5	25.5	25.5	25.5
Dextrin	252	180	130	25
DL-Methionine	3	3	3	3
Marker	0.1	0.1	0.1	0.1
Micro-ingredients	6.4	6.4	6.4	6.4
Diet Proximate Specifications				
Dry matter (g/kg)	923	912	913	893
Protein (g/kg DM)	442	441	451	474
Fat (g/kg DM)	237	213	219	242
Dextrin + Starch (g/kg DM)	298	247	193	79
Ash (g/kg DM)	44	59	63	73
Calcium (g/kg DM)	11.4	13.5	12.7	14.5
Phosphorus (g/kg DM)	9	10.4	10.5	11.3
Phytate	2	5	6.3	6.6
Soluble Fibre (g/kg DM)	0	8.5	13.2	15.2
Total Fibre (g/kg DM)	19.1	71.9	83.7	115.3
Apparent Digestibility				
Organic matter (%)	69.3	66.0	63.4	61.6
Nitrogen (%)	90.9	89.3	86.7	85.5
Fat (%)	96.2	98.0	93.1	85.7
Starch (%)	50.2	59.3	67.1	54.7
Phosphorus (%)	38.2	33.6	33.6	37.7
Faecal Dry Matter (g/kg)	119	109	82	69
Blood Glucose (mM)	8.3	7.8	7.7	6.6

Work by Refstie et al. (1999) examined the relative nutritional value of a range of soy protein products when included in diets for Atlantic salmon (*Salmo salar*) on an equivalent nitrogen/protein basis (Table A1.11). In this study solvent-extracted soybean meal was compared against and oligosaccharide reduced soybean meal, a soy protein concentrate and a soy protein isolate.

Significant improvements to organic matter and nitrogen digestibilities were observed with increased level of processing of the soybean meals. Notably the digestibility estimates presented by Refstie et al. (1999) differ markedly from those of Kaushik et al., (1995) who evaluated some similar meals, both in salmonid species. Interestingly recent data on the comparability of the different faecal collection methods used by different laboratories has shown some marked differences (Vandeberg and de la Noue, 2001). This is the most likely reason for these disparities. Some improvements to fat digestibility where observed with removal of oligosaccharides and some of the other NSP, but this was not consistent with the greatest levels of NSP removal as exhibited from the Protein Isolates treatment.

Other observations from the work of Refstie et al. (1999) included a minor improvement in the dry matter content of the faeces of the fish was observed with removal of the oligosaccharides, though greater improvements still were observed with increasing protein content of the soy product examined (Table A1.11). However, as there was no information on whether the concentrate and isolate were also oligosaccharide free it is difficult to ascertain how much of the improvement is due to the reduction in oligosaccharide levels or just removal of the NSP fraction of the soybean meal.

Work by Krogdahl et al. (1994) demonstrated that the protease inhibitors, notably trypsin inhibitor from soybeans had a clear negative impact on the intestinal trypsin activities and protein and amino acid digestibilities of rainbow trout. In a second study by this group Olli and Krogdahl (1995) examined the influence of some of the alcohol soluble components of soybeans on the digestibility of lipids by Atlantic salmon. These workers noted that there was a significant reduction in the total digestibility of lipid and also some specific fatty acids, notably the saturates. It was not clear from this study, but it was implied that these effects were due to the influence of soybean oligosaccharides and possibly also saponins, both of which were found in the alcohol soluble components of soybeans.

A study by Bureau et al. (1998) also examined the influence of some of the alcohol soluble components of soybeans on the digestibility of lipids by chinook salmon (*Oncorhynchus tshawytscha*) and rainbow trout. However these workers further purified some of the extracts to examine the specific effects of particular components. Notably the fractions they focused on where those containing the saponins, and they also included some purified Quillaja bark saponins (QBS) in the diets of the fish too. Clear effects where seen, with depressed growth and reduced feed intake, with the purified QBS also causing significant intestinal damage.

Summary

- *The research published on the use of soybean products in diets for aquaculture species is quite exhaustive and the volume eclipses by far that of any other feed ingredient.*
- *Typically soybean meals have been shown to have highly digestible protein and also good digestible energy values.*
- *The high protein content of soybean meals, coupled with their high levels of protein and energy digestibility make them a highly valuable ingredient for use in aquaculture diets.*
- *Protein concentrates prepared from soybean meals have been demonstrated to confer significant advantages as an ingredient in the aquaculture feeds sector. The trend in the published research on the use of soybean products in this industry sector is tending towards the use of more concentrates and isolates.*
- *Maximum potential inclusion levels vary between the different types of soybean products examined and species of fish. However, inclusion levels of up to 62% of soy protein concentrate of the diet and 24% with soyflour and solvent-extracted soybean meal have been achieved.*
- *Limitations to the use of non-processed soybean products have been suggested to arise from anti-nutritional factors such as oligosaccharides, saponins and in some cases protease inhibitors.*

A1.4.2 Lupin

There are numerous works that have studied the nutritional value of lupins when fed to a wide variety of aquaculture species. For a comprehensive review of this subject read Glencross (2001). However, the depth and quality of the reported work on lupins though is widely ranging and is certainly small compared to the volumes of work published on soybean products. More recently though there have been published some clearer evaluations of lupin meals with some key implications on how to best utilise these resources in aquaculture diets. Notably, there are three separate species of lupins which have been evaluated. These are *Lupinus albus, L.angustifolius* and *L. luteus*. Of these the majority of the international work is on *L. albus*, with the recent Australian work beginning to focus more on the other two species.

One of the earlier studies was that by Morales et al., (1994) who examined the apparent digestibility characteristics of a range of ingredients when fed to rainbow trout (Table A1.12). There were limitations to this work though in that the design did not allow the actual direct measurement of the digestibility of the test ingredients, but did allow an assessment of their imputed value in comparison to some reference ingredients. The diets in this study were formulated to include *L. albus* meal (cultivar or processing state not identified), corn gluten meal, casein and cottonseed meal at 40% of the total dietary protein as partial replacements for the fishmeal portion of iso-nitrogenous and iso-energetic diets. Additional diets in this study included a reference diet with fishmeal as the only protein resource, and another reference diet with casein as the only protein resource. The inclusion of fishmeal as the only protein resource in the reference diet allows some assessment if the nutritional value of the protein content of each of test diets and their ingredients as used in this study.

Assessment of the apparent digestible characteristics of each of the diets revealed that the apparent digestible dry matter of the lupin diet was lowest, as was the apparent digestibility of its organic matter and energy content. It is suspected that these observations are reflective of the relatively high levels of non-starch polysaccharides in the *L. albus* meal. Indeed evaluation of the digestibilities of the NFE and carbohydrate contents of the diet clearly support this, with the lupins having the lowest NFE and carbohydrate apparent digestibilites of all the ingredients evaluated in this study, by a considerable margin. Though it was not clearly stated it reasons that this work was based on the evaluation of a whole-seed *L. albus* meal.

In contrast to the poor energy and carbohydrate digestibilities the apparent protein digestibility of the lupin diet was higher than that of the cottonseed meal diet, but not as high as that of the corn gluten meal diet. It was though very comparable to the apparent protein digestibility of the fishmeal based reference diet (Table A1.12). Based on the determined digestibility value of the fishmeal protein it was calculated that the casein and corn gluten had apparent protein digestibilities of about 97% with the next highest being the *L. albus* meal (87%), which was slightly higher than that of the fishmeal (83%).

Table A1.12 Apparent digestibility of key dietary nutrients of a range of protein resources fed to rainbow trout. Data derived from Morales et al. (1994).

Diet digestibilities	Fishmeal	CA100%	CA40%	CO	LU	CG
Dry matter digestibility (%)	66.9	71.3	68.5	58.9	53.1	67.6
Organic matter digestibility (%)	73.2	72.8	74.4	64.7	56.3	71.8
Protein digestibility (%)	83.6	97.2	88.3	81.2	85.2	88.9
Energy digestibility (%)	74.3	77.8	77.4	68.7	62.7	75.6
Fat digestibility (%)	88.0	93.6	93.6	93.4	88.7	91.4
NFE digestibility (%)	54.5	41.4	53.9	35.5	11.7	44.8
Carbohydrate digestibility (%)	65.0	64.9	65.0	53.3	15.8	60.5

CA100%: Casein 100% of total protein diet, CA40%: Casein 40% of total protein diet, CO: Cottonseed meal diet, LU: Lupin meal diet, CG: Corn gluten meal diet

The influence of extrusion of *L. albus* meal was examined by Bangoula et al., (1993) in rainbow trout as a means of improving the nutritional value of the grain. These workers reported an improvement in the utilisation of the nitrogen-free extractives (NFE) by rainbow trout, and suggested that this response was related to a higher breakdown of cell walls, potentially allowing better access by digestive enzymes to nutritionally valuable cell components. It was also suggested that partial degradation of the α-galactosides may have taken place, potentially providing additional nutritional value. It has been reported that partial hydrolysis of the α-galactosides does occur during extrusion at high temperatures (Melcion, 1987). Though how the extrusion of lupin meal improves NFE utilisation, but does not deteriorate the protein utilisation was not discussed.

Table A1.13 Digestibility of *L. angustifolius* and *L. albus* whole and kernel meals at fed at variable inclusion levels to the silver perch. Data derived from Allan et al. (2000).

Nutrient	*L. angustifolius*		*L. albus*	
	Whole-seed	Kernel	Whole-seed	Kernel
Dry matter	50.3	67.6	64.7	77.8
Nitrogen	96.6	100.3	96.1	101.4
Energy	59.4	74.0	72.7	85.2
Phosphorus	71.8	80.1	77.5	73.8
Lysine	98.1	99.5	96.6	102.5
Methionine	83.9	91.7	92.2	97.3

A study by Allan et al. (2000) examined the influence of removing the seed coat (dehulling) of the lupin on its nutritional value when fed to the omnivorous species the silver perch (*Bidyanus bidyanus*). For this study these workers examined both *L. angustifolius* and *L. albus* varieties in both their whole-seed and kernel meal forms (Table A1.13).

Clear nutritional advantages of dehulling lupins were observed from the results of this study. Irrespective of lupin species evaluated improvements were seen in the digestibilities of dry matter, nitrogen, energy and some amino acids. Interestingly, improvements in phosphorus digestibility were observed with dehulling of *L. angustifolius*, but not *L. albus*.

While it is difficult to translate these results to that of most main-stream species such as trout and salmon and seabreams, it does provide a good robust account of the value of using kernel meals of lupins in fish diets. Notably, most studies since the mid to late nineties have focussed solely on the use of kernel meals or processed products from lupins.

Table A1.14 Digestibility specifications of key lupin species kernel meals and solvent-extracted soybean meal. Data derived from Glencross and Hawkins (2003).

Nutrient	White lupin (L. albus)	Sweet lupin (L. angustifolius)	Yellow lupin (L. luteus)	Soybean meal
Dry matter content (g/kg)	922	910	937	890
Crude protein	455	411	496	503
Total Nitrogen	73	66	79	80
Crude fat	137	60	55	12
Ash	36	32	38	88
Nitrogen-free extractives	405	497	410	397
Phosphorus	5	4	5	7
Gross energy (MJ/kg DM)	23.1	20.7	21.0	19.2
Alkaloids	0.08	0.18	0.03	0.01
Oligosaccharides	84	58	110	68
Rainbow trout (Stripping)				
Organic matter	59.3	58.0	55.5	65.5
Energy	64.0	62.4	64.9	75.1
Phosphorus	100.0	100.0	100.0	45.0
Nitrogen	88.3	93.1	95.3	86.8
Red seabream (Settlement)				
Organic matter	52.8	49.7	60.7	74.4
Energy	60.9	62.4	69.5	81.0
Phosphorus	100.0	100.0	90.9	81.7
Nitrogen	100.0	99.1	97.7	96.2

[a] Solvent-extracted high-protein soybean meal

Recently (Glencross and Hawkins, 2003) the digestibility of the kernel meals of all three species of lupin (*Lupinus albus*, *L. angustifolius* and *L. luteus*) was compared against each other and a reference ingredient of solvent extracted soybean meal, when fed to rainbow trout (*Oncorhynchus mykiss)*and red seabream (*Pagrus auratus*) (Table A1.14). The digestibility of protein of all lupin kernel meals was better than for the soybean meal for both fish species. The highest protein digestibility in trout was that from *L. luteus* kernel meal (95.3%), which at similar inclusion levels was better than that from kernel meals of both *L. albus* (88.3%) and *L. angustifolius* (93.1%) and also the soybean meal (86.8%).

The digestibility of dietary energy from each of the lupin kernel meals (range from 62.4% to 64.9%) was less than that obtained from soybean meal (75.1%). However, the higher gross energy content of the lupin kernel meals still resulted in both *L. luteus* and *L. albus* providing greater levels of digestible dietary energy with their inclusion in the diet than the inclusion of soybean meal would provide. Indeed it was suggested that both of these ingredients would already constitute appropriate replacements of any soybean meal used in diets for salmonids, particularly in lieu of the cost competitiveness.

The level of phosphorus digestibility was better in all lupin kernel meals than that from the soybean meal. Phosphorus digestibility was consistent between all lupins species at effectively 100% compared to the soybean meal with a phosphorus digestibility of 45%. This finding has important implications for the development of phosphorus limiting diets which are sometimes required in particular aquaculture farming systems.

However, the digestibility of organic matter was poorer from each of the lupin kernel meals relative to that from the soybean meal. In most cases, the increased inclusion of each of the lupin kernel and soybean meals in the diet also resulted in a decrease in the level of organic matter digestion. This observation constitutes the primary identified weakness of the lupin kernel meals and certainly efforts to improve their value will need to resolve this limitation.

A second study on the nutritional value of *L. angustifolius* kernel meals evaluated the influence of variability in the protein level of lupin (*L. angustifolius*) kernel meals when fed to rainbow trout (Glencross et al., 2003). In this study five kernel meals with a protein content ranging from 35% to 48% (on a dry matter basis) were incorporated into isonitrogenous diets to examine the digestibility of the protein and energy contents of these kernel meals (Figure A1.1). The results of this work identified that there was a strong correlation between protein content of a lupin kernel meal and the nutritional value of that protein. Notably the strongest correlation was that between kernel meal protein content and nitrogen digestibility ($R^2 = 0.981$).

The strong correlation between kernel meal protein content and its nitrogen digestibility had a direct effect on the relationship with energy digestibility. Notably, the kernel meal protein content had more influence on its energy digestibility than that of the nitrogen digestibility. It was suggested that this was a direct implication from the level of protein contribution of the kernel meal to its total and digestible energy content.

There was also a close relationship between NFE and dry matter digestibilities of the lupin kernel meals. Again this finding reiterates those earlier observations that it is the utilisation of the carbohydrate fraction of lupin kernel meals that is their key weakness as a feed grain in the aquaculture feeds sector. Clearly any resolution to improving the utilisation of this fraction of the grain will be seen as beneficial.

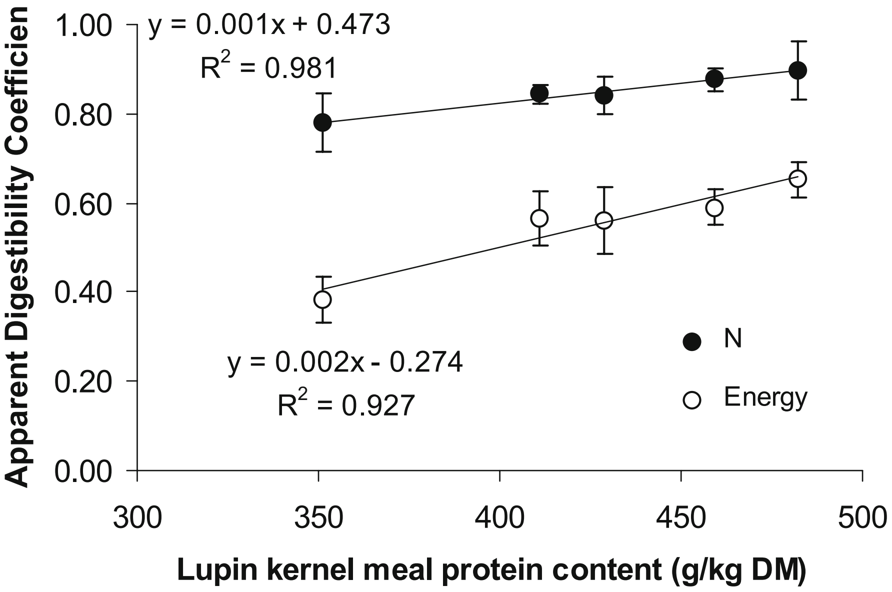

Figure A1.1 **Protein and energy digestibility as a function of crude protein content of lupin kernel meal, when fed to rainbow trout. Data derived from Glencross et al. (2003).**

The demonstrated relationship between lupin kernel meal protein content and its nutritional value provides a good support for the promotion of grain segregation by protein content and commodity pricing also according to protein content. It was suggested specifically this valuation should focus on the value per unit of digestible protein when used as an ingredient for fish diets. On this basis, some *L. angustifolius* kernel meals clearly warrant premium pricing. This basis of superior nutritional value as a function of protein content would also support promoting the increased production of *L. luteus* as a premium grain. Indeed based on digestible protein value, *L. luteus* kernel meals should be commanding at least a 26% premium compared with a 21% premium when determined on crude protein content of the kernel meal. Presently *L. luteus* seed are commanding only an 18% premium.

Some of the most conclusive work published to date evaluating lupins (*L. albus*) has been that by Burel et al., (1998) who conducted a series of studies examining the inclusion of a kernel meal in diets for rainbow trout. The first of these studies evaluated the inclusion of a kernel meal at 300 g/kg, 500 g/kg and 700 g/kg in diets that were designed to be isonitrogenous and isoenergetic. Another of the diets evaluated was a control diet in which no kernel meal was added (Table A1.15). The results of this study identified that *L. albus* kernel meal could be included in the diet of rainbow trout to a level of 500 g/kg with no loss in growth rate and significantly superior phosphorus retention. The inclusion of *L. albus* kernel meal to 700 g/kg however, resulted in poorer growth, feed efficiency and nitrogen and energy retention. Interestingly though, phosphorus retention improved further still with the higher inclusion level of *L. albus* kernel meal (Table A1.15). The loss in growth performance of fish fed the diets containing 700 g/kg of *L. albus* kernel meal was attributed to low feed intakes of this diet. It was suggested that high levels of *L. albus* kernel meal inclusion resulted in a loss of palatability of the diet

A second experiment conducted by Burel et al., (1998) examined further series of diets which were formulated to examine the high inclusion levels of *L. albus* kernel meal. In this study three diets contained 700 g/kg of the *L. albus* kernel meal, but with some of the diets also containing either a dietary ingestion stimulant (Finnstim™) or dietary iodine (Table A1.16). It was suggested that the poor growth of fish fed the 700 g/kg diet in the previous experiment was possibly a consequence of suppressed metabolic rate induced by anti-thyroidal anti-nutritional factors present in the *L. albus* kernel meal. The results of the second study however, showed no benefit of the inclusion of the iodine or the feed stimulant (Table A1.16). Of note though, fish fed any of the three 700 g/kg *L. albus* kernel meal diets had significantly poorer performance attributes (growth, feed utilisation efficiency and nitrogen retention) than those fish fed the control diet. This suggested that the problem encountered in the first study with poor performance with high inclusion levels of *L. albus* kernel meal had still not been overcome or identified.

A third experiment examined trout fed the control diet, the 500 g/kg and 700 g/kg diets, and a 700 g/kg diet containing Finnstim™. In this study the fish were allowed to self-regulate their own feed intake (Table A1.17). However, in this study considerably different results were obtained to those observed in the second study. In the third study no significant differences in the growth performance of fish fed each of the treatments was observed, though feed efficiency deteriorated with increasing inclusion of *L. albus* kernel meal. Examination of feed intake of the two 700 g/kg diets showed no benefit from the inclusion of the Finnstim™. However, despite the deteriorating feed efficiency and energy retention with increased inclusion of *L. albus* kernel meal, considerable improvements in phosphorus retention of fish fed the diets were observed.

Table A1.15 Influence of inclusion levels of extruded *L. albus* kernel meals fed to rainbow trout. Data derived from Burel et al. (1998).

	Reference	30% Lupin	50% Lupin	70% Lupin
Diet ingredients (g/kg)				
L. albus kernel meal	-	300	500	700
Fishmeal	530	350	205	65
Flaked corn	320	205	135	75
Fish oil	90	85	100	100
L-Methionine	-	-	-	2
Remains (uniform across treatments)	60	60	60	60
Fish performance criteria				
Initial weight (g)	23.1	23.1	23.1	23.1
Final weight (g)	90.6	108.1	94.2	53.8
DGC (%/d)	2.7	3.1	2.7	1.5
Feed intake (g/d/fish)	1.07	1.33	1.13	0.62
FCR (g fed/ g gain)	1.01	1.01	1.02	1.31
Nitrogen retention (%)	36.0	39.1	37.3	31.6
Phosphorus retention (%)	28.8	37.7	39.9	69.0

Table A1.16 Influence of attractants and iodine supplements on high inclusion levels of extruded *L. albus* kernel meals fed to rainbow trout. Data derived from Burel et al. (1998).

	Reference	Control	Attractant	Iodine
Diet ingredients (g/kg)				
L. albus kernel meal	-	700	700	700
Fishmeal	480	120	125	115
Pea meal	200	28	13	33
Pregelled starch	150	-	-	-
Fish oil	110	90	90	90
L-Methionine	-	2	2	2
Attractant	-	-	10	-
Potassium Iodide	-	-	-	65 x 10-5
Remains (uniform across treatments)	60	60	60	60
Fish performance criteria				
Initial weight (g)	34.0	34.0	34.0	34.0
Final weight (g)	104.2	86.4	89.5	85.3
DGC (%/d)	2.7	2.1	2.3	2.1
Feed intake (g/d/fish)	1.22	1.07	1.09	1.14
FCR (g fed/g gain)	0.86	1.03	1.11	1.25
Nitrogen retention (%)	40.0	29.9	31.0	25.9
Phosphorus retention (%)	22.5	29.5	39.1	43.7

Table A1.17 Influence of high inclusion levels of extruded L. albus kernel meals fed to rainbow trout. Data derived from Burel et al. (1998).

	Reference	50% Lupin	70% Lupin	70% + Attractant
Diet ingredients (g/kg)				
L. albus kernel meal	-	500	700	700
Fishmeal	480	230	120	125
Pea meal	200	60	28	13
Pregelled starch	150	60	-	-
Fish oil	110	90	90	90
L-Methionine	-	-	2	2
Attractant	-	-	-	10
Remains (uniform across treatments)	60	60	60	60
Fish performance criteria				
Initial weight (g)	27.0	27.0	27.0	27.0
Final weight (g)	79.0	80.0	87.7	84.0
DGC (%/d)	2.2	2.3	2.5	2.4
Feed intake (g/d/fish)	0.72	0.87	1.02	1.02
FCR (g fed/ g gain)	0.87	1.00	1.03	1.11
Nitrogen retention (%)	41.3	37.5	35.8	32.8
Phosphorus retention (%)	22.5	29.5	39.1	43.7

Summary

- *The digestible value of the protein content of lupins is generally very high, though there is some minor variability between lupin species. The digestibility of the energy content is limited primarily to that derived from digestion of the protein and lipid, with little or no nutritional value derived from the lupin carbohdyrates.*
- *Processing of lupin meals, specifically the removal of the seed coat, substantially improves their nutritional value.*
- *The nutritional value of the protein and energy content of lupins has been shown to be strongly related to the protein content of the kernel meal.*
- *Maximum potential inclusion levels vary between species of lupin, species of fish and processing state of the grain (kernel or whole-seed). However, inclusion levels up to 70% of the diet have been achieved.*
- *There is limited information available on anti-nutritional components of lupins when fed to fish. What information is available suggests that any anti-nutritional aspect of lupins is likely to be less than that of soybean meal.*

A1.4.3 Pea

Field peas are one ingredient that with nutritional potential that Australia also has some capacity to supply. However, the volume of published studies on the value of peas to aquaculture species is somewhat limited, even less than that of lupins. Field peas, although legumes from the same family as lupins and soybeans, differ considerably in their composition. Notably pea meals have a lower protein content (~250 g/kg), a very low fat content (~10 g.kg) and a high carbohydrate content (700 g/kg), which is predominantly starch .

Allan et al. (2000) in evaluating the nutritional value of a range of plant protein resources for the silver perch also examined the digestibility of field pea meal. These workers found that the dry matter digestibility of peas was better than that of lupins, but not quite as good as that of soybean meals (see Table A1.7 in section A1.4.1). The nitrogen/protein digestibility of the pea meal was considerably poorer than that of the soybean and lupin meals. This was also generally consistent with the digestibility observed of some essential amino acids. However, the energy digestibility of the pea meal was better than that of the lupin meal (67.0% cf. 59.4%), though not quite as good as that of the soybean meals (~80%). The observations of the dry matter and energy digestibilities are consistent with there being substantial digestion of the carbohydrate content of the pea meals, which contrasts that of both the lupin and soybean meals.

Gomes et al. (1995) in evaluating a suite of plant protein meals when fed to rainbow trout (see Table A1.8 in section A1.4.1) also evaluated the nutritional value of field pea meal. In this study the soybean meals generally had higher apparent dry matter digestibility than the field pea meal, which was similar to that of the lupin meal. The protein digestibility of the field pea meal was somewhat lower than that of the other plant protein meals at only 80.4% compared with the soybean meals which had on average a protein digestibility of 90% and lupin whole-seed meal at about 85%. Similarly the energy digestibility of the field pea meal was also one of the lowest examined at only 59.2%, it was similar to that of the lupins at 61.2%, though both were considerably below that of the soybean meals at about 80% to 90%.

Booth et al. (2001) also examined the nutritional value of a range of other plant legumes including field peas, faba beans, chick peas and vetch when fed to silver perch (Table A1.18). In this study each of the grains was also evaluated in whole-seed meal and kernel meal forms. Notably the dehulling process realised only a minor increase in protein content of the meal from about 25.5% to 27.7%. This level of increase in protein content was consistent among the legume meals evaluated. The fat content of each of the legume meals, field peas included was low, with most containing less than 2% fat and no increase in fat content was observed with dehulling.

The protein digestibility of the field pea meals was exceeded only by that of Faba bean meals. Improvements in protein digestibility of field peas were observed with dehulling. This effect was consistent across all of the legume meals examined, excepting chick pea meals.

Energy digestibilities of each of the plant legumes were improved with dehulling of the grains. Although digestibility of the Faba bean meals was an exception to this with a moderate decrease in energy digestibility with dehulling. Notably the carbohydrate content of each the meals examined I predominated by starch. Therefore it is likely the fish are obtaining significant energetic value of from the starch. This contrasts the observations of utilisation of the carbohydrate fraction of soybeans and lupins, which is dominated by non-starch polysaccharides.

Dry matter digestibilities of field peas were considerably improved with the removal of the seed coat (dehulling). This was a similar finding to that observed with lupins.

Table A1.18 Nutritional value of various plant legume meals to fed silver perch. Data derived from Booth et al. (2001).

	Ingredient Proximate Specifications (g/kg)				Ingredient Digestibilities		
	Protein	Fibre*	Energy(MJ)	Fat	Dry matter (%)	Protein (%)	Energy (%)
Field pea whole-seed	255	87	17.0	11	48.9	83.3	54.5
Field pea kernel-meal	277	28	17.3	10	62.0	88.1	67.0
Faba bean whole-seed	277	120	17.3	13	55.9	91.6	62.2
Faba bean kernel meal	313	33	17.6	13	58.2	96.6	58.8
Chickpea whole-seed	208	134	19.4	47	48.7	84.8	53.6
Chickpea kernel meal	242	25	19.3	50	58.4	81.2	60.2
Vetch whole-seed	309	72	17.9	9	41.5	74.9	55.5
Vetch kernel meal	323	41	18.6	9	78.3	87.7	81.8

* Fibre is acid detergent fibre (ADF).

Table A1.19 Utilisation of soybean, *L. angustifolius* kernel meal and pea meal by Atlantic salmon, (*Salmo salar*). Data derived from Carter and Hauler (1999).

	Fishmeal	Soybean	Lupin	Pea
Diet ingredients				
Fishmeal	615.0	369.0	369.0	369.0
Soybean meal	-	357.0	-	-
L. angustifolius kernel meal	-	-	424.0	-
Pea meal	-	-	-	449.0
DL-methionine	-	8.5	10.0	4.0
Fish oil	138.0	154.8	154.8	154.8
Bentonite	123.3	87.5	19.0	-
Remains (uniform across treatments)	23.2	23.2	23.2	23.2
Diet proximate specifications				
Dry matter (g/kg)	940	950	947	935
Crude protein (g/kg DM)	429	441	431	447
Gross energy (MJ/kg DM)	19.03	20.14	21.77	21.69
Fish performance criteria				
Initial weight (g)	34.7	34.7	34.8	34.8
Final weight (g)	78.9	75.9	78.6	75.1
DGC (%/d)	1.63	1.54	1.62	1.52
FCR (g fed / g gain)	1.7	1.7	1.4	1.6
Feed intake (g / fish / d)	1.11	1.07	0.96	0.99
Nitrogen retention (%)	44.5	44.2	52.7	46.2
Apparent Protein Digestibility (%)	90.5	92.0	91.3	93.8
Apparent Phosphorus Digestibility (%)	39.8	27.5	46.7	41.6
Apparent Energy Digestibility (%)	91.9	86.4	80.5	83.1

Carter and Hauler (1999) evaluated the nutritional value of diets fed to Atlantic salmon which included at 40% inclusion level, a field pea protein concentrate, *L. angustifolius* kernel meal and a soybean meal (Table A1.19). Each ingredient was included in the diet to constitute 40% of the total dietary protein. Digestibility of each of the diets was also measured, but not that of the discrete ingredients. From this study the highest nitrogen apparent digestibilities were those observed from the diets with pea meal (93.8%). Second highest apparent nitrogen digestibility was of the soybean meal diets (92.0%) with the apparent nitrogen digestibility of *L. angustifolius* kernel meal (91.3%) lower, but not significantly lower than either pea meal or soybean diets.

The apparent energy digestibilities of the pea meal diets (83.1%) were slightly lower than the diet that included soybean meal (86.4%), with the diet containing *L. angustifolius* kernel meal having the lowest energy digestibilities (80.5%). These observations are consistent with the lower protein and fat levels of pea and the substantial levels of digestible carbohydrates in the form of starch. Of particular note though were the phosphorus digestibilties. Highest in this regard was the diet containing the *L. angustifolius* kernel meal (46.7%), next highest was the diet containing the pea meal protein (41.6%). The diet containing soybean meal (27.5%) had considerably poorer phosphorus digestibility than the *L. angustifolius* kernel meal, pea meal and even the reference diet (39.8%).

Table A1.20 Utilisation of soybean, *L. angustifolius* protein concentrate and pea protein concentrate by the atlantic salmon (*Salmo salar*). Data derived from Carter and Hauler (2000).

	Control	S25	S33	LC25	LC33	PC25	PC33
Diet ingredients							
Fishmeal	601	451	400	451	400	451	400
Soybean meal	-	204	273	-	-	-	-
L. angustifolius protein concentrate	-	-	-	218	292	-	-
Pea protein concentrate	-	-	-	-	-	206	276
DL-methionine	-	3	5	4	6	5	6
Fish oil	155	160	159	157	156	167	169
Bentonite	48	-	-	-	-	-	-
Cellulose	50	36	17	24	-	43	27
Remains (uniform across treatments)	146	146	146	146	146	146	146
Diet proximate specifications							
Dry matter (g/kg)	941	948	943	925	910	927	933
Crude protein (g/kg DM)	419	413	413	425	425	413	406
Crude fat (g/kg DM)	263	258	268	272	260	260	258
Gross ash (g/kg DM)	130	80	80	80	70	70	60
Gross energy (MJ/kg DM)	21.9	22.7	22.9	22.8	22.6	22.8	22.9
Fish performance criteria							
Initial weight (g)	46.7	46.4	46.8	46.3	46.4	46.8	46.6
Final weight (g)	113.1	120.7	116.9	114.0	113.9	123.4	118.4
DGC (%/d)	1.96	2.14	2.04	2.00	1.99	2.18	2.08
FCR (g fed / g gain)	1.10	0.96	1.02	1.03	1.28	0.99	0.99
Feed intake (mg DM / g fish / d)	14.6	13.6	13.8	13.8	17.1	14.1	13.6
Nitrogen retention (%)	38.0	41.5	41.2	38.9	30.2	40.9	45.1
Apparent Protein Digestibility (%)	92.7	95.3	95.9	95.6	95.9	95.2	95.5
Apparent Energy Digestibility (%)	87.9	89.0	89.7	91.3	91.8	88.8	89.2

S25 and S33: soybean meal diets with 25% or 33% replacement of fishmeal protein; LC25 and LC33: lupin (*L. angustifolius* cv. Gungarru) protein concentrate diets with 25% or 33% replacement of fishmeal protein; PC25 and PC33: pea protein concentrate diets with 25% or 33% replacement of fishmeal protein

In addition to the nutritive value aspects to this study by Carter and Hauler (1999), these workers also examined the biological value of these diets (A1.19). In this part of the study, the nitrogen retention values of fish fed the pea seed meal (46.2%) or soybean meal (44.2%) diets or were significantly less than that of the *L. angustifolius* kernel meal diets (52.7%). This finding is interesting given that the diets were formulated primarily on a gross iso-nitrogenous and energetic basis.

In a second study Carter and Hauler, (2000) again evaluated the nutritional value of diets containing a pea protein concentrate, *L. angustifolius* protein concentrate and defatted soybean meal. The three protein resources were included in diets at either 25% or 33% replacement of the fishmeal protein content of the diet (Table A1.20).

The highest apparent nitrogen digestibilities were those observed from the diets in which the *L. angustifolius* protein concentrate replaced 33% of the fishmeal (Table A1.20). Second highest was the diet in which soybean replaced 33% of the fishmeal. Pea protein concentrate had the least influence on the nitrogen digestibility of the feeds. The apparent energy digestibilties as with the apparent nitrogen digestibility, the pea protein concentrate also had the least influence on the energy apparent digestibility of the feeds.

Assessment of the biological value of the diets fed to the juvenile Atlantic salmon in Carter and Hauler's, (2000) study supported that each diet maintained growth equal to that of the control/reference diet (Table A1.20). Food consumption of the pea protein concentrate diet was similar to that of both the reference and soybean diets and significantly lower than that in which 33% of the fishmeal was replaced by *L. angustifolius* protein concentrate. The fish fed the diet with 33% fishmeal replacement with pea protein concentrate (45.1%) also had similar nitrogen retention values as that of the 33% replacement soybean meal diet (41.2%). Both of these were significantly better that that of the 33% replacement *L. angustifolius* protein concentrate diet at only 30.2% nitrogen retention efficiency. Interestingly, both the pea protein concentrate and soybean meal treatments also had nitrogen retention values slightly higher than that of the fishmeal control, though only significantly so for the pea protein concentrate treatment at 33% replacement of the fishmeal.

The results of Carter and Hauler's (2000) second study contrasted those from earlier work by this group (Carter and Hauler, 1999). These differences were not fully explained, though it was suggested that the comparatively higher levels of non-starch polysaccharides in the *L. angustifolius* protein concentrate may have had an influence on its nutritional value. However, if this was the case then it could also be reasoned that addition of α-cellulose to some of the test diets should have had a similar effect. Furthermore, if the levels of NSP were the key reason for the relative deterioration in biological value of the diet, then this should have been more apparent in the earlier study by this group (Carter and Hauler, 1999) where a kernel meal, with higher NSP levels than the concentrate was used. Notably, in this earlier work, the reverse was observed, with greater biological value being attributed to the *L. angustifolius* kernel meal than that of either the soybean or pea protein resources.

There were no identified studies that have examined any of the anti-nutritional aspects of field pea meals when fed to an aquaculture species.

Summary

- *The proximate composition of field peas is considerably different to that of both soybean and lupin meals. Notably, they have a lower protein content, but their carbohydrate in contrast is predominated by starch.*
- *The digestible value of field peas is denoted by a slightly lower protein value than most other plant protein meals, though a relatively high energy digestibility which is due to the predominance of the digestible carbohydrate starch.*
- *The nutritional value of field peas was observed to be improved with the removal of the seed coat. Notably an increase in protein, energy and dry matter digestibilities have been observed.*
- *Similar to other plant protein resources the preparation of protein concentrates from field peas also results in a more usable and nutritionally valuable ingredient to fin-fish.*
- *Studies utilising a protein concentrate of field peas have shown that there potential to include this ingredient in diets for Atlantic salmon at up to27% with no loss in fish growth performance and up to 45% with minor loss in relative performance.*

A1.4.4 Canola

Canola and rapeseed are names used to describe the plants *Brassica napus* and *Brassica campestris*. The name "canola", is actually combination of two words – "Canadian" and "oil", based upon particular varieties developed by the Canadians. These varieties of the plant are notable in that the seeds yield oil with less than two per cent erucic acid, and the air-dried, oil free meal contains less than 30 micromoles of glucosinolates per gram (or 30 mmol/kg) (Anderson-Hafermann et al., 1993). Although the primary product from canola is its oil content, canola meal is also a valuable protein resource for use in the animal feed industries.

Burel et al. (2000) examined the nutritional value both solvent and heat-treated rapeseed meals and also that of extruded *L. albus* kernel meal and extruded peas (Table A1.21). From this study, Burel et al. (2000) reported the digestibility characteristics of the dry matter, protein, energy and phosphorus contents of each of the test ingredients. Key findings from the work of Burel et al., (2000) were the significantly higher protein digestibility of *L. albus* kernel meal (96.2%) in comparison to the pea (87.9%) and rapeseed (90.9% and 88.5%) meals. However, despite having relatively poor protein/nitrogen digestibility, the energy digestibility of the solvent-extracted rapeseed meal (76.4) was as good as that of the *L. albus* kernel meal (77.0%). Notably, the heat-treated rapeseed meal (70.0%) had poorer energy digestibility. This is probably a direct reflection of the markedly lower fat content (43 g/kg DM cf. 9 g/kg DM) of the heat-treated meal.

In most cases, the relative digestibility of the energy of each of the ingredients was a direct response to the protein content of the ingredient and the relative protein digestibility of that ingredient. Low levels of starch in both the rapeseed meals support that limited dietary energy would be obtained from carbohydrates in these ingredients, with the majority of the energetic value being derived from their protein content and the small levels of residual fat.

Table A1.21 Proximal composition and nutritional value of various plant meals to fed rainbow trout. Data derived from Burel et al. (2000).

	Extruded peas	Extruded Lupin	SE-Rapeseed	HT-Rapeseed
Ingredient Proximate Composition				
Dry matter (g/kg)	909	928	937	915
Crude protein (g/kg DM)	260	434	431	433
Crude fat (g/kg DM)	4.5	100	48	9
Ash (g/kg DM)	33	46	79	82
NFE (g/kg DM)	612	348	379	391
Phosphorus (g/kg DM)	4.4	5.4	14.9	15.6
Nutrient Apparent Digestibility				
Dry matter (%)	66.3	69.7	70.8	66.6
Protein (%)	87.9	96.2	90.9	88.5
Energy (%)	68.9	77.0	76.4	70.0
Phosphorus (%)	42.6	61.9	26.4	41.8

SE-Rapeseed: Solvent Extracted Rapeseed meal. HT-Rapeseed: Heat Treated Rapeseed meal.

Mwachireya et al. (1999) evaluated the nutritional value of a range of canola products derived from the physical, enzymatic and chemical processing of a commercially produced canola meal (Table A1.22). The products ranged in composition from a protein content of 407 g/kg DM in the unporcessed canola meal to a protein content of 935 g/kg DM in the canola protein isolate (CPI). Notably lysine content of the was markedly reduced. Energy content of each of the meals was relatively constant, though substantially enriched by the production of a protein isolate. Anti-nutritional content of the meals were also reduced with processing.

Digestibility of the protein of each of the canola meals varied only minimally. The greatest improvement in protein digestibility was observed of the CPI. This finding was clearly supportive of the negative nutritional influences of the variety of anti-nutritional factors found in canola meals. Digestibility of the energy content of each of the meals primarily reflected that of the protein digestibility. The exception was that of the methanol-ammoniated canola meal, which had a significantly lower energy digestibility.

Table A1.22 Nutritional value of canola meal and various canola meal processed options. Data derived from Mwachireya et al. (1999).

	CCM	SCM	GCM	ACM	PCM	CPI
Ingredient Composition						
Dry matter (g/kg)	892	895	981	882	990	971
Crude protein (g/kg DM)	407	415	446	443	487	935
Lysine (g/kg DM)	66	63	59	67	51	37
Crude fat (g/kg DM)	76	75	55	43	47	34
Gross Energy (MJ/kg DM)	22.0	21.8	20.5	21.8	19.8	25.1
Ash (g/kg DM)	123	125	100	144	116	57
Phytate (g/kg DM)	44	46	12	56	26	3
Crude Fibre (g/kg DM)	119	80	115	94	123	22
Phenolics (mmol/kg DM)	3.0	2.7	0.0	1.2	0.5	1.0
Glucosinolates (mmol/kg DM)	9.8	10.1	1.0	0.0	0.0	0.0
Nutrient Apparent Digestibility						
Protein (%)	88.1	85.8	84.2	83.8	84.4	97.6
Energy (%)	55.6	52.3	56.1	41.4	51.5	84.7

CCM: commercial canola meal, SCM: sieved canola meal, GCM: α-galactosidase treated canola meal, ACM: methanol-ammoniated canola meal, PCM: phytase-treated canola meal, CPI: canola protein isolate.

Some of the earliest work though, on the use of canola or rapeseed meals, was that by Higgs et al. (1982). In this study these workers evaluated the potential of two varieties of rapeseed meals and also that of a rapeseed protein concentrate (RPC) (Table A1.23). This work was notable in that it presented some of the earliest work on the use of protein concentrates being used in fish diets. The performance of fish fed diets with the RPC was good. In most cases the specific growth rate of fish fed these diets was equivalent or superior to that of fish fed the reference diet. However, it was observed that these fish generally had poorer nitrogen retention than that of fish fed the reference diet. This observation is unusual given the specific growth rate and feed conversion data presented (Table A1.23). There were also differences observed between the two varieties of canola meals. The protein content of the Candle rapeseed meal was marginally higher than that of the Tower rapeseed meal (410 g/kg cf. 381 g/kg). The levels of glucosinolates introduced into the test diets by each variety was also different, with the higher protein variety of Candle containing about 663 mg/kg of meal and the Tower variety containing 557 mg/kg of meal. At lower inclusion levels of rapeseed meal, no declines in growth performance of the fish were observed. However, with the highest inclusion levels of the Tower rapeseed meal, approximately 25% inclusion, a decline in growth (as denoted by nitrogen retention) was noted. It was suggested that this was influenced by the higher level of glucosinolates in that diet (T3). Interestingly though no such decline was observed with the highest inclusion level of the Candle rapeseed meal (C3), which is notable because this meal had a higher level of glucosinolates.

A second study by Higgs et al. (1983) focussed on the protein quality of the Altex variety of canola meal when fed to chinhook salmon. In this study Higgs et al. (1983) fed iso-energetic diets, varying in protein content of 290, 390 and 490 g/kg. The canola meal was included to provide 0%, 11.5% and 23% of the total dietary protein, essentially replacing the fishmeal content of the diet. A second series of diets in the experiment examined the value of including triiodothyronine (T_3) at either 0, 5 or 25 ppm.

These workers suggested that the nutritive value of canola and fishmeal protein were similar when evaluated in the 290 g/kg and 390 g/kg protein diets. However, when the canola meal was included in the 490 g/kg protein diet a decrease in growth performance of the fish was observed. Primarily this was a response to deterioration of appetite of the fish. Poorer protein utilisation was also observed with the deteriorating growth and appetite. The supplementation of T_3 was shown to stimulate growth, but only in the fish fed the 490 g/kg protein diets. Notably improvements in growth were correlated with increasing inclusion of the canola meal. It was inferred from this that the supplementation of T_3 was only useful when the glucosinolate content of the canola meals was influencing the metabolism of the fish. From this study Higgs et al. (1983) concluded that Altex canola meal could comprise up to 25% of the dietary protein content without adversely influencing the protein quality of the diet. However this limit was contingent on the total glucosinolate level of the diet being kept below 2.65 mmol/kg. They also supported that higher inclusion levels of canola meal (30%) could be used, provided at least 5 ppm of T_3 was added to the diet.

These two studies by Higgs et al. (1982; 1983) provided good support that canola meals could be useful ingredients when included in diets for fish. They identified that glucosinolates were a potential problem with high inclusion levels, but that there was some potential for aversion of this by using dietary additives such as T_3. There was also some indication that there was inherent variability between different meal varieties, but that the development of protein concentrates offered some of the best potential.

A study by Teskered et al. (1995) examined the inclusion of normal and dephytinized rapeseed protein concentrate (RPC) when fed to rainbow trout. Three different sources of RPC were evaluated as partial or total replacements of fish meal in practical, iso-nitrogenous (430 g/kg) and isoenergetic (21.6 MJ/kg GE) diets. The three sources of RPC included undephytinized RPC, undephytinized solvent-treated control RPC and dephytinized RPC. Each was included in test diets at 19%, 39% and 59% of the total diet, which effectively allowed replacement of 33%, 66% and 100% of the dietary protein content.

Growth, survival, feed intake, feed efficiency of the fish was unaffected with the inclusion of either undephytinized RPC or dephytinized RPC at up to 66% replacement of the fishmeal. Although 100% replacement of the fishmeal did not influence feed intake of the fish, it did significantly reduce growth and accordingly feed efficiency. The authors concluded that undephytinized RPC, undephytinized solvent-treated control RPC or dephytinized RPC could replace fishmeal at 39% inclusion in the diet without loss of performance. Notably though the authors did not demonstrate either the relative or actual contributions of the protein content of the RPC's to growth of the fish. Ideally this study needed to be conducted with either protein limiting diets, to ensure efficiency of protein use, and/or include negative control treatments to demonstrate relative utilisation of the RPC's.

Table A1.23 Evaluation of different varieties of canola meal and a canola protein concentrate when fed to juvenile chinhook salmon, *Oncorhynchus tshawytscha*. Data derived from Higgs et al. (1982).

	Reference	T1	T2	T3	C1	C2	C3	B1	B2	B3
Ingredients (g/kg)										
Cottonseed meal	94	-	-	-	-	-	-	-	-	-
Canola meal (Tower)	-	120	159	297	-	-	-	-	-	-
Canola meal (Candle)	-	-	-	-	129	171	320	-	-	-
RPC (Bronowski)	-	-	-	-	-	-	-	80.6	106	199
Fishmeal	489	489	489	407	489	489	407	489	489	407
Dried whey	24	24	-	-	24	-	-	24	-	-
Wheat germ meal	43	43	-	-	43	-	-	43	-	-
Wheat middlings	96	61	84	10	70	96	30	110	149	130
DL-Methioine	4.3	3.2	2.5	2.2	2.6	3.4	2.0	4.5	4.6	4.9
Other ingredients	249.7	259.8	265.5	283.8	242.4	240.6	241	248.9	251.4	259.1
Diet Proximate Composition										
Dry matter (g/kg)	900	913	905	905	902	903	915	904	920	910
Crude protein (g/kg DM)	548	539	538	536	553	556	534	558	558	549
Crude fat (g/kg DM)	181	170	172	170	164	167	168	162	160	175
Ash (g/kg DM)	130	134	138	134	141	136	133	137	133	126
NFE	141	158	151	160	142	141	164	144	150	149
Gross Energy (MJ/kg DM)	22.4	22.0	22.0	22.0	21.9	22.0	22.0	21.9	22.0	22.3
Isothiocynates (mg/kg DM)	0	46	61	113	68	90	168	0	0	0
Oxazolidnethione (mg/kg DM)	0	21	28	52	18	23	44	0	0	0
Total Glucosinolates (mg/kg DM)	0	67	89	165	86	113	212	0	0	0
Growth performance										
Specific Growth Rate (%/d)	1.8	1.8	1.9	1.9	1.8	1.8	1.8	1.9	2.0	2.1
Nitrogen retention (%)	30.0	31.0	32.0	25.0	31.0	33.0	30.0	20.0	27.0	35.0
Food Conversion Ratio (g/g)	5.6	5.3	4.8	6.3	5.6	4.8	5.3	5.6	5.3	4.2

RPC: rapeseed protein concentrate. NFE: Nitrogen free extractives.

Table A1.24 Influence of dietary phytase on nutritive value of a canola protein concentrate when fed to rainbow trout. Data derived from Forster et al. (1999).

	Basal Diet	CPC	Phytase 500	Phytase 1500	Phytase 4500
Ingredients (g/kg)					
CPC	0	415.8	415.8	415.8	415.8
Fishmeal	520	173.2	173.2	173.2	173.2
Preglled wheat starch	80	92.5	92.4	92.2	91.6
Raw wheat starch	90.9	0	0	0	0
Phytase	0	0	0.1	0.3	0.9
$CaHPO_4$	0	10	10	10	10
DL-Methionine	1.4	3.7	3.7	3.7	3.7
Cellulose	20	0	0	0	0
Other ingredients	287.7	304.8	304.8	304.8	304.8
Diet Proximate Composition					
Dry matter (g/kg)	910	918	914	914	913
Crude protein (g/kg DM)	449	449	450	451	450
Crude fat (g/kg DM)	197	187	188	194	189
Ash (g/kg DM)	108	103	113	110	117
Phytate (g/kg DM)	n.d.	23.4	23.4	23	23
Gross Energy (MJ/kg DM)	22.6	21.9	22.4	22.3	22.3
Nutrient Apparent Digestibility					
Organic matter (%)	86.2	84.6	84.6	83.7	84.4
Protein (%)	92.6	95.5	95.7	95.7	95.7
Energy (%)	88.8	87.4	88.0	87.4	87.6
Phosphorus (%)	44.3	37.4	42.7	40.1	51.0
Phytate (%)	-	4.8	20.6	26.8	45.4
Growth performance					
Initial weight (g)	18.5	17.4	18.2	17.8	17.6
Final weight (g)	94.3	83.2	91.1	86.1	81.4
Gain (g)	75.8	65.8	72.9	68.3	63.8
DGC (%/d)	2.27	2.11	2.23	2.15	2.06
FCR	0.82	0.85	0.85	0.83	0.88

CPC: canola protein concentrate. DGC: daily growth coefficient. FCR: food conversion ratio.

A study by Forster et al. (1999) examined the potential of the exogenous enzyme phytase to improve the nutritional value of a canola protein concentrate (CPC). In this study improvements in the digestibility of protein in the diets were observed with the inclusion of CPC in the diet (Table A1.24). However, no improvements in the digestibility of organic matter or dietary energy content were observed with inclusion of CPC. The resultant effects of these changes in nutritional value on the growth performance of the fish were minimal, suggesting if anything only a slight deterioration in growth.

The addition of phytase to the diet resulted in significant improvements in the digestibility of phytate, and at the highest inclusion level of phytase, improvements in phosphorus digestibility were also noted. Although differences in growth were observed, notably at the highest phytase inclusion levels a decrease in growth was observed, generally these were not consistent with any pattern, but instead appeared random differences.

The specific effects of rapeseed glucosinolates were only examined recently (Burel et al., 2001). In a study using rainbow trout, Burel et al. (2001) fed either of two rapeseed meals containing glucosinolates at concentrations of 26 mmol/kg or 40 mmol/kg. Each of the rapeseed meals were included in diets at incremented levels. Inclusion of T_3 in a second experiment was also examined, as was the injection of thyroid stimulating hormone (TSH) into the fish in a third experiment. Changes in the levels of plasma triiodothyronine (T_3) and thyroxine (T_4) were examined from fish fed incremented levels of rapeseed meal. Baseline levels of T_3 and T_4 were 3.5 and 5.8 ng/mL respectively. This experiment is clearly summated by examination of the influences of the highest inclusion level of rapeseed meal. Inclusion of 30% rapeseed meal in the diet (~7.8 mmol/kg of diet), resulted initially in increases in T_3 levels at day 7 that were followed by declines in the plasma T_3 levels and by day 58 they had reduced to 6.0 ng/mL. This was significantly less that those of the control fed fish at 8.0 ng/mL. However, the levels of T_4 in the plasma of the fish were more influenced by the inclusion of rapeseed meal in the diet than those of the T_3 levels. By day 58 the level of plasma T_4 in fish fed the 30% rapeseed meal diet had declined to only 3.8 ng/mL. In addition to the changes in thyroid hormone levels, poorer growth and feed utilisation was also observed from fish in the rapeseed meal fed treatments.

In a second aspect to this study T_3 was added at either of two levels to a rapeseed meal diet. The inclusion of T_3 in the diet significantly improved the levels of plasma T_3 in fish fed the rapeseed meal diet. No changes in plasma T_4 levels were noted. Despite these improvements in plasma T_3 levels, growth and feed utilisation were still poorer than that of fish in the control treatment.

A third experiment in the study of Burel et al. (2001) examined the influence of thyroid stimulating hormone (TSH) injection on the thyroid hormone levels of fish fed the control and rapeseed meal diets. Injection of fish with TSH significantly increased the plasma levels of T_4 within 24 h, but no changes in T_3 levels were noted. Injection of TSH into rapeseed fed fish had no influence on either T_4 or T_3 levels. These workers claimed that the influence of rapeseed meal glucosinolates on the thyroid axis of fish was elicited primarily by the blockage of the activity of the thyroid follicles. This was clearly supported by the evidence of suppression of TSH influence on T_4 production.

Summary

- *The nutritional value of canola meals was observed to be similar to that of many other plant resources, with good dry matter, energy digestibilities and average protein digestibilities. Notably, an increase in protein, energy and dry matter digestibilities have been observed with the removal of anti-nutritional factors and the development of protein concentrates*
- *Evaluation of the inclusion level of some canola meals has shown that up to 30% inclusion can be achieved without goitrogenic problems, though this depends on the meal variety. More specifically limits to inclusion have been suggested to be restricted, not by canola meal per se, but rather the level of glucosinolates in the diet. A critical threshold of 2.65 mmol/kg of diet has been suggested.*
- *The more recent studies have generally focussed more on the development and use of a protein concentrate. Such CPC's have been included in diets in excess of 40% of the diet without problem.*
- *The key anti-nutritional problems with canola and rapeseed meals lie in their content of glucosinolate and their breakdown products of such as isothyocyanates, nitriles and thiocyanates. Other anti-nutritionals such as phytate and crude fibre content have been identified and alleviation strategies developed. However management of glucosinolate related problems remain a key issue.*

A1.6 The Next Step Forward

A1.6.1 Technology options for plant protein meal use in aquaculture feeds

For plant protein meals to gain greater acceptance and to be more actively considered by the aquaculture feed processing industry sector, several issues need to be resolved. The nature of these issues are considerable and varied.

From the perspective of the aquaculture feeds industry, the primary value in most plant protein meal commodities is in its protein value. Although these products will also generally contribute some dietary lipids and energy, it is the protein value for which they are primarily sought. Because of this, any increase in the protein content of the grain or meals thereof, will substantially increase the value of those products to the aquaculture sector. Ideally, the value of the grain to the aquaculture sector should be proportional to the digestible or available protein or energy content. The development of higher protein and digestible energy varieties of each of the feed grains could achieve considerable improvements to returns to the grain sector.

Determination of the level of nutritional value within most grain varieties is a critical aspect of being able to attribute economic value to the grain. The variability of nutritional value of a such commodity can also impact on its perceived value, with reduced variability levels being favoured in that they allows greater confidence in formulating diets closer to the animals requirements. Assessment of the variability in the chemical composition can be readily obtained using standard analytical techniques, and recent developments in the use of Near-Infra-Red Reflectance (NIR) spectrometry have allowed the development of some rapid assessment systems (Aufrere et al., 1996), However, determination of nutritional value and the assessment of its variability has been a comparatively more difficult and slower parameter to assess. Presently there is a paucity of knowledge on the intrinsic nutritional variability within a variety of different feed grains, from different regions, kept under different storage conditions or even of variable age. This is even more so the case for nutritional assessment in aquaculture species.

With an increased focus on the environmental responsibilities of aquaculture industries worldwide, the potential attributes of several feed grains as an aquaculture feed commodity are considerably strengthened. Notably, this could add considerable value to their use, particularly in diets fed to species in phosphorus sensitive environments.

While the nutrient composition of plant meals is often the positive selling point of these ingredients, it is clearly the anti-nutritional factor content that is a major “Achilles heel”. The overall level of research and understanding of the influences of the key anti-nutritional factors on fish is far from comprehensive let alone satisfactory. Presently detailed research has examined the influences of protease inhibitors, saponins and phytate. Some suggestive studies on the influence of glucosinolate breakdown products and oligosaccharides have been examined, though they are far from conclusive. Other anti-nutritionals such as alkaloids and tannins have been relatively neglected.

Another potential limitation, however, to the use of most feed grain products in diets for aquaculture species is the level of non-starch polysaccharides (NSP) in the grain. Effectively, NSP in most aquaculture species has no nutritional value, and acts little more than as fibre. Furthermore, there are potentially adverse effects from too high levels of fibre in some aquaculture species, with the potential for the reduction of the value of other nutrients in the diet (Saxby et al., 2001).

To address the issue of NSP levels in lupins there are several options. One option is that of physical processing of the grain to remove as much fibre and NSP as possible. This effectively creates protein concentrates, with reduced NSP levels. Methods such as air classification and solvent extraction to cost-effectively create such protein concentrates have already been reported (Evans, 1998), though there is limited information on their nutritional influences. Another option is the use of supplementary enzymes, both as a preliminary processing method of the grain/meal and as a dietary addition. Key enzymes for use in such scenarios include the α galactosidases and xylanases, though phytase use has already been shown to be effective in some species, and in some instances had accessory attributes that have added further value.

While considerable benefits have been identified from the dehulling process to produce a protein enriched kernel meal, only a few studies have shown benefit from the evaluation of protein concentrates or isolates. This observation contravenes the rationale of the importance of protein level in the grain, which is generally well accepted. In many instances where the potential value of protein concentrates has not been identified, the studies have frequently lacked the appropriate controls or not had an appropriate design. This observation places potentially a more positive speculation on the value of protein concentrates, provided they can be made to be cost-effective on a per unit protein basis. Therefore, co-use production, where value is also identified or made of the NSP or lipid fraction, needs to be developed. This capacity to be able to attribute value to both portions of the grain is important in being able to spread cost-recovery of the processing.

Presently, most modern, intensive fish farming diets contain some plant protein meal resources. In most cases this niche is filled by soybean meal, or some other soybean meal derived products. Based on the data presented in this review there is no consistent, clear evidence to suggest that soybean meal is nutritionally superior in quality to any of the other plant protein meals to which it has been compared. However, the overall protein content of soybean meals no doubt plays a dominant role in its popularity as a feed ingredient in aquaculture diets. Provided the other plant protein meals can prove to be cost competitive on a per unit digestible protein and/or energy basis, then they should have the potential to gain a greater level of market acceptance and use in this feeds sector.

A1.7 References

Allan, G.L., Parkinson, S., Booth, M.J., Stone, D.A.J., Jantrarotai, W. (2000) Replacement of fish meal in diets for Australian silver perch *Bidyanus bidyanus*: Digestibility of alternative ingredients. Aquaculture, 186, 293-310.

Anderson-Hafermann, J.C., Zhang, Y. and Parsons, C.M. (1993). Effects of processing on the Nutritional Quality of Canola Meal. Poultry Science. 72, 326-333.

Arnesen, P., Brattas, L.E., Olli, J. and Krogdahl, A. (1989) Soybean carbohydrates appear to restrict the utilization of nutrients by Atlantic salmon (*Salmo salar* L.). In: Takeda, M, Watanabe, T. (Eds), The Current Status of Fish Nutrition in Aquaculture. The Proceedings of the Third International Symposium on Nutrition and Feeding in Fish. Tokyo University of Fisheries, Toba, Japan, pp. 273-280.

Aufrere, J., Graviou, D., Demarquilly, C., Perez, J.M. and Andrieu, J. (1996) Near infrared reflectance spectroscopy to predict energy value of compound feeds for swine and ruminants. Animal Feed Science and Technology 62, 77-90.

Austreng, E., Skrede, A. and Eldegard, A. (1979) Effect of dietary fat source on the digestibility of fat and fatty acids in rainbow trout and mink. Acta Agriculture Scandinavia 29, 119-126.

Bangoula, D., Parent, J.P., Vellas, F. (1993) Nutritive value of white lupin (*Lupinus albus* var Lutop) fed to rainbow trout (*Oncorhynchus mykiss*). Effects of extrusion cooking. *Reproduction and Nutrition Developments* 33, 325-334.

Booth, M., Allan, G.L., Frances, J. and Parkinson, S. (2001) Replacement of fishmeal in diets of silver perch: VI. Effects of dehulling and protein concentration on the digestibility of four Australian grain legumes in diets for silver perch (*Bidyanus bidyanus*). *Aquaculture* 196: 67-85.

Bureau, D.P., Harris, A.M. and Cho, C.Y. (1998) The effects of purified alcohol extracts from soy products on the feed intake and growth of chinhook salmon (*Oncorhynchus tshawystcha*) and rainbow trout (*Oncorhynchus mykiss*). Aquaculture 161, 27-43.

Burel, C., Boujard, T., Corraze, G., Kaushik, S.J., Boeuf, G., Mol, K.A., Van der Geyten, S. and Kuhn, E.R. (1998) Incorporation of high levels of extruded lupin in diets for rainbow trout (*Oncorhynchus mykiss*): nutritional value and effect on thyroid status. Aquaculture 163, 325-345.

Burel, C., Boujard, T., Tulli, F. and Kaushik, S. (2000a) Digestibility of extruded peas, extruded lupin, and rapeseed meal in rainbow trout (*Oncorhynchus mykiss*) and turbot (*Psetta maxima*). Aquaculture 188, 285-298.

Burel, C., Boujard, T., Kaushik, S., Boeuf, G., Van Der Geyten, S., Mol, K.A., Kuhn, E.R., Quinsac, A., Krouti, M., Ribailler, D. (2000b) Potential of plant-protein sources as fishmeal substitutes for turbot (*Psetta maxima*): growth, nutrient utilisation and thyroid status. Aquaculture 188, 285-298.

Burel, C., Boujard, T., Kaushik, S.J., Boeuf, G., Mol, K.A., Van der Geyten, S., Darras, V.M., Kuhn, E.R., Pradet-Balade, B., Querat, B., Quinsac, A., Krouti, M. and Ribaillier, D. (2001) Effects of rapeseed meal glucosinolates on thyroid metabolism and feed utilisation in rainbow trout. General and Comparative Endocrinology 124, 343-358.

Carre, B., Brillouet, J.M. and Thibault, J.T. (1985) Characterisation of polysaccharides from white lupin (*Lupinus albus* L.) cotyledons. Journal of Agricultural and Food Chemistry, 33, 285-292.

Carter, C.G. and Hauler, R.C. (1999) Evaluation of phytase in Atlantic salmon, *Salmo salar* L., feeds containing fishmeal and different plant meals. In: Fishmeal replacement in Aquaculture Feeds for Atlantic salmon. Project 93/120. Fisheries Research and Development Corporation, Canberra, Australia. pp 34-45.

Carter, C.G. and Hauler, R.C. (2000) Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. *Aquaculture* 185, 299-311.

Fenwick, G.R., Price, K.R., Tsukamato, C. and Okubo, K. (1991) Saponins. In: Toxic Substances in Crop Plants (Ed. J.P.F. Dmello, C.M. Duffus and J.H. Duffus). The Royal Society of Chemistry. Cambridge. pp. 285-328.

Forster, I., Higgs, D.A., Dosanjh, B.S., Rowshandeli, M. and Parr, J. (1999) Potential for dietary phytase to improve the nutritive value of canola protien concentrate and decrease phosphorus output in rainbow trout (*Oncorhynchus mykiss*) held in 11C fresh water. Aquaculture 179, 109-125.

Gdala, J., Jansman, A.J.M., Buraczewska, L., Huisman, J. and van Leeuwen, P. (1997) The influence of -galactosidase supplementation on the ileal digestibility of lupin seed carbohydrates and dietary protein in young pigs, Animal Feed Science and Technology 67(2-3): 115-125.

Glencross, B.D. (2001) Feeding lupins to fish – A review of the nutritional and biological value of lupins in aquaculture feeds. http://www.wa.gov.au/westfish/res/broc/report/lupin. pp 126.

Glencross, B.D., Curnow, J.G. and Hawkins, W.E. (2003). Evaluation of the variability in chemical composition and digestibility of different lupin (*Lupinus angustifolius*) kernel meals when fed to rainbow trout (*Oncorhynchus mykiss*). Animal Feed Science and Technology. IN PRESS

Glencross, B.D., and Hawkins, W.E. (2003). A comparison of the digestibility of several lupin (*Lupinus* spp.) kernel meal varieties when fed to either rainbow trout (*Oncorhynchus mykiss*) or red seabream (*Pagrus auratus*). Aquaculture Nutrition. ACCEPTED

Gomes, E.F., Rema, P. and Kaushik, S.J. (1995) Replacement of fish meal by plant proteins in the diet of Rainbow Trout (*Oncorhynchus mykiss*): digestibility and growth performance. *Aquaculture* 130, 177-186.

Griffiths, D.W. (1991) Condensed tannins. In: Toxic Substances in Crop Plants (Ed. J.P.F. Dmello, C.M. Duffus and J.H. Duffus). The Royal Society of Chemistry. Cambridge. pp. 180-201.

Harris, D.J. and Jago, J. (1984) Report on chemical composition of sweet lupin seed from Western Australia. Government Chemical Laboratories. Perth. pp 12.

Higgs, D.A., McBride, J.R., Markert, J.R., Dosanjh, B.S., Plotnikoff, M.D. and Clarke, W.C. (1982) Evaluation of Tower and Candle rapeseed protein concentrate as protein supplements in practical dry diets for juvenile chinook salmon (*Oncorhynchus tshawytscha*). Aquaculture 29, 1-31.

Higgs, D.A., Fagerlund, U.H.M., McBride, J.R., Plotnikoff, M.D., Dosanjh, B.S., Markert, J.R. and Davidson, J. (1983) Protein quality of Altex canola meal for juvenile chinook salmon (*Oncorhynchus tshawytscha*) considering dietary protein and 3,5,3’-triiodo-L-thyronine content. Aquaculture 34, 213-238.

Kaushik, S.J., Cravedi, J.P., Lalles, J.P., Sumpter, J., Fauconneau, B. and Laroche, M. (1995) Partial or total replacement of fishmeal by soybean protein on growth, protein utilisation, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 133, 257-274.

Krogdhal, A., Lea, T.B. and Olli, J.J. (1994) Soybean proteinase inhibitors affect intestinal trypsin activities and amino acid digestibilities in rainbow trout (Oncorhynchus mykiss). Comparative Biochemistry and Physiology 107A, 215-219.

Melicion, J.P., Zerniki, W. and Conan, L. (1987) L’aliment extrude colza-pois: une nouvelle matiere premiere? Review de Alimention au Animales. 2, 129-136.

Morales, A.E., Cardenete, G., De la Higuera, M. and Sanz, A. (1994) Effects of dietary protein source on growth, feed conversion and energy utilisation in rainbow trout, *Oncorhynchus mykiss*. Aquaculture 124: 117-126.

Mwachireya, S.A., Beames, R.M., Higgs, D.A. and Dosanjh, B.S. (1999) Digestibility of canola protein products derived from the physical, enzymatic and chemical processing of commercial canola meal in rainbow trout, *Oncorhynchus mykiss* (Walbaum) held in fresh water. Aquaculture Nutrition 5, 73-82.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M. (2000) Effect of aquaculture on world fish supplies. Nature 405, 1017 – 1024.

Petterson, D.S. (2000) The use of Lupins in Feeding Systems – Review. Asian Australian Journal of Animal Science, 13 (6), 861-882.

Petterson, D.S., Sipsa, S. and Mackintosh, J.B. (1997) The Chemical Composition and Nutritive Value of Australian Pulses. Grains Research and Development Corporation, Canberra, Australia. pp 65.

Refstie, S., Storebakken, T. and Roem, A.J. (1998) Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture 162, 301-312.

Refstie, S., Svihus, B., Shearer, K.D. and Storebakken, T. (1999) Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soyabean products. Animal Feed Science and Technology 79, 331-345.

Robaina, L., Izquierdo, M.S., Moyano, F.J., Socorro, J., Vergara, J.M., Montero, D. and Fernandez-Palacios, H. (1995) Soybean and lupin seed meals as protein sources in diets for gilthead seabream (*Sparus aurata*): nutitional and histological implications. Aquaculture 130, 219-233.

Saxby, S.A., Williams, I.A. and Glencross, B.D. (2001). *Dietary fillers reduce growth and apparent digestibilities of pink snapper*. In: Recent Advances in Animal Nutrition in Australia, Volume 13 (J. Corbett ed.). The University of New England, Armidale. p 10A.

Storebakken, T., Shearer, K.D. and Roem, A.J. (1998) Availability of protein, phosphorus and other elements in fish meal, soy-protein concentrate and phytase-treated soy-protein-concentrate-based diets to Atlantic salmon, *Salmo salar*. Aquaculture 161, 365-379.

Tacon, A.G.J. (1990) Standard Methods : For the Nutrition and Feeding of Farmed Fish and Shrimp. Argent Laboratories Press. Redmond, Washington, USA.

Teskered, Z. Higgs, D.A., Dosanjh, B.S., McBride, J.R., Hardy, R.W., Beames, R.M., Jones, J.D., Simell, M., Vaara, T. and Bridges, R.B. (1995) Assessment of undephytinized and dephytinized rapeseed protein concentrate as sources of dietary protein for juvenile rainbow trout (*Oncorhynchus mykiss*). Aquaculture 131, 261-277.

USDA (United States Department of Agriculture) (2001) Internet site: http://www.usda.gov.

van Barneveld, R.J. (1999) Understanding the nutritional chemistry of lupin (*Lupinus* spp.) seed to improve livestock production efficiency. Nutrition Research Reviews 12: 203-230.

Vandenberg, G.W., de la Noue, J. (2001) Apparent digestibility comparison in rainbow trout (*Oncorhynchus mykiss*) assessed using three methods of faeces collection and three digestibility markers. Aquaculture Nutrition 7, 237-245.

White, C.E., Campbell, D.R. and McDowell, L.R. (2000) Effects of dry matter content on trypsin inhibitors and urease activity in heat treated soya beans fed to weaned piglets. Animal Feed Science and Technology 87, 105-115.

Appendix 2 – Valuing Australian grains and grain products in the international aquaculture feeds sector

A2.1 Introduction

The Australian grains industry has been considering the opportunities and potential for gaining access to domestic and international markets in the aquaculture[1] feeds sector. The challenges involved for the Australian grains industry to achieve these goals are similar to those pressures faced by many other countries and indeed many other resource sectors, attempting to take market share of any particular market. In addition, there are key issues specific to the aquaculture feeds sector that need to be made aware of to capitalise on these opportunities, either domestically or internationally.

Fundamentally, aquaculture feed manufacturers, like all businesses respond primarily to the pressure to make a profit. In the feeds sector this is achieved through two main avenues, by obtaining maximum market share and price and secondly by minimising production costs. For the purposes of this document we will assume that operating costs (excluding purchase of raw materials) are constant. In this scenario, market share will be driven by a range of variables including such features as proximity of supplier to market, customer loyalty, quality of product and price of product. The second avenue, of reducing production costs can also play a prominent role in obtaining and maintaining market share. This achieved by allowing the manufacturer to keep the product cost competitive through keeping the costs of the product to a minimum.

The key to minimising production costs is the economic sourcing of raw materials; namely the supply of feed ingredients. Traditionally the use of fishmeal has been central to the commercial manufacture of aquaculture feeds. Indeed, not only is fishmeal an ideal source of protein and energy for aquaculture diets, but in many instances it is also more cost effective than many alternatives. This will become more apparent later in the review.

However, fishmeal tends to be something of a volatile commodity. Notably there is considerable influence of climatic effects, such as that of El nino events, on global fishmeal production and subsequently on fish meal supply and price. In addition to this volatility, the total fish meal and fish oil supply is relatively static at 6 million and 1 million tonnes per annum respectively (Figure A2.1).

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[1] In this article, the term fish and aquaculture is used to cover just fin-fish (primarily those fed high-nutrient dense diets), as the nutrient requirements of most invertebrate species are often quite different and require different formulation strategies.

Therefore, with increasing global production of aquaculture feeds (presently increasing at 10-15% per annum), and all fish meal resources presently accounted for, the need for alternative protein resources in aquaculture feeds is clear and present. There are also other considerable incentives to use alternative ingredients in aquaculture diets. These incentives range from economic, social and political reasons (Naylor et al., 2000).

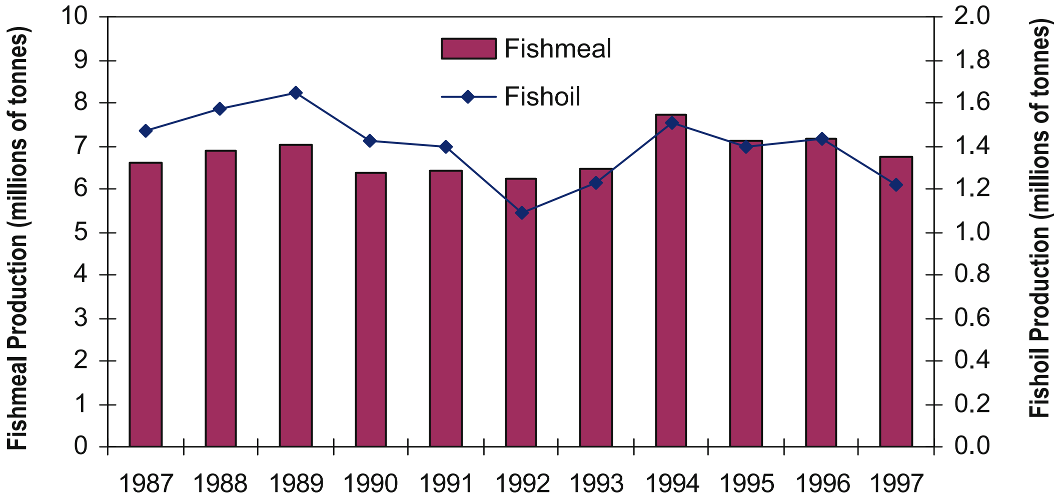

Figure A2.1 Global production of fishmeal and oil over the past decade (data derived from http://apps.fao.org).

A2.2 Alternative protein resources in aquaculture feeds

The identification and development of alternatives to the use of fishmeal in aquaculture diets remains a high priority for improving the sustainability of aquaculture. To improve resource security and reliability for aquaculture feeds, one option has been to increase the use of plant protein meals. Numerous studies have been undertaken on a range of plant protein resources with mixed results (Moyano et al., 1992; Gomes et al., 1995; Allan et al., 1997; Carter et al., 2000).

In Appendix I, the evaluation and use of alternatives to fishmeal were discussed in detail, as were some of the alternatives to fish oil use. There are numerous issues presently limiting the inclusion of plant protein meals in aquaculture diets. Some of these issues also have a direct bearing on the value of the plant protein meals to the aquaculture feed sector. One of these perceived issues is the less appropriate amino acid composition of the plant protein meals relative to fishmeal. While the amino acid composition of protein resources is important to their value in diets for terrestrial monogastric animals, like pigs and poultry, it is considerably less important to aquaculture species.

A2.3 Placing a value on plant protein commodities

Using least-cost linear formulation techniques and a range of hypothetical plant protein resources it was possible to examine a range of factors that influence the relative value/price of those hypothetical plant protein resource. To do this the software package Feedmania™ (ABRI, University of New England, NSW, Australia) was used with emphasis on the effective price each resource would have to be for it to be considered to be cost effective under a range of formulation constraints.

Issues examined in this study include the influence of plant protein (PP) resource protein content, fish meal price, diet energy content and diet phosphorus content. In evaluating the influence of these factors on the prospective price of each diet, the diet specifications used were based on those required by a technically advanced, modern high-energy feed for Rainbow trout and Atlantic salmon (PIVOT Aquaculture, 2000).

A2.4 Relative irrelevance of amino acid composition of the plant protein resource

Fundamentally, all animals require both amino acids and energy from which to grow. Without exception, the essential amino acids are obtained from the protein content of an animal's diet. Therefore it would seem reasonable that the amino acid composition of the protein source is critical to its efficient use. This is the case in the nutrition of many species, notably so with pigs and poultry. With most fin-fish however, unlike that with pigs and poultry, dietary energy supply can only rely minimally on that provided by carbohydrates, with the primary energy sources being those from fat and protein. This difference requires that a certain level of protein use inefficiency to be catered for, so as to allow for metabolism of protein for energetic reasons. Because of this the total level of dietary protein, and therefore most essential amino acids, in most fish diets is surplus to most amino acid requirements. It is only through the substitution of alternative protein resources for fishmeal and at considerable levels, that the potential for amino acid limitation is even a consideration.

In addition to the issues associated with minimal amino acid limitations encountered through routine formulation, aquaculture feeds, like those of pigs and poultry have also shown good capacity to utilise supplementary crystalline amino acids (Rodehutscord et al., 2000). What this implies is that even if the use of novel protein resources introduces amino acid limitations, then these can be easily and cost-effectively ameliorated through the use of crystalline amino acids.

In Tables A2.1 and A2.2 several diets were formulated to allow evaluation of the influence of a range of plant protein resources on the amino acid composition of the diets. Each diet was formulated to provide the same gross nutrient specifications. The specifications catered to are those typically used in a modern salmonid diet. The proximate specifications of each test ingredient used in the formulations are also detailed. Notably, the requirements for lysine and methionine, usually the two first limiting amino acids, in each diet are also indicated. These values are determined based on the total dietary protein content and the relative proportions of that protein required as either lysine or methionine (Kaushik, 1998).

In each of the diets the objective of each formulation was to formulate to the required specifications, by blending only fishmeal and the plant meal of object. Fish oil content was also modified to accommodate the required level of dietary fat. Diets were not formulated on a least-cost basis, as this requires the somewhat arbitrary assignment of a preordained value to each commodity that would have countered the argument being examined. In undertaking these formulations a range of outcomes are observed. Notably, the absolute level of inclusion of the plant protein resource is strongly linked to that of its relative protein level. In the case of the common plant protein resources evaluated it is noted that soybean meal (48% protein) had the highest inclusion level. This point of the level of influence of protein level is further noted with the hypothetical use of Lupin Protein Concentrates (LPC). In the examples presented in this study, two LPC's of 50% and 60% protein are evaluated. LPC was used as an example in preference to other potential protein concentrates because of its amino acid composition was more likely the induce a limitation in diet amino acid composition.

Table A2.1. Ingredient specifications used in the examination of dietary amino acid limitations using formulations containing plant protein meal components.

	Fishmeal	SE Canola	Lupin Kernel	SE Soybean	LPC50	LPC60
Dry matter	900	920	910	920	900	910
Fat	90	25	65	25	50	15
Protein	650	350	380	480	500	600
Carbohydrate	0	477	460	352	320	255
Phosphorus	25	7	3	6	4	3
Ash	150	68	5	63	30	40
Lysine	52.6	16.8	14.6	29.6	19.2	23.1
Threonine	26.8	15.1	10.9	18.4	14.3	17.2
Methionine	18.5	6.8	2.0	6.6	2.6	3.2
Isoleucine	30.0	13.8	12.2	23.0	16.1	19.3
Leucine	50.0	24.0	21.2	37.0	27.9	33.5
Tryptophan	8.1	4.2	3.1	7.0	4.1	4.9
Valine	34.0	17.6	11.7	23.6	15.4	18.5
Phenylalanine	26.2	13.9	11.8	23.7	15.5	18.6
Histidine	16.3	9.0	7.9	12.2	10.4	12.5
Arginine	50.0	19.3	35.9	35.8	47.2	56.7

SE: Solvent-Extracted, LPC: Lupin Protein Concentrate

Table A2.2. Examination of dietary amino acid limitations using formulations containing plant protein meal components.

		Reference	SE Canola	Lupin Kernel	SE Soybean	LPC50	LPC60	LPC60+dIMET
DIET SPECIFICATIONS (%)								
Pre-mix vitamins		0.5	0.5	0.5	0.5	0.5	0.5	0.5
Fish oil		16.2	16.6	16.0	17.3	16.8	19.3	19.3
Wheat flour		20.0	10.0	10.0	10.0	10.0	10.0	10.0
Soybean meal		0.0	0.0	0.0	25.7	0.0	0.0	0.0
Canola meal		0.0	17.0	0.0	0.0	0.0	0.0	0.0
Lupin kernel		0.0	0.0	19.9	0.0	0.0	0.0	0.0
LPC 50%		0.0	0.0	0.0	0.0	30.4	0.0	0.0
LPC 60%		0.0	0.0	0.0	0.0	0.0	46.4	46.1
Fish meal		63.3	55.9	53.6	46.5	42.3	23.8	23.8
DL-Methionine		0.0	0.0	0.0	0.0	0.0	0.0	0.3
Dry matter (g/kg)		915	919	917	921	920	922	919
Protein (g/kg)		450	450	450	450	450	450	450
Fat (g/kg)		220	220	220	220	220	220	220
Carbohydrate (g/kg)		147	155	165	164	179	192	188
Phosphorus (g/kg)		16	15	14	13	12	8	8
Gross Energy (MJ/kg)		21.8	22.0	22.2	22.1	22.3	22.6	22.6
	REQD							
Lysine	19.8	33.3	32.3	31.1	32.1	28.1	23.2	23.1
Methionine	7.3	11.7	11.5	10.3	10.3	8.6	5.9	7.4

REQD: Required level of amino acids based on diet protein and energy specifications

What is notable about the highest protein level LPC is that it is only when this resource is used at a high inclusion level that an amino acid limitation in the diet is encountered (Table A2.1). However, it is also shown that such a limitation is easily averted with small amounts of supplementary crystalline DL-Methionine.

All of these factors combine to suggest, that in most instances, the amino acid composition of plant protein meals is largely irrelevant to aquaculture diets. Indeed it should be reiteratied that nutritionists are seldom "looking" for the perfect ingredient, but rather a suite of complementary ingredients that can be blended together to provide the required nutrients, in a functional pellet, at the lowest cost (van Barneveld, 1998). In effect, because of this relative lack of importance of the amino acid composition of the plant protein resources, all subsequent modeling of the value of plant protein resources and factors influencing that value, in this study are determined solely on the basis of the protein content of a hypothetical plant protein resource.

A2.5 Influence of plant meal protein content on plant meal resource value

Fundamentally, PP resources are included in aquaculture diets for their protein value. Notably, the higher the protein content of the meal, the greater potential it has to replace the fishmeal portion of the diet. Therefore it reasons that the closer the protein content of the plant meal to that of the fishmeal then also the closer the value of the plant meal to that of fishmeal.

To examine this concept a series of diets were formulated and the hypothetical PP resources included as options, despite being priced above that of fishmeal. In doing so, the software used in this study allowed the determination of the effective price that the PP would have to be reduced to for it to be considered a viable option to include in the diet. By varying the price of the fishmeal component of the diet the effect of this variable could also be examined.

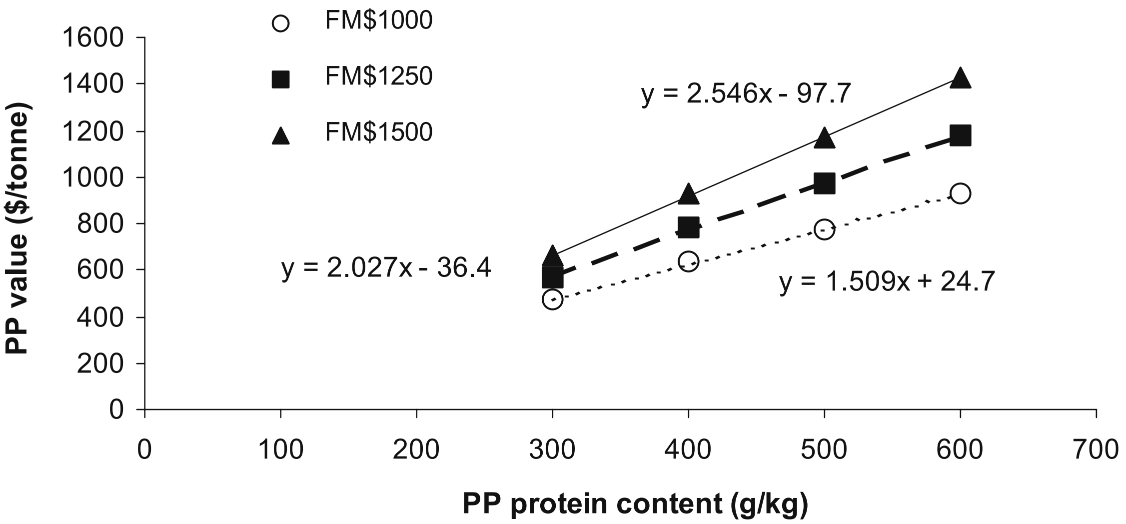

Figure A2.1 Effect of plant protein meal protein content on the theoretical value of that plant protein resource including influences of fishmeal price.

A direct linear response between the protein content of a plant meal and its value was determined (Figure A2.1). Typically the value of the PP resources increased with increasing protein content. For example, an increase in value of the PP resource from $472 a tonne to $930 a tonne, when fishmeal was $1000 a tonne was observed. Similarly, an increase in value of the PP resource from $660 a tonne to $1430 a tonne, when fishmeal was $1500 a tonne was also observed.

However, it should be noted that this model does not take into account competition among PP resources and relative inclusion levels of various ingredients under current pricing levels. This will be discussed later in this document, with a focus on competition based around the use of high-protein solvent extracted soybean meal.

It should also be noted that such modeling also does not take into account price depressing effects of any anti-nutritional factors or undesirable high fibre levels in the PP resource.

However, it can be noted that the extent of the relationship varies as a function of the cost of the fishmeal. The rate at which the value of the PP resources increases with increasing protein content occurs at a greater rate when the fishmeal price is higher. This relationship is discussed further below.

A2.6 Influence of fishmeal price on plant protein resource value

The potential price payable for PP resources used in aquaculture feeds depends strongly on the price paid for fishmeal (Figure A2.2). This becomes something of an issue because of the volatility in price and supply of this commodity. Notably in the modeling of the effects of this on the value of PP resources in this study, three price options for fishmeal were included. The data here also clearly shows that there is a direct relationship between the value of the PP resources and the price of fishmeal. For example, an increase in value of the PP resource from $639 a tonne to $933 a tonne, when the PP resource had 400 g/kg protein was observed as the cost of the fishmeal increased from $1000 a tonne to $1500 a tonne. Similarly, an increase in value of the PP resource from $930 a tonne to $1430 a tonne when the PP resource had 600 g/kg protein was observed over the same range of fishmeal prices.

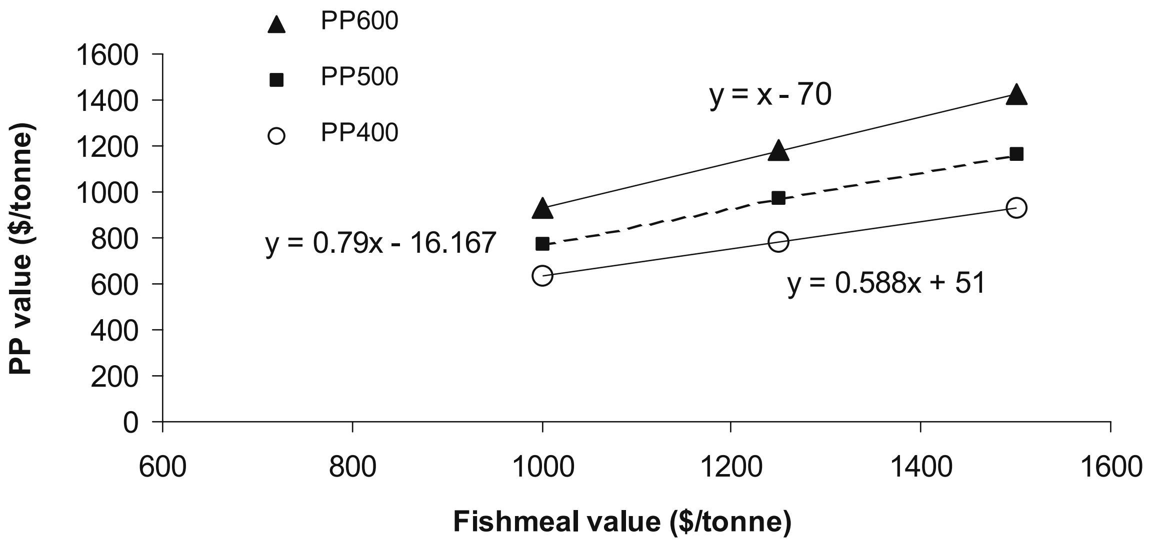

Figure A2.2 **Effect of fishmeal value on the value of plant protein (PP), including influences of variable protein content of plant protein.**

A2.7 Combined influence of multiple factors

The two previous sections have shown that the potential price payable for PP resources used in aquaculture feeds depends strongly on both the price paid for fishmeal and also the protein content of the PP resource. Because of this clear inter-relationship between the PP resource protein content and fish meal price on the value of the PP resource, this relationship is better described as a three-dimensional one using the following equation: PP$ per tonne = -897.092 + 2.027x (PP protein content) + 0.688 x (fishmeal $ per tonne).

What is observed from this equation, other than those details mentioned previously, is that the influence of PP resource protein content has a greater influence of the PP resource value than the influence of the fishmeal price on PP resource value. This finding has many implications on defining the value of PP resources and will also be broached again in the discussion of the influence of other plant commodities on the value of plant protein resources. Fundamentally this relationship details that theoretically, the influence of protein content is of greater importance than that of the value of other competitor resources.

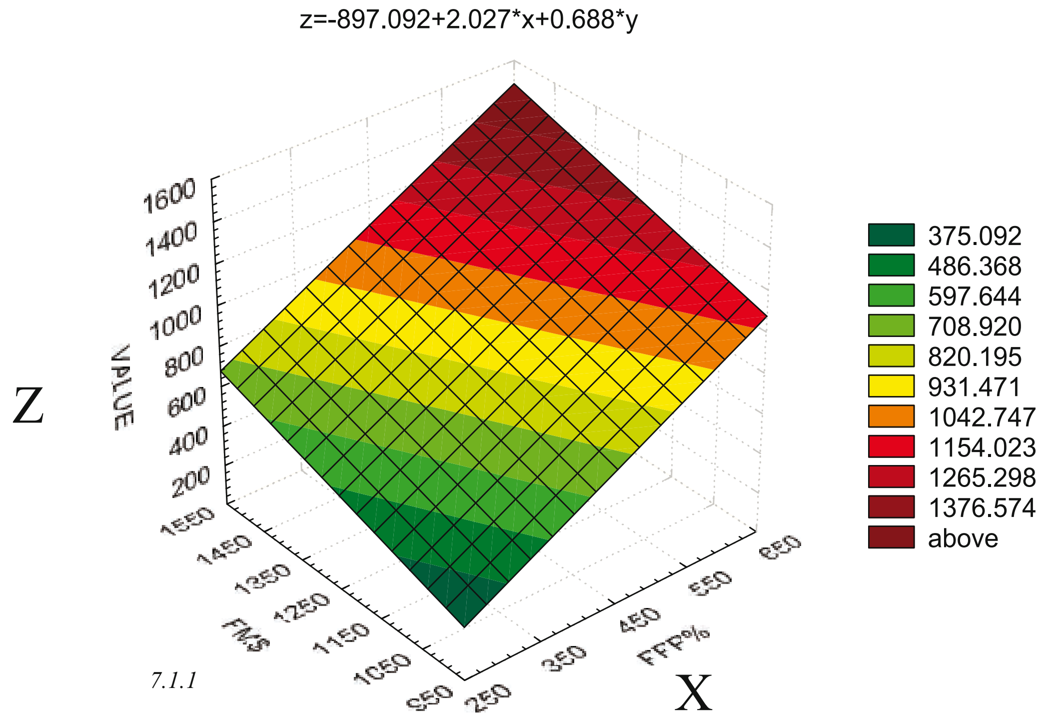

Figure A2.3 The theoretical three dimensional relationship between plant protein resource (Z; PPP%) protein content , fishmeal price (Y; FM$) and the value(Z; VALUE) of the plant protein resource.

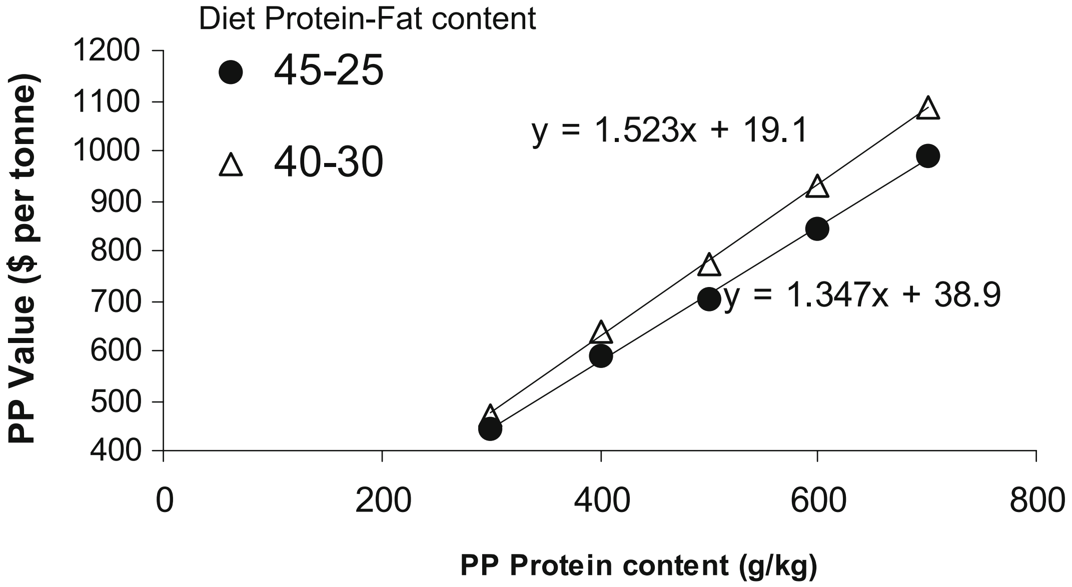

Figure A2.4 Influence of diet energy (fat) content on the effective value of a plant protein (PP) resource with increasing level of protein content in the PP resource.

A2.8 Influence of other diet formulation constraints on plant protein resource value

In examining the influence of diet energy content on the effective price of PP resources three modern high-nutrient dense diets, presently being used in the salmonid industry were used as the basis from which to formulate the model diets. These three diets were (protein% - fat% - gross energy MJ/kg) 45-22-22.0, 45-

25-22.7 and 40-30-23.9. Of these diet specifications, no differences in the theoretical value of the PP resources were encountered when comparing between the 45-22-22.0 and 45-25-22.7 diets. However, with the decrease in diet protein content, concomitant with the increase in diet fat and gross energy content, differences in the prospective value of the PP resources were observed (Figure A2.4). Notably, as the energy content of the diet increased the effective value of the PP resources also increased. The value of the PP resources also increased in a linear relationship as their protein content increased. The rate of this increase in value with increasing protein content of the PP resources was greater in the higher-energy diets. This is primarily a response to the limitation of opportunity in the formulations to accommodate "filler" nutrients that provide limited nutritional value.

In some aquaculture industries there is environmental pressure to reduce discharges of nutrients such as phosphorus. The primary way to manage this is through dietary manipulation. The influences of diet phosphorus content constraints on the value of PP resources were also considerable. Normally a standard formulation for a 45-22-22.0 diet comprises wheat, fishmeal and fish oil and has a total phosphorus content of about 19.2 g/kg. It was also noted, that to get an effective diet (45-22-22.0) formulated, such that it contained less than about 16 g/kg of phosphorus, then the PP resources begin to become compulsory inclusions. Effectively this "ransoms" the formulation to the lowest priced plant protein commodity per unit protein. For example a diet was formulated to contain a maximum of 10 g/kg of phosphorus and with options of narrow-leaf lupin kernel meal ($350/tonne), soybean meal ($500/tonne) and a range of protein concentrates with protein levels of 60%, 70% and 80% are included. Not only was one of the protein concentrates also "ransomed" to be included, but if the 60% protein commodity is valued at $1000 per tonne, then the effective prices of the 70% and 80% concentrates are $1174 and $9502 a tonne respectively. This example clearly shows the extent of the influence of this formulation constraint on the potential value of protein concentrates.

A2.9 Value adding prospects

A variety of protein concentrates made from plant protein products are already available to the stockfeeds industry. The aquaculture feeds sector, as is supported from some of the data presented (Figure A2.3 and Table A2.2), has considerable potential to gain from the use of such products. Indeed there are existing products already being actively used in the aquaculture feeds market. Some of these products include HP300 (www.hamlet-protein.com) and Isolated Soy Proteins (ISP) (www.admworld.com) and SUPRO EX (www.protein.com). The proximate specification of these products varies, with products such as HP300 being about 55% protein, 2.5% fat and 25% carbohydrates. At the high-end of the spectrum are the plant protein isolate products that typically have a composition of about 90% protein, 1% fat and 1% carbohydrates.

Consultation with the Australian aquaculture feeds industry has indicated that a range of plant protein concentrates and isolates are used, ranging from relatively low-protein meals (30% protein) to soy protein isolates with protein levels at 85% and greater. The high-value (> $500/tonne) part of this range is exclusively secondary processed meals, which tend to be priced according to the protein content. Typically, most of those products presently used are soybean-based products. The potential for such a product to be made in Australia, from an Australian grown grain will be contingent on the identification of a cost-effective raw material and processing technique being identified. In contrast, consultation with the pig and poultry feed sectors has supported that there is little interest in the use of such concentrates and protein isolates (D. Goussac - WESFEEDS, pers. comm. 2001). This contrast is a likely outcome of the difference in primary protein source used in each feed sector and the base level of dietary protein that is being targeted by each. Notably the key difference being that the diets in the aquaculture feeds sector are tending to be > 40% protein, were as those in the pig and poultry feed sectors are typically < 30% protein.

A2.10 Competition among plant protein commodities

Several Australian feed grain products are already cost-competitive products against soybean meal in the aquaculture feeds sector. Notable among these are some of the processed lupin products, such as the kernel meals of the narrow-leaf (*Lupinus angustifolius*) and yellow (*L. luteus*) varieties (Table A2.2). However, at no stage did the canola meal become a competitive commodity and the *L. angustifolius* kernel meal and soybean meal only became competitive in the absence of *L. luteus* kernel meal and LPC60. These results are driven primarily by the crude protein content of each of the PP resources. This value could also be effectively interpreted as the gross energy contribution, thereby also accommodating for the contribution of crude fat content from each PP resource. This later issue is of note given that present fish oil prices are generally exceeding those of fish meal prices.

Table A2.2 Competitive ingredient pricing amongst some typical plant protein commodities available in Australia when considered in aquaculture feed formulations.

Ingredient Composition	Solvent Ex Canola	Narrow-leaf lupin kernel	Yellow lupin kernel	Solvent Ex Soybean	LPC 60%	Fish Meal
Dry matter	920	910	910	920	910	900
Fat	25	65	65	25	15	90
Crude Protein	350	380	480	480	600	680
DCP coefficient	0.85	0.95	0.95	0.85	0.95	0.85
DCP	300	360	460	410	570	578
Carbohydrates	477	435	325	352	255	0
Phosphorus	7	3	3	6.5	3	25
Ash	68	30	40	63	40	130
INPUT COST $/TONNE	300	350	450	500	1000	1200
Diet Formulation						
Fish oil	16.1	19.1	24.6	16.9	19.6	25.1
Wheat flour	10.0	10.0	10.0	10.0	10.0	10.0
Soybean meal						
Canola meal - Solvent Extract						
Lupins (ASLK)	22.9	14.5	14.1			
Lupins (YLK)				51.4	32.5	31.4
LPC60						
Fish meal	51.0	56.4	51.3	21.7	37.9	33.5
Effective Ingredient Price						
Fish oil	24392	24392	24392	25250	25250	25250
Wheat flour	180	180	180	180	180	180
Soybean meal	450	450	450	-322	-322	-322
Canola meal - SE	67	67	67	-1084	-1084	-1084
Lupins (ASLK)	406	406	406	-531	-531	-531
Lupins (YLK)	nc	nc	nc	676	676	676
LPC60%	nc	nc	nc	908	908	908
Fish meal	1549	1549	1549	1385	1385	1385
DIET PRICE ($/tonne)	983	1066	1085	780	938	961
Diet Composition						
Crude Protein	45	45	40	45	45	40
Digestible Protein	38	38	35	38	38	35
Crude Fat	22	25	30	22	25	30
Fixed constraints	10% wheat	10% wheat	10% wheat	10% wheat	10% wheat	10% wheat

nc: Not Considered as a formulation option.

Discussions with aquaculture feed companies in Australia suggest that the aquaculture feeds sector in Australia, and presumably elsewhere, is aware of the presence and suitability of some of these commodities. However, this sector is largely unaware of the cost-competitiveness, availability or even of many of the subtle differences in value of the commodities available. It would appear that promotion of these grain commodities to this industry sector could be improved. Notably, the acceptance and use of soybean meal and soy products by this sector is comparatively high. This use of soybean meal and soy products has been driven primarily by promotion, supply and price issues.

One of the strengths of soybean meal, which few other commodities can compete with, is the volume of availability of this product. Some 100 million tonnes of soybean meals are produced worldwide annually (www.fas.usda.gov). This sheer magnitude of volume means that this commodity has considerable economic weight, particularly in the price sensitive, high-volume low-specification end of the market. Typically, feed sectors in this niche include pig, poultry, tilapia and shrimp feed markets.

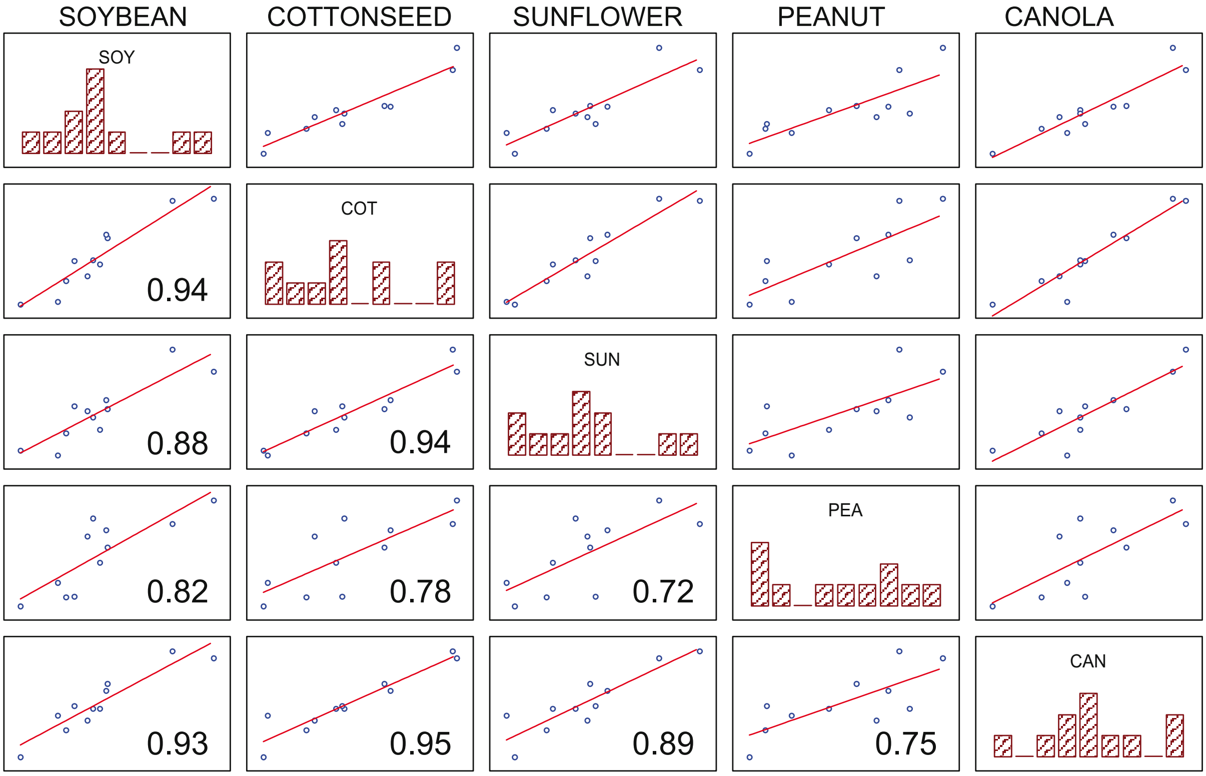

Figure A2.4 **Relationship between soymeal (48% protein) and other plant protein meal prices over the past decade (1991 – 2001). Indicated is the correlation coefficient of each relationship (data sourced from www.fas.usda.gov).**

There are a range of factors that influence the global soybean meal price and also the price of many other plant protein commodities. Notably there is close relationship among the long-term prices of many of these meals (Figure A2.4). An examination of fishmeal and soybean meal prices over the past decade also shows some correlation (R^2 <0.5) between the prices of these two commodities, though this is not as strong as that between the other plant protein resources.

The relationships shown in figure A2.4 are also quite consistent with the reputed pricing structures of Australian grown feed grains such as lupins, which are also supposed to be based on that of the soybean meal value (M. Tucek – Grain Pool of WA, pers. comm. 2000). Examination of the relationships of relative value between each of the commodities is also somewhat consistent with the relationship of relative protein content between each of the commodities. Similarly, lupins (*L. angustifolius*) command a price of about 65% of that of soybean meal (48% protein: US$165 cf. AUD$220 for lupin grain, 32% protein; Countryman, November 2001).

In a paper by Petterson et al. (2000) the notion of basing the value of the feed grain commodity on the amount of digestible, not crude protein was introduced. Glencross et al. (2001) compared the pricing structure of *L. angustifolius* kernel meal, a static value, against the theoretical value when examined on a digestible and crude protein basis and concluded that because the actual value of the resource was that of the digestible protein, or more correctly the available lysine, then the value of the resource should also reflect this. It was also suggested that a similarly valuable assessment basis could be achieved based on available energy content of the feed resources (Cho and Bureau, 1998). Although there are indications that the feeds industries are aware of this concept, there is limited reflection in the price paid for the various commodities according to this concept.

Clearly, for Australian grain commodities to obtain greater penetration and take a greater market share of the aquaculture feeds sector, several approaches need to be taken. Of these approaches, included are an improved marketing effort, an improvement to the basis by which the value of protein resources is assigned and also the development of mechanisms for increasing the protein content of what grain resources are to be developed and promoted. Notably, this last option particularly suits the Australian industries because of their lack of an ability to compete on a market volume basis and therefore a need to focus more on low-volume, high-value sectors.

A2.11 Conclusions

This discussion paper has considered the potential and a range of influencing factors affecting the use of alternative plant protein resources in aquaculture feeds. Considered were also a range of issues that influence the current valuing basis of plant protein commodities in the aquaculture feeds sector. While it was shown, that ultimately market competition among resources plays an important part in this process, it was also shown that the value and supply of soybean meal was also an important factor.

However, in the process of evaluating the relative value of plant protein resources to the aquaculture feeds industry it was necessary to demonstrate the relative irrelevance of the amino acid composition of the plant protein resource to their value in this sector. This was because of the need for high levels of protein inclusion required in modern aquaculture diets and that there was a need for accommodating a level of protein use inefficiency that meant that seldom where amino acid limitations in diets encountered.

It was also shown that in spite of the influences of market competition, that the value of plant protein resources in the aquaculture feeds sector was also influenced by their protein content and the value of the primary protein source used to make most aquaculture diets. In most cases this protein source is fishmeal. A three-dimensional equation based on both the plant resource protein content and the fishmeal value was determined to describe this relationship. The concept of a valuing mechanism based on useable nutrients, including protein, amino acids or energy content of ingredients was also highlighted.

Several opportunities for Australian grain commodities were also identified as being presently competitive. However, the most positive opportunities for the Australian grains industry to capitalise on in the aquaculture feeds sector appears to lie with value-adding prospects. Notably a range of soybean based products are already used in this feeds sector, where they command an attractive premium. It is this high-value, low volume end of the feeds commodity market that Australian grain commodities can potentially benefit from most.

A2.12 References

Allan, G.L. (1997) Alternative feed ingredients for intensive aquaculture. *Recent Advances in Animal Nutrition in Australia* 98-109.

Carter, C.G. and Hauler, R.C. (2000) Fish meal replacement by plant meals in extruded feeds for Atlantic salmon, *Salmo salar* L. *Aquaculture* 185, 299-311.

Cho, C.Y. and Bureau, D.F. (1998) Development of bioenergetic models and the Fish-PrFEQ software to estimate production, feeding ration and waste output in aquaculture. *Aquatic Living Resources* 11, 199-210.

Food and Agricultural Organisation (FAO) (2001). http://apps.fao.org/

Glencross, B.D., Curnow, J.C. and Hawkins, W.E. (2001). Assessment of the variability of WA lupins as an aquaculture feed ingredient. Final Report to the Grains Research Committee. AN: 11820(1-3), pp 57.

Gomes, E.F., Rema, P. and Kaushik, S.J. (1995) Replacement of fish meal by plant proteins in the diet of Rainbow Trout (*Oncorhynchus mykiss*): digestibility and growth performance. *Aquaculture* 130, 177-186.

Kaushik, S.J. (1998) Whole body amino acid composition of European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) and turbot (*Psetta maxima*) with an estimation of the IAA requirement profiles. *Aquatic Living Resources* 11, 355-358.

Moyano, F.J., Cardenete, G., De la Higuera, M. (1992) Nutritive value of diets containing a high percentage of vegetable proteins for trout, *Oncorhynchus mykiss*. *Aquatic Living Resources* 5:23-29.

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C.M., Clay, J., Folke, C., Lubchenco, J., Mooney, H., Troell, M. 2000. Effect of aquaculture on world fish supplies. *Nature* 405, 1017 – 1024.

Petterson, D., Glencross, B. and Kingwell, R. (2000) An economic assessment of the inclusion of Australian sweet lupin (ASL) seed (grain) and kernel meal in aquaculture diets. In: Final Report of Project DAW555, “Evaluation of legume and oilseed meals in the diet of marine finfish”. Grain Research and Development Corporation, Canberra, Australia. pp 19.

PIVOT Aquaculture (2000) Feed Specification Guide. PIVOT Aquaculture, Cambridge, Tasmania. pp 44.

Rodehutscord, M., Borchert, F., Gregus, Z., Pfeffer, E. 2000. Availability and utilisation of free lysine in rainbow trout (*Oncorhynchus mykiss*) 2. Comparison of L-Lysine.HCl and L-Lysine sulphate. *Aquaculture* 187, 177-183.

van Barneveld, R.J. (1998) Influence of oil extraction method on the nutritional value of canola meal for growing pigs. Project DAS38-1188. Pig Research and Development Corporation, Canberra, Australia. pp 46.