Proceedings of the Second Workshop for

Seeding a Future for Grains in Aquaculture Feeds

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CLIMA Centre for Legumes in Mediterranean Agriculture


Aquaculture Feed Grains Program

Government of Western Australia

GRDC Grains Research & Development Corporation

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New Ideas, New Products, New Issues

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Introduction

In early 2003 the Grains R&D Corporation commenced investment in a Centre for legumes in Mediterranean Agriculture (CLIMA) project to examine the potential for the production of value-added grain products intended for the aquaculture feeds market. Initial progress was rapid and at the first workshop of "Seeding a Future for Grains in Aquaculture Feeds", (Glencross, 2003a), in May 2003, it was supported that the project should proceed to an expanded second phase.

Expanding the Project Format

To progress towards the project's second phase additional investment has been sought from the Fisheries R&D Corporation (FRDC) and three separate commercial investors/stakeholders. In the second phase of the project the objectives have also expanded and additional researcher collaborators become involved in the project. Notably, each of the commercial and research partners contributes a special set of skills considered essential to the successful progress of the projects objectives. These partners are:

- Skretting Australia
- Weston Technologies
- CBH Group
- AKVAFORSK (Norway)
- University of Tasmania – School of Aquaculture
- CSIRO Marine Research

The nature of the collaborative involvement of each of these new partners is anticipated to substantially improve the capacities of the existing CLIMA team (Department of Fisheries, Department of Agriculture and Chemistry Centre). The nature of these additional skills will be detailed further in these proceedings under each research collaborators individual contribution. The nature of the commercial partner's involvement is clearly to assist with progression of the commercialisation of the research and help test the realities of the research findings under specific market sector conditions.

Which Markets and Why?

As the project enters its second phase, two key prospective markets for the value-added grain products have been identified. These are the salmonid and prawn feed markets. These two markets have been chosen on the basis that they are the technically most advanced aquaculture feed markets in the world. Together they constitute about 3.6 million tonnes of feed each year (Table 1).

Although lupins have been shown to be able to be included in diets up to 40% (Farhangi and Carter, 2001; Glencross et al., 2004) without palatability or growth problems, there is little practical application for such high inclusion levels. Typically more realistic commercial inclusion levels for salmonid feeds are of the order of 10% to 20% depending on price and protein content.

While higher inclusion levels would be feasible in diets for tilapia and catfish species this is not earmarked as a target market. Although significant volume exists in these markets, the feeds are low-protein and low-energy and are therefore made to a very low-cost and therefore cost-sensitivity of ingredient choice is high (Table 2). Conversely salmonid feeds are high in protein, very energy dense and have little formulation flexibility. In addition to this there are further aspects to ingredient choice, such as ingredient functionality,

which are also important and can result in some ingredients having an identified "point-of-difference" with respect to other competitor products. This allows increased marketability of such products and an increase in the value per unit protein or energy.

Table 1. Production of key aquaculture species in 2001 and feed use estimates.

	Salmonids	Prawns	Tilapia	Misc. FW Fish
Production (Mt)	1.782	1.271	1.385	2.986
FCR	1.0	1.5	2.0	2.0
Feed (Mt)	1.782	1.906	2.770	5.972

Mt: Million tonnes. Misc. FW Fish: Miscellaneous freshwater fish, includes catfish, but not carps. Data from www.fao.org

It is also worth noting that global production of lupins annually is around 1.5 million tonnes, compared to annual soybean meal production of 125 million tonnes (Figure 1). If you were to consider the prospect for that of the lupin kernel meal market, then this sector volume decreases further to a capacity of 1 million tonnes, based on total processing of global lupin production. Clearly the opportunity for lupin value-added products is not one on a high-volume basis and is therefore better targeted to niche markets where value can be attributed to its "point-of-difference" aspects.

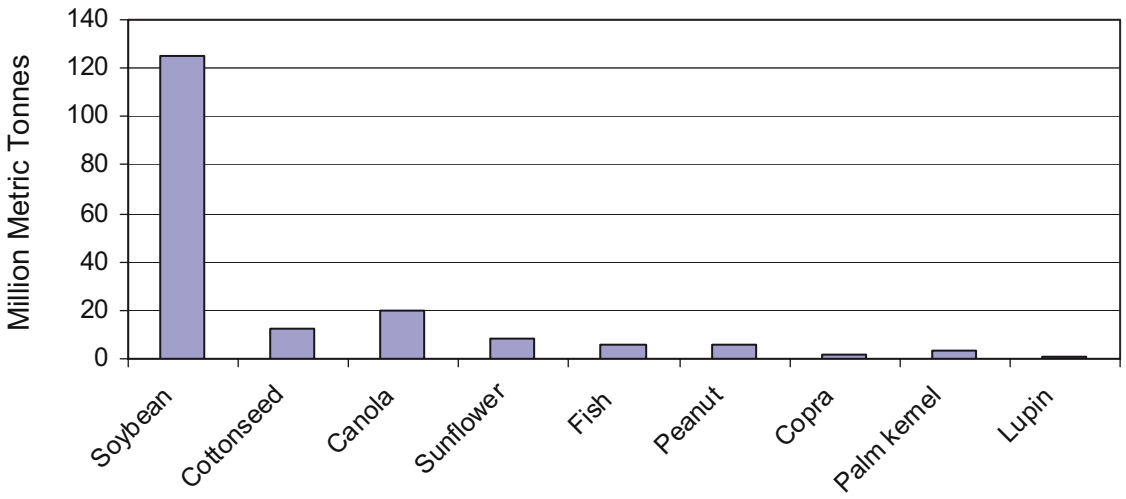

Figure 1. Volume of production of key plant protein meals in 2001/2002.

Such a "point-of-difference" between lupin kernel meals and soybean meal use in the salmonid aquaculture feed sector has been identified and it is hypothesised that there may be other "point-of-difference" aspects that are yet to be identified. Additional details on these initiatives will be discussed later.

Table 2. Theoretical diet formulations for key species showing diet protein and fat levels, likely ingredient inclusion levels and ingredient costs and overall formulation costs.

Species	Catfish	Tilapia	Shrimp	Salmonid
Diet Protein	250	300	450	450
Diet Fat	70	80	90	250
Fish meal ($1,200)	9.0	11.0	33.0	42.2
Crustacean Meal ($1,500)	0.0	0.0	10.0	0.0
Fish oil ($1,00)	0.5	2.0	3.5	20.1
Rice Bran ($200)	45.0	33.0	0.0	0.0
Wheat ($250)	15.0	15.0	18.0	9.0
Soybean Meal ($500)	14.0	17.0	17.0	0.0
Corn Gluten ($900)	0.0	5.0	0.0	13.6
Lupin Kernel Meal ($400)	15.0	15.0	16.0	14.6
Formulation cost ($/t)	395	470	800	936

Formulations only approximate and not showing minor additives

Revised Project Objectives

The objectives of the expanded project were altered to allow some focus on current commercial needs. Notable were the additional foci on the evaluation of the functional properties of the products and also the quality variability in lupin kernel meals, the key product currently being used by the industry. The specific revised objectives are:

1. Identification of processes enabling the production of value-added grain protein product for use in the animal feeds sector.
2. Evaluation of the nutritional value and functional characteristics of a range of value-added grain protein products when fed to fish (salmonids and prawns).
3. Commercial transfer of intellectual property for development of new grain product(s).

To address these objectives key studies are being targeted by each of the research collaborators. Discussion of the background to and the plans for these studies is part of the purpose of this workshop. From this it is hoped that the collective wisdom can assist with the best definition of the issues to address and how to optimise the outcomes from the project. The nature of the background issues and the proposed research strategies to address them are also detailed in subsequent sections of these proceedings. In addition to that there are further papers on new technologies and economic scenarios from the various value-added technologies.

New Products

Based on a series of theoretical planning exercises and modelling studies the optimal composition of a value-added protein product for the aquaculture feeds industry was identified to be between 50% and 65% protein, price contingent (Glencross, 2003b; Sipsas, 2003). Following the development of a series of prototype products in early 2003, an upscaling of the protein concentrate production was undertaken in 2004. The focus in 2004 was to evaluate the influence of industrial drying technologies on the products and the implications of heat damage to the product. As with the 2003 products the 2004 products were also based on the use of *L. angustifolius* and *L. luteus* kernel meals allowing some evaluation of the influences of initial protein content on the viabilities of the processes.

New Issues

Following the redevelopment of the objectives of the project and the realisation that the aquaculture feeds industry is now using lupin kernel meals in its formulations the issue of variability in product quality was highlighted as an important area warranting additional research. Earlier research has shown that there is substantial variability in nutritional value among the different *L. angustifolius* cultivars (Glencross et al., 2003). It appears that the variability in response by fish to the digestion of kernel meals from these cultivars is strongly related to the variability in protein content of the grain. Because of this relationship, the development of calibrations based on composition in relation to digestible protein, amino acids and energy value would be of value to the aquaculture feeds industry.

In this regard the use of Near-Infrared Reflectance Spectroscopy (NIRS) has been identified as a priority in developing an assessment of lupin kernel meals for digestible protein, amino acids and energy from this feed ingredient. While the use of this technology is relatively new in the aquaculture feeds sector, its application in the terrestrial feeds sector is better established and as such this workshop will also examine where research in that field has gone in recent years.

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Getting the value from the grain - Protein, protein and more protein

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Introduction

In general, a key component of the value of many feed grains, like lupins, is their protein content. It is recognised that the higher the protein content of an ingredient then the higher its potential value. However a plant derived protein product for the feed industry including aquaculture 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. To assess a new ingredient's potential to be adopted, the prospective protein levels at which it will to be cost-effective to both produce and to use need to be determined.

Kingwell (2003) presented a paper with market analysis of prices paid for feeds of different protein content revealing that the marginal value of protein content in feeds is an increasing function of protein content. *"In other words. Low protein feeds attract small premiums for any increase in their protein content (i.e. $6 per every % increase) while higher protein feeds receive large premiums for their further improvement in protein content ($22 per every % protein improvement)* Kingwell (2003). Often these higher premiums are a reflection of the higher processing costs involved in the production of these very high protein products.

In this study, a simplistic evaluation (least-cost linear formulation) was made with a hypothetical diet (Table 3 and Table 4) to show the relationship between likely inclusion levels, and likely cost of the final diet with increasing protein content of the test protein product (in this case lupin).

Table 3. Base hypothetical diet used to determine the influence of increasing protein content in and ingredient rates and diet costs.

Formulation		Ingredient cost	Cost of diet
		A$ per tonne	
Pre-mix vitamins	0.50	1000	5
Fish oil	18.90	1200	227
Wheat flour	9.00	260	23
Poultry meal	22.00	600	132
Sweet lupin kernel (35% product)	10.80	315	34
Fish meal	38.80	1350	524
Total	100		945

Composition of Diet	g/kg
Dry matter	920
Protein	450
DCP	405
Fat	250
Carbohydrate	120
Phosphorus	19
Ash	106
Gross Energy (MJ/Kg)	22.57
Estimated Digestible Energy (MJ/Kg DM)	18.95
Dry matter Gross Energy (MJ/Kg DM)	24.54

The potential price payable of plant protein resources used in aquaculture feeds depends strongly on the price paid for fishmeal. However in this case we have chosen to use 90 cents per kilo of protein as the neutral cost value of plant protein ingredient (Kingwell 2003). Figure 2. Shows what the relative values of both lupin (narrow leafed and yellow) based protein products as well as soybean meals. It is apparent that at the lower end the values fall close to commercial reality, however as the protein content increases the products are undervalued.

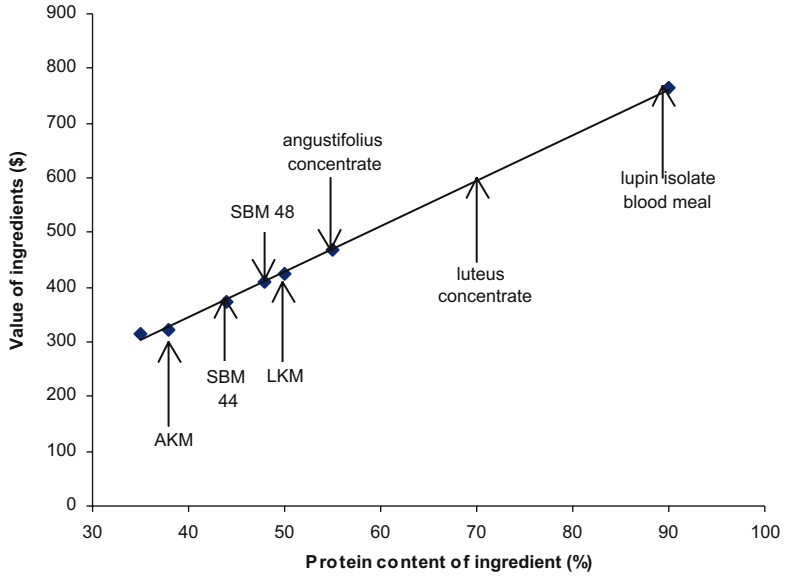

Figure 2. Value of ingredient as a function of protein content, valued at $0.90 per kg/tonne.

Table 4. Influence of increasing protein content in a plant protein on inclusion levels, diet costs and potential ingredient calue, using the base diet in Table 3.

Increasing protein content of ingredient (lupin)	35%	38%	40%	42%	44%	48%	50%	52%	55%	58%
Likely Inclusion (%)	10.8	12.0	13.0	14.1	15.4	19.1	21.6	24.2	32.0	46.0
Scenario 1: Linear increase of price @ 90¢/kg										
A: Ingredient cost	315	342	360	378	396	432	450	468	495	522
Protein price A$ per kg	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90	0.90
Diet cost	945	936	929	920	910	882	863	843	783	676
Scenario 2: Maximum ingredient cost - keeping Diet cost constant.										
B: Ingredient cost	315	419	485	555	623	765	831	889	999	1108
Protein price A$ per kg	0.90	1.10	1.21	1.32	1.42	1.59	1.66	1.71	1.82	1.91
Diet cost static	945	945	945	945	945	945	945	945	945	945
Scenario 3: Increasing ingredient cost and decreasing diet cost (splitting the difference)										
C: Ingredient cost	315	354	383	415	448	524	567	614	690	776
Protein price A$ per kg	0.90	0.93	0.96	0.99	1.02	1.09	1.13	1.18	1.25	1.34
Diet cost	945	937	932	925	918	900	888	879	846	793

Glencross (2003) suggested that the influence of plant protein content, had a greater influence of on the value of the ingredient than the influence of fishmeal price. Table 4. Details three varying scenarios, looking at the influence of increasing protein content on inclusion levels and diet costs and potential ingredient value. A hypothetical diet (Table 3) is used. Only fishmeal and lupin are the changing variables in the inclusion rates.

Scenario 1- As the protein content increases so too does the inclusion rate. By costing the lupin ingredient at a constant rate of 90 cents per kilo, this effectively drops the cost of the diet. However producing protein products above 44% at these prices, start to become unfeasible.

Scenario 2- was designed to investigate the maximum value the ingredient could attain, without changing the cost of the diet, as protein and inclusion rates were increased. Scenario 3 – looks at apportioning the costs between the producer of the ingredient and the feed formulator. Therefore as protein is increasing the cost of the ingredient is increased but at an exponential rate rather then linearly. It appears that there is great scope for producing lupin protein products (40-50% crude protein, %CP), at cost competitive prices.

The next question then – which is the better starting material?.

Table 5. Yield, protein and seed characteristics of modern cultivars.

Variety / line	Relative yield	Protein % 'as is'	Protein % kernels 'as is"	Relative seed size	Seed coat (%)
Belara	114	30.2	40.0	107	24.5
Walan 2141	115	31.0	40.7	107	23.8
Tanjil	107	31.4	41.7	99	24.8
Kalya	105	31.6	41.7	99	24.4
Quilinock	117	31.9	41.7	112	23.5
Merrit	100	32.4	42.6	100	24.1
Tallerack	95	33.2	43.6	95	24.1
Myallie	97	33.4	43.6	101	23.4
Walan 2173*	108	34.1	43.8	114	22.3
Wodjil	70	38	52		27

*Planned release 2005

Table 5. Shows the most relevant specifications of our most modern lupin cultivars. Taking the two extremes in protein content Belara (30.2%) and Walan2173 (34.1%) of the narrow leafed varieties and also the yellow lupin variety Wodjil at 38%CP it has the highest protein content of any commercially available lupin variety. An analysis of the profit margins a processor in the business of producing lupin kernels meals is likely to make using the three different cultivars and given varying scenarios (Table 6). Dehulling is the first step in the process of producing a 'protein enriched lupin product', however dehulling 1 tonne of Belara and dehulling 1 tonne of Wodjil is going to cost the same. The kernel values for protein will influence what your return will be and it seems wiser to spend money turning a good product into a great product, rather than a moderate product into a good product, given the exponential nature of the returns on protein. As can be clearly seen it appears that even at a '$100' premium Wodjil would still be the starting material of choice.

The aquaculture feeds sector, as is supported from the data presented (Table 4), has considerable potential to gain from the development and use of products with varying degrees of refinement from *L. angustifolius* and in particular *L. luteus*. The potential for such a product to be made in Australia, from Australian grown grain will be contingent on the identification of a **'cost-effective'** raw material, not necessarily the **'cheapest'** raw material.

Table 6. Prospective processing margins from use of different initial grain varieties.

	NLL-Belara		NLL - 2173		YL-Wodjil	
whole seed protein % 30			34		38	
Purchase tonnes	1 000		1 000		1 000	
Price $(t) : Grain Pool	220		232		250	
Price	220 000		232 000		250 000	
Processor :Scenario 1, linear increase in protein @ 90c per kg						
Seed coat	24,8		22,3		27,0	
Dehulling kernel yeild	75 %		78 %		73 %	
	kernel (t)	hulls(t)	kernel (t)	hulls(t)	kernel (t)	hulls(t)
yield	752	248	777	223	730	270
Protein in kernels	42 %		44 %		52 %	
Price $(t)@90c/kg	378	90	396	90	468	90
Value of kernel/hulls	284 256	22 320	307 692	20 070	341 640	24 300
Gross	306 576		327 762		365 940	
dehull/mill costs per $45(t)	-45 000		-45 000		-45 000	
minus purchase price	239 256		262 692		296 640	
	220 000		232 000		250 000	
	19 256		30 692		46 640	
margin / per tonne	***19***		***31***		***47***	
Scenario 2, as above but changing original seed prices to affect the same final margin						
Price; seed per tonne:	220		244		278	
margin / tonne	***19***		***19***		***19***	
Scenario 3, using increasing protein prices as per Table2 (scenario 3C), and also adjusting seed prices to reflect most probable industry reality.						
Price; seed per tonne:	220		250		320	
Price $(t) Table 2 (3C)	378		448		614	
margin / tonne	***19***		***53***		***83***	

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Drying technologies - applications and limitations

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Introduction

One of the key limiting stages to the development of lupin protein concentrates was identified as the step of product drying (Kingwell, 2003). There are several different industrial drying technologies that have been identified as having potential application in the drying of lupin protein concentrates. Among these are:

- Fluid-bed-dryers
- Band-dryers
- Rotary-dryers
- Ring or Flash-dryers
- Drum-dryers
- Spin-flash-dryers
- Spray-dryers

Essentially, these drying processes can be categorised based on whether they operate on a batch or continuous process (Invensys-APV, 2003). Clearly, the most economic process is likely to be one where the process can be based on a large through-put, and therefore a continuous process is far more likely to be a suitable industrial scale technology. The different drying technologies also vary in the basic method of heat transfer, whether it is convection, conduction or radiation. In the processing industries, most dryers employ forced convection in association with continuous operation (Invensys-APV, 2003). Drum and indirectly heated rotary dryers are the exceptions in that they use heat transferred by conduction.

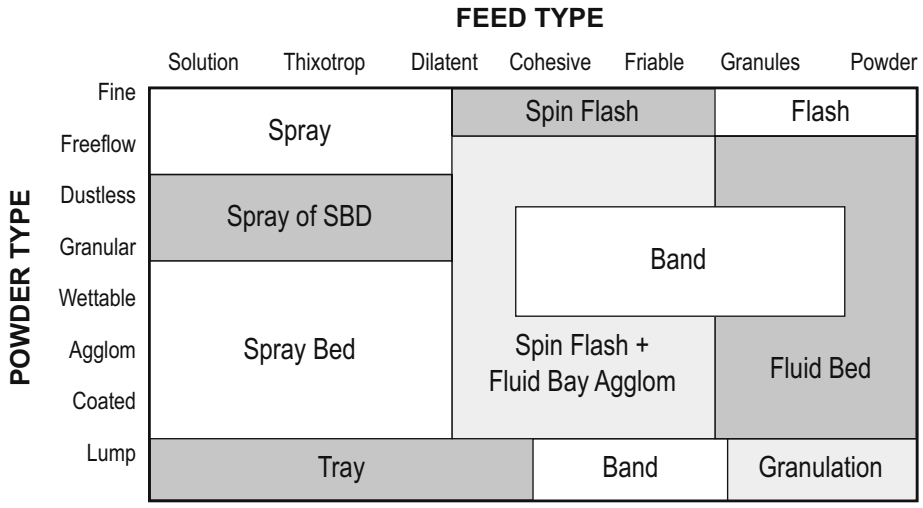

Figure 3. A guide to dryer selection. From Invensys-APV (2003).

For drying of liquids or liquefied concentrates, the evaporator of choice is usually either a drum-drier or spray-drier. This was supported by the matrix in Figure 3, where key industrial processes were categorised based on the initial and final product characteristics. From this and other industry advice it was suggested that three drying technologies, drum-drying, ring-drying and spray-drying, would have suitable potential as drying options for the production of lupin protein concentrates.

Drum-Drying

In drum-dying the liquefied product is poured onto a heated metal drum. On contact with the heated drum, the water flashes off and as the drum turns, the dried product is scraped from the drum to a collection point. Drum dryers are usually steam heated, although use of other heating methods has been developed (Invensys-APV, 2003). Typically drum dryers can be classified as either single or double-drum operations. This technology dries the product very quickly (seconds), but can cause some quality damage to products. Drum driers also have limitations in regard to throughput and are usually use in a batch drying application.

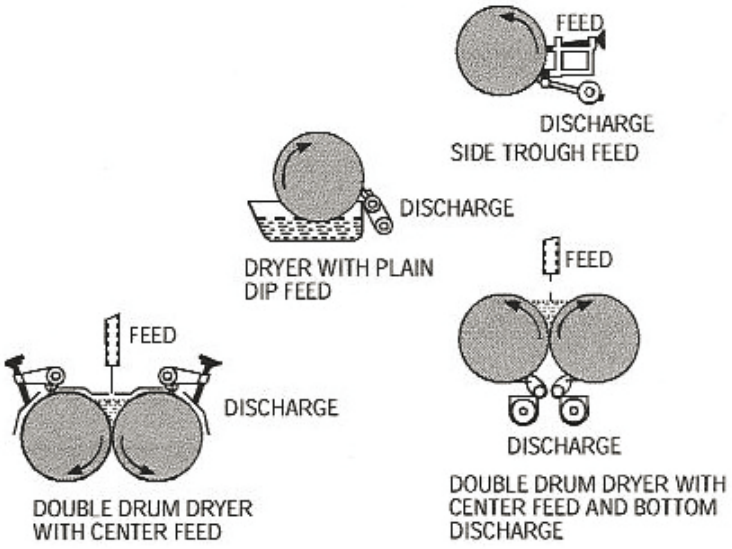

Figure 4. Configurations for some typical drum-drying operations.

Ring-Drying

The ring-drying process works on the product being conveyed in a hot air stream, with a centrifugal classifier in-line to select and recycle semi-dry products, while the smaller, lighter and drier products are removed with the exhaust air. The process usually provides a rapid, even and gentle drying process, removing both surface and internal moisture of the product. Ring-drying technology has been widely used in the starch and gluten production industries (Invensys-APV, 2003).

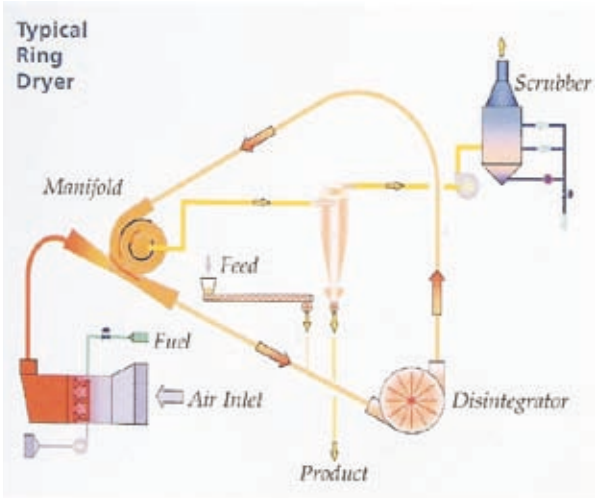

Figure 5. Configuration for standard ring-drying operations.

Spray-Drying

Spray-drying technology is one of the more widely used processes used in most modern drying facilities (Invensys-APV, 2003). Products such as chemicals, minerals, ceramics, milk powders, coffee, fruit-juice, protein isolates and protein concentrates are frequently dried using spray-dry technology. Fundamentally the process is a simple one. A liquefied product is atomised into a spray of droplets and the droplets are then contacted with hot air in a drying chamber. The evaporation of moisture from the product occurs under control temperature and airflow conditions. Powder is discharged continuously from the drying chamber. There are many different configurations for spray-dryers, widely varying in drying characteristics depending on product quality requirements.

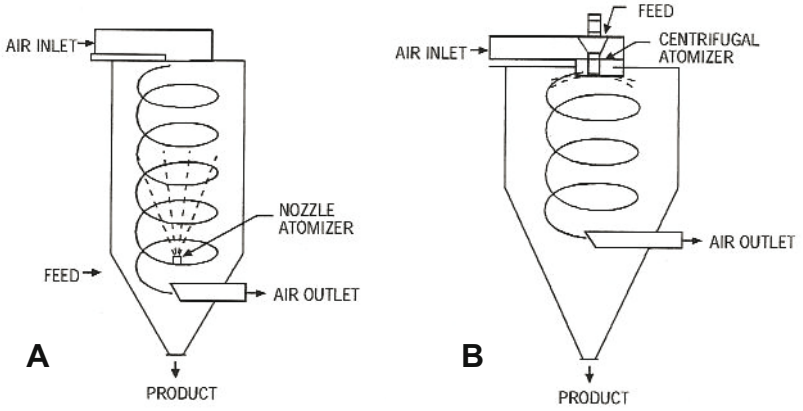

Figure 6. Configuration for standard spray-drying operations (A) mixed-flow configuration, (B) co-current configuration.

Spray drying offers much more versatility than other drying technologies, is capable of very large though-puts and offers the gentlest industrial drying option available, making it the usual preference for food industry applications (Invensys-APV, 2003). However, it is a far more costly operation to implement, with quotes for a 2500 kg/hr output, spray-drier at ~ $6 million, when compared with a ring-drier with the same output quoted at ~ $3 million. Both quotes were based on supply of equipment and construction of plant only, excluding plant-house and civil works. Operational costs for each system are similar. Quotes for drum-driers were difficult to obtain, as the technology is largely being replaced by spray-drying operations. However, it is anticipated that the supply costs would be significantly lower than that of a spray drier or ring-drier, with similar or marginally lower operational costs.

Drying the Lupin Protein Concentrates

As previously stated three drying processes were short-listed for evaluation, based on production characteristics and likely cost scenarios. These were drum-drying, ring-drying and spray drying. It was decided that it would be most appropriate to use industrial scale equipment, where possible, to test the potential for drying the two lupin protein concentrates (*L. luteus* cv. Wodjil and *L. angustifolius* cv. Myallie) using these drying options.

While several facilities were identified for each of these three drying systems, actually having the opportunity to use the facilities at a reasonable cost proved difficult. In the end, our liquid concentrates were freighted to Saurin Technologies pilot-scale drying facility, in the Dandenong region of Victoria. At their facility we managed to test the viability of both spray-drying and ring-drying. We are still pursuing opportunities for drum-drying.

The spray-drying technique proved very useful in drying the liquefied concentrates. Few problems were encountered in the processing, and two hundred kilograms of product was produced. However, ring-drying

proved problematic in regard to the feed-in of the liquefied concentrate to hot air stream of the drier. The liquefied concentrate did not become entrained in the hot air stream and tended to clump, and form rubberised-like conglomerates, making the ring-drying process ineffective. Further pursuit of this drying technology was abandoned due to these problems and time constraints.

To examine the influence drying technologies have on the nutritional value of lupin protein concentrates, two additional drying techniques were also evaluated. Samples of the same wet concentrate were also freeze-dried and oven dried (100°C for 15 hrs, with a further 24 hrs at 140°C, then a further 5hr at 150°C). These treatments were used as positive and negative controls respectively. The composition of all 2nd stage prototype products produced from these processes is presented in Table 7. It is planned to evaluate the digestible nutrient and energy value of all these products in rainbow trout. Earlier work by the group has shown that heat/processing damage can severely limit the nutritional value of plant protein products when fed to fish, and this work is planned to examine the extent of such damage and potential cost/value implications (Glencross et al., 2004).

Table 7. Composition of 2nd-stage prototype ingredients evaluated. Details are on a dry matter basis (g/kg DM) unless otherwise specified.

INGREDIENTS	LKM	MKM	LPCD	MPCD	LPCS	MPCS	LPCF	MPCF
Dry Matter (g/kg)	924	914	958	974	n/a	n/a	928	964
Protein	549	423	789	744	n/a	n/a	801	746
Fat	79	80	39	90	n/a	n/a	113	133
Ash	43	35	42	39	n/a	n/a	34	30
Organic Matter	957	965	958	961	n/a	n/a	966	970
Phosphorus	6	5	7	6	n/a	n/a	7	6
Energy (MJ/kg DM)	19.37	18.65	22.68	23.46	22.80	23.45	21.88	23.13

LKM: *L. luteus* (cv. Wodjlil) kernel meal; MKM: *L. angustifolius* (cv. Myallie) kernel meal; LPCD: *L. luteus* protein concentrate oven-dried; MPCD: *L. angustifolius* protein concentrate oven-dried; LPCS: *L. luteus* protein concentrate spray-dried; MPCS: *L. angustifolius* protein concentrate spray-dried; LPCF: *L. luteus* protein concentrate freeze-dried; MPCF: *L. angustifolius* protein concentrate freeze-dried EHC: Enzymatically-hydrolysed casein. n/a : data not yet available.

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Progress with new species and varieties

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Background

The Department of Agriculture has an experienced and innovative lupin breeding program that has supported the lupin industry with new varieties for over 30 years. The breeding program is responsible for developing new varieties in narrow-leafed lupins (*Lupinus angustifolius*), yellow lupins (*L. luteus*) and albus lupins (*L. albus*). These species are all commercial crops in Western Australia. The breeding objectives include yield, disease resistance, herbicide tolerance and quality. While in the past most emphasis has been on yield, disease resistance and maintaining a level of quality the new breeding objectives include increasing the quality of lupins, making them easier to de-hull and trying to differentiate lupins in the market for their particular unique properties.

Narrow-leafed Lupin (*L. angustifolius*)

In 2004 there will be a new variety released, WALAN2141, which has improved yield, superior resistance to the fungal disease anthracnose, better tolerance of the herbicide metribuzin and slightly higher protein than the variety it replaces. All these attributes will reduce input costs for farmers and increase potential income through greater yield thus making the lupin part of the farming system more reliable. While the protein levels are only slightly higher than the lowest cultivar, Belara, it begins the trend for an upward movement in protein levels for future varieties. WALAN2141 production should grow to about 40% of deliveries within 4 years, and should replace Belara, most of the Tanjil and Wonga, and all of the minor varieties.

In 2006, WALAN2173M will be released as a high protein variety with good yield, herbicide tolerance and disease resistance. This will be the first variety that highlights the quality character rather than the usual agronomic characters. WALAN2173M has about 3 - 4% more protein than the most popular varieties, Tanjil and Belara (Table 8). The level of production of WALAN2173M will be related to the price offered for its superior level of protein as there are other varieties in the system that farmers can make more money out of through yield.

Table 8. Comparison of narrow-leafed cultivars and new varieties.

	% of production*	Yield	Anthracnose resistance score **	Protein %db	Oil %db	Seed weight mg
WALAN2141	(40)	121	6	35.1	6.7	155
WALAN2173M	(10)	106	6	38.7	6.9	165
Belara	26	112	3	34.4	7.3	155
Tanjil	25	100	7	35.7	7.1	145
Wonga	14	100	7	35.7	7.1	145
Kalya	12	94	5	35.8	6.7	145
Merrit	8	90	4	36.9	6.6	145
Quilinock	1	107	3	36.1	6.2	165

* values in parenthesis indicate estimates. ** where score of 1 = very susceptible and 9 = very resistant.

Yellow Lupins

The yellow lupin industry in Western Australia started in 1997, with the release of the cultivar Wodjil but

has not progressed greatly because of aphid susceptibility and low yields. Developing aphid resistant lines has proved elusive but we now know more about the alkaloids that are essential for resistance and the level that is needed in the plant. GRDC has funded a three year project to further develop yellow lupins and to use the current knowledge on aphid resistance to develop lines with appropriate levels of alkaloids. Two new experimental varieties will be released this year to give the industry a fresh start and to allow it to look at better ways of controlling aphids in the crop. While in-crop control comes at a cost, the returns from yellow lupins will depend upon yield and price. The two new varieties have better yields than Wodjil under difficult conditions but are similar under good conditions. Protein levels have been maintained. Yellow lupin has higher protein content and better amino acid balance than narrow-leafed lupin making it a preferred feed for poultry, pigs and in aquaculture.

Albus Lupins

The albus lupin industry collapsed in 1997 after the fungal disease anthracnose devastated crops in 1996 and 1997. Since then efforts have been made to develop resistant varieties that could be used to re-establish this industry in the north. A new variety will be released this year that has improved resistance. The resistance is adequate for protection in the lower rainfall areas or where there is no external sources of fungal infection and it will be a few years yet before the industry can be established back around the high incidence zone near Geraldton. COGGO is now funding the albus breeding program.

Pearl Lupins

GRDC has funded a three year project to explore the potential of the Pearl Lupin in the Western Australian environment. The pearl lupin is seen as the soybean of the lupin species because of its very high protein (45%) and oil (18%) content. While there is no doubt about its quality there is little knowledge of its adaptation to a Mediterranean climate. The following summarises last season's discoveries

- It performed best on the more fertile soils where albus lupins and chickpeas are adapted, although the pH needs to be acid to neutral rather than alkaline. It definitely does not grow well on sandy, infertile soils
- Erratic podding has been noted over most environments and it seems to prefer moderate temperatures at podding. Hot spells in the north and cold spells in the central wheatbelt interrupt pod set.
- Without modification and breeding the pearl lupin could be productively grown in the Avon Valley - Moore area on the red soils and the Mt Barker area on forest gravels and loamy soils.

Some breeding has been done to incorporate low alkaloids into this species and there is now a line being bulked up that is fully domesticated.

Developing an *in vitro* assessment method for quality determination.

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Introduction

Amino acids are characterised by the **-CH(NH_2)COOH** substructure. Nitrogen and two hydrogens comprise the amino group, **-NH_2**, and the acid entity is the carboxyl group, **-COOH**. Amino acids link to each other when the carboxyl group of one molecule reacts with the amino group of another molecule, creating a peptide bond **-C(=O)NH-** and releasing a molecule of water (**H_2O**). Amino acids are the basic building blocks of enzymes, some hormones, proteins, and body tissues.

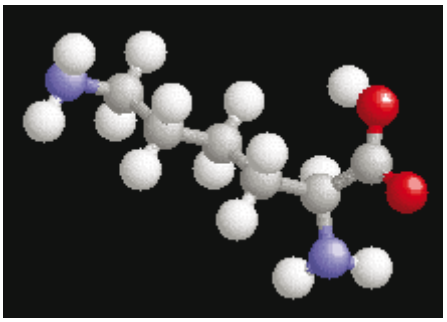

Figure 7. Molecular structure of the amino acid lysine.

Lysine (Figure 7) is an essential amino acid and it is well established that it can be a limiting component in diets (Moughan and Rutherford, 1996). It is known that during processing lysine can react with other components, typically carbohydrates and become unavailable to the animal (Hurrell and Carpenter, 1981). This is particularly pronounced where the processing has involved heating, either direct or to a lesser extent by steam or significant storage times of components.

This work aims to develop a robust method of assessing feed quality by measuring the reactive lysine in a variety of feed blends and ingredients.

There are many assay techniques available that determine chemically active lysine content in foodstuffs. However heat treatment may convert the chemically active lysine to a form that unable to be absorbed in the gut of animals. A method that is predictive of the feed trial outcomes and ingredient quality will save considerable time and money.

The Traditional method

The reactive groups in amino acids include -NH2 and -COOH groups and groups present on side chains. In peptides and proteins only the side chain is available for reactions (besides amino and carboxylic groups at the terminal ends). In lysine compounds reacting with amino groups can affect both the amino group at N-terminal end and the epsilon-amino group.

The traditional method of determining chemically reactive lysine is known as the FDNB method. This uses FDNB (1 fluor- 2, 4, dinitrobenzene, commonly called Sangers Reagent) which combines with the free NH2 group of the NH2-terminal in the lysine producing a yellow colour. This reaction is problematic in samples where the protein may have undergone Malliard reactions (particularly after heating) leading to discoloration

that may interfere with the FDNB determination. Additionally the FDNB may overestimate the amount of reactive lysine that is available for use by the animal (Booth, 1971; http://utenti.lycos.it/Pasquale_Petrilli/aastruc/aareac.htm).

FDNB-Lysine has some significant shortfalls. The correction factor is different for different materials, which makes it difficult to apply to unknown samples. The yellow colour can be overestimated due to a reaction with carbohydrate during hydrolysis.

Other methods, including enzymatic methods (Schaesteen et al., 2002) have been developed but many are fraught with problems that are not easily overcome in mixed component samples such as feeds.

The Chemistry Centre Method

This method is based on that of Moughan and Rutherfurd (1996) with the additional benefit of recent advances in technology, most notably the liquid chromatograph mass spectrometry (LC-MS).

The LC-MS allows low levels of detection in complex sample matrices without the need for excessively complex chemical pre-treatments or derivatisations (Figure 8). The LC-MS is a valuable tool that is finding many applications across the breadth of Chemistry Centre activities.

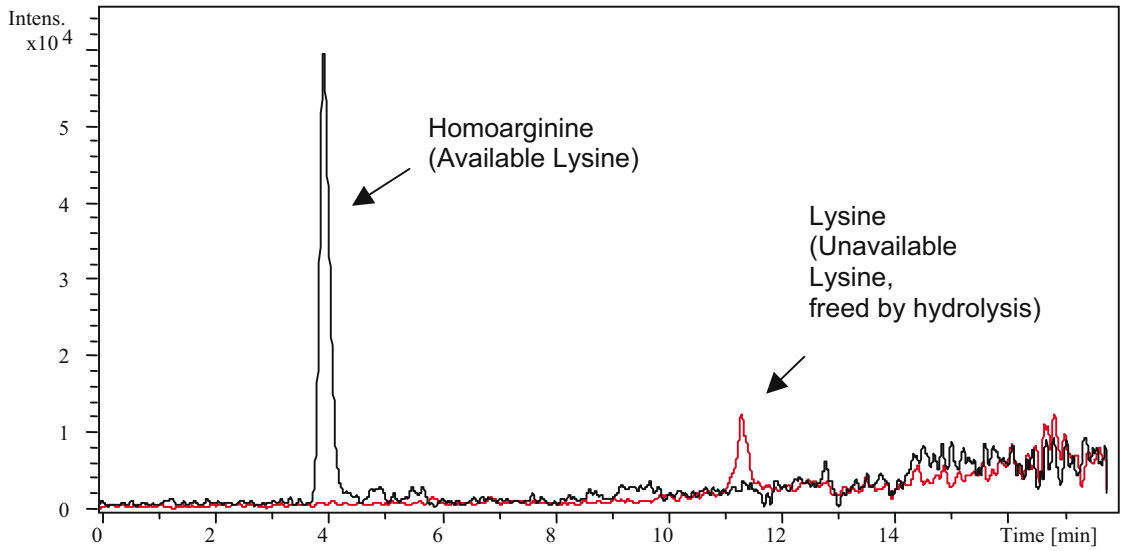

Figure 8. A typical chromatogram of reactive (available) and unreactive (unavailable) Lysine in Lupin Protein Isolate.

The material is finely ground and a homogenous sample is treated with o-methylisourea (OMIU). The reaction is allowed to proceed at room temperature for six days in a sealed container. The resulting sub-sample is then hydrolysed by adding an equal volume of hydrochloric acid and heating at 110°C for 24 hours. The hydrolysed product is then presented to the LC-MS. The sample is quantified against standards of known concentration. Recoveries of samples and internal standards are also calculated.

The method has been optimised at each step to ensure acceptable recoveries. The methodology was also modified to ensure that the relatively small amount of sample in the fish trials (Glencross et al., 2004) was amenable to the procedure. Additionally the procedure has been found to have a dynamic range that allows it to continue to be used in lupin seeds (typically 1-2% lysine), protein concentrates with much higher lysine contents and variable formulation feeds.

Conclusions

The small number of trial analyses carried out to date has indicated that the technique is sufficiently robust to accurately provide a good measure of the reactive lysine in samples of feed and ingredients. Additional work is still to be carried out on faecal material.

Additional work will be carried out as a result of the feeding trials currently being performed in Pemberton. This will allow for a correlation of the *in vitro* work with the biological observations. The technique will also be used to quantify the potential protein profile redistribution as a result of the lupin concentrate process. It may be possible to manipulate the concentrate production technique to avoid losses of lysine during production and processing.

It is hoped that the data obtained from the current feeding trials will also allow for a future assessment of the feed quality by NIRS (near infrared spectroscopy). NIRS has been used internationally for a number of years for commercial testing the quality of feed such as silage and grains (Sprague et al., 2003). However it is not likely that the technique has been used to determine the quality of aquaculture feeds containing lupin protein concentrates. The NIR technique is a derivative technique, which allows for calibration against many variables (such as crude protein, dry digestible matter and fibre). It may be possible to use the data from this experiment to calibrate against the RLA and biological factors. If successful the NIRS technique is a rapid and relatively simple technique that can be used. The potential down side to the pursuit of the NIRS technique will be its robustness. The technique requires a relatively constant (or at least comprehensive) matrix for meaningful results.

Future Work

Additional work required to establish the correlation of the RLA method with the results of animal feeding trials. Also effects of protein redistribution resulting from lupin protein concentrate production. Lysine will be lost during this process, where a NaOH extraction of the lupin kernel meal will lead to losses of lysine. This may countered in the feed by the addition of synthetic lysine.

Avoid NIRS – canola experience with the NIRS has suggested difficulties. A good predictor of the extent of the Malliard reaction but not necessarily RLA, difficulties expected in variable component mixes of feeds. May be useful in conjunction with other tests e.g. residual protein after ENDF.

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Economic opportunities for *Lupinus luteus* – Yellow lupins?

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Introduction

At the last workshop (Kingwell, 2003) preliminary estimates of profits from isolating and selling nutrient components of *L. angustifolius* and *L. luteus* were presented and compared to profits from traditional grain marketing. The analysis showed that the major sources of value in *L. luteus* were its protein components whereas in *L. angustifolius* fibre and protein components were of importance.

The analysis suggested there were higher returns from isolate production involving *L. luteus* compared to traditional grain marketing; although the same could not be as easily said for *L. angustifolius*. What has happened since?

Current Situation

Commercial players appear to have agreed that the prospects of returns from isolate production are sufficiently attractive for them to have made initial investments. As outlined at Crop Update 2004, in late 2003 Cooperative Bulk Handling Ltd (CBH) and George Weston Foods Pty Ltd (GWF) announced their intention to form a 50-50 joint venture company to build and operate a lupin dehulling plant. The plant will be located adjacent to CBH's Metro Grain Centre and is anticipated to process 50,000 tonnes of lupins in its first year, increasing to 200,000 tonnes in subsequent years. CBH and GWF will independently develop markets for kernel and hull products from the plant. GWF also proposes to establish a lupin protein extraction plant, adjacent the dehulling plant, within the next three years. The initial outlet for the high value added lupin kernel protein and kernel fibre products produced will be through GWF's worldwide affiliation with Associated British Foods.

Analysis of Protein Isolate Returns

The previous analysis of protein isolation involving *L. luteus* has been revised and subject to a sensitivity analysis. The key variables and typical levels are listed in Table 9. Employing an assumption of normally distributed variables with coefficients of variation ranging from 10 to 20 per cent and introducing technical constraints such as component percentages summing to 98% (i.e. 2% wastage), the distribution of the processing and selling margin (based on a fixed lupin whole grain purchase price of $250) is as shown in Figure 9. By contrast Figure 10 shows the price distribution that could be offered for seed of *L. luteus* while preserving a profit margin of 15 per cent of the gross value of isolates. The distributions were generated by 2000 random samples of the distributions of key variables.

The analysis suggests fairly attractive returns to production of protein isolates using *L. luteus*; even where growers are offered a gross price of $250/t. Further, the size of margins suggests that growers, under a range of scenarios, could receive well over $250/t for their *L. luteus* grain. Such a finding is consistent with the observations of Glencross *et al* (2003 & 2004) who examined *L. luteus*' role and value as a particular aquaculture feed and concluded that "On a price per unit protein basis, the measure used in the aquaculture feed market, yellow lupin kernel meals should command about a 30 per cent to 40 per cent premium over narrow-leaf lupin kernel meals."

Table 9. Costs, returns and margins associated with nutritional component isolation for *L. luteus*

Isolation Cost Efficiencies - *L. luteus*						
Fraction	**Amount**	**Protein**	**Protein Component**	**Unit Value ($/t)**	**Value of Component ($/t of seed)**	
Hulls	30%	10%	3%	$150	$45	
Fibre	19%	5%	1%	$1,000	$190	
LPC	32%	65%	21%	$1,000	$320	
Albumin	4%	90%	4%	$2,500	$100	
LPI	13%	90%	12%	$2,000	$260	
	98%		40%		$915	Gross revenue
				Dehulling	$20	
				Milling	$15	
				Sieving and Extraction	$50	
				Repairs and maintenance	$5	Drying cost per tonne
				Drying LPC	$32	$100
				Drying LPI & Fibre	$252	$700
				Operating costs	374	
				Overheads and depreciation (10%)	$92	
				Margin @ 15% of component gross value	$137	
				Potential offer price for luteus ($/t)	$312	

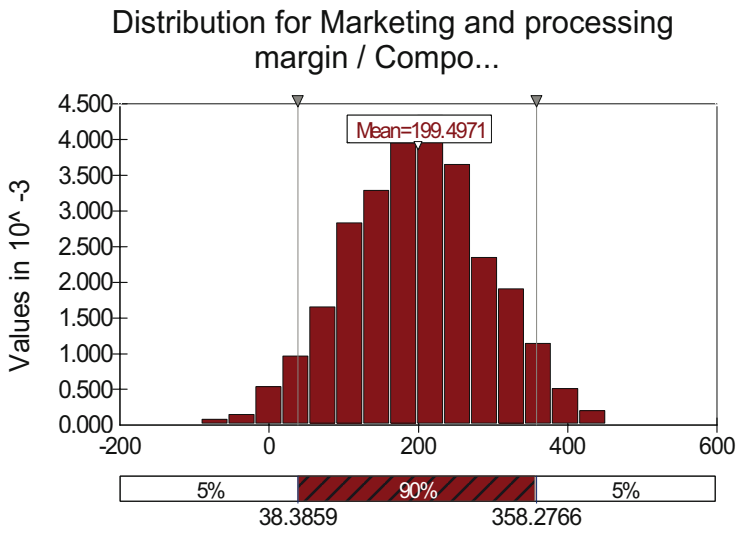

Figure 9. Distribution of profit margin from processing and sale of isolates ($/tonne of seed input).

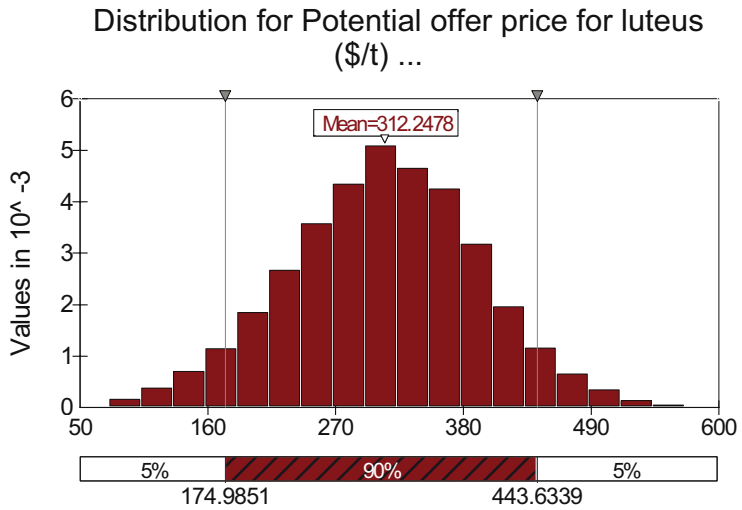

Figure 10. Distribution of the offer price for whole lupin seed based on a fixed percentage profit margin from processing and sale of isolates ($/tonne of seed input).

The value of breeding and technical change in altering *L. luteus* characteristics regarding its yield of protein isolates is shown in Table 10. The value is expressed as the upper price that could be paid to growers while preserving the fixed percentage profit margin from processing and sale of isolates. In effect this assumes that the extra value from the changes in *L. luteus* are shared between the processor/marketer and the grower. The combination of reducing the hull as a proportion of the seed weight and reducing fibre are suggested to be valuable alterations.

Table 10. Impacts on grower price of altering characteristics of *L. luteus*.

Scenario	Grower price ($/t)
Possible base *L. luteus* price	313
Hull proportion ↓10%	324
Fibre ↓10%	326
LPC ↓10%	300
Albumin ↑10%	318
LPI ↑10%	318
Hull proportion ↓10% & Fibre ↓10%	341

Not shown in Table 10 are other important technical innovations such as low-cost drying methods that would greatly improve the profit margin from component isolation.

Historically *L. luteus* have been priced at around $30 per tonne above the above the *L. angustifolius* price. The preceding analysis suggests that this price differential could or should change to make *L. luteus* production more attractive.

Farming systems models can be used to identify the trigger prices that would encourage a grower to consider planting *L. luteus*. Previous analyses of narrow-leaf lupin (*L. angustifolius*) in low rainfall farming systems have shown they remain as a profitable option on good sandplain soils. By contrast analyses of *L. luteus* often have concluded that they do not generally form part of most profitable farm plans. At current prices and yields, an examination of land use alternatives on the Wodjil soils in the eastern wheatbelt reveals that currently a gross price of around $225 per tonne (= $188/t farm-gate) would be sufficient to cause a profit-maximising farmer to begin to consider growing *L. luteus* on their Wodjil soil. This analysis assumes an insecticide cost of $25/ha, 90 kg/ha seeding rate, and yield of *L. luteus* in the range of 0.675 to 0.735 t/ha on the Wodjil soils. The

current indicator pool price from the Grain Pool for season 2004/5 for *L. angustifolius,* based on 32% protein content, is between $200 to $220 per tonne. This price rises for protein premiums up to a maximum of 40% protein. If the gross price for *L. luteus* is thus $250 per tonne or more, then this will be attractive to some growers with Wodjil soils.

The farming system models can be used to show how responsive is farm profit to increases in the price of *L. luteus* (see Figure 11).

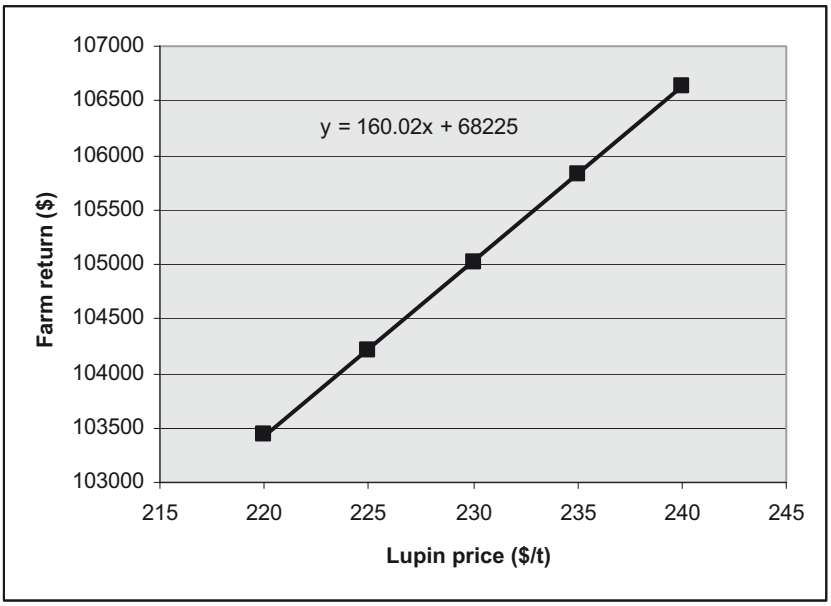

Figure 11. Eastern wheatbelt expected farm profit as a function of the price of *L. luteus*.

Every dollar increase in the gross price of *L. luteus* increases farm returns by around $160 or around 60 cents per hectare of lupins grown or by only 4 cents per hectare of farm area. The implications are that for the average eastern wheatbelt farmer, production of *L. luteus* is very much a second-order issue at prices in the range $220 to $240 per tonne. However, at prices above $260 per tonne then on certain farm types *L. luteus* production emerges as a more important enterprise, with a larger share of farm area being switched into *L. luteus* production.

To increase *L. luteus* production one or a combination of the following need to occur:

(i) the yield differential between *L. angustifolius* and *L. luteus* narrows,

(ii) the costs of production of *L. luteus* relative to those *L. angustifolius* lessen (e.g. more aphid resistant *L. luteus*),

(iii) the price differential between *L. luteus* and *L. angustifolius* widens in favour of *L. luteus or*

(iv) the yield reliability and ease of growing *L. luteus* improves.

A Reflection

When advocating a new industry or promoting some promising innovation, caution is usually merited because often important limitations or competing scenarios are overlooked. For example, consider the views a decade ago of leading plant scientists. Both seem destined to be wide of the mark (see figure 12). The latest Crop Variety Sowing Guide shows that *L. luteus* only accounted for 0.06 per cent of the area sown to lupins in season 2003 (i.e. around 30,000 ha).

> "I anticipate there will be 100,000 hectares of yellow lupins grown in 10 years time." (p. 33) (Cowling, 1995)

"I believe that in 2005 we will be producing no more than 4 million ha of cereals, perhaps 1 million ha of lupins and about 1 million ha of other broadleaf crops." (p.96) (Perry, 1995)

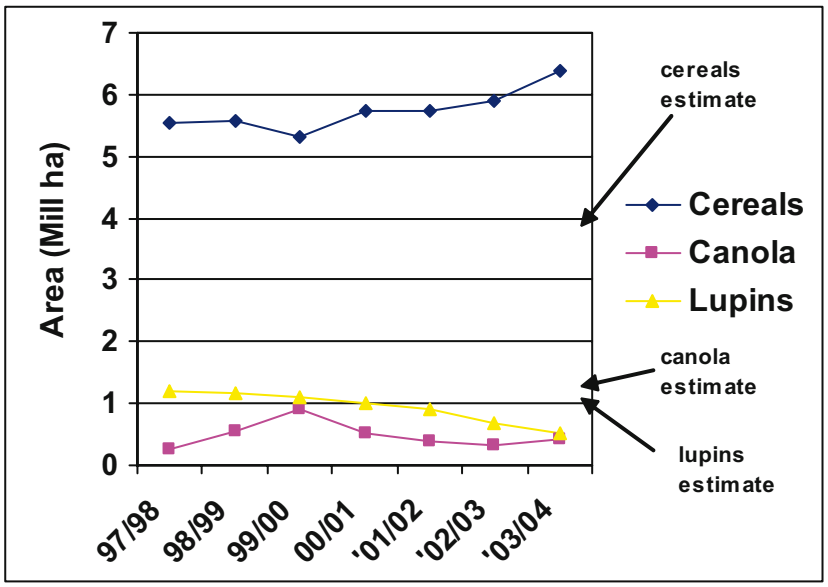

Figure 12. Industry expert projections of a decade ago.

In the case of *L. luteus* predicting a surge in its production due to a likely increase in the price premium for *L. luteus* is fraught with unforeseen issues that often tend to dampen prospects. Hence, although the signs are favourable for resurgence in *L. luteus* production, other competing changes are likely to diminish this growth.

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Application of near infra-red spectroscopy (NIRS) to manage the nutritional quality of aquaculture feed ingredients

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Introduction

Cost-effective use of available feed ingredients is fundamental to profitability of any livestock industry, including the aquaculture sector. To effectively utilise limited feed resources, it is essential that we identify those factors that can influence ingredient quality and subsequently develop techniques for the assessment of all ingredients prior to inclusion in compound feeds. To date, our ability to achieve this has been restricted, with techniques available to nutritionists and feed manufacturers limited to measurement or prediction of chemical composition of feed ingredients, or book values based on *in vivo* measurements. Given the variation that exists in the nutritional and physical quality of feed ingredients, this approach is far from adequate and alternative techniques for the rapid assessment of nutritional quality must be identified.

Near infrared spectroscopy (NIRS) represents a rapid, cost-effective, repeatable and accurate means of assessing the nutritional quality of feed ingredients and, in some cases, complete feeds. The procedure is based on the fact that when exposed to specific wavelengths of infrared light, components within a foodstuff, such as protein, moisture, starch and oil, have characteristic NIR absorption bands (Figure 13). Using this principle, calibrations between characteristic NIR spectra and nutritional quality for any ingredient can be developed to optimise the final quality of aquafeeds, but first it is necessary to define the primary drivers of nutritional quality for aquafeed ingredients.

The application of NIRS for the assessment of the nutritional quality of feed ingredients for aquaculture species is not as advanced as it is for other monogastrics (e.g. pigs and poultry). This is due to the fact that we have less knowledge about the nutritional requirements of many aquaculture species, and there are other factors that must be considered when producing many aquatic feeds such as water stability and binding capacity.

The aim of this paper is to identify where the greatest gains are to made through the application of NIRS in aquafeed production and to highlight some recent developments in the application of NIRS that may be improve the production of aquafeed.

Defining nutritional quality of ingredients for aquafeeds

The “nutritional quality” of aquafeed ingredients reflects their comparative ability to supply specific nutrients to the target species via a specific diet form (eg. semi-moist, steam, or extruded pellet) while being free of chemical, physical and microbiological contaminants. Our capacity to measure the nutritional quality of an ingredient prior to diet manufacture will influence:

- The match of diet specifications to the nutrient requirements of the target fish species;
- Variation in fish performance through consistency of nutrient supply over time;
- The overall quality of the processed diet in terms of pellet integrity and the influence of processing on nutrient supply;
- The health status of the fish through elimination of feed contaminants.

Further to this, measurement of key macro-nutrients (protein, moisture, fat) in complete feeds can represent an important quality assurance tool that ensures all ingredients have been correctly assembled and that the processing conditions did not comprise overall diet quality.

With the above in mind, the following outlines the most appropriate applications of NIRS to define the nutritional quality of ingredients for use in aquafeeds.

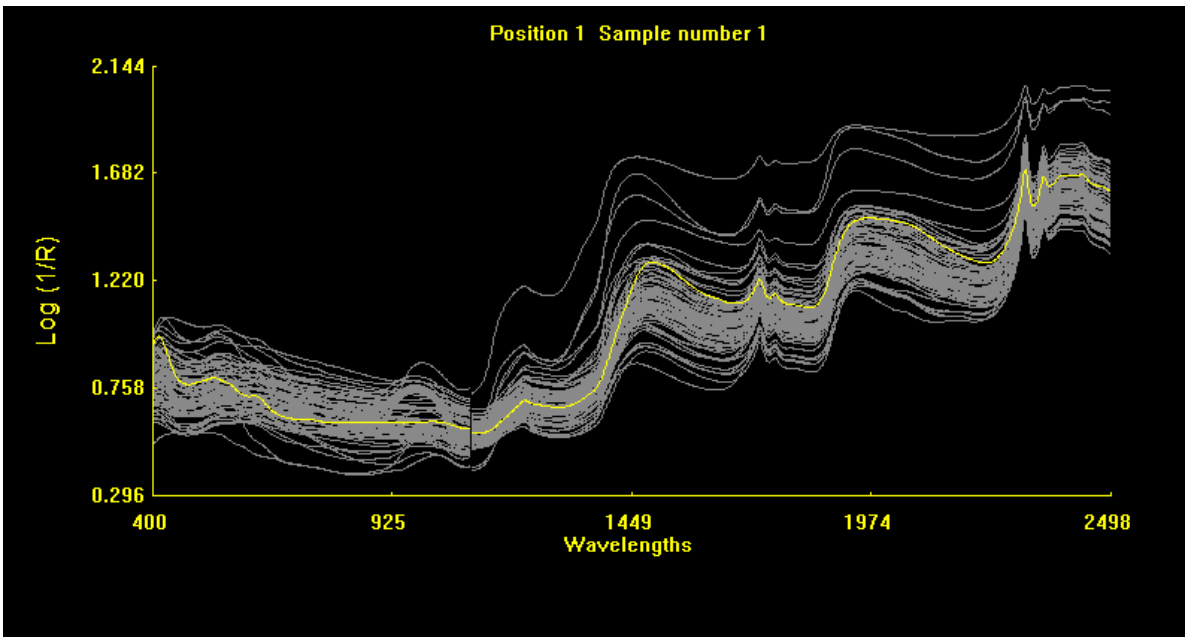

Figure 13. NIRS spectra can be used to detect differences in the composition of aquafeed ingredients and can vary significantly for ingredients of apparently consistent quality.

Measurement of macro-nutrients or components using NIRS

Macro-nutrients such as crude protein, moisture and crude fat and ingredient components such as starch content (often critical to pellet binding and the capacity of a processed feed to float or sink) and oil content represent a useful means of assessing ingredient consistency, and hence the "complimentary additivity" of that ingredient when it is incorporated into a processed feed. In addition, they provide a useful guide to the supply of nutrients from that ingredient. While definition of "available" or "digestible" nutrients provides a more accurate match of diet specifications to fish requirements, in most cases gross chemical composition is adequate for aquafeed production, with this discussed in more detail later in this paper.

NIRS calibrations for ingredient components such as crude protein, moisture, fat and starch can be developed relatively inexpensively. Only *in vitro* wet chemistry is required (as opposed to *in* vivo experiments with target fish species) to complete measurements on a wide range of ingredients, and NIRS is well established in the measurement of these parameters in a wide variety of feeds. Gross chemical composition can also be applied to a wide range of fish species, and in some cases, correlates can be used to define "available" nutrient supply. In cases where there are few changes to the combination of ingredients in a processed feed, NIRS can be applied to measure processed feed quality as well as ingredient quality.

An example of where NIRS can be applied to maintain the supply of nutrients and to maintain manufactured feed quality is the farmed southern bluefin tuna industry. Farmed tuna are currently fed baitfish as their primary source of nutrients while manufactured diets being developed for tuna and other fish species may also incorporate a fresh baitfish component. As the quality of baitfish used in tuna production varies significantly, our capacity to measure quality is important if we are to improve the efficiency of farmed tuna production. A survey of baitfish being used to feed farmed tuna revealed large variation in the crude protein, crude fat, free fatty acid and peroxide value of the baitfish on offer (Table 11). To account for this, van Barneveld (2001a) demonstrated that the crude protein, moisture, crude fat and free fatty acid content of bait fish can be adequately screened using NIRS on samples of processed frozen, processed thawed and processed freeze-dried bait fish, the latter being the most accurate (Table 12).

Table 11. Typical variation in the composition and quality of baitfish fed to southern bluefin tuna.

Parameter	Range
Crude protein (%, DM)	49.4-75.3
Crude fat (%, DM)	1.9-36.5
Free fatty acids (%, DM)	2.9-53.4
Peroxide value (meg/kg, DM)	<0.1-598.0

Table 12. Calibrations statistics for chemical constituents of baitfish (1100-2500 nm).

	N	Mean	RSQ	SECV	1-VR
Moisture (%)	72	73.13	0.98	0.64	0.96
Crude protein (%)	75	17.99	0.85	0.58	0.73
Crude fat (%)	73	2.07	0.96	0.68	0.88
Free fatty acids (%)	71	6.61	0.84	1.49	0.69

RSQ, r-squared; SECV, standard error of cross validation; VR, variance ratio.

Measurement of available nutrient supply

In mature livestock sectors such as the pig and poultry industries, measurement of available nutrient supply is critical to optimising production efficiency because it accounts for losses that occur during digestion and metabolism. Diet specifications and available ingredients change on a daily basis, and a wide range of ingredients are utilised to produce the diets. Compared with the terrestrial livestock sectors, there is less information on the nutrient requirements of many aquaculture species, or we have less capacity to change the performance of the fish during the production cycle through a diet change. As a consequence, the need for NIRS calibrations that allow the prediction of nutrient supply on a routine basis is diminished. In addition:

- There is less capacity in aquaculture systems to control the intake of individual fish. As a consequence, the variation in intake in a commercial system is far more likely to exceed the variation in nutrient supply that may exist between individual batches of feed ingredients.
- We utilise a more defined range of feed ingredients in aquafeeds, particularly semi-moist and extruded aquafeeds due to the influence of ingredient changes on overall pellet quality. In many cases, when an ingredient change occurs, significant pilot scale production runs are required to define new manufacturing parameters. This makes it difficult to respond to NIRS measurements that relate to nutrient supply as opposed to ingredient consistency.
- In general, feed ingredients used in aquafeeds are of higher quality than those used by the terrestrial stockfeed industries, and as a consequence, less variation in likely to exist between individual batches of ingredients. NIRS represents a useful tool to ensure that this variation is in fact minimal, but it is unlikely that significant variation will exist in available nutrient supply.
- When the carbohydrate component of an aquafeed is minimal, the potential variation in nutrient supply and the need for routine assessment of available nutrient supply is also likely to be less.
- The high capacity of many carnivorous fish to digest protein and fat will result in minimal variation in available nutrient supply and will decrease the need for routine NIRS measurement of available nutrient supply. The lack of variation will also make it very difficult to collate a suitable dataset for use in calibration development.

For the above reasons, once an ingredient source and its corresponding nutritional quality has been established using *in vivo* experimentation and a process has been defined to incorporate that ingredient into a mixed diet, it is more important to utilise NIRS to measure consistency rather than nutrient supply *per se*. Certainly, where the definition of nutritional requirements is advanced for a particular aquaculture species, where the species is known to respond to minor changes in the nutrient content of the diet, and where significant flexibility exists in terms of the range and source of ingredients for a particular aquafeed (such as shrimp feeds), then NIRS calibrations should be developed to measure nutrient supply from particular ingredients. Given the costs associated with developing NIRS calibrations for nutrient supply, it is suggested that in the short term, there are better applications of NIRS technology in the aquafeed sector than prediction of available nutrient supply.

Assessment of processing responses using NIRS

In animal (fish meals, meat meals) and vegetable proteins (soybean meal, canola meal) that have undergone processing, heat damage can result in an overestimation of nutritional quality due to reactions between the ε-amino group of lysine with other compounds. Rutherfurd et al. (1997) developed the digestible reactive lysine assay as a means of assessing heat damage in feed ingredients and it was subsequently demonstrated by van Barneveld et al. (1999) that reactive lysine per se could be used as a measure of heat damage. To assess the potential for NIRS to measure total and reactive lysine in heat treated protein sources, van Barneveld (2001b) subjected samples of canola meal to a structured range of dry and autoclaved heat treatments to create sample sets of 60 for each protein source. In addition to this, random samples of canola meal were included in the sample set, prior to development of NIRS calibrations for both total and reactive lysine (Tables 13).

Table 13. Performance indicators for NIRS calibrations developed for the prediction of total and reactive lysine (g/kg, as received) in cold-pressed and solvent-extracted canola meal samples (van Barneveld, 2001b).

Constituent	SEL	SECV	SD	SECV/SEL	SECV/SD
Total lysine	0.40	0.42	4.64	1: 1.05	0.09
Reactive lysine	0.60	0.76	4.46	1: 1.27	0.17

SEL, Standard error of laboratory reference; SECV, Standard error of cross validation; SD, Standard deviation.

Based on the above data, there is no reason why NIRS calibrations could not be developed for both heat-treated feed ingredients commonly used in aquafeed production and processed aquafeeds themselves. This routine quality assurance procedure could significantly enhance the consistency and quality of aquafeeds.

In addition to heat damage, development of NIRS capacity for the measurement of changes in starch properties (gelling temperatures etc) with increased heat application could be very useful in defining the capacity to incorporate a new ingredient into extruded aquafeeds.

Measurement of contaminants

NIRS is routinely applied in the pharmaceutical and other industries for the detection of contaminants. Providing the contaminant in question has a characteristic NIR absorption spectra, accurate calibrations can be developed relatively easily using “spiked” samples.

A bigger issue associated with the measurement of contaminants in aquafeeds and aquafeed ingredients is the accuracy of the sampling procedure. Unless a representative sample can be routinely collected, little value will be gained through rapid and objective analysis using NIRS. In particular, it is unlikely that an adequate sampling protocol for moulds and mycotoxins will be practically feasible in a commercial feedmill or ingredient receival point. With this in mind, it appears that prevention of these contaminants through correct storage and the strategic application of mould inhibitors is by far the best course of action, and where this has proved difficult to achieve or frequently ineffectual, then application of effective mycotoxin binders should be considered.

Conclusions

The need to improve our capacity to manage the nutritional quality of aquafeed ingredients increases with improvements in the efficiency of aquaculture production systems, definition of the nutritional requirements of these species and scrutiny from consumers of the inputs to these systems. Rapid, accurate and cost-effective analysis of feed ingredients using NIRS represents a useful way to monitor ingredient quality by the aquafeed sector.

In aquafeed production systems, primary contributors to the management of nutritional quality of feed ingredients include the assessment of macro-nutrients such as protein, fat and moisture, and key components of feed ingredients including starch and oil. Capacity to detect over-processing or heat damage, changes in starch properties with heat application and contaminants will also contribute to reduced variation in feed quality. In the longer term, as knowledge of nutrient requirements for target species improves, as the capacity to manipulate the composition of diets increases, and as the capacity to control the intake of individual fish in a commercial production system increases, the need to measure the "available" or "digestible" nutrient supply using NIRS will increase.

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Product evaluation update - Rainbow trout

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Introduction

Over the past 12 months the majority of the nutritional evaluation work on the grain protein concentrates and isolates produced in the project have used rainbow trout, primarily as a "laboratory rat" species, to evaluate the products. As with earlier work by the CLIMA research group on grain product evaluation the products have been studied with view to providing answers to three central issues:

a. Defining the digestibility of key nutrients from the ingredients.

b. Evaluating the palatability of each product when fed to an aquaculture species.

c. Defining the influence of ingredient use on the aquaculture species capacity to utilise the nutrients from the ingredient for growth.

In addition to this, over the past 12 months ingredient functionality has also been targeted as an additional issue to examine and progress has already been made to assess this with many of the products. The evaluation process has examined some of the new products produced, although much of the previous 12 months work has focussed on completing the evaluation process of the first "prototype" products.

Digestibility of prototype-1 products

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".

Table 14. 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
NFE	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	22.2	22.2	22.6	22.6	19.6	20.3	23.0	21.2

LKM: *L. luteus* kernel meal; AKM: *L. angustifolius* kernel meal; LPC: *L. luteus* protein concentrate; APC: *L. angustifolius* protein concentrate; LPI: *L. luteus* protein isolate; API: *L. angustifolius* protein isolate; SBM: Solvent-extracted soy bean meal; SPC: Soy protein concentrate; SPI: Soy protein isolate; EHC: Enzymatically-hydrolysed casein. NFE: Nitrogen-Free Extract (approximates carbohydrate content).

High digestible value of protein and energy for all protein meals and concentrates was observed (Table 15). 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 (Glencross et al., 2005a). Given the substantial differences observed between the two methods, particularly with ingredients high in NFE, it was deemed more appropriate to use stripping as a standard method for future studies.

Table 15. Apparent digestibility coefficients of first generation protein concentrate and isolate products produced from sweet and yellow lupin varieties when assessed using stripping faecal collection methods. Digestibility coefficients determined using the method of Sugiura et al. (1998).

Ingredients	LKM	AKM	LPC	APC	LPI	API	SBM	SPC	SPI	EHC
Stripping										
Nitrogen/Protein	88.6	85.3	102.1	98.4	99.4	95.1	92.1	97.9	98.2	92.2
Phosphorus	183.3	346.0	131.5	138.5	67.5	120.9	27.7	76.3	54.0	92.3
Energy	64.2	53.1	94.4	84.2	92.4	91.3	72.1	87.3	95.6	91.5
Organic Matter	57.5	44.6	92.8	70.7	88.3	87.6	61.0	67.2	96.4	89.1
Settlement										
Nitrogen/Protein	97.2	97.2	99.3	101.0	101.1	98.6	99.0	106.9	97.8	96.0
Phosphorus	175.9	272.2	70.7	87.2	52.9	71.7	56.7	58.9	42.2	85.4
Energy	83.8	70.5	92.3	86.6	91.7	93.8	83.3	85.6	93.1	98.8
Organic Matter	80.9	64.8	94.1	76.7	90.0	94.8	77.3	82.0	95.2	98.5

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 of prototype-1 products

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 six weeks an effect of both of the positive controls was evident, but no specific effects that were attributable to inclusion of either of the protein concentrates. Re-examination of the feed intake data of the first ten days of the study using a paired t-test identified that the 40% APC diet had a significantly lower feed intake than the reference diet, as did both negative controls (Figure 14).

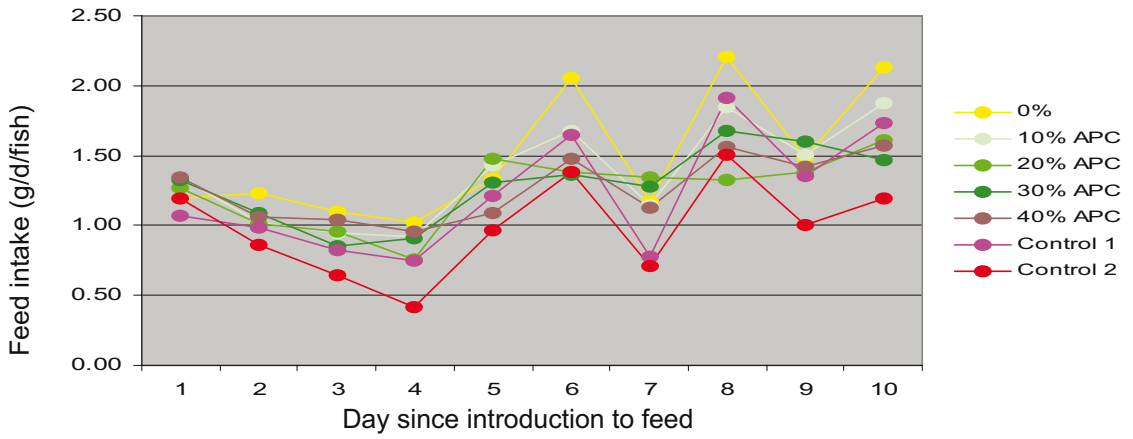

Figure 14. Variation in feed intake over the first 10 days of feeding various APC inclusion levels and control diets.

Table 16. Growth experiment data.

	0%	10%-L	20%-L	30%-L	40%-L	10%-A	20%-A	30%-A	40%-A	C1	C2	COM
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	35.7
Final weight (g)	126.8	127.4	130.5	130.1	128.0	126.4	123.0	124.0	126.4	117.4	97.8	132.6
DGC (%/d)	4.13	4.14	4.25	4.23	4.16	4.12	4.00	4.04	4.11	3.83	3.12	4.30
Gain (g)	91.2	91.7	95.0	94.4	92.4	90.8	87.2	88.4	90.8	81.9	62.0	96.9
FCR	0.95	0.91	0.88	0.88	0.90	0.90	0.87	0.92	0.89	0.91	0.94	0.87
FCE	1.14	1.19	1.22	1.22	1.20	1.20	1.24	1.17	1.20	1.18	1.14	1.24
Feed intake (g/fish/d)	1.89	1.82	1.83	1.83	1.81	1.78	1.66	1.78	1.78	1.64	1.28	1.84
Survival	100%	100%	99%	100%	100%	99%	99%	100%	100%	98%	100%	100%
N retention (%)	34.4	39.1	42.2	41.1	39.5	38.8	41.0	38.0	36.5	35.9	33.7	35.5
P retention (%)	28.6	29.3	29.4	37.7	36.3	33.7	33.4	36.4	38.6	29.6	25.9	35.1
E retention (%)	44.0	46.0	50.4	48.8	52.2	48.9	49.2	47.8	48.4	45.5	40.0	50.9

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. COM: Commercial diet, Skretting Pacific LE. DGC: Daily Growth Coefficient. FCR: Food Conversion Ratio (feed:gain). FCE: Feed Conversion Efficiency (gain:feed).

Growth by fish fed diets with increasing levels of either the LPC or the APC both showed that these ingredients had few palatability problems long-term and sustained good growth by rainbow trout (Glencross et al., 2005b). Notably growth was equivalent to that obtained with an extruded commercial formulation. Growth, feed intake and feed utilisation efficiencies were influenced in the control treatments, thereby demonstrating that the experiment had the capacity to detect real effects, should they have been present (Table 16). Feed conversion/efficiency was not significantly affected by the inclusion of the protein concentrates. Phosphorus retention was improved with the inclusion of either protein concentrate, which combined with the known high digestibilities of these products will mean that they have an advantage in being able to limit soluble phosphorus release with their inclusion in the diet.

Utilisation value

One of the problems that can result from the use of plant protein resources is the introduction of anti-nutritional factors or non-metabolisable or usable nutrients. These types of issues with feed ingredients can have the potential to reduce the value of the ingredient. To examine this issue in fish an experimental approach called the protein-limited-restrictively-fed design was used.

In this experiment, diets were formulated with each test ingredient included at either 20% or 40% of the diet and the diets all kept to the same level of digestible protein (333 g/kg) and energy (15.8 MJ/kg). The diets were formulated with the same amount of limiting protein to ensure that protein quality became a point of sensitivity in comparison of the treatments. The reference diet was based on fish meal as the only protein source. The diets were then fed to each treatment at the same amount, restrictively to ensure that there was no feed intake variation between the treatments. This ensures that the fish cannot vary their feed intake to compensate for any inadequacies in the diets and by inference, quality of the ingredients. Because of these experimental constraints any deficiencies in the diets are clearly exhibited as growth effects. A series of control treatments where 20% and 40% of the diet was replaced by cellulose, were also included in the study to examine the effects of similar levels of addition of something to the reference diet, but of no nutritional value. This experimental 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., 2003a).

The results of this study demonstrated that both APC and LPC could replace fish meal at up to 40% with no deleterious effect on nutrient utilisation from the test ingredients (Table 16). Similar high levels of performance were also observed from *L. luteus* kernel meal and a soy protein concentrate. The cellulose control diets both performed significantly poorer than every other treatment. The 20% cellulose addition diet treatment performed

better than the 40% cellulose addition diet treatment. This study completed the tripartite evaluation of the prototype lupin protein concentrate products, with comprehensive sets of data on nutrient and energy digestibility, palatability and nutrient utilisation (Glencross et al., 2005b).

Table 17. PLRF Growth experiment data.

	0%	20% A	40% A	20% L	40% L	20% C	40% C	20% S	40% S	20% K	40% K
Initial weight (g)	114.1	113.3	114.1	114.6	113.9	113.0	113.4	113.8	113.7	112.3	113.6
Final weight (g)	208.4	213.2	202.7	213.5	206.5	170.5	137.7	206.9	199.0	210.2	215.5
DGC (%/d)	2.57	2.70	2.44	2.66	2.53	1.69	0.77	2.54	2.37	2.67	2.74
Gain (g)	94.2	99.9	88.6	98.9	92.6	57.5	24.4	93.1	85.3	97.9	101.9
% increase	183%	188%	178%	186%	181%	151%	121%	182%	175%	187%	190%
FCR	0.99	0.92	1.05	0.93	1.01	1.58	3.30	0.99	1.08	0.94	0.91
Feed intake (g/fish)	92.0	92.0	93.0	92.0	93.3	90.5	79.6	92.0	92.0	92.0	93.0
Survival	100%	100%	98%	100%	97%	100%	98%	100%	100%	100%	98%

0%: Reference Diet. 20%A and 40%A: 20% and 40% inclusion of APC respectively. 20%L and 40%L: 20% and 40% inclusion of LPC respectively. 20%C and 40%C: 20% and 40% inclusion of cellulose respectively. 20%S and 40%S: 20% and 40% inclusion of soy protein concentrate respectively. 20%K and 40%K: 20% and 40% inclusion of *L. luteus* kernel meal respectively.DGC: Daily Growth Coefficient. FCR: Feed Conversion Ratio (feed:gain).

Digestibility of prototype-2 products

Based on the findings from the evaluation of the prototype-1 products and the identification that drying costs would be one of the key limiting factors to economic success of such products, protein concentrates were again made from *L. angustifolius* (cv. Myallie) and *L. luteus* (cv. Wodjil) (Kingwell, 2003). This time the concentrates produced were dried using a variety of laboratory and industrial techniques. These included freeze-drying (positive control), oven-drying (negative control), spray-drying and ring-drying. The spray-dried and ring-dried products were dried using industrial scale facilities. While a viable product was produced using the spray-drying technology, the ring-drying technology proved to not be suitable to drying the product. An experiment evaluating the digestible protein, amino-acid, phosphorus and energy values of these products was undertaken in March 2004. Samples are presently being analysed to allow determination of these parameters.

Table 18. Composition of 2nd-stage prototype ingredients evaluated. Details are on a dry matter basis (g/kg DM) unless otherwise specified.

INGREDIENTS	LKM	MKM	LPCD	MPCD	LPCS	MPCS	LPCF	MPCF	EHC
Dry Matter (g/kg)	924	914	958	974	n/a	n/a	928	964	916
Protein	549	423	789	744	n/a	n/a	801	746	839
Fat	79	80	39	90	n/a	n/a	113	133	11
Ash	43	35	42	39	n/a	n/a	34	30	70
Organic Matter	957	965	958	961	n/a	n/a	966	970	930
Phosphorus	6	5	7	6	n/a	n/a	7	6	9
Energy (MJ/kg DM)	19.37	18.65	22.68	23.46	22.80	23.45	21.88	23.13	20.09

LKM: *L. luteus* (cv. Wodjlil) kernel meal; MKM: *L. angustifolius* (cv. Myallie) kernel meal; LPCD: *L. luteus* protein concentrate oven-dried; MPCD: *L. angustifolius* protein concentrate oven-dried; LPCS: *L. luteus* protein concentrate spray-dried; MPCS: *L. angustifolius* protein concentrate spray-dried; LPCF: *L. luteus* protein concentrate freeze-dried; MPCF: *L. angustifolius* protein concentrate freeze-dried EHC: Enzymatically-hydrolysed casein. n/a : data not yet available.

Plans for the upcoming 12 months

A single growth trial is planned for July/August 2004 to complete the evaluation of the 2nd-stage prototype products. It is proposed that this trial will compare the metabolisable protein and energy values of the MPCS and MKM products to determine if the efficiency of utilisation of these ingredients is equivalent to the same parameters from fish meal or soybean meal.

Although there is good information on the variability among lupin species, there is only limited data on the variability within lupin kernel meal species (Glencross and Hawkins, 2004). Of the information on variability within a lupin species (*L. angustifolius*), primarily among different cultivars, substantial variability in the digestible protein and energy value was identified (Glencross et al 2003b). In order to maximise the value of this ingredient to the aquaculture feeds industry, attempts to reconcile this variability and develop methods to predict it are considered a priority. Accordingly, much of the future activities of the research program are being directed towards establishing methods for such quality assurance assessment. This is likely to entail several digestibility trials evaluating the digestible nutrient and energy values of many lupin kernel meal samples in order to begin the development of Near-Infrared-Reflectance Spectroscopy (NIRS) calibrations for lupin kernel meal use in salmonid diets. Three to four digestibility trials are planned for the latter half of 2004 to assess the variability in lupin kernel meal digestibilities.

While short-term research plans are to complete the evaluation of the Generation-2 prototype products, beyond this activities are likely to centre on lupin kernel meal evaluation and assessment of commercially produced products from partner organisations.

A single growth trial is planned for March/April 2005 to further the evaluation of the lupin kernel meal products. It is proposed that this trial will compare the metabolisable protein and energy values of different kernel meal products, based on their digestibility and functionality differences, to determine if the efficiency of utilisation of these ingredients is equivalent to the same parameters from fish meal or soybean meal.

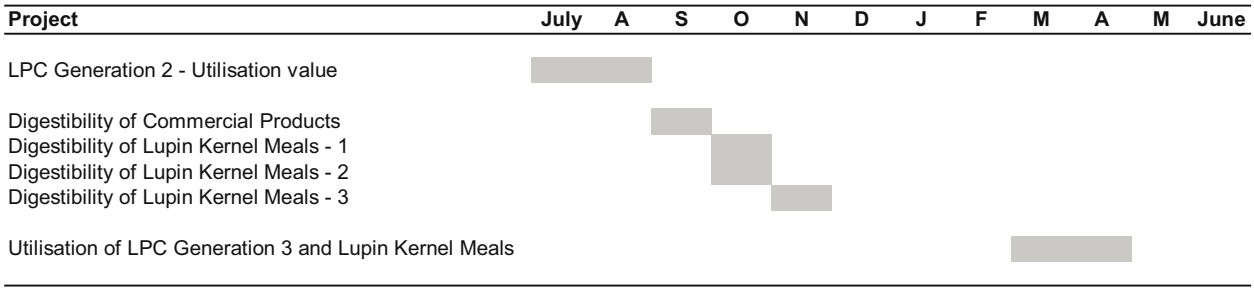

Figure 15. Gannt chart of proposed experiment activities for 2004/2005.

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Product evaluation update - Atlantic salmon (Winter Salmon)

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Introduction

The Norwegian salmon farming industry is the largest industrialised aquaculture industry in the world. It annually produces a harvest in excess of 500,000 tonnes of fish. To support this more than 600,000 tonnes of fish feed is used each year. Fish meals are traditionally the major protein ingredients in fish feed. However, 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.

For these reasons, the use of protein rich grains and vegetable protein concentrates is steadily increasing in diets for Atlantic salmon, although such ingredients may be rich in indigestible material (Bach-Knudsen, 1997). The most widely used vegetable protein sources at this point are maize gluten, wheat gluten, various soy protein concentrates, and extracted or full fat soybean meal. The protein price of soybean meals is relatively low compared to these other alternatives. However, extensive use of soybean meal to salmon has been limited by the presence of potent anti-nutritional factors (ANFs) in soy (reviewed by Storebakken et al., 2000; Francis et al., 2001). Furthermore, due to strict GMO regulations and low customer acceptance of GMO foods, GMO soy and maize gluten is not used in European salmon feeds.

Australian lupin kernel meals have begun to be used by commercial feed companies in Norway as a supplement or alternative to soybean meal. This is due to high protein content, but also because of potentially less problems with ANFs. It is therefore of value for the industry there to increase their understanding of how these ingredients are best utilised.

Feed digestion at cold water temperatures

Both lipid and protein digestion have been observed to be lower at the colder water temperatures in Atlantic salmon (Bendiksen et al., 2003). Dietary soybean meals have also been shown to affect the overall digestibility of lipid in salmon (Refstie et al., 1998, 2001, 2002). Because of these reasons it is important to evaluate the digestible value of feeds and ingredients at a range of temperatures. In Norway, winter water temperatures in some aquaculture areas approach close to 0°C.

It is planned to undertake an evaluation of the digestibility of 6 test ingredients when fed to Atlantic salmon in salt water at 6°C (Anderson et al., 1993). The apparent digestibility of protein, energy and essential amino acids in selected grain protein meals and/or grain protein concentrates developed as part of the linked CLIMA-GRDC project will be undertaken. The final choice of which products will be evaluated will be undertaken in close consultation with the grains processing and aquaculture feed industries. However, several key grain products have already be ear-marked as potential options. These include:

1. *L. luteus* (cv. Wodjil) protein concentrate – spray dried
2. *L. angustifolius* (cv. Myallie) protein concentrate – spray dried
3. *L. angustifolius* (cv. Myallie) kernel meal
4. *L. angustifolius* (cv. Belara) kernel meal
5. *L. luteus* (cv. Wodjil) kernel meal
6. Solvent-extracted soybean meal

The two cultivars of *L. angustifolius* kernel meals (Myallie and Belara) show substantially different viscosity profiles when examined using rapid-viscosity analysis (RVA). It is suspected that this viscosity is related to differences in the non-starch polysaccharide (NSP) composition between the two lupin cultivars. The implications of these differences in viscosity on the digestion of these grains in Atlantic salmon are unknown.

The digestibility of the grain products will be determined using the reference diet substitution method. Ytterbium oxide will be used as a digestibility marker, each added to the diets at 0.05% on a dry matter basis. Diet preparation and digestibility experiment protocols will be as described by the FRDC Aquaculture Nutrition Subprogram Methods Manual. Collection of faecal material by stripping will start after a three-week conditioning period and will continue until sufficient faecal is available for the whole range of analyses (crude protein, energy, amino acids and Yb). The six treatment and one reference diets will be randomly allocated amongst 21 tanks (groups of fish) to enable the collection of three replicates for each diet, based on a Latin-square design. Hopefully, the same diets as fed to the warm-water Atlantic salmon studies will be used in this study, allow some direct comparisons between the two sets of studies.

Gut health in Salmon

The most potent ANF(s) in soy are antigens that cause inflammation in the intestinal mucosa (van den Ingh et al., 1991; Baeverfjord and Krogdahl, 1996; Krogdahl et al., 2000, 2003). Notably, soybean meals have been observed to cause enteritis problems in the distal intestine of Atlantic salmon at even low inclusion levels (Krogdahl et al., 2003). For this reason, the Norwegian salmon industry is reluctant to use more that 5-10% soybean meal in salmon feed. Alcohol washing of soybean meal to produce soy protein concentrate largely eliminated this antigenicity problem (van den Ingh et al., 1991, 1996). Thus, it is believed that antigenic soy peptides and/or proteins induce these responses in Atlantic salmon.

It is unknown if lupin kernel meals cause the same enteritis problems in the distal intestine of Atlantic salmon. Thus, a series of comparisons of the enteritic effects of lupin kernel meals and lupin protein concentrates, with soybean meal, when fed to Atlantic salmon is planned, starting with the cold-water experiment outlined above. This work will examine the relative changes in histology of the fish's proximal and distal intestines. An enzymological evaluation of these tissues will also be undertaken. Using these approaches a comprehensive approach to understanding the antigenic influences of lupin kernel meals when fed to Atlantic salmon will be gained.

This work will be done in collaboration with the Gut Health Group of the Aquaculture Protein Centre (APC). APC is a Centre of Excellence initiated by the Research Council of Norway, and is devoted to developing basic nutritional, physiological and technological knowledge needed to optimise the use of protein in feed for farmed fish.

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Product evaluation update - Atlantic salmon (Summer Salmon)

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Introduction

In 2003 the first steps were made towards assessing the feasibility of the new generation lupin protein concentrates and isolates when fed to Atlantic salmon (Glencross et al., 2004 -Appendix 1). This built on earlier work examining the use of air-fractionated lupin protein concentrates and lupin kernel meals when fed to Atlantic salmon (Carter and Hauler, 2000; Farhangi and Carter, 2001; Carter et al., 2002). In the second phase of the project special components are being included on ingredient digestibility by Atlantic salmon in warm (15°C) and cold (6°C) water temperatures and also the effects of lupin products on gut transit rates by Atlantic salmon.

Salmon versus Trout

The 2003 lupin protein concentrate trials were largely based on comparing the differences in digestibility assessments between Atlantic salmon and rainbow trout when fed the same diets, using the same faecal collection methods. This study found that for the protein concentrates and isolates that there was little difference in the findings between the two fish species for protein, energy and organic matter digestibilities, although major differences in phosphorus digestibilities were noted. However, substantial differences were noted in the capacity of the two species to deal with lupin kernel meals. Notably, trout dealt much better with the higher levels of non-starch polysaccharides than did the Atlantic salmon (Table 19), as was noted from the significant differences in organic matter digestibility.

Table 19. Digestibility coefficients of new generation lupin products and existing soybean products when fed to either rainbow trout or Atlantic salmon.

	LKM	LPC	LPI	SBM	SPC	SPI	EHC	Pooled SEM
TROUT								
N	0.972	1.010	0.986	0.990	1.069	0.978	0.960	0.006
P	2.722	0.872	0.717	0.567	0.589	0.422	0.854	0.118
E	0.705	0.866	0.938	0.833	0.856	0.931	0.988	0.014
OM	0.648	0.767	0.948	0.773	0.820	0.952	0.985	0.018
SALMON								
N	1.304	1.087	0.969	0.944	0.901	0.974	1.124	0.0295
P	0.260	0.081	0.226	0.081	-0.204	0.242	0.914	0.0805
E	0.696	1.059	1.045	0.890	1.012	1.174	1.093	0.0332
OM	0.553	0.818	0.958	0.734	0.783	0.979	1.012	0.0342

Data from Glencross et al. (2004)

Mineral and trace element digestibilities were compared in diets containing *Lupinus albus* and *L. angustifolius* meals at high inclusion levels (Ward & Carter, in preparation). Differences in composition between the two ingredients were reflected by differences in digestibility and carcass composition for some, but not all, of the minerals and trace elements. There were no differences in the growth performance of Atlantic salmon fed the two meals.

As the expanded CLIMA-GRDC project enters its second phase, with a renewed focus on quality assurance issues for use of lupin kernel meals in the aquaculture feeds industry, the need to include a special component on Atlantic salmon is clear given the subtle differences between the two salmonid species. This is especially so for lupin kernel meals. This new component is forming part of the FRDC funded component of the expanded project.

Why two water temperatures?

It has been recognised by the international aquaculture feeds industry that Atlantic salmon digestion differs when the water is at their upper thermal ranges or their lower thermal ranges (Bendiksen et al., 2003). Notably, both lipid and protein digestion was lower at the colder water temperatures. Because of this reason it is important to evaluate the digestible value of feeds and ingredients at a range of temperatures. In Tasmania however, the lower ranges of water temperatures are rarely reached. However, in summer water temperatures in the Tasmanian salmon farming industry reach the upper limits of the species. Therefore it makes more sense for the upper thermal range work to be undertaken in Tasmania.

It is planned to undertake an evaluation of the digestibility of 5 test ingredients when fed to Atlantic salmon in salt water at 15°C. This is the standard temperature that has been used in numerous experiments on Atlantic salmon conducted at School of Aquaculture, TAFI. The apparent digestibility of protein, energy and essential amino acids in selected grain protein meals and/or grain protein concentrates developed as part of the linked CLIMA-GRDC project will be undertaken. The final choice of which products will be evaluated will be undertaken in close consultation with the grains processing and aquaculture feed industries. The digestibility of the grain products will be determined using the reference diet substitution method. Ytterbium oxide will be used as a digestibility marker, each added to the diets at 0.05% on a dry matter basis. Diet preparation and digestibility experiment protocols will be as described by the FRDC Aquaculture Nutrition Subprogram Methods Manual. Collection of faecal material by stripping will start after a one-week conditioning period and will continue until sufficient faecal is available for the whole range of analyses (crude protein, energy, amino acids and Yb). The six diets will be rotated amongst six tanks (groups of fish) to enable the collection of four replicates for each diet, based on a Latin-square design.

Why gut transit?

Current research is assessing the effects of lupin carbohydrates on gastric and digestive tract evacuation rates. Other research has shown that certain carbohydrates within lupins affect the digestion and gut metabolism processes, and it is likely that this will also be the case with Atlantic salmon, but the significance of such a potential effect is not known (Glencross et al., 2003a). Substantial variability in the different varieties of lupins has also been observed on the digestion process and the implications of this to Atlantic salmon are also not known (Glencross et al., 2003b). This part of the research project will develop an approach for further assessment of grain products in order to understand their effects on digestive processing.

As part of the digestibility experiment a study will be carried out to determine the influence of each product on the gastric evacuation rate by the fish. In this study two batches of each experimental diet will be made to contain one of two markers (ytterbium or yttrium based). One marked feed will be fed over approximately one week to achieve high feed intake and then replaced by the other marked feed. Faeces will be collected at regular intervals prior to the change and up to a week following the change. Analysis of the faeces will be used to show the time frame of the replacement of one marker by the other, which would be used to establish the rate of gastric evacuation. Faeces will have to be collected by settlement since handling fish is likely to influence gastric evacuation.

The effects of different carbohydrates on gastric evacuation and digestion were investigated in Atlantic salmon, principally to compare starch or cellulose with lupin non-starch polysaccharides (Irwin 2003). These three carbohydrates were added to diets containing fish meal or fish meal plus lupin protein concentrate. A final diet containing lupin kernel meal instead of the lupin protein concentrate plus non-starch polysaccharides was

also used. Diets containing lupin non-starch polysaccharides had significantly lower digestibility than those containing starch and were little different to diets with cellulose for DM and crude protein digestibility. Gastric evacuation fitted an exponential model, these predicted that time for 90% of contents to be evacuated were between about 33 h for fish meal + starch diet and 70 h for fish meal + cellulose diet. The kernel meal (33 h) appeared to have a shorter gastric evacuation time than the lupin concentrate + non-starch polysaccharides (42 h). Further exploration of gastric evacuation will be conducted in the next year of the research.

Acknowedgements

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Value-added lupin products for prawn feeds

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Introduction

Initial research into the use of lupins in aquaculture feeds for prawns was carried out as part of the FRDC Fishmeal Replacement Sub-program. This research determined the digestibility of well whole and dehulled lupin meal (lupin kernel meal, LKM) from the most widely grown cultivar of lupins (*L. angustifolius* cv Gungurru) when used in feeds for the black tiger prawn, *Penaeus monodon* (Smith et al., 1998). Further research suggested that LKM could be included at up to 30% in prawn feeds without a detrimental effect on production, but that there was a loss of performance at higher inclusion levels (Smith and Tabrett, 2003).

In 2000, the GRDC funded a three-year research project to identify the factors that limited the use of lupins in prawn feeds and to develop methods to improve the efficacy of lupins as fishmeal replacements. This project identified a number of factors in lupin meal that affected the digestibility and the nutritional value of the feed (Smith, 2002). It also found that the lupin protein was highly digestible - more digestible than the protein in fishmeal. Though attempts were made to improve the nutritional value of LKM so that more of it could be included in the feed without loss of performance, the results were inconclusive. However, recent research at CSIRO (Smith and Tabrett, unpublished data) has suggested that there is a significant difference between the growth rate of prawns fed diets containing the *L. angustifolius* cultivar, Gungurru, used in the original FRDC Fishmeal Replacement Sub-program, (and held as the standard since then) and those fed diets containing equivalent levels of LKM derived from some of the more recently developed varieties or cultivars.

A new research proposal, "Development of value-added plant protein products for the aquaculture feeds sector", submitted by Centre for Legumes in Mediterranean Agriculture in Western Australia with the Western Australian Department of Fisheries, University of Tasmania and CSIRO Marine Research, is currently being considered by the FRDC. The research outlined in this proposal is designed to complement the current GRDC research into the development of new grain-based feed ingredients for aquaculture. In the CSIRO Marine Research component of the FRDC project, it is proposed that the grain-based ingredients (mostly lupin products) that are produced by the GRDC program will be evaluated in feeds for the black tiger prawn, and that strategies will be developed to offset any limitations that are identified in the use of these products.

Proposed Research

1. Determine the nutritional value of selected grain products developed as part of the linked CLIMA-GRDC project when included in feeds for Black tiger prawns

A digestibility experiment will be carried out at the start of the project in 2004, to determine the apparent digestibility of dry matter, protein, energy and essential amino acids in selected grain protein meals and/or grain protein concentrates developed as part of the linked CLIMA-GRDC Project. A further digestibility experiment will be conducted 12 months later to evaluate products produced by the CLIMA-GRDC Project in the intervening period. The final choice of which products will be evaluated in these digestibility studies will be made in close consultation with the grains processing and aquaculture feed industries. The digestibility of the grain products will be determined using the reference diet substitution method. Our previous research (GRDC CSM1) has shown that increasing levels of lupin non-starch polysaccharides have an adverse effect on diet protein digestibility. Therefore ingredient digestibility may be determined at two substitution levels: 30 and 50%, with 6 replicates /treatment. The value of this option versus an option involving an increased number of ingredients evaluated at only one substitution level (30%), will also be considered in consultation with the Principal Investigator and stakeholders.

2. Determine the nutritional value of selected grain products for black tiger prawns

In consultation with the Principal Investigator and stakeholders, some of the grain products evaluated in the digestibility studies, will be evaluated further in 6 to 8 week growth assays, two in 2004/05 and with the option of a third experiment in 2005/06. The growth assays will typically involve the evaluation of one product in each experiment. The dietary treatments in the experiment will consist of a basal diet and five other diets in which fishmeal in the basal formulation is serially replaced with the grain product. Adjustments will made to dietary starch, fish oil and vegetable oil to maintain the digestible protein and lipid content and the fatty acid composition constant across diets. The inclusion of the grain products will be up to the point where at least 50% of the digestible protein of marine origin is replaced with grain protein. This approach provides dose response data which enables the maximum inclusion level to be determined, and the point at which there is a change in response due to the inclusion of the grain product in the feed. This information is valuable for feed formulation and from a quality assurance perspective. A commercial prawn feed will also be included among the treatments as a control.

3. Consolidation Experiments

From the results of the digestibility study and the growth assays, and in consultation with the Principal Investigator and industry stakeholders, a decision will be made on the priority issue to be addressed in the third growth assay experiment. At this stage it appears that it would be to address one of the following issues: a comparison of soybean meal with the priority grain product, an investigation into the effect of lupin oligosaccharides on prawn growth response or an investigation into the effect of the low methionine content of lupin protein

3.1. Comparison between solvent-extracted soybean meal and the priority grain product

The pricing of lupins is generally linked to the price of soybean meal, being approximately two thirds of its price. Due to the high price of soybean meal, feed mills are starting to look for economical alternatives that can be used in its place. In this study, it is proposed that a growth assay, as described above, be carried out with solvent-extracted soybean meal in one series of diets and a selected lupin product to compare the responses due to fishmeal replacement with soybean meal and with the lupin product. This data will provide a realistic basis for the pricing of the lupin products for the aquaculture feed market.

3.2. Effect of lupin oligosaccharides on diet digestibility and nutritional value

Lupin oligosaccharides have been implicated as anti-nutritional factors that appear to be indigestible in the stomach and small intestine of monogastric animals. High levels of raffinose series oligosaccharides are also believed to interfere with the digestion of other nutrients in the stomach. It is important to determine the effect of oligosaccharides in the diet of prawns, so quality assurance specifications on the maximum acceptable oligosaccharide content of prawn feeds may be determined. This would be valuable additional information that will allow feed manufacturers to use lupin products in prawn feeds with confidence.

A dose response experiment will be carried out to determine the effect of serial inclusions of lupin oligosaccharides on digestibility and growth response obtained with a reference diet. Oligosaccharides will be extracted from *L. angustifolius* kernel meal using aqueous ethanol. The oligosaccharide content of the extract will be determined and aliquots of the extract added to a reference diet that is known to be highly digestible and result in a good growth response. The apparent digestibility of dry matter, protein, energy of the diets will be measured and the growth response of prawns fed the diets will be measured over 6 weeks. There will be six inclusion levels of oligosaccharide with 6 replicates per treatment. A commercial prawn feed will used as a control treatment. Data will be analysed using regression analysis and/or broken stick analysis as appropriate.

3.3. Essential amino acid deficiency in lupins

Lupin protein has a particularly low level of the essential amino acid, methionine, such that the combined methionine+cystine level in diets could be limiting growth of prawns when lupin kernel meal is used at an inclusion level of >300 g/kg of diet, replacing fishmeal on a equivalent crude protein basis. At this inclusion level digestible lupin protein replaces about half of the digestible protein of marine origin (fishmeal, squid meal, crustacean meal) in a practical diet formulation.

The objective of this study would be to determine whether the low methionine content of lupin protein is a major factor causing a reduction of growth rate and FCR of prawns fed diets containing high inclusion levels of lupins. The experiment will be designed to demonstrate any difference in the response of prawns fed diets containing a lupin protein with and without supplementation with methionine. In this study we propose to use 2 to 3 g black tiger prawns and carry out a 6 to 8 week growth assay with dietary treatments constituting a 2 x 6 factorial experiment with 4 replicates/treatment. The diets will be formulated to contain 0 and 40% inclusion of lupin protein concentrate, at the expense of fishmeal, corn starch and with the addition of fish oil such that they all contain the same concentration of digestible protein and lipid, and have a similar fatty acid composition. Each of these diets will be supplemented with serial inclusions of methionine (e.g. at 0, 0.5, 1.0, 1.5, 2.0 and 2.5% of diet) such that the apparent methionine deficiency will be partially supplemented, fully supplemented or provided in excess within the series. Methionine will be provided either in the free crystalline form or in a microencapsulated form. Though free crystalline methionine is highly soluble and significant losses from the diet would occur through leaching, at this stage, it is the only practical way for commercial feed manufacturers to add supplementary methionine to a feed. To provide a measure of the leaching loss, the methionine content of the diets will be determined 'as prepared' and after 30 min and 60 min immersion in water. The growth rate, apparent feed intake and survival of prawns in the study will be recorded. Data will be analysed using regression analysis and as a two-way ANOVA.

Conclusion

A key objective of the proposal is to provide grain producers, grain processors, aquaculture feed manufacturers and the prawn and salmon aquaculture industries with information about the nutritional characteristics and quality assurance criteria of the products so that they can be marketed and used with confidence in aquaculture feed formulations. The results of the research will be communicated directly to industry stakeholders at an annual workshop. In addition, an annual update focusing on the prawn component of the project will be provided to the Australian Prawn Farmers Association by CSIRO Marine Research.

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Product evaluation update - Functionality

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Introduction

Irrespective of how good an ingredient may be nutritionally if it is to be a useful feed ingredient then it must also be conducive to being used in extrusion processing. Typically all modern fish diets are produced using extrusion technology. In this process the ingredients are blended, moistened and they cooked whilst under pressure. The process allows the generation of improved binding properties in the final product and also an increase in the porosity of the product.

In high-energy salmonid feeds this porosity is useful in that under both passive and vacuum infusion processes it is possible to further increase the oil content of the pellet. The increase in binding strength of the pellet is also a benefit as it reduces the amount of fines from the product and also increases the durability of product relative to compression produced pellets.

Extrusion of lupin kernel meals

The primary purpose for using extrusion processing in the manufacture of aquaculture feed is to produce a stable pellet with physical attributes appropriate for the target species. The design of a feed for any given species requires consideration of a range of physical characteristics such as sinking rate, dry stability, wet stability, mechanical strength, size, shape, texture, oil and water absorption.

Lupin kernel meal (*L. angustifolius*) has been shown to be a well accepted and nutritionally valuable ingredient in many aquaculture diet applications. The protein and lipid components of lupin kernel meal constitute most of the digestible energy of this valuable feed ingredient with the lupin protein often superior in digestible value to that of other plant and animal protein sources (Glencross 2003).

Previous preliminary studies have investigated the processing of lupins in diets for fin-fish and salmonoids using extrusion technology (Evans 1999). Resulting diets had a range of characteristics depending on the processing conditions used. Notably the pellets tended to have higher bulk densities, greater durability, faster sinking rates, and reduced oil absorption. A limitation of these studies was their single inclusion rate of lupin kernel meal of 30%, not allowing for 'dose response' trends.

To determine the effect of a *L. angustifolius* kernel meal replacement of fishmeal on pellet quality a series of kernel meal inclusion levels of 0%, 5%, 10%, 15%, 20%, 25% and 30% (Table 20) were extruded. The extruder used was a pilot scale twin screw extruder model APV MFP 19 with intermeshing, co- screws. The extruder barrel was smooth walled, open clam type design having dimensions 19mm x 475mm (diameter x length).

Lupin inclusion increased the Radial Expansion, Pellet Volume (total expansion) and Oil Absorption in a non linear fashion until the 20% inclusion rate and then decreased below the control at the 30% inclusion rate. Bulk density was the mirror image of Radial expansion, decreasing until the 20% inclusion rate and then increasing again until at the 30% inclusion rate the Bulk density is higher than the control. Sink rate followed the Bulk density trend decreasing until 20% then increases again.(Figures 16a,b,c d & e). There was a linear correlation found between Pellet volume and Oil Absorption (r^2 =0.81).

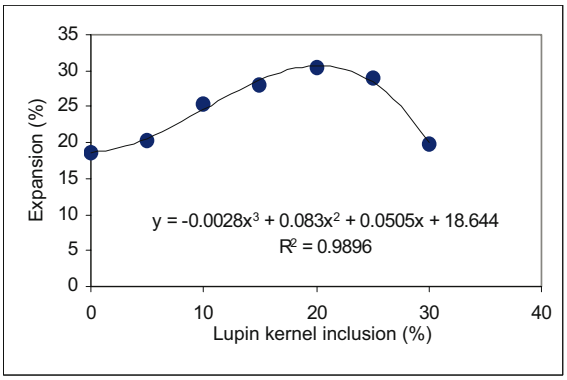

Fig. 16a. Effect of lupin inclusion on radial expansion ie pellet diameter.

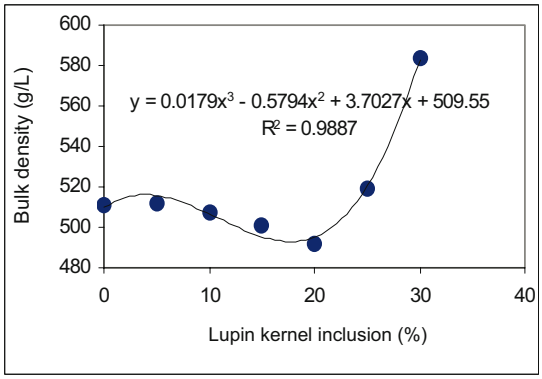

Fig. 16b. Effect of lupin inclusion on bulk density.

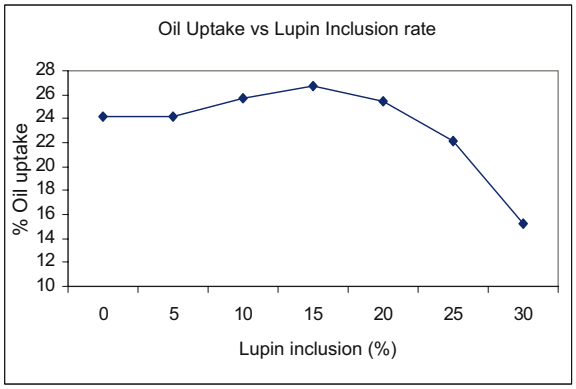

Fig. 16c. Effect of lupin inclusion on Oil Absorption.

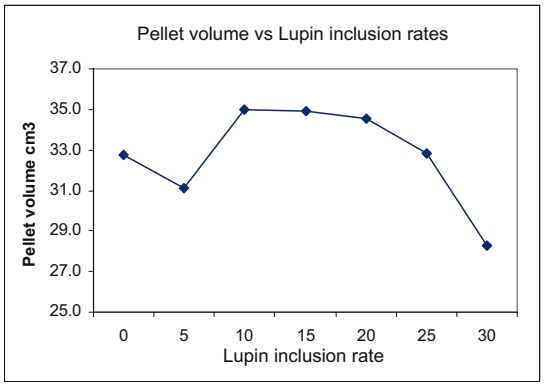

Fig. 16d. Effect of lupin inclusion on Pellet Volume ie total expansion.

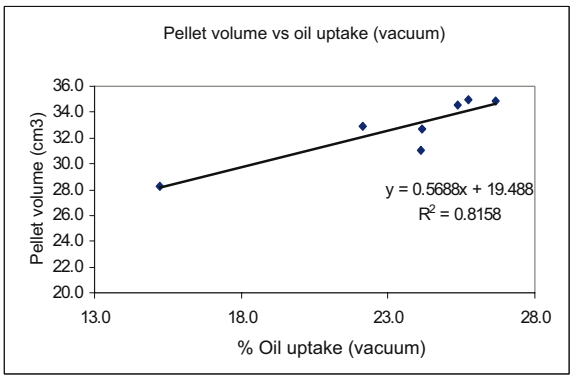

Fig. 16e. Effect of lupin inclusion on Oil Absorption.

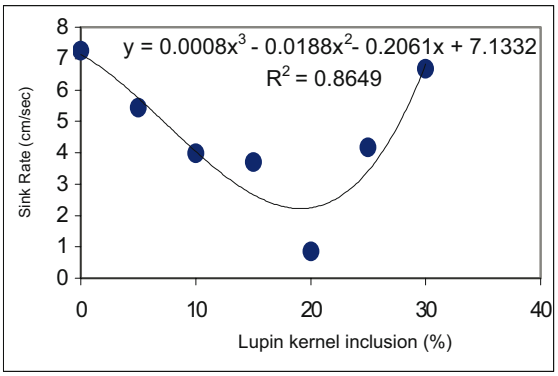

Fig. 16f. Effect of lupin inclusion on Sink Rate.

Figure 16.

Expansion is due to the temperature and pressure difference at the extruder die causing water vapour to flash off and stretch the starch molecules. It appears that at inclusion levels under 20% the lupin non-starch polysaccharides are taking up extra hydration in the extruder and on exiting the die – this water is flashed off, allowing for a porous open structure in the pellet. However when the inclusion levels are higher than 20% then active competition for the water between the starch molecules and the lupin NSP's impact on starch gelatinisation which is the most important factor in expansion and pellet binding. However torque seems to steadily decrease with increasing lupin inclusion until the 20% rate and then plateaus along these lines.

However, experimental work with extruders can be costly and time consuming. These restrictions limit the throughput of treatments and therefore limit the amount of data that can be generated. Rapid Viscosity Analysis is a relatively new technique adopted from the food technology industries where it is used in assessing gelatinisation and agglutination properties of foods.

Table 20. Formulations for complete diets and extrusion mashes and intended final composition of the complete diet formulation.

	REFERENCE DIET		LUPIN KERNEL MEAL DIET		DIET COMPOSITION	g/kg
	Complete	Extruder	Complete	Extruder		
Pre-mix vitamins	0.5	0.60%	0.5	0.60%	Dry matter	922
Cellulose	11.1	13.33%	0.0	0.00%	Protein	417
Wheat starch	2.0	2.40%	2.0	2.40%	DCP	360
Fish oil	17.0	0.36%	16.7	0.00%	Fat	220
Wheat gluten	4.4	5.28%	4.4	5.28%	Carbohydrate	199
Wheat flour	10.0	12.00%	10.0	12.00%	Phosphorus	14
Fish meal	55.0	66.03%	36.4	43.70%	Ash	85
Sweet lupin kernel	0.0	0.00%	30.0	36.01%	Gross Energy (MJ/kg)	22.0
					Estimated Digestible Energy (MJ/kg)	18.5
					Dry matter Gross Energy (MJ/kg)	23.8

Rapid Viscosity Analysis of Ingredients

Rapid Viscosity Analysis (RVA) is a technique used to assess the rheology of whatever sample is being assessed. It measures the resistance to a small rotor inside a cup in which a sample is contained. The moisture content and thermal management of this cup can also be varied to allow some mimicry of conditions as they occur under extrusion. Typically the thermal regimes used are one of 50°C for 2 minutes, ramping temperature up to 95°C over 3 minutes, holding at 95°C for 3 minutes before cooling to 50°C over 3 minutes, then holding at 50°C for a further 2 minutes (Figure 17). Ideally the RVA data need to be correlated with extrusion data to provide meaningful interpretation, but relative hydration responses and peak viscosities provide an indication of prospective extrusion energy and water demands.

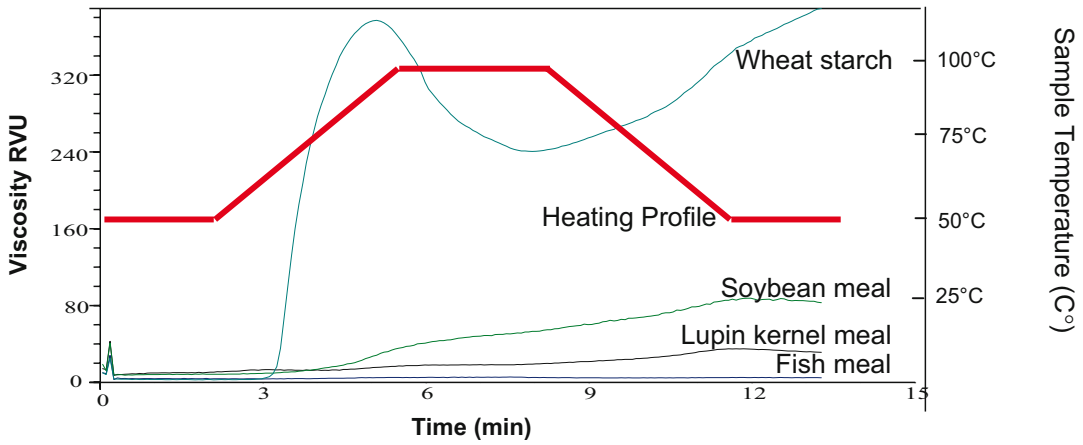

Figure 17. RVA profile of wheat starch, soybean meal, lupin kernel meal and fish meal on a standard heat-hold-cool program.

Rapid Viscosity Analysis of Feed Ingredient Mixtures

Whilst RVA evaluation of individual ingredients provides information about the functionality of that ingredient in isolation, in practicality ingredients are always processed and extruded as mixtures. To examine the influence of a L. angustifolius kernel meal on the RVA profile of a feed mixture two diets were formulated to the same nutrient specification and blended to make a series of diets with lupin kernel meal inclusion levels of 0%, 5%, 10%, 15%, 20%, 25% and 30% (Table 20). Formulations were mixed without their oil components to make an extruder mash.

The RVA profile of each of the extruder mashes and their blends was also evaluated using the standard heat-hold-cool protocol (Figure 18). It was noted that with increasing inclusion of lupin both the initial peak (A) and final (B) viscosities increased. A preliminary hydration response (C) was also observed and this also increased with increasing lupin inclusion. However the rate of increase in each of the viscosities was not linear, with dramatic increases in viscosity seen from

the 5% to the 10% inclusion level and from the 25% to the 30% inclusion level (Figure 19). These observations are notable in that they are also consistent with the attributes of the pellets produced from extrusion of the same mash.

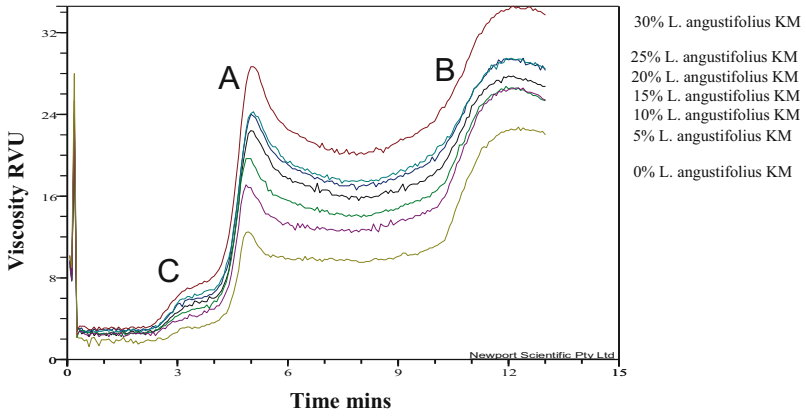

Figure 18. RVA profiles of increasing the lupin kernel meal content of a extrusion mix for a salmonid diet. The initial peak (A) and final (B) viscosities and the preliminary hydration response (C) are indicated.

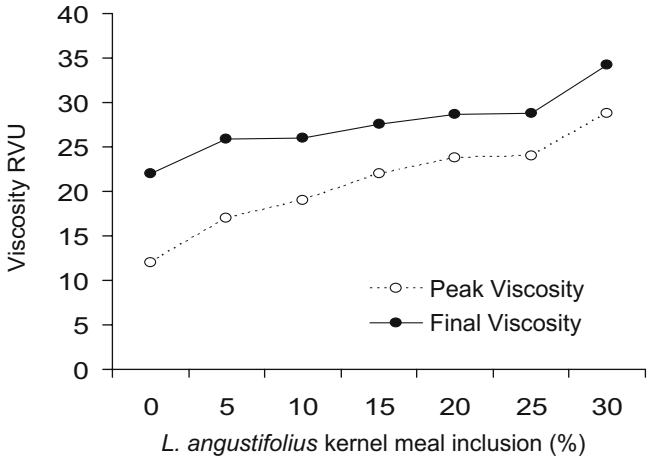

Figure 19. Relative viscosities of the initial peak and final RVA profiles of extruder mashes with increasing content of *L. angustifolius* kernel meal.

Rapid Viscosity Analysis of Lupin Kernel Meals

There are about 200 different species of lupins although only a handful of species are actually grown as crop species (Gladstones, 1998). Of those species grown *Lupinus angustifolius* constitutes the bulk of production. However, within this species there are also numerous cultivars varieties, each with different characteristics.

An evaluation was made on the variability between the lupin species with respect to their RVA profile (Figure 20). From this study it was shown that *L. atlanticus* kernel meal had the highest peak and final viscosities. It also has the lowest protein content of the kernel meal varieties examined. *L. luteus* kernel meal had the lowest peak and final viscosities. Subtle differences were also noted between two different cultivars of *L. angustifolius*. Because of this observed variability within this species further evaluations on the RVA profiles were made on a suite of cultivars of *L. angustifolius* (Figure 21). Within this study highest peak and final viscosities were observed from the Belara variety and the lowest peak and final viscosities from the Myallie variety.

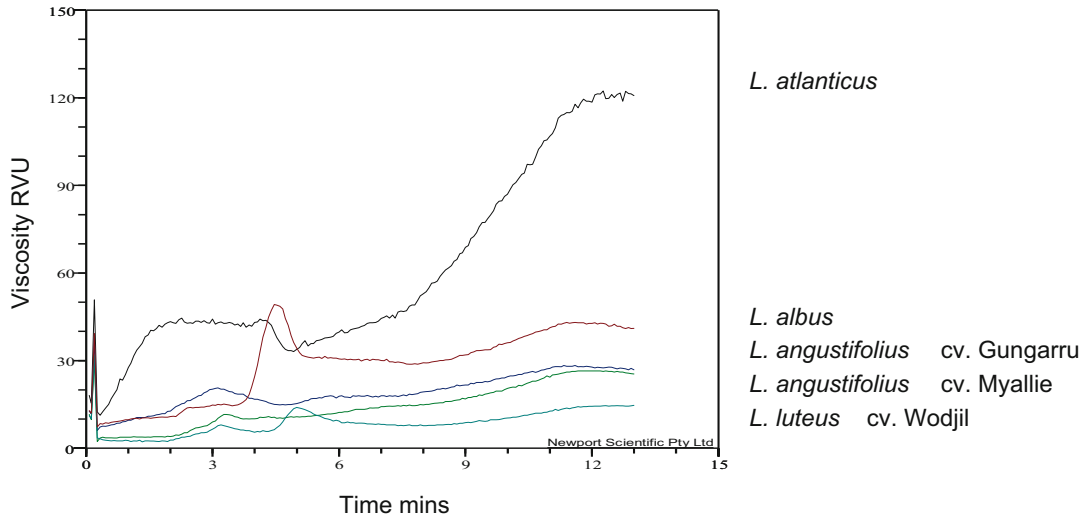

Figure 20. RVA profiles of various lupin species kernel meals.

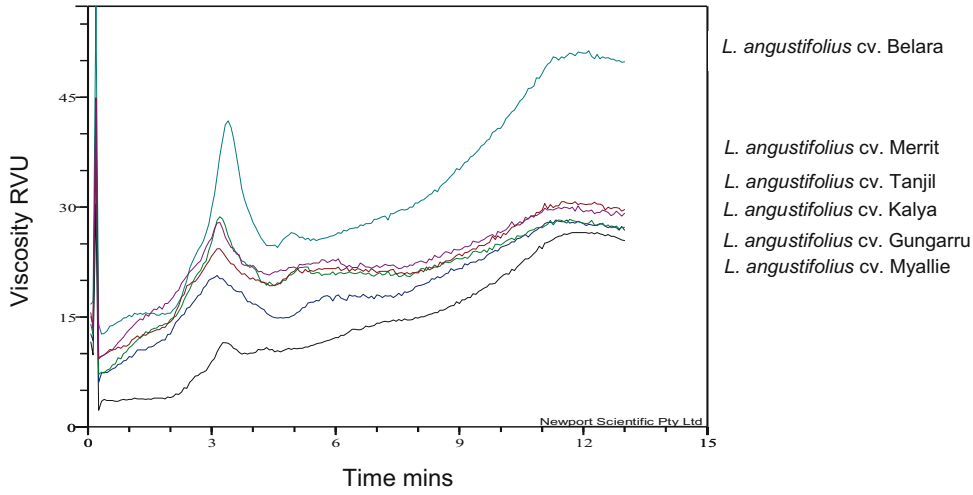

Figure 21. RVA profiles of various L. angustifolius kernel meal varieties/cultivars.

Rapid Viscosity Analysis of Lupin Protein Concentrates

An evaluation was made on the variability between the different value-added lupin products with respect to their RVA profile (Figure 22). From this study it was shown that the kernel meal had the highest initial peak viscosity, but that the protein concentrate had a higher final viscosity. With further removal of the non-starch polysaccharide (NSP) components of the meals a further decrease in both initial peak and final viscosities was observed. From this observation it was concluded that some of the NSP fractions of lupin kernel meals are the main contributing factors to functionality of the ingredient, but that some aspects of this functionality may still be related to the protein composition.

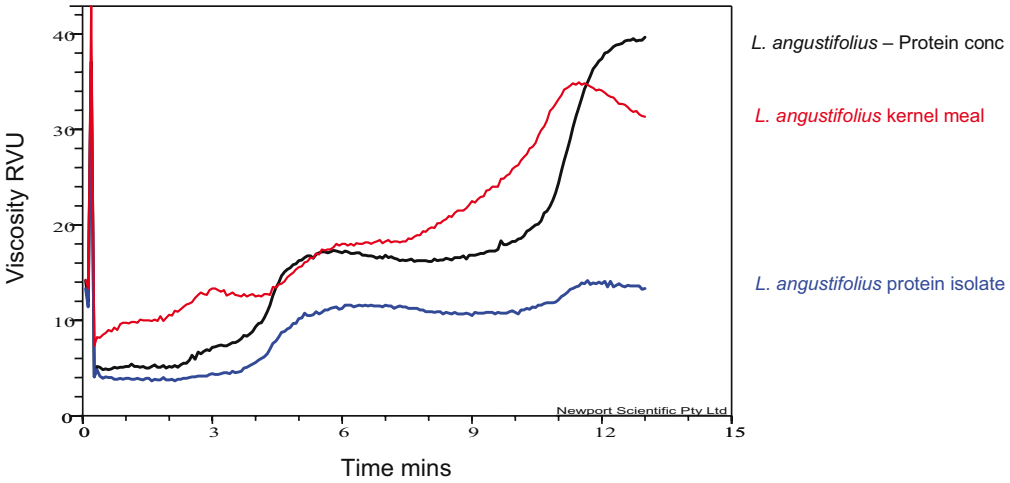

Figure 22. RVA profiles of *L. angustifolius* kernel meal, protein concentrate and protein isolate.

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Gladstones, J.S. 1998. Distribution, Origin, Taxonomy, History and Importance. In: Lupins and Crop Plants: Biology, Production, and Utilisation (J.S. Gladstones, C.A. Atkins and J. Hamblin Eds.) CABI Publishing, Cambridge, UK. pp 475.

Glencross, B.D. (Ed) 2005. *Proceedings of the first workshop for Seeding a Future for Grains in Aquaculture Feeds - 28 May 2003*, Fisheries Occasional Publications No. 22, Department of Fisheries, Western Australia, 79p.

Appendix 1 – Project Publications

- Glencross, B.D., Carter, C.G., Duijster, N., Evans, D.R., Dods, K., McCafferty, P., Hawkins, W.E., Maas, R., Sipsas, S. (2004) A comparison of the digestibility of a range of lupin and soybean protein products when fed to either Atlantic salmon (*Salmo salar*) or rainbow trout (*Oncorhynchus mykiss*). Aquaculture 237, 333-346.

Appendix 2 – Other Relevant Publications

- Glencross, B., Curnow, J., Hawkins, W. (2003) Evaluation of the variability in chemical composition and digestibility of different lupin *(Lupinus angustifolious)* kernel meals when fed to rainbow trout *(Oncorhynchus mykiss*) Animal Feed Science and Technology 107, 117-128.
- Glencross, B., Evans, D., Hawkins, W., Jones, B. (2004) Evaluation of dietary inclusion of yellow lupin (*Lupinus luteus*) kernel meal on the growth, feed utilisation and tissue histology of rainbow trout (*Oncorhynchus mykiss*) Aquaculture 235, 411-422.